

**ATTACHMENT 1: STATEMENT OF WORK  
FOR TASK ORDER: 75N93021F00002**

**PRECLINICAL DEVELOPMENT SERVICES FOR AIDS THERAPEUTICS  
CONTRACT NUMBER: HHSN272201400006I**

**TASK AREA 3 – PHARMACOLOGY AND TOXICOLOGY: ADDITION OF FINAL  
BIOANALYTICAL LONG-TERM STABILITY TIME POINTS FOR GLP STUDIES OF**  
**IN RATS AND DOGS**

Proprietar  
y

Proprietary Info

**STATEMENT OF WORK**

**A. BACKGROUND**

Proprietary Info is an injectable liposomal nanoformulation of three antiretrovirals Proprietary Info  
Proprietary Info. In 2019, under Task Order No. HHSN27200008 of this contract, the Contractor completed GLP toxicity studies of Proprietary Info in rats (Study M398-18, Bioanalytical Validation Report B181-18) and dogs (Study M397-18, Bioanalytical Validation Report B185-18). Upon completion of these studies, plasma samples were collected and stored below -60°C at the contractor's site to determine long term stability and the maximum length of storage of Proprietary Info plasma samples, as required by the FDA.

**B. SCOPE**

The Contractor shall conduct final long-term stability time points of Proprietary Info in rat and dog plasma from the two previously completed GLP studies under Task Order No. HHSN27200008 of this contract, and provide Amended Final Reports related to these two completed studies, which shall include the data from the final long-term stability time points of Proprietary Info in rat and dog plasma associated with this task order.

**C. TECHNICAL REQUIREMENTS**

Independently and not as an agent of the Government, the Contractor shall furnish all the necessary services, qualified personnel, materials, equipment, and facilities, not otherwise provided by the Government under the terms of the Task Order, as needed, to complete the activities listed below. Specifically, the Contractor shall:

1. Conduct final long-term stability time points for plasma samples of Proprietary Info from the studies M398 -18 and M397-18 that are currently being stored at SRI below -60°C.
2. Amend Final Reports for Bioanalytical Validation Studies of Proprietary Info in rat plasma (B181-18) and dog plasma (No. B185-18) to include the final stability data.
3. Amend the Final Reports for Studies M397-18 and M398-18 to include a reference to the revised Validation Study reports.
4. Audit the amended Final Reports by the Quality Assurance Unit to confirm GLP compliance of the additions.

#### **D. PROJECT MANAGEMENT**

The Contractor shall provide the basic infrastructure to successfully manage the following activities during the performance of this task order:

1. Plan, initiate, implement, manage, and coordinate study activities conducted under this Task Order. Monitor overall progress within this Task Order and coordinate with the base contract's management team in fulfilling its requirement to provide Monthly Progress Reports that summarize progress across all awarded Task Orders.
2. Submit Study Protocols, in accordance with the Reporting Requirements and Deliverables section of this Task Order. Unless indicated otherwise, Study Protocols will be requested by the COR on a case by case basis and shall describe the experimental design and methodology proposed for all work to be conducted under an individual study. Study Protocols are subject to technical review by the COR, and recommended revisions shall be incorporated, as needed, prior to the initiation of the proposed studies.
3. Provide task order-related information and updates of study-related activities to the COR upon request.
4. The Contractor shall prepare and submit the following reports and other deliverables in accordance with the DELIVERIES Article in SECTION F of this Task Order:
  - a. Study Protocol(s)
  - b. Study Report(s)
  - c. Copies of Task Order-related Information and Updates of Study-related Activities
  - d. Draft Amended Final Reports (Final Reports from Task Order HHSN27200008, which are amended to include all data from the work associated with this current task order).
  - e. Final Versions of the Amended Final Reports (Final Reports from Task Order HHSN27200008, which are amended to include all data from the work associated with this current task order).

**[END OF STATEMENT OF WORK]**



**ATTACHMENT 1: STATEMENT OF WORK  
PRECLINICAL DEVELOPMENT SERVICES FOR AIDS THERAPEUTICS**

**Contract No. HHSN272201400006I/Task Order No. HHSN27200008**

**PHARMACOLOGY AND TOXICOLOGY STUDIES OF [Proprietary Info]  
[Proprietary Info] AND [Proprietary Info] AND TO SUPPORT THE DEVELOPMENT OF  
MITOCHONDRIAL TOXICITY ASSAYS**

**STATEMENT OF WORK**

**A. SCOPE**

The Contractor shall conduct pharmacology and toxicology studies of [Proprietary Info] [Proprietary Info] and to support the development of mitochondrial toxicity assays.

**B. TECHNICAL REQUIREMENTS**

Independently and not as an agent of the Government, the Contractor shall furnish all the necessary services, qualified personnel, materials, equipment, and facilities, not otherwise provided by the Government under the terms of the contract, as needed, to perform the activities listed below. Specifically, the Contractor shall:

1. Conduct a Good Laboratory Practice (GLP) 28-Day Repeat Dose Toxicity Study in dogs with [Proprietary Info]. The study design and the required evaluation endpoints are described in the attached SRI Study Protocol (Attachment A).
2. Conduct a 5-week repeat dose non-Good Laboratory Practice (non-GLP) dose-range-finding and toxicokinetics study in dogs with [Proprietary Info] a fixed-dose-combination (FDC) of [Proprietary Info] which is being developed for the treatment of HIV infection. The study design and required evaluation endpoints are described in the SRI Study Plan/Protocol (Attachment B).
3. Conduct a GLP 5-week GLP repeat dose toxicity study in rats with [Proprietary Info] a fixed-dose-combination (FDC) of [Proprietary Info] which is being developed for the treatment of HIV infection. The study design and required evaluation endpoints are described in the SRI Study Plan/Protocol. (Attachment C).
4. Conduct a non-GLP, toxicity pharmacokinetic study in C57BL/6 mice with [Proprietary Info] [Proprietary Info]. The study design and required evaluation endpoints are described in the SRI Study Plan/Protocol (Attachment D).
5. Conduct a study to assess in vitro mitochondrial toxicity of potential therapeutic agents. The Contractor shall incorporate the following activities into the study:
  - a. Continue the study to assess in vitro mitochondrial toxicity of potential anti-TB agents, described in the previous contract year. Specifically, in collaboration with an outside collaborator, continue the assessment of in vitro mitochondrial toxicity of [Proprietary Info] and its analogues in [Proprietary Info]. A total of ~ 150 cell samples will be tested.

- b. Continue development and validation of mitochondrial assays, and assessment of the in vitro mitochondrial toxicity of potential in antiretroviral drugs, initiated in the previous contract year. Specifically,
  - i. set up a 3-dimensional (3D) cell culture in up to 2 cell lines,
  - ii. optimize and identify culture conditions,
  - iii. determine cytotoxicity in the 3D cell culture in 2 control drugs with exposure times of up to 28 days;
  - iv. Compare the results of 3D culture, 2-dimensional (2D) culture, and/or with hollow-fiber reactor. A total of ~150 samples will be tested.
  - v. Evaluate mitochondrial toxicity profiles of 3 antiretroviral drugs for up to 28-day exposure, in vitro in the selected cell lines of 2D, 3D cell culture. A total of ~100 samples will be tested.
  - vi. Submit results to scientific conference and/or peer-reviewed journal, as appropriate.
6. Prior to commencing any work involving laboratory animals, the Contractor shall submit all protocols to the COR for review/approval, using the appropriate format, following current FDA and/or ICH guidelines for study conduct, and obtain Institutional Animal Care and Use Committee (IACUC) approval for each animal study listed above, and provide the COR and Contracting Officer with documentation of each approval.
7. Submit Study Reports to the Contracting Officer's Representative (COR) and the Contracting Officer, at the conclusion of each of the five (5) studies detailed above. Please note the above two GLP studies (#1 and #3) and the two non-GLP studies (#2 and #4) shall provide data of suitable quality and integrity, in order to support applications to the U.S. Food and Drug Administration (FDA) and other regulatory agencies. The GLP studies shall be performed in accordance with the U.S. FDA "Good Laboratory Practice for Nonclinical Laboratory Studies" (GLP), as described in 21 CFR Part 58, and the Contractor shall retain all records, as required, under GLP guidelines. The Contractor shall submit a summary of the results of each study to the COR, using the format specified in the SRI International Protocols document. Work under this Task Order shall be deemed complete upon technical review and acceptance by the COR and the Contracting Officer of the Final Report.

### **C. PROJECT MANAGEMENT**

1. Plan, initiate, implement, manage, and coordinate study activities conducted under this Task Order. Monitor overall progress under this Task Order and coordinate with the base contract's management team, in fulfilling its requirement to provide Monthly Progress Reports that summarize progress achieved across all awarded Task Orders.
2. Submit Study Protocols, in accordance with the Reporting Requirements and Deliverables section of this Task Order. Study Protocols will be requested by the COR on a case by case basis and shall describe the experimental design and methodology proposed for all work to be conducted under an individual study. Study Protocols are subject to technical review by the COR, and recommended revisions shall be incorporated, as needed, prior to the initiation of the proposed studies.
3. Provide task order-related information and updates of study activities to the COR upon request.

### **END OF STATEMENT OF WORK**



Final Report • May 15, 2019

# MAXIMUM TOLERATED DOSE AND PHARMACOKINETIC STUDY OF Proprietary Info FOLLOWING A SINGLE DOSE ADMINISTRATION TO MALE AND FEMALE C57BL/6 MICE

**Author:**Redacted by agreement**Testing Facility:**

SRI International  
Biosciences Division  
333 Ravenswood Avenue  
Menlo Park, CA 94025

**SRI Study Number:** B173-18  
**SRI Project Number:** P25035.413

**Study Initiation:** October 1, 2018

**Experimental Work Performed:**

**Start:** October 2, 2018  
**Finish:** February 4, 2019

**Study Completion:** May 15, 2019

**Sponsor:**

National Institute of Allergy and Infectious Disease  
Division of AIDS  
5601 Fishers Lane, Redacted by agreement  
Bethesda, MD 20892

**Sponsor's Representative:**Redacted by agreement

**DAIDS Contract Number:** HHSN272201400006I, Task Order #8

Maximum Tolerated Dose and Pharmacokinetic Study of Proprietary Info  
Following a Single Dose Administration to Male and Female C57BL/6 Mice  
SRI Study No. B173-18

APPROVAL SIGNATURES

Written By:

Personal Info

Redacted by agreement

Study Director

05/15/19  
Date

Personal Info

Redacted by agreement

Pharmacokineticist

5-15-19  
Date

Approved By:

Personal Info

Redacted by agreement

5-15-19  
Date

SRI International  
Biosciences Division  
333 Ravenswood Avenue  
Menlo Park, CA 94025



**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

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**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**SUMMARY**

The objective of this study was to determine the tolerability and the plasma pharmacokinetics of Proprietary Info following an intravenous (iv), intraperitoneal (ip) or oral gavage (po) administration. Male and female C57BL/6 mice were given a single dose administration of the test article.

This study was conducted in two phases. During Phase A, male and female mice (1/sex/group) were administered Proprietary Info as a single dose at 20 mg/kg (iv), 20 mg/kg (ip), or 40 mg/kg (po) and observed immediately postdose, 2-4 hr and 24 hr postdose for signs of toxicity. Based on normal clinical observations noted in each dose group, the dose levels were increased, and new mice were treated at 40 mg/kg (iv), 40 mg/kg (ip) or 100 mg/kg (po). At

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The Phase B dose levels selected in consultation with the Sponsor were 20 mg/kg (iv), 40 mg/kg (ip) and 100 mg/kg (po). During Phase B, male and female mice (12/sex/group) were given a single dose administration. Blood was collected for drug plasma levels at 0.083, 0.25, 0.5, 1, 2, 4, 8 and 24 hr postdose (iv, ip) and 0.25, 0.5, 1, 2, 4, 6, 12 and 24 hr postdose (po). Clinical observations were documented immediately postdose and prior to the last blood collection. Proprietary Info

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**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
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**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**KEY PERSONNEL**

**Name**

**Functional Role**

Redacted by agreement

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**Maximum Tolerated Dose and Pharmacokinetic Study of**  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

## **I. PURPOSE OF STUDY**

The purpose of this study was to provide data that can be used to support research efforts. It was exploratory and not within the scope of U.S. Food and Drug Administration (FDA) "Good Laboratory Practice for Nonclinical Laboratory Studies" (GLP) as described in 21 CFR Part 58. Nevertheless, the study was planned, performed, recorded and reported in accordance with standard practices to ensure data quality and integrity.

## **II. OBJECTIVE OF STUDY**

The objective of this study was to determine the tolerability and the plasma pharmacokinetics of Proprietary Info following an intravenous (iv), intraperitoneal (ip) or oral gavage (po) administration. Male and female C57BL/6 mice were given a single dose administration of the test article.

The protocol and amendments are presented in Appendix A.

## **III. EXPERIMENTAL DESIGN**

### **Phase A: Maximum Tolerated Dose (MTD)**

<b>Group</b>	<b>Treatment <sup>a</sup></b>	<b>Dose Route</b>	<b>Dose Level (mg/kg) <sup>b</sup></b>	<b>Dose Conc. (mg/ml) <sup>b</sup></b>	<b>Dose Volume (ml/kg)</b>	<b>No. of Animals</b>
1	Proprietary Info	iv	20	4	5	1M/1F
2		iv	40	8	5	1M/1F
3		iv	20	4	5	1M/1F
4		ip	20	2	10	1M/1F
5		ip	40	4	10	1M/1F
6		ip	40	4	10	1M/1F
7		po	40	4	10	1M/1F
8		po	100	10	10	1M/1F
9		po	100	10	10	1M/1F

<sup>a</sup> Phase A was conducted using escalating or de-escalating dose levels (e.g., escalating to 40, 100 mg/kg, etc. until clinical effects were seen, then doing one more escalation or de-escalation based on observations seen at the previous dose level).

<sup>b</sup> The dose level and dose concentration were based on the free base form of the test article. The dose formulations were prepared based on the salt content; with a correction factor of 1.10.

### **Species and Strain**

Mouse and C57BL/6

### **Route of Administration**

iv (via tail vein given over 30 seconds), ip or po (via gavage)

**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
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**Frequency**

Single dose starting on Day 1; mice were treated as indicated in the table above.

Clinical observations were performed immediately postdose, 2-4 hr postdose and prior to euthanasia at 24 hr. All surviving mice were euthanized at 24 hr postdose.

**Dosing Volume**

iv: 5 ml/kg

ip and po: 10 ml/kg

Dose volumes were calculated based on each animal's Day 1 body weight

**Duration of In-Life Phase**

24 hr per dose group

**Phase B: Pharmacokinetics (PK)**

Group	Treatment	Dose Route	Dose Level (mg/kg) <sup>a, d</sup>	Dose Conc. (mg/kg) <sup>a</sup>	Dose Volume	No. of Animals <sup>b</sup>	Blood Collection Time Points <sup>c</sup>
1	Proprietary Info	iv	20	4	5	12M/12F	0.083, 0.25, 0.5, 1, 2, 4, 8 and 24 hr
2		ip	40	4	10	12M/12F	0.083, 0.25, 0.5, 1, 2, 4, 8 and 24 hr
3		po	100	10	10	12M/12F	0.25, 0.5, 1, 2, 4, 6, 12 and 24 hr

<sup>a</sup> The dose level and dose concentrations were based on the free base form of the test article. The dose formulations were prepared based on the salt content; with a correction factor of 1.10.

<sup>b</sup> Blood was collected and processed to plasma from 3M/3F untreated mice (not included in table above) for baseline control samples.

<sup>c</sup> Two blood samples were collected from each mouse; three mice were assigned for each time point.

<sup>d</sup> Dose levels were determined based on the Phase A MTD results.

**Species and Strain**

Mouse and C57BL/6

**Route of Administration**

iv (via tail vein given over 30 seconds), ip or po (via gavage)

**Frequency**

Single dose on Day 1



**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Dosing Volume**

iv: 5 ml/kg

ip and po: 10 ml/kg

Dose volumes were calculated based on each animal's Day 1 body weight

**Duration of In-Life Phase**

24 hr

**IV. MATERIALS AND METHODS**

**A. Test and Control Articles**

**1. Test Article**

Proprietary Info

Proprietary Info

**Physical Description**

Yellow solid

**Storage Conditions**

Room temperature, 18.5° - 25.5°C

**Characterization of Test Article**

The Sponsor was responsible for characterization and stability of the test article and provided a Chemical Data Sheet to SRI for inclusion in the final report (Appendix B).

Proprietary Info

**Maximum Tolerated Dose and Pharmacokinetic Study of  
Following a Single Dose Administration to Male and Female C57BL/6 Mice  
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2.

Proprietary Info

**Physical Description**

Clear, colorless solution

**Storage Conditions**

Room temperature, 18.5° - 25.5°C

**Characterization of Vehicle**

Information on the identity, purity and stability of the vehicle was obtained by recording all of the pertinent information provided on the CofA provided by the supplier (Appendix B).

**3. Preparation of Dose Formulations**

The dose formulations were corrected for salt content, using a correction factor of 1.10.

Dose formulations were prepared aseptically by combining the appropriate amount of test article in the vehicle to achieve the target concentration. The formulations were mixed well using a vortex mixer for 2 min or by magnetic stirrer for 15 min. On each dosing day, the highest concentration was prepared first, and lower concentrations were diluted from the higher concentration. Dose formulations were clear, yellow solutions.

**4. Storage of Dose Formulations**

**Phase A (MTD):** Each dose formulation was prepared fresh prior to dose administration.

**Phase B (PK):** The dose formulations were prepared fresh on Day 1 and maintained at room temperature until use.

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**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
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**6. Test Article Handling**

At a minimum, personnel handling the test article formulations wore eye protection, gloves and a protective smock or laboratory coat.

**7. Disposition**

All bulk test article was retained.

Residual dose formulations will be discarded when the final report is submitted.

**8. Method for Assuring Correct Dosing**

The administration of each dose formulation was properly documented, and the amount administered to each animal was recorded.

**B. Test System**

**Species**

Mouse

**Strain**

C57BL/6

**Supplier**

Proprietary Info

**Number of Animals**

**Phase A:** 18

**Phase B:** 72

**Sex**

**Phase A:** 9 males and 9 females

**Phase B:** 36 males and 36 females (+ 3 controls per sex)

**Age at First Dose**

8-10 weeks

This deviation had no effect on the study's validity or integrity as Phase A was designed to last over 9 days, and the animals' ages were expected to be greater than the protocol-specified age range of 7-8 weeks.



Proprietary Info

**Maximum Tolerated Dose and Pharmacokinetic Study of  
Following a Single Dose Administration to Male and Female C57BL/6 Mice  
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**Weight Range at First Dose**

**Phase A:** 22.6-27.8 g (males); 19.5-23.9 g (females)

**Phase B:** 21.2-26.7 g (males); 16.9-20.5 g (females)

This deviation had no effect on the study's validity or integrity as the differences between the estimated weight ranges of 22-30 g (males) and 17-25 g (females) and the actual body weights of the animals were minimal.

**Animal Care**

General procedures for animal care and housing were in accordance with the current Association for assessment and Accreditation of Laboratory Animal Care (AAALAC) in recommendations, current requirements stated in the *Guide for the Care and Use of Laboratory Animals* (National Research Council), and current requirements as stated by the U.S. Department of Agriculture through the Animal Welfare Act and Animal Welfare regulations (November 2013).

**Quarantine**

**Phase A:** 3 days

**Phase B:** 1 day; the quarantine period was reduced from 3 days to 1 day for in-life scheduling purposes. This deviation had no effect on the study's validity or integrity as animals were considered healthy by the attending veterinarian prior to dose administration.

**Housing**

1 per cage

**Cages**

Microisolator cages with hardwood chip bedding

**Light Cycle**

12 hr light/12 hr dark

**Temperature**

70-73°F

**Humidity**

40-59%

**Ventilation**

At least 10 room volumes per hour, with no recirculation of air

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**Food**

Envigo Teklad Certified Global 18% Protein Rodent Diet, #2018C. Food was provided *ad libitum*. Feed was analyzed periodically to ensure that contaminants known to be capable of interfering with the study and reasonably expected to be present in such feed were not present at levels that would affect the study. Documentation of feed analyses is maintained at SRI for reference. A copy of the lot specific reports provided by the supplier will be maintained in the study records.

**Water**

Water (purified, reverse osmosis) was provided *ad libitum*. Based on previous reports, no contaminants that could interfere with and affect the results of the study were expected to be present in the water. Copies of annual analysis reports are maintained at SRI for reference.

**Assignment of Animals to Study**

**Day**

**Phase A (MTD):** Animals were assigned 1-8 days prior to initiation of treatment. This deviation did not affect the validity or integrity of the study as the animals were treated according to their body weight on Day 1 for each dose group.

**Phase B (PK):** One day prior to the initiation of treatment

**Randomization**

Animals were randomly assigned to treatment groups via a computerized body weight stratification procedure (Phase A: Provantis 9.3.1.1, Phase B: Provantis 10.1.0.1). Two Phase A animals were excluded based on health (exhibited fight wounds).

**Identification**

Animals were individually identified by a unique ear punch.

**Welfare of the Animals**

Every effort was made to minimize, if not eliminate, pain and suffering in all animals in this study. No animals were required to be euthanized prior to their scheduled sacrifice during this study due to their health status.

**C. Experimental Procedure (In-Life Evaluations)**

**1. Preparation of Animals**

Animals were not fasted before dose administration.

**2. Dose Administration**

Intravenous (iv), intraperitoneal (ip) or oral (po); formulations were vortexed or inverted prior to administration to each animal.

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**3. Mortality/Morbidity**

Animals were checked at least once daily.

**4. Clinical Observations**

**Phase A:** Recorded immediately postdose on day of treatment, 2-4 hr postdose, and prior to euthanasia at 24 hr postdose.

**Phase B:** Recorded immediately postdose on the day of treatment and prior to the last blood collection.

Animals were examined for any altered clinical signs, including gross motor and behavioral activity, and observable changes in appearance.

**5. Body Weights**

Body weights were measured for randomization and on Day 1 for dose administration calculations.

**6. Plasma Drug Levels – Phase B Only (PK)**

**Method of Collection**

Blood from the retro-orbital sinus of mice under isoflurane anesthesia were collected in tubes containing K<sub>3</sub>EDTA. Sodium fluoride (NaF) was added promptly to the blood samples by transferring 225 µl of whole blood from the K<sub>3</sub>EDTA tube into an Eppendorf tube containing 25 µl of 1000 mM NaF. These mixtures were kept on wet ice and processed to plasma within 30 min of collection. Plasma was prepared by centrifuging treated blood samples at 2-8°C and transferred to cryovials kept on dry ice. All plasma samples were stored frozen at ≤ -60°C until analysis. NaF was used to prevent further breakdown of Proprietary Info during blood sample processing and storage.

**Volume**

Maximum ~300 µl whole blood (~120 µl of plasma) per sample.

**Frequency**

Group 1 (iv): 0.083, 0.25, 0.5, 1, 2, 4, 8 and 24 hr postdose

Group 2 (ip): 0.083, 0.25, 0.5, 1, 2, 4, 8 and 24 hr postdose

Group 3 (po): 0.25, 0.5, 1, 2, 4, 6, 12 and 24 hr postdose

Blood was collected and processed to plasma from 3M/3F untreated mice for baseline control samples.

Two blood samples were collected from each mouse; three mice were assigned for each time point.

**Method of Analysis**

Drug levels of Proprietary Info and its major metabolite Proprietary Info were determined in collected plasma samples, using a bioanalytical method developed at SRI (Appendix D).



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**Pharmacokinetic Analysis**

The plasma drug level data were analyzed using Phoenix<sup>®</sup> WinNonlin<sup>®</sup> (version 6.4) software to perform noncompartmental analysis and sparse modeling. The dose administered was input to the program as mg/kg, and as a result no additional corrections for individual body weights of the animals were necessary.

The following parameters and constants were determined: maximal plasma concentration ( $C_{max}$ ; mean  $\pm$  SE), time to maximum plasma concentration ( $T_{max}$ ), area under the plasma concentration time curve to the last time point ( $AUC_{last}$ , mean  $\pm$  SE), and terminal elimination half-life ( $t_{1/2}$ ). The plasma concentration extrapolated to time zero ( $C_0$ ) was also calculated for the group administered Proprietary Info by the iv route. If the terminal phase had less than three data points or the  $r^2$  was less than 0.8, then the  $t_{1/2}$  could not be calculated. Bioavailability (F) of Proprietary Info was calculated using  $AUC_{last}$  values adjusted for dose administered.

**Disposition**

Residual bioanalytical samples will be discarded on submission of the final report.

**D. Necropsy**

Necropsy was not performed on this study.

**Euthanasia**

An overdose of sodium pentobarbital was administered via intraperitoneal injection.

**E. Control of Bias**

While evaluating the responses of the animals and conducting the analyses, the technical staff were aware of the treatment history of each animal and sample. Based on the relatively objective endpoints to be examined, bias did not influence the results of the study.

**V. REGULATORY COMPLIANCE**

**A. Good Laboratory Practice Compliance**

This study did not require compliance with U.S. FDA "Good Laboratory Practice for Nonclinical Laboratory Studies," as described in 21 CFR Part 58. Nevertheless, the study was planned, performed, recorded and reported in accordance with standard practices to ensure data quality and integrity.

**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**B. Retention of Records and Study Samples**

A copy of the final report, including all appendices and raw data will be retained and stored by SRI International for a period of one year. At the end of the retention period, the Sponsor will be contacted for instructions regarding disposition of the raw data.

**VI. RESULTS**

**A. Mortality/Morbidity and Clinical Observations**

Clinical observations are summarized in Table 1. Mortality/morbidity and individual animal clinical observations are presented in Appendix C.

Proprietary Info

**B. Plasma Drug Concentrations of** Proprietary Info

Individual animal plasma concentration data are presented in Appendix D. Figures 1- 3 illustrate the plasma concentrations of Proprietary Info after iv, ip and po administration of Proprietary Info respectively.

Proprietary Info

**Maximum Tolerated Dose and Pharmacokinetic Study of [Proprietary Info]  
Following a Single Dose Administration to Male and Female C57BL/6 Mice  
SRI Study No. B173-18**

**C. Pharmacokinetics Analysis**

The results of the pharmacokinetics data analysis are presented in Tables 2 and 3.

Proprietary Info

Proprietary Info




**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**VII. DISCUSSION AND CONCLUSIONS**

Proprietary Info

**Maximum Tolerated Dose and Pharmacokinetic Study of [Proprietary Info]**  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

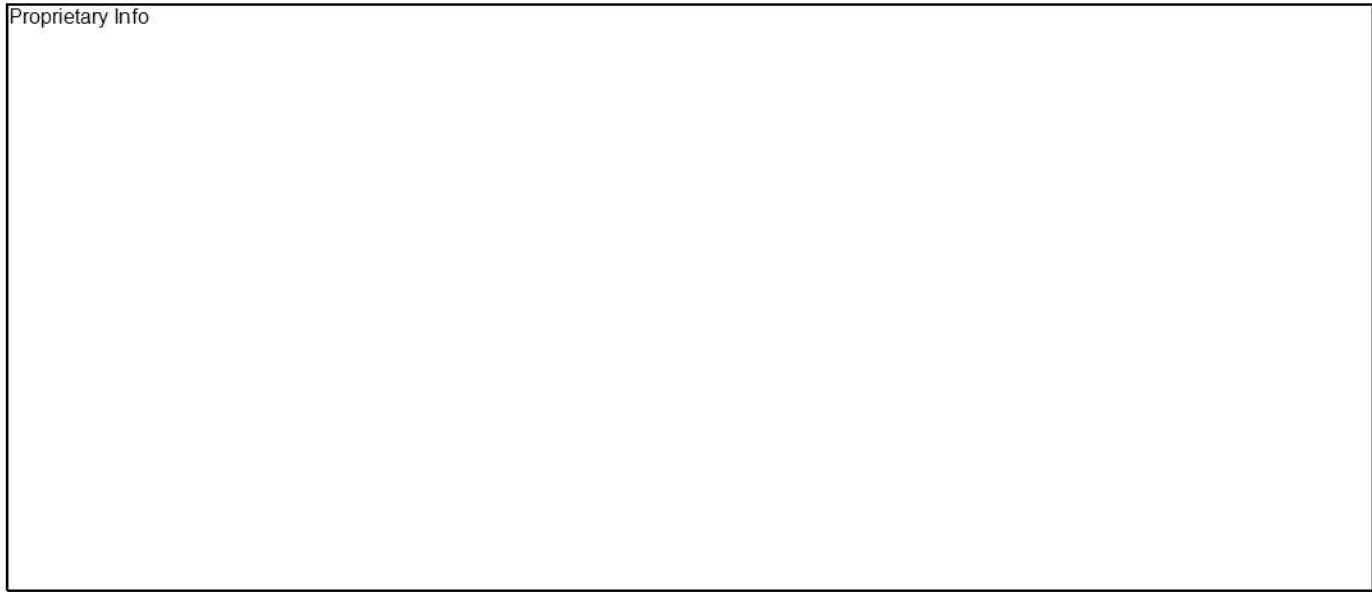
Proprietary Info



**Figure 1.** Plasma concentrations of [Proprietary Info] after iv administration of [Proprietary Info] (20 mg/kg) in male and female C57BL/6 mice.

**Maximum Tolerated Dose and Pharmacokinetic Study of [Proprietary Info]**  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

Proprietary Info



**Figure 2.** Plasma concentrations of [Proprietary Info] and [Proprietary Info] after ip administration of [Proprietary Info] (40 mg/kg) in male and female C57BL/6 mice.

**Maximum Tolerated Dose and Pharmacokinetic Study of [Proprietary Info]**  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

Proprietary Info



**Figure 3.** Plasma concentrations of [Proprietary Info] and [Proprietary Info] after po administration of [Proprietary Info] (100 mg/kg) in male and female C57BL/6 mice.



Table 1a  
Clinical Observations Summary  
(Phase A)

12/18/2018 12:31:35PM

Page: 1 of 4

Proprietary Info



"-" indicates Not Applicable  
Provantis version 10.1.0.1

Obtained via FOIA by White Coat Waste Project

Table 1a  
Clinical Observations Summary  
(Phase A)

12/18/2018 12:31:35PM

Page: 2 of 4

Proprietary Info

"-" indicates Not Applicable  
Provantis version 10.1.0.1

Obtained via FOIA by White Coat Waste Project

Table 1a  
Clinical Observations Summary  
(Phase A)

12/18/2018 12:31:35PM

Page: 3 of 4

Proprietary Info



"-" indicates Not Applicable  
Provantis version 10.1.0.1

Obtained via FOIA by White Coat Waste Project

Table 1a  
Clinical Observations Summary  
(Phase A)

12/18/2018 12:31:35PM

Page: 4 of 4

B173-18 - Maximum Tolerated Dose and Pharmacokinetic Study of Proprietary Info  
Following a Single Dose Administration to Male and Female C57BL/6 Mice

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Key Page

**General Footnotes**

"-" indicates Not Applicable  
Provantis version 10.1.0.1

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings</u>		
1	<span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> (20;IV)	Control	Group 1	20 mg/kg	IV
2	<span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> (40;IV)	Dose	Group 2	40 mg/kg	IV
3	<span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span>	Dose	Group 3	20 mg/kg	IV
4	<span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> (20;IP)	Dose	Group 4	20 mg/kg	IP
5	<span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> (40;IP)	Dose	Group 5	40 mg/kg	IP
6	<span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span>	Dose	Group 6	40 mg/kg	IP
7	<span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> 40;PO	Dose	Group 7	40 mg/kg	PO
8	<span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> 100;PO	Dose	Group 8	100 mg/kg	PO
9	<span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> 100;PO	Dose	Group 9	100 mg/kg	PO



Table 1b  
Clinical Observations Summary  
(Phase B)

12/18/2018 12:33:41PM

Page: 1 of 2

Proprietary Info

"-" indicates Not Applicable  
Provantis version 10.1.0.1

Obtained via FOIA by White Coat Waste Project

Table 1b  
Clinical Observations Summary  
(Phase B)

12/18/2018 12:33:41PM

Page: 2 of 2

B173-18 - Maximum Tolerated Dose and Pharmacokinetic Study of Proprietary Info  
Following a Single Dose Administration to Male and Female C57BL/6 Mice

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Key Page

**General Footnotes**

"-" indicates Not Applicable  
Provantis version 10.1.0.1

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings</u>		
1	<span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> (20)IV	Control	Group 1	20 mg/kg	IV
2	<span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> (40)IP	Dose	Group 2	40 mg/kg	IP
3	<span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> (100)PO	Dose	Group 3	100 mg/kg	PO

**Maximum Tolerated Dose and Pharmacokinetic Study of [Proprietary Info]  
Following a Single Dose Administration to Male and Female C57BL/6 Mice  
SRI Study No. B173-18**

**Table 2**

**PHARMACOKINETICS OF [Proprietary Info] IN MALE AND FEMALE C57BL/6 MICE AFTER INTRAVENOUS,  
INTRAPERITONEAL, AND ORAL ADMINISTRATION**

Proprietary Info

**Maximum Tolerated Dose and Pharmacokinetic Study of [Proprietary Info]  
Following a Single Dose Administration to Male and Female C57BL/6 Mice  
SRI Study No. B173-18**

**Table 3**

**PHARMACOKINETICS OF [Proprietary Info] IN MALE AND FEMALE C57BL/6 MICE AFTER INTRAVENOUS,  
INTRAPERITONEAL, AND ORAL ADMINISTRATION OF [Proprietary Info]**

Proprietary Info



**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Appendix A**

**PROTOCOL AND AMENDMENTS**

**I. MAXIMUM TOLERATED DOSE AND PHARMACOKINETIC STUDY OF**  
**Proprietary Info FOLLOWING A SINGLE DOSE ADMINISTRATION TO**  
**MALE AND FEMALE C57BL/6 MICE**

**II. SRI STUDY NUMBER: B173-18**

**III. SPONSOR**

National Institute of Allergy and Infectious Disease  
Division of AIDS  
5601 Fishers Lane, Redacted by agreement  
Bethesda, MD 20892

Contract Number: HHSN272201400006I

Sponsor's Representative:

Redacted by agreement

**IV. TESTING FACILITY**

SRI International  
Biosciences Division  
333 Ravenswood Avenue  
Menlo Park, CA 94025

Study Director:

Redacted by agreement

**V. PROPOSED IN-LIFE SCHEDULE**

Phase A:

Start of In-Life (first dose): October 2, 2018

Termination (last observation): October 10, 2018

Phase B:

Start of In-Life (first dose): TBD

Termination (final collection): TBD

**VI. APPROVALS**

Redacted by agreement

Personal Info

Redacted by agreement

SRI Study Director

Date

Date

**Maximum Tolerated Dose and Pharmacokinetic Study of [Proprietary Info]**  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

## VII. PURPOSE OF STUDY

The purpose of this study is to provide data that can be used to support research efforts. It is exploratory and not within the scope of U.S. Food and Drug Administration (FDA) "Good Laboratory Practice for Nonclinical Laboratory Studies" (GLP) as described in 21 CFR Part 58. Nevertheless, the study will be planned, performed, recorded and reported in accordance with standard practices to ensure data quality and integrity.

## VIII. STUDY OBJECTIVE

The objective of this study is to determine the tolerability and the plasma pharmacokinetics of [Proprietary Info] following an intravenous (iv), intraperitoneal (ip) or oral gavage (po) administration. Male and female C57BL/6 mice will be given a single dose administration of the test article.

## IX. EXPERIMENTAL DESIGN

### Phase A: Maximum Tolerated Dose (MTD)

Group	Treatment <sup>a</sup>	Dose Route	Dose Level (mg/kg) <sup>b</sup>	Dose Conc. (mg/ml) <sup>b</sup>	Dose Volume (ml/kg)	No. of Animals
1	[Proprietary Info]	iv	20	4	5	1M/1F
2	[Proprietary Info]	iv	TBD	TBD	5	1M/1F
3	[Proprietary Info]	iv	TBD	TBD	5	1M/1F
4	[Proprietary Info]	ip	20	2	10	1M/1F
5	[Proprietary Info]	ip	TBD	TBD	10	1M/1F
6	[Proprietary Info]	ip	TBD	TBD	10	1M/1F
7	[Proprietary Info]	po	40	4	10	1M/1F
8	[Proprietary Info]	po	TBD	TBD	10	1M/1F
9	[Proprietary Info]	po	TBD	TBD	10	1M/1F

<sup>a</sup> Phase A will be conducted using escalating or de-escalating dose levels (e.g., escalating to 40, 100 mg/kg, etc., until clinical effects are seen, then doing one more escalation or de-escalation based on observations seen at the previous dose level).

<sup>b</sup> The dose level and dose concentration is based on the free base form of the test article. The dose formulation will be prepared based on the salt content; with a correction factor of 1.10.

### Species and Strain

Mouse and C57BL/6

### Route of Administration

iv (via tail vein given over 30 seconds), ip or po (via gavage)

**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Frequency**

Single dose starting on Day 1; mice will be treated as indicated in the table above.

Clinical observations will be performed immediately post dose, 2-4 hr post dose and prior to euthanasia at 24 hr. All surviving mice will be euthanized at 24 hr post dose.

**Dosing Volume**

iv: 5 ml/kg

ip and po: 10 ml/kg

Dose volumes will be calculated based on each animal's Day 1 body weight

**Duration of In-Life Phase**

24 hours per dose group

**Phase B: Pharmacokinetics (PK)**

Group	Treatment	Dose Route	Dose Level (mg/kg) <sup>a</sup>	Dose Conc. (mg/kg) <sup>a</sup>	Dose Volume	No. of Animals <sup>b</sup>	Blood Collection Time Points <sup>c</sup>
1	<span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span>	iv	TBD <sup>d</sup>	TBD	5	12M/12F	0.083, 0.25, 0.5, 1, 2, 4, 8 and 24 hr
2	<span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span>	ip	TBD	TBD	10	12M/12F	0.083, 0.25, 0.5, 1, 2, 4, 8 and 24 hr
3	<span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span>	po	TBD	TBD	10	12M/12F	0.25, 0.5, 1, 2, 4, 6, 12 and 24 hr

<sup>a</sup> The dose level and dose concentration is based on the free base form of the test article. The dose formulation will be prepared based on the salt content; with a correction factor of 1.10.

<sup>b</sup> Blood will be collected and processed to plasma from 3M/3F untreated mice (not included in table above) for baseline control samples.

<sup>c</sup> Two blood samples will be collected from each mouse; three mice will be assigned for each time point.

<sup>d</sup> TBD = dose levels to be determined based on the Phase A MTD results. Actual dose levels and concentrations will be documented in an amendment.

**Species and Strain**

Mouse and C57BL/6

**Route of Administration**

iv (via tail vein given over 30 seconds), ip or po (via gavage)



**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Frequency**

Single dose on Day 1

**Dosing Volume**

iv: 5 ml/kg

ip and po: 10 ml/kg

Dose volumes will be calculated based on each animal's Day 1 body weight

**Duration of In-Life Phase**

24 hours

**X. MATERIALS AND METHODS**

**A. Test and Control Articles**

**1. Test Article**

Proprietary Info

**Supplier**

Proprietary Info

**Manufacturer**

Proprietary Info

**Lot Number**

Proprietary Info

**Molecular Weight**

Proprietary Info

**Physical Description**

Yellow solid.

**Storage Conditions**

Room temperature, 15° - 30°C.

**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Characterization of Test Article**

The Sponsor is responsible for characterization and stability of the test article and will provide a Certificate of Analysis (CofA), or equivalent documentation, to SRI for inclusion in the final report. The raw data generated by the Sponsor in support of this CofA or its equivalent will not be verified or maintained by SRI.

**2. Vehicle Control**

Proprietary Info

**Supplier**

Proprietary Info

**Manufacturer**

Proprietary Info

**Lot Number**

Proprietary Info

**Physical Description**

Clear, colorless solution

**Storage Conditions**

To be documented in the final report.

**Characterization of Vehicle**

Information on the identity, purity and stability of the vehicle may be obtained by recording all of the pertinent information provided on the container labels or in a CofA provided by the supplier.

**3. Preparation of Dose Formulations**

The dose formulations will be corrected for salt content, using a correction factor of 1.10.

Dose formulations will be prepared aseptically by combining the appropriate amount of test article in the vehicle to achieve the target concentration. The formulations will be mixed well using a vortex mixer and/or a sterile stir bar and/or sonication. Visual inspection of each formulation will be documented. Details of dose preparation will be included in the final report.

**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**4. Storage of Dose Formulations**

**Phase A (MTD):** Each dose formulation will be prepared fresh prior to dose administration.

**Phase B (PK):** The dose formulation will be prepared fresh on Day 1 and maintained at room temperature until use.

**5. Characterization of Dose Formulations**

Stability, homogeneity and concentration analysis of the test article in the vehicle will not be performed for this study.

**6. Test Article Handling**

At a minimum, personnel handling the test article formulations will wear eye protection, gloves and a protective smock or laboratory coat.

**7. Disposition**

All bulk test article will be retained. The Sponsor will be contacted regarding disposition of the test article.

Residual dose formulations will be discarded when the final report is submitted.

**8. Method for Assuring Correct Dosing**

The administration of each dose formulation will be properly documented and the amount administered to each animal will be recorded.

**B. Test System**

**Species**

Mouse

**Strain**

C57BL/6

**Supplier**

Charles River Laboratories or other reputable supplier

**Number of Animals**

Phase A: 18

Phase B: 72

**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Sex**

Phase A: 9 males and 9 females

Phase B: 36 males and 36 females (+ 3 controls per gender)

**Age at First Dose**

7–8 weeks

**Weight Range at First Dose**

Approximately 22–30 g (males); 17–25 g (females)

**Animal Care**

General procedures for animal care and housing will be in accordance with the current Association for assessment and Accreditation of Laboratory Animal Care (AAALAC) in recommendations, current requirements stated in the *Guide for the Care and Use of Laboratory Animals* (National Research Council), and current requirements as stated by the U.S. Department of Agriculture through the Animal Welfare Act and Animal Welfare regulations (November 2013).

**Quarantine**

At least 3 days

**Housing**

Up to 3 per cage

**Cages**

Microisolator cages with hardwood chip bedding

**Light Cycle**

12 hr light/12 hr dark

**Temperature**

68–79°F

**Humidity**

30–70%. Brief excursions outside this range may occur; excursions of less than 4 hr/day will not be considered deviations from the protocol.

**Ventilation**

At least 10 room volumes per hour, with no recirculation of air.



**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Food**

Envigo Teklad Certified Global 18% Protein Rodent Diet, #2018C or Purina Certified Rodent Chow #5002 or equivalent. Food will be provided *ad libitum*. Feed is analyzed periodically to ensure that contaminants known to be capable of interfering with the study and reasonably expected to be present in such feed are not present at levels that would affect the study. Documentation of feed analyses is maintained at SRI for reference. A copy of the lot specific reports provided by the supplier will be maintained in the study records.

**Water**

Water (purified, reverse osmosis or untreated tap water) will be provided *ad libitum*. Based on previous reports, no contaminants that could interfere with and affect the results of the study are expected to be present in the water. Copies of annual analysis reports are maintained at SRI for reference.

**Assignment of Animals to Study**

**Day**

Phase A (MTD): No more than 6 days before initiation of treatment.

Phase B (PK): No more than 2 days before initiation of treatment.

**Randomization**

Animals will be randomly assigned to treatment groups via a computerized body weight stratification procedure. Animals may be excluded based on health, behavior or inappropriate weight.

**Identification**

Animals will be individually identified by a unique ear punch or by another approved method.

**Welfare of the Animals**

Every effort will be made to minimize, if not eliminate, pain and suffering in all animals in this study. Moribund animals and animals experiencing undue pain and suffering will be euthanized at the discretion of the Study Director, attending veterinarian, or other qualified person. The Study Director will make every effort to protect the scientific validity of the study.

**C. Experimental Procedure (In-Life Evaluations)**

**1. Preparation of Animals**

Animals will **not be fasted** before dose administration.

**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**2. Dose Administration**

Intravenous (iv), intraperitoneal (ip) or oral (po); formulations will be vortexed or inverted prior to administration to each animal.

**3. Mortality/Morbidity**

Animals will be checked at least once daily.

**4. Clinical Observations**

Phase A: Recorded immediately post dose on day of treatment, 2-4 hr post dose and prior to euthanasia at 24 hr post dose.

Phase B: Recorded immediately post dose on the day of treatment and prior to the last blood collection.

Animals will be examined for any altered clinical signs, including gross motor and behavioral activity, and observable changes in appearance.

**5. Body Weights**

Body weights will be measured for randomization and on Day 1 for dose administration calculations.

**6. Plasma Drug Levels – Phase B Only (PK)**

**Method of Collection**

Blood from the retro-orbital sinus of mice under isoflurane anesthesia will be collected in tubes containing K<sub>3</sub>EDTA, processed to plasma, and stored frozen at  $\leq -60^{\circ}\text{C}$ ).

**Volume**

Maximum ~300  $\mu\text{l}$  whole blood (~120  $\mu\text{l}$  of plasma) per sample.

**Frequency**

Group 1 (iv): 0.083, 0.25, 0.5, 1, 2, 4, 8 and 24 hr post dose

Group 2 (ip): 0.083, 0.25, 0.5, 1, 2, 4, 8 and 24 hr post dose

Group 3 (po): 0.25, 0.5, 1, 2, 4, 6, 12 and 24 hr post dose

Blood will be collected and processed to plasma from 3M/3F untreated mice for baseline control samples.

Two blood samples will be collected from each mouse; three mice will be assigned for each time point.

**Maximum Tolerated Dose and Pharmacokinetic Study of [Proprietary Info]  
Following a Single Dose Administration to Male and Female C57BL/6 Mice  
SRI Study No. B173-18**

**Method of Analysis**

Drug levels of [Proprietary Info] and its major metabolite [Proprietary Info] will be determined in collected plasma samples, using a bioanalytical method developed at SRI.

**Pharmacokinetic Analysis**

The plasma drug level data will be analyzed using Phoenix<sup>®</sup> WinNonlin<sup>®</sup> (version 6.4 or higher) software to perform noncompartmental modeling. The dose administered will be input to the program as mg/kg, and as a result no additional corrections for individual body weights of the animals will be necessary.

The following parameters and constants will be determined if the data allow: maximal plasma concentration ( $C_{max}$ ), time to maximum plasma concentration ( $T_{max}$ ), area under the plasma concentration time curve (AUC), and terminal elimination half-life ( $t_{1/2}$ ).

Other methods of analysis may be used or other parameters calculated, as appropriate, based on the plasma drug level data.

**Disposition**

Residual bioanalytical samples will be discarded on submission of the final report.

**D. Necropsy**

Necropsy will not be performed on this study.

**Euthanasia**

An overdose of sodium pentobarbital will be administered via intraperitoneal injection.

**E. Control of Bias**

While evaluating the responses of the animals and conducting the analyses, the technical staff will be aware of the treatment history of each animal and sample. Based on the relatively objective endpoints to be examined, bias is not expected to influence the results of the study.

**XI. REGULATORY COMPLIANCE**

**A. Good Laboratory Practice Compliance**

This study does not require compliance with U.S. FDA "Good Laboratory Practice for Nonclinical Laboratory Studies," as described in 21 CFR Part 58.



**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

Nevertheless, the study will be planned, performed, recorded and reported in accordance with standard practices to ensure data quality and integrity.

**B. Standard Operating Procedures (SOPs)**

All operations pertaining to this study, unless specifically defined in this protocol, will be performed according to the SOPs of the laboratory. All deviations from any SOP and the reasons for the deviations will be documented and acknowledged by the Study Director.

**C. Protocol Amendments and Deviations**

All changes or revisions made to the approved protocol by any involved party and the reasons for the changes and revisions will be documented, signed and dated by the Study Director and the Sponsor's Representative. Amendments will be maintained with the protocol. Verbal or email approval for changes in the protocol may be granted by the Sponsor's Representative, but a written amendment as described above will follow.

All deviations from the protocol and the reasons for the deviations will be documented and acknowledged by the Study Director. The Sponsor's Representative will be informed of the occurrence of any deviations that might affect the results of the study, and any corrective actions taken.

**D. Retention of Records and Study Samples**

A copy of the final report, including all appendices, raw data will be retained and stored by SRI International for a period of one year. At the end of the retention period, the Sponsor will be contacted for instructions regarding disposition of the raw data.

**XII. REPORTING**

The final report will accurately and completely describe the study design, procedures and findings. An analysis and summary of the data followed by the conclusions derived from the analyses will also be included. A draft report will be issued prior to submission of the final report.

# **PROTOCOL AMENDMENT NO. 1**

**PROTOCOL TITLE** Maximum Tolerated Dose and Pharmacokinetic Study of  
 [Proprietary Info] Following a Single Dose Administration to Male and  
 Female C57BL/6 Mice

**SRI Study Number:** B173-18

**Sponsor:** National Institute of Allergy and Infectious Disease  
 Division of AIDS  
 5601 Fishers Lane, [Redacted by agreement]  
 Bethesda, MD 20892

**Sponsor's Representative:** [Redacted by agreement]

**SRI Study Director** [Personal Info]

This amendment modifies the following lines/sections of the study protocol. Additions are in bold and italics: ***addition***. Deleted text has been struck through: ~~deleted~~.

## **Section IX. EXPERIMENTAL DESIGN, page 2-3:**

### **Phase A: Maximum Tolerated Dose (MTD)**

<b>Group</b>	<b>Treatment <sup>a</sup></b>	<b>Dose Route</b>	<b>Dose Level (mg/kg) <sup>b</sup></b>	<b>Dose Conc. (mg/ml) <sup>b</sup></b>	<b>Dose Volume (ml/kg)</b>	<b>No. of Animals</b>
1	[Proprietary Info]	iv	20	4	5	1M/1F
2	[Proprietary Info]	iv	<del>TBD</del> <b>40</b>	<del>TBD</del> <b>8</b>	5	1M/1F
3	[Proprietary Info]	iv	<del>TBD</del> <b>20</b>	<del>TBD</del> <b>4</b>	5	1M/1F
4	[Proprietary Info]	ip	20	2	10	1M/1F
5	[Proprietary Info]	ip	<del>TBD</del> <b>40</b>	<del>TBD</del> <b>4</b>	10	1M/1F
6	[Proprietary Info]	ip	<del>TBD</del> <b>40</b>	<del>TBD</del> <b>4</b>	10	1M/1F
7	[Proprietary Info]	po	40	4	10	1M/1F
8	[Proprietary Info]	po	<del>TBD</del> <b>100</b>	<del>TBD</del> <b>10</b>	10	1M/1F
9	[Proprietary Info]	po	<del>TBD</del> <b>100</b>	<del>TBD</del> <b>10</b>	10	1M/1F

<sup>a</sup> Phase A will be conducted using escalating or de-escalating dose levels (e.g., escalating to 40, 100 mg/kg, etc., until clinical effects are seen, then doing one more escalation or de-escalation based on observations seen at the previous dose level).

<sup>b</sup> The dose level and dose concentration is based on the free base form of the test article. The dose formulation will be prepared based on the salt content; with a correction factor of 1.10.



## PROTOCOL AMENDMENT NO. 1

## Phase B: Pharmacokinetics (PK)

Group	Treatment	Dose Route	Dose Level (mg/kg) <sup>a</sup>	Dose Conc. (mg/kg) <sup>a</sup>	Dose Volume	No. of Animals <sup>b</sup>	Blood Collection Time Points <sup>c</sup>
1	Proprietary Info	iv	TBD <sup>d</sup> 20	TBD 4	5	12M/12F	0.083, 0.25, 0.5, 1, 2, 4, 8 and 24 hr
2	Proprietary Info	ip	TBD 40	TBD 4	10	12M/12F	0.083, 0.25, 0.5, 1, 2, 4, 8 and 24 hr
3	Proprietary Info	po	TBD 100	TBD 10	10	12M/12F	0.25, 0.5, 1, 2, 4, 6, 12 and 24 hr

<sup>a</sup> The dose level and dose concentration is based on the free base form of the test article. The dose formulation will be prepared based on the salt content; with a correction factor of 1.10.

<sup>b</sup> Blood will be collected and processed to plasma from 3M/3F untreated mice (not included in table above) for baseline control samples.

<sup>c</sup> Two blood samples will be collected from each mouse; three mice will be assigned for each time point.

<sup>d</sup> TBD = dose levels to be determined based on the Phase A MTD results. Actual dose levels and concentrations will be documented in an amendment.

**Reason for Change:** The dose levels and dose concentrations used in the MTD Phase of the study was documented, and the dose levels and dose concentrations selected in consultation with the Sponsor to be used in the PK Phase is documented.

**Effect on the Study:** None.

**Effective Date:** 10/15/18

## APPROVALS

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Date

10/23/18  
Date

**PROTOCOL AMENDMENT NO. 2**

**PROTOCOL TITLE** Maximum Tolerated Dose and Pharmacokinetic Study of  
[Proprietary Info] Following a Single Dose Administration to Male and  
Female C57BL/6 Mice

**SRI Study Number:** B173-18

**Sponsor:** National Institute of Allergy and Infectious Disease  
Division of AIDS  
5601 Fishers Lane, [Redacted by agreement]  
Bethesda, MD 20892

**Sponsor's Representative:** Marina Protopopova, PhD

**SRI Study Director** [Redacted by agreement]

---

This amendment modifies the following lines/sections of the study protocol. Additions are in bold and italics: ***addition***. Deleted text has been struck through: ~~deleted~~.

**Section V. PROPOSED IN-LIFE SCHEDULE, page 1:**Phase B:Start of In-Life (first dose): ***November 29, 2018***Termination (final collection): ***November 30, 2018*****Section X. MATERIALS AND METHODS, C. Experimental Procedure (In-Life Evaluations), page 9:****6. Plasma Drug Levels – Phase B Only (PK)****Method of Collection:**

Blood from the retro-orbital sinus of mice under isoflurane anesthesia will be collected in tubes containing K<sub>3</sub>EDTA. ***Sodium fluoride (NaF) will be added promptly to the blood samples by transferring 225 µl of whole blood from the K<sub>3</sub>EDTA tube into an Eppendorf tube containing 25 µl of 1000 mM NaF. These mixtures will kept on wet ice and processed to plasma within 30 minutes of collection. Plasma will be prepared by centrifuging treated blood samples at 2-8°C and transferred to cryovials kept on dry ice. All plasma samples will be stored frozen at ≤ -60°C until analysis. NaF will be used with the intention of preventing further breakdown of [Proprietary Info] during blood sample processing and storage. Plasma samples will be, processed to plasma, and stored frozen at ≤ -60°C.***

## PROTOCOL AMENDMENT NO. 2

**Reason for Change:** 1. To document the Phase B (PK) in-life start and end dates.  
2. To document the method of plasma collection from the treated mice in the Phase B (PK) portion of the study. Bioanalytical method development was performed in order to determine the best way to process the upcoming plasma samples for drug level determination.

**Effect on the Study:** None.

**Effective Date:** 11/14/18

---

**APPROVALS**

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ate

Redacted by agreement

Date

11/14/18

**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Appendix B**

**CERTIFICATE OF ANALYSIS**

DATA SHEET

Molecular Formula

Proprietary Info

Label Information

Lot No. Proprietary Info

CAS unassigned

Chemical Name

Caution: Limited by United States Law  
To Investigational Use Only.  
Not for use in humans.

Proprietary Info

Structural Formula

Proprietary Info

Amount Shipped:

2.89 g

Reference Sample:

10 mg

Send to: Redacted by agreement

Solubility:

Soluble in DMSO, MeOH, and H<sub>2</sub>O.

Attached Spectral Data:

<sup>1</sup>H NMR - 600 MHz (DMSO-d<sub>6</sub>)Mass - Electrospray (ES<sup>+</sup>)UV - λ<sub>max</sub> 221, 254, 357, 369 nm

Melting Point:

279-282°C (dec.) (uncorrected)

HPLC - 96.5 area% purity

Analytical Data:

Thin Layer Chromatography (R<sub>f</sub> value):

Element	Calcd <sup>*</sup>	Obsd
C	56.11	56.11
H	6.81	6.50
N	9.74	9.87
Cl	8.87	8.63

0.26 (MeOH:CH<sub>2</sub>Cl<sub>2</sub>, 1:9, silica gel)<sup>\*</sup>Calcd for C<sub>33</sub>H<sub>39</sub>N<sub>5</sub>O<sub>5</sub>·1.8 HCl·0.3 EtOH·3.0 H<sub>2</sub>O

Adjusted FW = 719.2

Drying:

in vacuo at RT to  
constant weight

Appearance:

yellow solid

Storage: (Long term storage)

Store in a well-closed container under  
argon at -20°C (freezer).

Date: August 27, 2018



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B173-18

JW 12/3/18

ICU MEDICAL  
3900 Howard Lane  
Austin, TX. 78728-6599

## Certificate of Analysis

Product Name: LACTATED RINGER'S INJECTION, USP  
Product Number: 0409-7953-02  
Lot Number: 85027JT List Number: 79530463  
Date of Manufacture: 1-21-18 Expiration Date: 07/01/2019

STM	Test Description	Final Limits		Result	Sub Batch	Pass/Fail
		Lower	Upper			
75145	Meets the requirements of Drug Code 75145.					Pass
B-0693	BET: NMT 0.50 EU/mL			<0.06	01	Pass
				<0.06	03	Pass
				<0.06	04	Pass
				<0.06	05	Pass
				<0.06	06	Pass
C-0002	Potassium Identification				01	Pass
					03	Pass
					04	Pass
					05	Pass
					06	Pass
C-0003	Chloride Identification				01	Pass
					03	Pass
					04	Pass
					05	Pass
					06	Pass
C-0005	Calcium Identification				01	Pass
					03	Pass
					04	Pass
					05	Pass
					06	Pass
C-0021	pH Determination	6.0	7.5	6.7	01	Pass
		6.0	7.5	6.8	03	Pass
		6.0	7.5	6.8	04	Pass
		6.0	7.5	6.8	05	Pass
		6.0	7.5	6.8	06	Pass

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## Certificate of Analysis

**Product Name:** LACTATED RINGER'S INJECTION, USP  
**Product Number:** 0409-7953-02  
**Lot Number:** 85027JT **List Number:** 79530463  
**Date of Manufacture:** 1-21-18 **Expiration Date:** 07/01/2019

STM	Test Description	Final Limits		Result	Sub Batch	Pass/Fail
		Lower	Upper			
C-0040	Total Chloride Assay (mg/mL)	3.680	4.080	3.813	01	Pass
		3.680	4.080	3.813	03	Pass
		3.680	4.080	3.815	04	Pass
		3.680	4.080	3.811	05	Pass
		3.680	4.080	3.764	06	Pass
C-0078	Potassium Assay (mg/mL)	0.142	0.173	0.153	01	Pass
		0.142	0.173	0.153	03	Pass
		0.142	0.173	0.152	04	Pass
		0.142	0.173	0.152	05	Pass
		0.142	0.173	0.153	06	Pass
C-0078	Sodium Assay (mg/mL)	2.850	3.150	2.913	01	Pass
		2.850	3.150	2.916	03	Pass
		2.850	3.150	2.910	04	Pass
		2.850	3.150	2.899	05	Pass
		2.850	3.150	2.918	06	Pass
C-1501	Calcium (mcg/mL)	49.0	60.0	53.0	01	Pass
		49.0	60.0	52.7	03	Pass
		49.0	60.0	53.2	04	Pass
		49.0	60.0	53.1	05	Pass
		49.0	60.0	52.5	06	Pass
C-1520	Lactate Assay (mg/mL)	2.900	3.300	3.017	01	Pass
		2.900	3.300	3.007	03	Pass
		2.900	3.300	3.003	04	Pass
		2.900	3.300	2.997	05	Pass
		2.900	3.300	3.003	06	Pass
C-1520	Lactate Identification					

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Date of Manufacture: 1-21-18 Expiration Date: 07/01/2019

STM	Test Description	Final Limits		Result	Sub Batch	Pass/Fail
		Lower	Upper			
					01	Pass
					03	Pass
					04	Pass
					05	Pass
					06	Pass
C-1648	Sodium Identification				01	Pass
					03	Pass
					04	Pass
					05	Pass
					06	Pass
C-2371	Ag NMT 5 ng/mL			ND	01	Pass
				ND	03	Pass
				ND	04	Pass
				ND	05	Pass
				ND	06	Pass
C-2371	As NMT 7.5 ng/mL			<LOQ	01	Pass
				ND	03	Pass
				ND	04	Pass
				ND	05	Pass
				ND	06	Pass
C-2371	Au NMT 50 ng/mL			ND	01	Pass
				ND	03	Pass
				ND	04	Pass
				ND	05	Pass
				ND	06	Pass
C-2371	Ba NMT 350 ng/mL			<LOQ	01	Pass
				<LOQ	03	Pass

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Date of Manufacture: 1-21-18 Expiration Date: 07/01/2019

STM	Test Description	Final Limits		Result	Sub Batch	Pass/Fail
		Lower	Upper			
				< LOQ	04	Pass
				< LOQ	05	Pass
				< LOQ	06	Pass
C-2371	Cd NMT 1.0 ng/mL			< LOQ	01	Pass
				< LOQ	03	Pass
				ND	04	Pass
				ND	05	Pass
				ND	06	Pass
C-2371	Co NMT 2.5 ng/mL			< LOQ	01	Pass
				ND	03	Pass
				< LOQ	04	Pass
				< LOQ	05	Pass
				ND	06	Pass
C-2371	Cr NMT 550 ng/mL			< LOQ	01	Pass
				< LOQ	03	Pass
				< LOQ	04	Pass
				< LOQ	05	Pass
				< LOQ	06	Pass
C-2371	Cu NMT 65 ng/mL			ND	01	Pass
				ND	03	Pass
				ND	04	Pass
				ND	05	Pass
				ND	06	Pass
C-2371	Fe NMT 650 ng/mL			< LOQ	01	Pass
				< LOQ	03	Pass
				< LOQ	04	Pass
				< LOQ	05	Pass

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Lot Number: 85027JT List Number: 79530463  
Date of Manufacture: 1.21.18 Expiration Date: 07/01/2019

STM	Test Description	Final Limits		Result	Sub Batch	Pass/Fail
		Lower	Upper			
				< LOQ	06	Pass
C-2371	Hg NMT 0.75 ng/mL			ND	01	Pass
				ND	03	Pass
				ND	04	Pass
				ND	05	Pass
				ND	06	Pass
C-2371	Ir NMT 5 ng/mL			< LOQ	01	Pass
				ND	03	Pass
				ND	04	Pass
				ND	05	Pass
				ND	06	Pass
C-2371	Li NMT 125 ng/mL			< LOQ	01	Pass
				< LOQ	03	Pass
				< LOQ	04	Pass
				< LOQ	05	Pass
				< LOQ	06	Pass
C-2371	Mn NMT 125 ng/mL			< LOQ	01	Pass
				ND	03	Pass
				ND	04	Pass
				ND	05	Pass
				ND	06	Pass
C-2371	Mo NMT 45 ng/mL			< LOQ	01	Pass
				< LOQ	03	Pass
				< LOQ	04	Pass
				< LOQ	05	Pass
				< LOQ	06	Pass



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Lot Number: 85027JT List Number: 79530463  
Date of Manufacture: 1.21.18 Expiration Date: 07/01/2019

STM	Test Description	Final Limits		Result	Sub Batch	Pass/Fail
		Lower	Upper			
C-2371	Ni NMT 10 ng/mL			< LOQ	01	Pass
				< LOQ	03	Pass
				< LOQ	04	Pass
				< LOQ	05	Pass
				< LOQ	06	Pass
C-2371	Os NMT 5 ng/mL			ND	01	Pass
				ND	03	Pass
				ND	04	Pass
				ND	05	Pass
				ND	06	Pass
C-2371	Pb NMT 2.5 ng/mL			< LOQ	01	Pass
				ND	03	Pass
				ND	04	Pass
				ND	05	Pass
				ND	06	Pass
C-2371	Pd NMT 5 ng/mL			< LOQ	01	Pass
				ND	03	Pass
				ND	04	Pass
				ND	05	Pass
				ND	06	Pass
C-2371	Pt NMT 5 ng/mL			ND	01	Pass
				ND	03	Pass
				ND	04	Pass
				ND	05	Pass
				ND	06	Pass
C-2371	Rh NMT 5 ng/mL			ND	01	Pass

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Lot Number: 85027JT List Number: 79530463  
Date of Manufacture: 1-21-18 Expiration Date: 07/01/2019

STM	Test Description	Final Limits		Result	Sub Batch	Pass/Fail
		Lower	Upper			
				ND	03	Pass
				ND	04	Pass
				ND	05	Pass
				ND	06	Pass
C-2371	Ru NMT 5 ng/mL			<LOQ	01	Pass
				ND	03	Pass
				ND	04	Pass
				ND	05	Pass
				ND	06	Pass
C-2371	Sb NMT 45 ng/mL			ND	01	Pass
				<LOQ	03	Pass
				ND	04	Pass
				ND	05	Pass
				<LOQ	06	Pass
C-2371	Se NMT 40 ng/mL			ND	01	Pass
				ND	03	Pass
				ND	04	Pass
				<LOQ	05	Pass
				ND	06	Pass
C-2371	Sn NMT 300 ng/mL			ND	01	Pass
				ND	03	Pass
				ND	04	Pass
				ND	05	Pass
				ND	06	Pass
C-2371	Tl NMT 4 ng/mL			<LOQ	01	Pass
				ND	03	Pass
				ND	04	Pass

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Lot Number: 85027JT List Number: 79530463  
Date of Manufacture: 1-21-18 Expiration Date: 07/01/2019

STM	Test Description	Final Limits		Result	Sub Batch	Pass/Fail
		Lower	Upper			
				ND	05	Pass
				ND	06	Pass
C-2371	V NMT 5 ng/mL			<LOQ	01	Pass
				<LOQ	03	Pass
				ND	04	Pass
				<LOQ	05	Pass
				ND	06	Pass
C-2371	Zn NMT 650 ng/mL			<LOQ	01	Pass
				<LOQ	03	Pass
				<LOQ	04	Pass
				<LOQ	05	Pass
				<LOQ	06	Pass
M-0477	Sterility- Must meet cycle minimum and maximum parameters.				01	Pass
					03	Pass
					04	Pass
					05	Pass
					06	Pass
M-0477	Sterility- Must meet key and critical parameters as defined in the applicable S-Specification.				01	Pass
					03	Pass
					04	Pass
					05	Pass
					06	Pass
M-0477	Sterility-Must meet chemical indicator testing requirements.				01	Pass
					03	Pass
					04	Pass
					05	Pass
					06	Pass

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# Certificate of Analysis

Product Name: LACTATED RINGER'S INJECTION, USP  
Product Number: 0409-7953-02  
Lot Number: 85027JT List Number: 79530463  
Date of Manufacture: 1-21-18 Expiration Date: 07/01/2019

STM	Test Description	Final Limits		Result	Sub Batch	Pass/Fail
		Lower	Upper			
P-0416	Solution must be clear.				01	Pass
					03	Pass
					04	Pass
					05	Pass
					06	Pass
P-0416	Solution must not contain one or more particles which are visible upon attentive examination.				01	Pass
					03	Pass
					04	Pass
					05	Pass
					06	Pass
P-0452	10.0 micron Sub-Visual Particulate	0	25	1,1,1,0,2,1,0,1,1,1	01	Pass
		0	25	1,2,2,2,1,1,2,1,1	03	Pass
		0	25	1,1,1,1,3,1,1,3,1,2	04	Pass
		0	25	3,2,2,2,2,1,1,2,2,2	05	Pass
		0	25	1,1,2,2,1,2,2,1,1,1	06	Pass
P-0452	25.0 micron Sub-Visual Particulate	0	3	0,0,0,0,0,0,0,0,0,0	01	Pass
		0	3	0,0,0,0,0,0,0,0,0,0	03	Pass
		0	3	0,0,0,0,0,0,0,0,0,0	04	Pass
		0	3	0,0,0,0,0,0,0,0,0,0	05	Pass
		0	3	0,0,0,0,0,0,0,0,0,0	06	Pass
P-0759	Volume: 250 mL to 300 mL			271 mL	01	Pass
				271 mL	03	Pass
				271 mL	04	Pass
				271 mL	05	Pass
				271 mL	06	Pass

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3900 Howard Lane  
Austin, TX. 78728-6599

# Certificate of Analysis

Product Name: LACTATED RINGER'S INJECTION, USP  
Product Number: 0409-7953-02  
Lot Number: 85027JT List Number: 79530463  
Date of Manufacture: 1-21-18 Expiration Date: 07/01/2019

STM	Test Description	Final Limits		Result	Sub Batch	Pass/Fail
		Lower	Upper			

This product has been manufactured and tested in current Good Manufacturing Practices (cGMP) facilities in accordance with appropriate regulations. This product meets applicable specifications, applicable Regulatory Submissions or Marketing Authorizations and, where appropriate, Compendial requirements. No Class 1, Class 2, Class 3 or other solvents used. Drug product testing is not required. The undersigned certifies this to be a true representation of the results.

Personal Info

Quality Cer

Date:

5-29-18

5/30/18



**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Appendix C**

**INDIVIDUAL CLINICAL OBSERVATIONS**

**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Appendix C-1**

**INDIVIDUAL CLINICAL OBSERVATIONS (PHASE A)**

Individual Clinical Observations  
(Phase A)

12/18/2018 12:30:08PM Page: 1 of 20

Proprietary Info



X=Present; K=Scheduled Removal

Individual Clinical Observations  
(Phase A)

12/18/2018 12:30:08PM Page: 2 of 20

Proprietary Info

X=Present; S=Slight; K=Scheduled Removal

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Individual Clinical Observations  
(Phase A)

12/18/2018 12:30:08PM Page: 3 of 20

Proprietary Info

X=Present; K=Scheduled Removal



Individual Clinical Observations  
(Phase A)

12/18/2018 12:30:08PM Page: 4 of 20

Proprietary Info

X=Present; K=Scheduled Removal

Individual Clinical Observations  
(Phase A)

12/18/2018 12:30:08PM Page: 5 of 20

Proprietary Info

X=Present; K=Scheduled Removal

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Individual Clinical Observations  
(Phase A)

12/18/2018 12:30:08PM Page: 6 of 20

Proprietary Info

X=Present; K=Scheduled Removal

Individual Clinical Observations  
(Phase A)

12/18/2018 12:30:08PM Page: 7 of 20

Proprietary Info

X=Present; K=Scheduled Removal

Individual Clinical Observations  
(Phase A)

12/18/2018 12:30:08PM Page: 8 of 20

Proprietary Info



X=Present; K=Scheduled Removal



Individual Clinical Observations  
(Phase A)

12/18/2018 12:30:08PM Page: 9 of 20

Proprietary Info

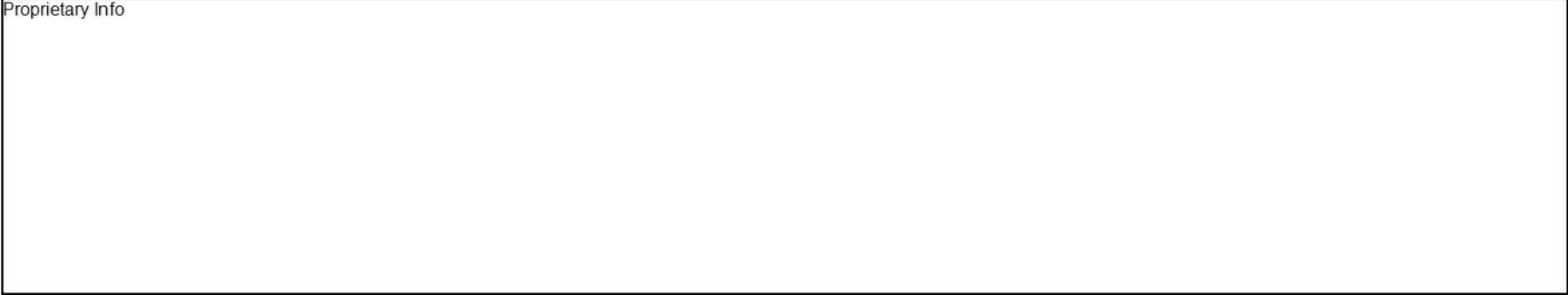
X=Present; K=Scheduled Removal

Obtained via FOIA by White Coat Waste Project

Individual Clinical Observations  
(Phase A)

12/18/2018 12:30:08PM Page: 10 of 20

Proprietary Info

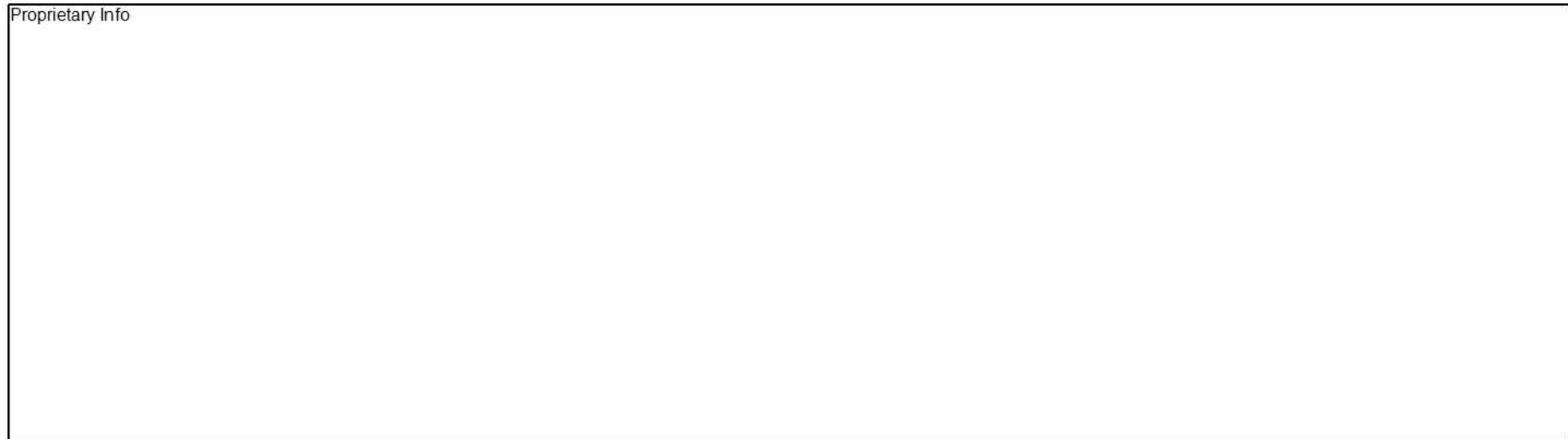


X=Present; K=Scheduled Removal

Individual Clinical Observations  
(Phase A)

12/18/2018 12:30:08PM Page: 11 of 20

Proprietary Info



!=Result comment recorded against 1 or more clinical observations. E=Extreme; X=Present; S=Slight; D=Unscheduled Removal; M=Moderate; K=Scheduled Removal

Individual Clinical Observations  
(Phase A)

12/18/2018 12:30:08PM Page: 12 of 20

Proprietary Info

X=Present; K=Scheduled Removal

Individual Clinical Observations  
(Phase A)

12/18/2018 12:30:08PM Page: 13 of 20

Proprietary Info



X=Present; K=Scheduled Removal



Individual Clinical Observations  
(Phase A)

12/18/2018 12:30:08PM Page: 14 of 20

Proprietary Info

X=Present; K=Scheduled Removal

Individual Clinical Observations  
(Phase A)

12/18/2018 12:30:08PM Page: 15 of 20

Proprietary Info

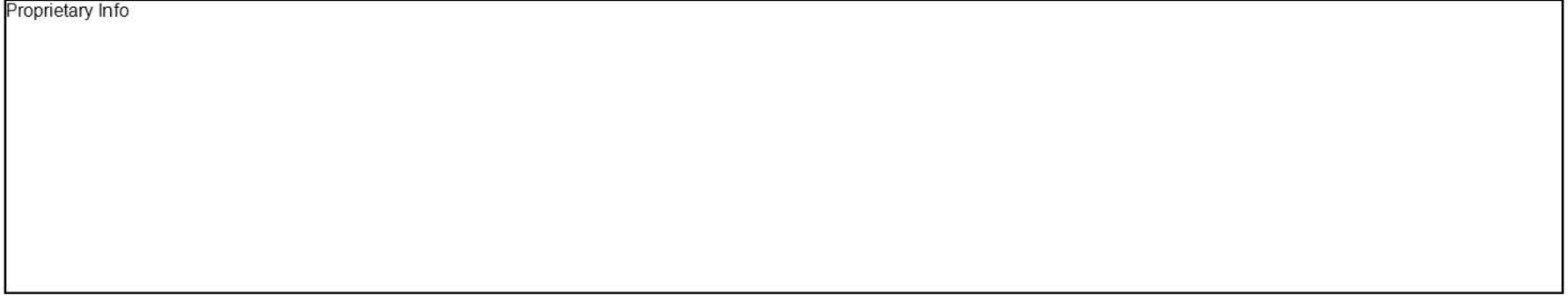


X=Present; K=Scheduled Removal

Individual Clinical Observations  
(Phase A)

12/18/2018 12:30:08PM Page: 16 of 20

Proprietary Info



X=Present; K=Scheduled Removal

Individual Clinical Observations  
(Phase A)

12/18/2018 12:30:08PM Page: 17 of 20

Proprietary Info

X=Present; K=Scheduled Removal

Individual Clinical Observations  
(Phase A)

12/18/2018 12:30:08PM Page: 18 of 20

Proprietary Info

X=Present; K=Scheduled Removal



Individual Clinical Observations  
(Phase A)

12/18/2018 12:30:08PM Page: 19 of 20

Proprietary Info

Individual Clinical Observations  
(Phase A)

12/18/2018 12:30:08PM Page: 20 of 20

B173-18 - Maximum Tolerated Dose and Pharmacokinetic Study of [Proprietary Info]  
Following a Single Dose Administration to Male and Female C57BL/6 Mice

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Key Page

**General Footnotes**

Provantis version 10.1.0.1  
"." indicates Not Applicable

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings</u>		
1	[Proprietary] (20;IV)	Control	Group 1	20 mg/kg	IV
2	[Proprietary] (40;IV)	Dose	Group 2	40 mg/kg	IV
3	[Proprietary Info]	Dose	Group 3	20 mg/kg	IV
4	[Proprietary] (20;IP)	Dose	Group 4	20 mg/kg	IP
5	[Proprietary] (40;IP)	Dose	Group 5	40 mg/kg	IP
6	[Proprietary Info]	Dose	Group 6	40 mg/kg	IP
7	[Proprietary] 40;PO	Dose	Group 7	40 mg/kg	PO
8	[Proprietary] 100;PO	Dose	Group 8	100 mg/kg	PO
9	[Proprietary] 100;PO	Dose	Group 9	100 mg/kg	PO

**Timeslot Definition**

<u>Abbreviation</u>	<u>Description</u>
IPD	Immediate post dose
2-4	2-4 hr post dose

**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Appendix C-2**

**INDIVIDUAL CLINICAL OBSERVATIONS (PHASE B)**

Individual Clinical Observations  
(Phase B)

12/18/2018 12:21:40PM Page: 1 of 10

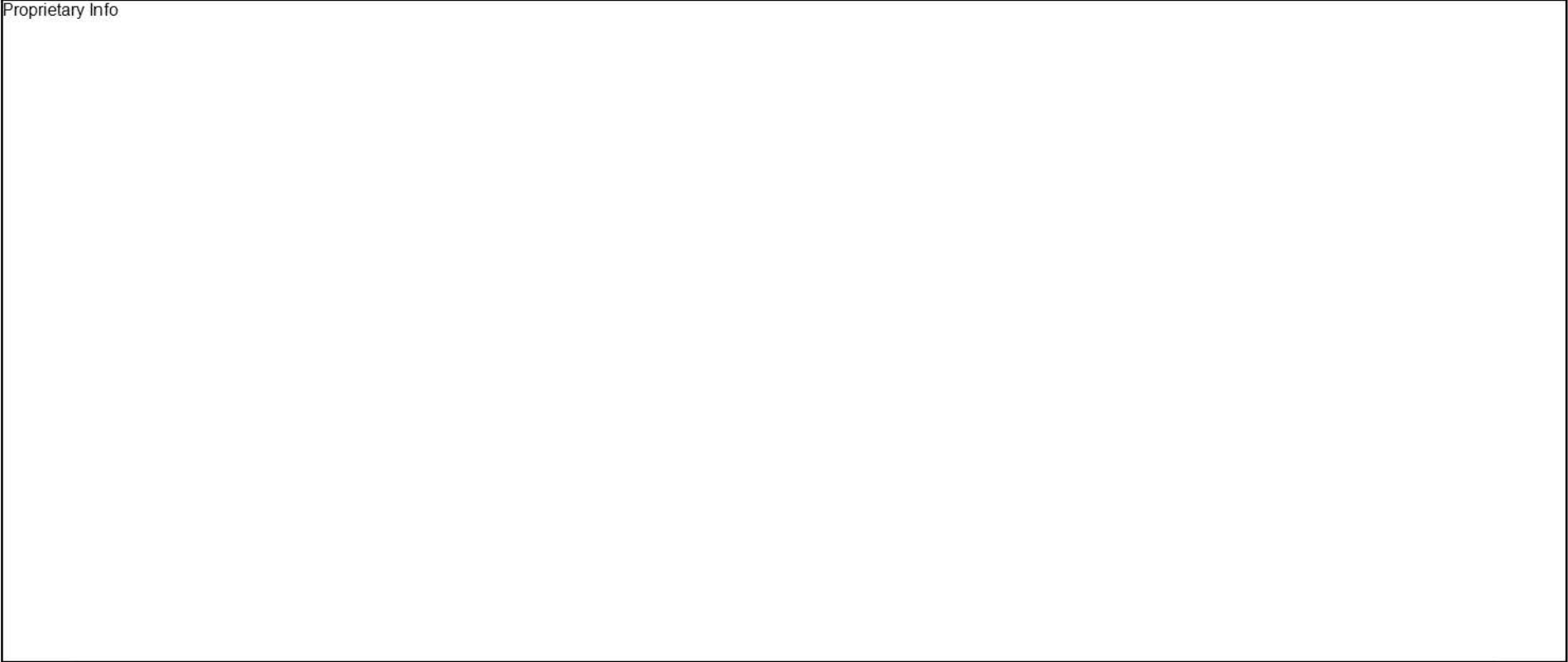
Proprietary Info



Individual Clinical Observations  
(Phase B)

12/18/2018 12:21:40PM Page: 2 of 10

Proprietary Info



K=Scheduled Removal; X=Present; S=Slight; M=Moderate; E=Extreme

Obtained via FOIA by White Coat Waste Project

Individual Clinical Observations  
(Phase B)

12/18/2018 12:21:40PM Page: 3 of 10

Proprietary Info





Individual Clinical Observations  
(Phase B)

12/18/2018 12:21:40PM Page: 4 of 10


Proprietary Info



Individual Clinical Observations  
(Phase B)

12/18/2018 12:21:40PM Page: 5 of 10

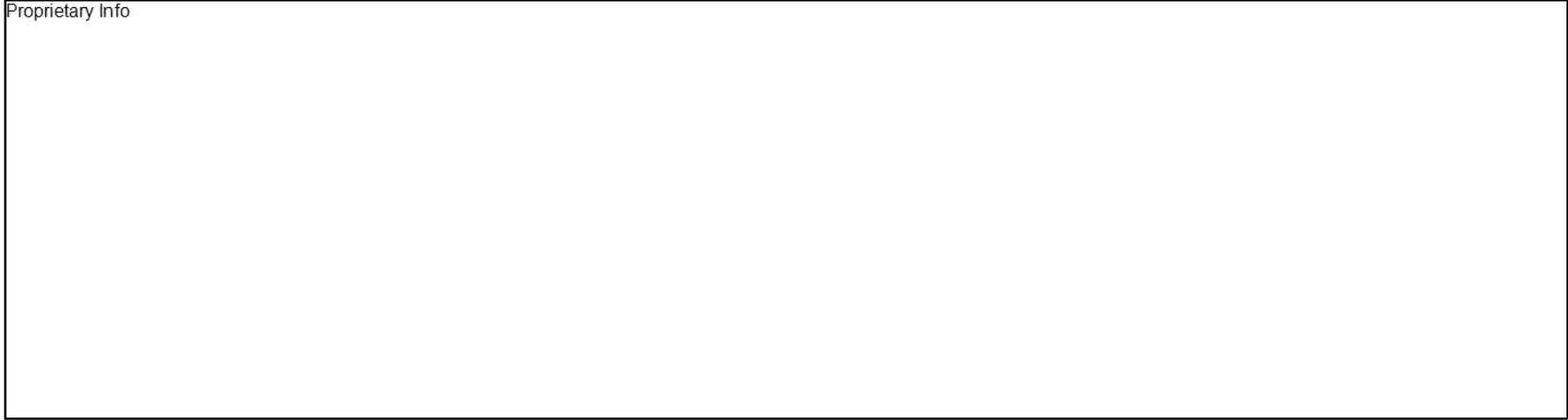
Proprietary Info



Individual Clinical Observations  
(Phase B)

12/18/2018 12:21:40PM Page: 6 of 10

Proprietary Info




K=Scheduled Removal; X=Present; S=Slight

Proprietary Info



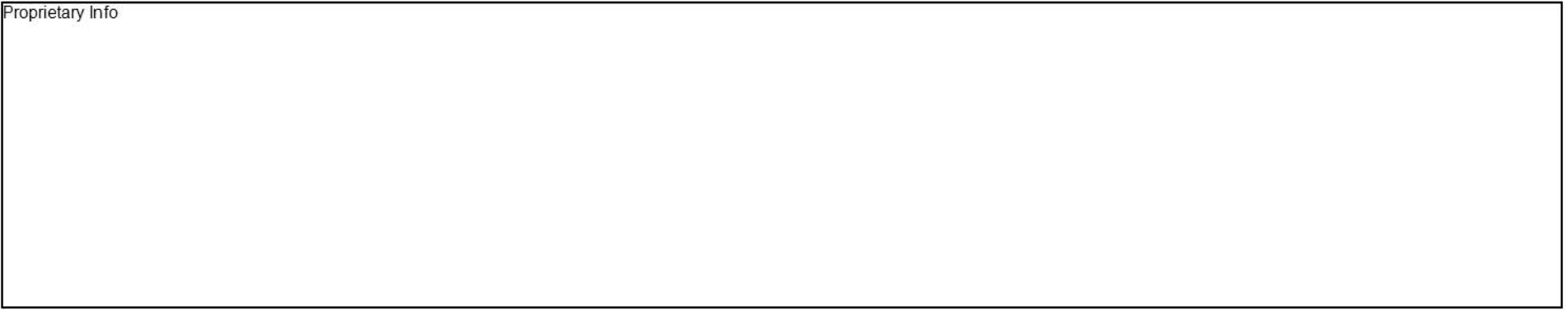
Proprietary Info



Individual Clinical Observations  
(Phase B)

12/18/2018 12:21:40PM Page: 9 of 10

Proprietary Info



Individual Clinical Observations  
(Phase B)

12/18/2018 12:21:40PM Page: 10 of 10

B173-18 - Maximum Tolerated Dose and Pharmacokinetic Study of Proprietary Info  
Following a Single Dose Administration to Male and Female C57BL/6 Mice

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Key Page

**General Footnotes**

Provantis version 10.1.0.1  
"." indicates Not Applicable

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings</u>		
1	<span style="border: 1px solid black; padding: 0 2px;">Proprietary</span> (20)IV	Control	Group 1	20 mg/kg	IV
2	<span style="border: 1px solid black; padding: 0 2px;">Proprietary</span> (40)IP	Dose	Group 2	40 mg/kg	IP
3	<span style="border: 1px solid black; padding: 0 2px;">Proprietary</span> (100)PO	Dose	Group 3	100 mg/kg	PO

**Timeslot Definition**

<u>Abbreviation</u>	<u>Description</u>
IPD	Immediate post dose
P-e	Pre-euthanasia



Maximum Tolerated Dose and Pharmacokinetic Study of Proprietary Info  
Following a Single Dose Administration to Male and Female C57BL/6 Mice  
SRI Study No. B173-18

Appendix D

BIOANALYTICAL CHEMISTRY

Written by:

Personal Info

Redacted by agreement

May 15, 2019  
Date

Approved by:

Personal Info

Redacted by agreement

5/15/2019  
Date

Biosciences Division  
SRI International

**Maximum Tolerated Dose and Pharmacokinetic Study of [Proprietary Info]**  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

## **I. INTRODUCTION**

The objective of this study was to measure the plasma drug levels of [Proprietary Info] and [Proprietary Info] in male and female C57BL/6 mouse plasma samples collected in SRI Study No. B173-18 after an intraperitoneal (ip), intravenous (iv) or oral gavage (po) administration of [Proprietary Info]. Sample analysis was not performed in accordance with the U.S. FDA "Good Laboratory Practice for Nonclinical Laboratory Studies," as described in 21 CFR Part 58. Methods of collection of plasma are outlined in the main study protocol. Specific bioanalytical methods and results of plasma analyses are presented in the sections that follow.

## **II. MATERIALS AND METHODS**

### **A. Materials**

Acetonitrile, isopropanol, and methanol were purchased from Avantor (Center Valley, PA). Pooled-gender C57BL/6 mice plasma was purchased from Bioreclamation, IVT (Hicksville, NY). Dimethyl sulfoxide and formic acid were purchased from Sigma-Aldrich (Milwaukee, WI). Reference standards [Proprietary Info] and internal standard [Proprietary Info] were obtained from the Chemical Repository at SRI. Chromatography for [Proprietary Info] was achieved with a Synergi Polar RP 80 Å column (50 x 4.6 mm; 4 µ) purchased from Phenomenex (Torrance, CA).

### **B. Bioanalytical Method**

[Proprietary Info] and [Proprietary Info] **Calibration Standards.** Calibration standards (25, 100, 250, 500, 1000, 1750, 2500, and 4000 ng/ml and Quality Control (QC) samples (50, 750, and 3500 ng/ml) were prepared in blank pooled C57BL/6 mice plasma as follows. A primary stock solution of 1.0 mg/ml [Proprietary Info] was prepared by accurately weighing out compound and adding dimethyl sulfoxide to achieve a concentration of 1.0 mg/ml in solution. This 1.0 mg/ml solution was then used to prepare various spiking solutions by dilution with dimethyl sulfoxide. One volume of spiking solution was added to 99 volumes of blank C57BL/6 mouse plasma to attain the nominal concentrations of standards with a final non-plasma matrix of 1.0%. These calibration standards and QCs were prepared and stored at -70°C (nominal), thawed, and analyzed in triplicate on each day of study sample analysis.

**Sample Preparation for [Proprietary Info] and [Proprietary Info] Plasma Analysis.** The bioanalytical method for analysis and quantification of [Proprietary Info] and [Proprietary Info] in mouse plasma (sample volume 50 µl) entailed the addition of 200 µl internal standard solution (100 ng/ml of [Proprietary Info] in acetonitrile) to the standards, QCs, and study samples. The tubes were vortexed for 10 min, and then centrifuged for 10 min at 18000g to remove the precipitated proteins. All the supernatants were transferred to clean tubes, and the solvent was evaporated under vacuum. The dried residues were reconstituted with 60 µl of 50% acetonitrile with 0.1% formic acid. The reconstituted samples were then vortexed for 5 min on a multi-tube vortex mixer at one half speed, clarified by

**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

centrifugation (18000 g, 5 min), and then transferred to HPLC vials fitted with glass inserts for LC-MS/MS analysis. Study samples were quantitated using a set of calibration standards prepared in blank matrix that were processed in parallel.

**Chromatographic Analysis.** Samples were analyzed by LC-MS/MS and the details of the method are provided below.

**LC Conditions**

HPLC Instrument: Waters 2795 Alliance Integrated System  
Autosampler Temp.: 10°C  
Column: Synergi Polar RP 80 Å column (50 x 4.6 mm; 4 µ)  
ColumnTemp.: 20°C  
Mobile phase: A= Milli-Q-Water with 0.1% formic acid  
B= Acetonitrile with 0.1% formic acid  
Elution Mode: Gradient;

<u>Time (min)</u>	<u>%A</u>	<u>%B</u>	<u>Flow rate (ml/min)</u>
0	95	5	0.3
1.0	95	5	0.3
10.0	5	95	0.3
12.0	5	95	0.3
12.2	95	5	0.3
15.0	95	5	0.3

Flow Split: none  
Flow Divert: none

Injection volume: 20 µl  
Needle Wash: 75:25:0.1(v:v:v) acetonitrile:isopropanol:formic acid  
Purge Solution: 10:90 methanol:Milli-Q-Water with 0.1% formic acid

**MS Conditions**

Instrument: Micromass Quattro LC  
Ionization: Electrospray ionization, positive ion mode  
Detection: Multiple Reaction Monitoring (MRM);

<u>Analyte</u>	<u>MRM Transition (m/z)</u>	<u>Collision Energy</u>	<u>Retention Time (min)</u>
<span style="border: 1px solid black; padding: 2px;">Proprietary Info</span>	586.30→344.80	50 eV	~5.36
	392.20→362.10	35 eV	~5.43
	348.00→318.00	35 eV	~5.63

Dwell Times: 0.333 sec  
Resolution: Unit mass resolution at Q1 and Q3  
Capillary Voltage: 3.50 kV  
Cone Voltage: 45 V



**Maximum Tolerated Dose and Pharmacokinetic Study of [Proprietary Info]  
Following a Single Dose Administration to Male and Female C57BL/6 Mice  
SRI Study No. B173-18**

Desolvation Temp.: 450°C  
Source Temp.: 115°C  
Quantitation: Integration and Quantitation by the Quanlynx portion of Masslynx Software ver.4.1.

[Proprietary Info] **Data Analysis.** The method to quantitate [Proprietary Info] in plasma samples using calibration standards was as follows: Peak areas of the [Proprietary Info] were divided by the peak area of the [Proprietary Info] (internal standard) to yield peak area ratios (PAR). The calibration standard curve for the drug being assayed was prepared by performing weighted (1/y) linear regression for the peak area ratio (PAR) as the dependent variable (y-axis) and concentration as the independent variable (x-axis). The regression yields an equation of the form:

$$\text{Peak Area Ratio} = m \cdot [\text{Proprietary Info}] + b$$

Where m is the slope of the regression and b is the y-intercept. [Proprietary Info] concentrations in the QC standards and the study experimental samples then were calculated as follows:

$$[\text{Proprietary Info}] = (\text{PAR} - b)/m$$

These calculations are performed by the QuanLynx portion of the Masslynx software. The goodness of fit of this standard curve is indicated by the coefficient of determination ( $r^2$ ) obtained from the quadratic regression, with perfect fit yielding an  $r^2$  value of 1.000.

[Proprietary Info] **Data Analysis.** The calibration standard curves for [Proprietary Info] were prepared by performing weighted (1/x) quadratic regression of the peak area ratio (PAR) of [Proprietary Info] to that of [Proprietary Info] (internal standard) as the dependent variable (y-axis), and [Proprietary Info] concentration as the independent variable (x-axis). The regression yields an equation of the form:

$$\text{PAR} = a [\text{Proprietary Info}]^2 + c$$

To solve for the concentration of [Proprietary Info] in the samples, the quadratic equation was first made equal to zero by subtracting y (peak area ratio of [Proprietary Info]) from both sides of the equation, which results in a new constant (c-y), and then solving the equation below:

$$\text{Concentration of } [\text{Proprietary Info}] (x) = \frac{-b \pm \sqrt{b^2 - 4a(c-y)}}{2a}$$

These calculations are performed by the QuanLynx portion of the Masslynx software. The goodness of fit of this standard curve is indicated by the coefficient of determination ( $r^2$ ) obtained from the quadratic regression, with perfect fit yielding an  $r^2$  value of 1.000.

**Maximum Tolerated Dose and Pharmacokinetic Study of [Proprietary Info]**  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

[Proprietary Info] **Lower Limit of Quantitation (LLOQ).** The LLOQ for this standard is defined as the lowest concentration that can be measured yielding method precision values of relative standard deviation (RSD)  $\pm 20\%$  and accuracy values of  $\pm 20\%$  of the nominal value. For this study we set 25 ng/ml as the lowest concentration point on our calibration curve. The mean back-calculated concentration for the [Proprietary Info] LLOQ standards was  $26.2 \pm 2.24 \mu\text{g/ml}$  ( $n=5$ ), which corresponds to % CV of 8.53%, and mean % accuracy of 105%. This indicates that the accuracy and precision are acceptable for a bioanalytical method at the set LLOQ for the scope of these preliminary investigations.

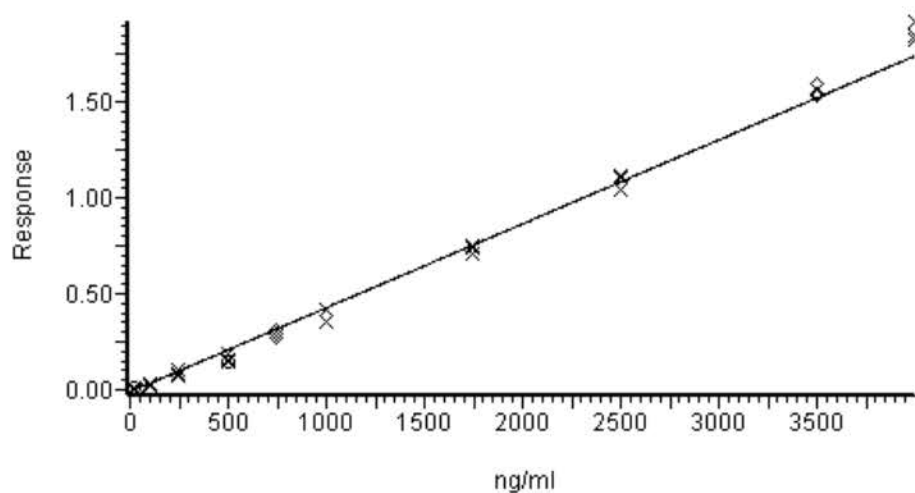
[Proprietary Info] **Lower Limit of Quantitation (LLOQ).** The LLOQ for this standard is defined as the lowest concentration that can be measured yielding method precision values of relative standard deviation (RSD)  $\pm 20\%$  and accuracy values of  $\pm 20\%$  of the nominal value. For this study we set 25 ng/ml as the lowest concentration point on our calibration curve. The mean back-calculated concentration for the [Proprietary Info] LLOQ standards was  $24.9 \pm 3.62 \mu\text{g/ml}$  ( $n=3$ ), which corresponds to % CV of 14.6%, and mean % accuracy of 99.5%. This indicates that the accuracy and precision are acceptable for a bioanalytical method at the set LLOQ for the scope of these preliminary investigations.

### III. ANALYSIS OF PLASMA SAMPLES

Calibration curve data for all days of analysis are presented in Table D-1 for [Proprietary Info] and Table D-2 for [Proprietary Info]. A representative standard curve for the sample assay is presented in Figure D-1 for [Proprietary Info] and Figure D-2 for [Proprietary Info]. Results of the QC samples over the course of the analytical runs in this study are summarized in Table D-3 for [Proprietary Info] and Table D-4 for [Proprietary Info]. The experimental samples from this study were analyzed with the bioanalytical method described, and the results of these analyses for the individual mouse at each of the plasma samples are presented in Table D-5 for [Proprietary Info] Table D-6 for [Proprietary Info]

**Maximum Tolerated Dose and Pharmacokinetic Study of [Proprietary Info]  
Following a Single Dose Administration to Male and Female C57BL/6 Mice  
SRI Study No. B173-18**

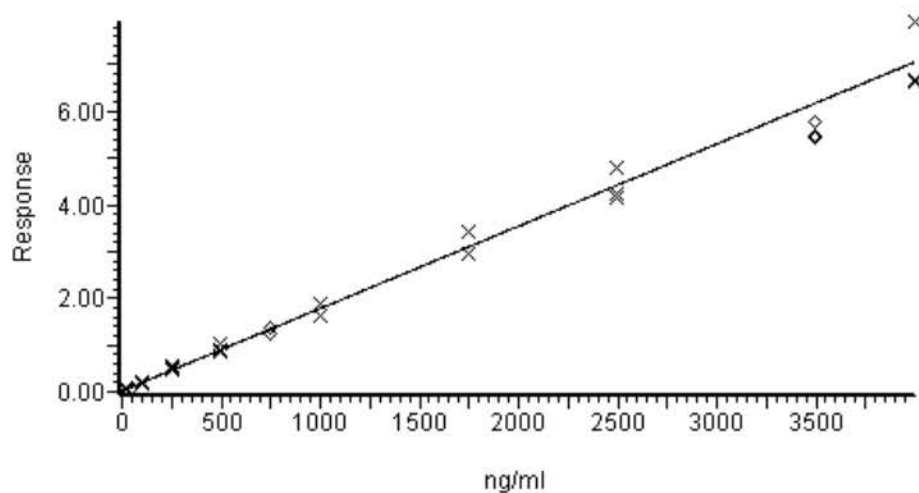
Compound name: [Proprietary Info]  
Correlation coefficient:  $r = 0.996370$ ,  $r^2 = 0.992753$   
Calibration curve:  $0.000438172 * x + -0.0109461$   
Response type: Internal Std ( Ref 2 ), Area \* ( IS Conc. / IS Area )  
Curve type: Linear, Origin: Exclude, Weighting:  $1/y$ , Axis trans: None



**Figure D-1.** Plasma standard curve for analysis of [Proprietary Info]. Calibration standards are depicted with an X, while QC samples are depicted with a diamond.

**Maximum Tolerated Dose and Pharmacokinetic Study of [Proprietary Info]**  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

Compound name: [Proprietary Info]  
Coefficient of Determination:  $R^2 = 0.992602$   
Calibration curve:  $-1.19141 \times 10^{-9} * x^2 + 0.00176455 * x + 0.0206801$   
Response type: Internal Std (Ref 2), Area \* (IS Conc. / IS Area)  
Curve type: 2nd Order, Origin: Exclude, Weighting: 1/x, Axis trans: None



**Figure D-2.** Plasma standard curve for analysis of [Proprietary Info]. Calibration standards are depicted with an X, while QC samples are depicted with a diamond.



**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Table D-1**

Proprietary Info **CALIBRATION CURVE DATA USED DURING SAMPLE ANALYSIS**

<b>Run Date</b>	<b>Slope</b>	<b>Intercept</b>	<b>Coefficient of Determination (<math>r^2</math>)</b>
<b>1/21/2018</b>	7.26E-04	2.97E-02	0.989
<b>1/29/2019</b>	4.38E-04	-1.09E-02	0.993

**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Table D-2**

Proprietary  
Info

**CALIBRATION CURVE DATA USED DURING SAMPLE ANALYSIS**

<b>Run Date</b>	<b>a</b>	<b>b</b>	<b>c</b>	<b>Coefficient of Determination (<math>r^2</math>)</b>
1/21/2018	1.19E-09	1.76E-03	0.0207	0.993

**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Table D-3**

**QC DATA OBTAINED DURING SAMPLE ANALYSIS**

**Accuracy and Precision of C57BL/6 Mouse Plasma QC Samples**

Proprietary Info

**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Table D-4**

**QC DATA OBTAINED DURING SAMPLE ANALYSIS**

**Accuracy and Precision of C57BL/6 Mouse Plasma QC Samples**

Proprietary Info

**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Table D-5**

**MEAN PLASMA DRUG LEVELS OF** Proprietary Info

**Group: Baseline**

Proprietary Info
------------------

<sup>a</sup>LLOQ = 50 ng/ml

<sup>b</sup>NA = Not Applicable

**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Table D-5 (continued)**

**MEAN PLASMA DRUG LEVELS OF** Proprietary Info

**Group 1**  
**Dose 20 mg/kg IV**  
**Males**

Proprietary Info

<sup>a</sup>LLOQ = 25 ng/ml  
<sup>b</sup>NA = Not Applicable

**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Table D-5 (continued)**

**MEAN PLASMA DRUG LEVELS OF** Proprietary Info

**Group 1**

**Dose 20 mg/kg IV**

**Females**

Proprietary Info

<sup>a</sup>LLOQ = 25 ng/ml

<sup>b</sup>NA = Not Applicable



## Proprietary Info

Proprietary Info

Proprietary Info

<sup>b</sup>NA = Not Applicable

## Proprietary Info

Proprietary Info

Proprietary Info

<sup>b</sup>NA = Not Applicable

**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Table D-5 (continued)**

**MEAN PLASMA DRUG LEVELS OF** Proprietary Info

**Group 3**

**Dose 100 mg/kg PO**

**Males**

Proprietary Info

<sup>a</sup>LLOQ = 25 ng/ml

<sup>b</sup>NA = Not Applicable

**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Table D-5 (concluded)**

**MEAN PLASMA DRUG LEVELS OF** Proprietary Info

**Group 3**  
**Dose 100 mg/kg PO**  
**Females**

Proprietary Info

<sup>a</sup>LLOQ = 25 ng/ml

<sup>b</sup>NA = Not Applicable

**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Table D-6**

**MEAN PLASMA DRUG LEVELS OF**

Proprietary Info

**Group: Baseline**

Proprietary Info

<sup>a</sup>LLOQ = 25 ng/ml

<sup>b</sup>NA = Not Applicable

## Proprietary Info

Proprietary Info

Proprietary Info

Male

Proprietary Info

<sup>b</sup>NA = Not Applicable

**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Table D-6 (continued)**

**MEAN PLASMA DRUG LEVELS OF**

Proprietary Info

**Group 1**  
**Dose 20 mg/kg IV**  
**Female**

Proprietary Info

<sup>a</sup>LLOQ = 25 ng/ml

<sup>b</sup>NA = Not Applicable



**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Table D-6 (continued)**

**MEAN PLASMA DRUG LEVELS OF** Proprietary Info

**Group 2**  
**Dose 40 mg/kg IP**  
**Male**

Proprietary Info

<sup>a</sup>LLOQ = 25 ng/ml

<sup>b</sup>NA = Not Applicable

**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Table D-6 (continued)**

**MEAN PLASMA DRUG LEVELS OF** Proprietary Info

**Group 2**  
**Dose 40 mg/kg IP**  
**Females**

<span style="border: 1px solid black; padding: 2px;">Proprietary Info</span>	
--	--

<sup>a</sup>LLOQ = 25 ng/ml

<sup>b</sup>NA = Not Applicable

## Proprietary Info

10

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10

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10

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**Maximum Tolerated Dose and Pharmacokinetic Study of** Proprietary Info  
**Following a Single Dose Administration to Male and Female C57BL/6 Mice**  
**SRI Study No. B173-18**

**Table D-6 (concluded)**

**MEAN PLASMA DRUG LEVELS OF** Proprietary

**Group 3**

**Dose 100 mg/kg PO**

**Female**

Proprietary Info

<sup>a</sup>LLOQ = 25 ng/ml

<sup>b</sup>NA = Not Applicable

Final Report • October 29, 2019

# METHOD VALIDATION REPORT FOR THE QUANTITATIVE ANALYSIS OF Proprietary Info IN K<sub>2</sub> EDTA DOG PLASMA

**Validation Scientist:**Redacted by agreement**Testing Facility:**

SRI International  
Biosciences Division  
333 Ravenswood Avenue  
Menlo Park, CA 94025

**SRI Study Number:** B185-18  
**SRI Project Number:** P25035.411

**Sponsor:**

National Institute of Allergy and Infectious Diseases  
Division of AIDS  
5601 Fishers Lane, Redacted by agreement  
Bethesda, MD 20892-9830

**Sponsor's Representative:**Redacted by agreement

**NIAID Contract Number:** HHSN272201400006I/TO- HHSN27200008

**Method Validation Report for the Quantitative Analysis of Lopinavir, Ritonavir, and  
Tenofovir in K<sub>2</sub> EDTA Dog Plasma  
SRI Study No. B185-18**

**APPROVAL SIGNATURES**

Validation Scientist:

Personal Info

Redacted by agreement

10/29/19  
Date

Approved by:

Personal Info

Redacted by agreement

10/25/2019  
Date

Personal Info

Redacted by agreement

10-25-19  
Date

SRI International  
Biosciences Division  
333 Ravenswood Avenue  
Menlo Park, CA 94025

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**SUMMARY**

A bioanalytical method was validated for the quantitative analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA dog plasma. Full details of the validated analytical method are provided in SRI Test Method 106.201 (Appendix A). The validation demonstrated that the method is appropriate for quantitation of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA dog plasma.

This validation was initiated following method development, which included determination of the assay range, selectivity, intra-batch accuracy and precision, sensitivity, recovery, matrix effect on ionization, matrix effects using 6 unique lots of matrix, and stability of the analyte in the matrix. The parameters investigated during this validation were based on the results obtained from these method development experiments. There were no significant changes made to the methodology between method development and validation. Results from the method development experiments will not be reported here.

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**QUALITY ASSURANCE UNIT**

**Final Report and  
Conflict of Interest Statement**

SRI's Quality Assurance Unit assures that the non-GLP study - Method Validation Report for the Quantitative Analysis of Proprietary Proprietary and Proprietary in K<sub>2</sub> EDTA Dog Plasma, SRI Study No. B185-18-- has been reviewed for consistency with the U.S. Food and Drug Administration Good Laboratory Practice Regulations (21 CFR Part 58).

The following inspections were conducted during this study:

<u>Phase Inspected</u>	<u>Date of Inspection</u>	<u>Date Findings Reported to Management/Study Director</u>
Bioanalytical Method Validation	05-20-19	05-20-19
Raw Data	07-31-19	07-31-19
Draft Final Report	07-31-19	07-31-19
Final Report Verification	10-29-19	10-29-19

This statement certifies that the personnel listed below participated in the inspections and audit of this study. These personnel have not been involved in the generation or evaluation of the data. Participation by the individuals listed below poses no conflict of interest.

Redacted by agreement

Redacted by agreement

10/29/19  
Date

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**KEY PERSONNEL**

<b>Name</b>	<b>Functional Role</b>
Redacted by agreement	



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**SUMMARY OF VALIDATION PARAMETERS**

Analytes	[Proprietary] [Proprietary] [Proprietary]
Internal standards	[Proprietary Info] [Proprietary Info] [Proprietary Info]
Matrix	K <sub>2</sub> EDTA dog plasma
Quantitation range	5.00-1000 ng/ml [Proprietary] and [Proprietary] 25.0-5000 ng/ml [Proprietary Info]
Sample volume	0.0200 ml
Extraction procedure	Methanol protein precipitation
Analytical method	LC-MS/MS
Regression type, weighting	Linear, 1/x [Proprietary] and [Proprietary] Linear, 1/x <sup>2</sup> [Proprietary Info]
Maximum batch size	93 samples
Correlation Coefficient	≥ 0.9992 [Proprietary Info] ≥ 0.9993 [Proprietary] ≥ 0.9992 [Proprietary Info]
Intra- and inter-assay QC levels	
LLOQ	5.00 ng/ml [Proprietary] and [Proprietary] 25.0 ng/ml [Proprietary Info]
Low	15.0 ng/ml [Proprietary] and [Proprietary] 75.0 ng/ml [Proprietary Info]
Mid	400 ng/ml [Proprietary] and [Proprietary] 2000 ng/ml [Proprietary Info]
High	800 ng/ml [Proprietary] and [Proprietary] 4000 ng/ml [Proprietary Info]
Dilution QC level	5000 ng/ml [Proprietary] and [Proprietary] 25000 ng/ml [Proprietary Info] diluted 10-fold, 50-fold
Intra-batch precision	1.1% to 5.5% [Proprietary Info] 1.0% to 5.5% [Proprietary] 0.6% to 6.0% [Proprietary Info]
Intra-batch accuracy	91.4% to 105.1% [Proprietary Info] 94.7% to 106.8% [Proprietary] 94.5% to 109.0% [Proprietary Info]
Inter-batch precision	2.6% to 7.3% [Proprietary Info] 2.2% to 6.3% [Proprietary] 2.2% to 6.3% [Proprietary Info]
Inter-batch accuracy	98.4% to 99.3% [Proprietary Info] 96.5% to 100.4% [Proprietary] 96.6% to 102.5% [Proprietary Info]
Mean recovery of Analyte	95.4% [Proprietary Info] 94.6% [Proprietary] 100.1% [Proprietary Info]
Mean recovery of Internal Standard	108.9% [Proprietary Info] 107.8% [Proprietary Info] 105.4% [Proprietary Info]



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Mean matrix effect on ionization Analyte	110.0% [Proprietary Info] 142.2% [Proprietary] 99.0% [Proprietary Info]
Mean matrix effect on ionization Internal Standard	111.9% [Proprietary Info] 143.7% [Proprietary Info] 98.4% ([Proprietary Info])
Selectivity	6 out of 6 lots satisfied acceptance criteria
Matrix effects	6 out of 6 lots satisfied acceptance criteria
Analyte carryover	<20% of mean LLOQ peak area for [Proprietary] and [Proprietary] Some BI/BI samples showed evidence of carryover for [Proprietary] Refer to the Carryover section of this report for additional information.
Internal Standard carryover	<5% of mean internal standard peak area
Room temperature stability	26 hours established (all analytes)
Freeze thaw stability	5 cycles established (all analytes)
Reinjection (autosampler) stability	188 hours (refrigerated) established
Post-preparative extract stability	85 hours (refrigerated) established (all analytes)
Whole blood processing stability	4 hours (all analytes)
Effect of hemolysis	0.5% and 2% hemolysis; no impact (all analytes)
Refrigerated stock stability	40 days
Room temperature stock stability	23 hours
Long term matrix storage stability	Interim 21 days established ( $\leq -60^{\circ}\text{C}$ ) (all analytes). Stability is ongoing.

## I. INTRODUCTION

A bioanalytical method was validated for the quantitative analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA dog plasma, over the concentration ranges 5.00-1000 ng/ml [Proprietary] and [Proprietary] and 25.0 ng/ml-5000 ng/ml [Proprietary Info] with 0.0200 ml sample volumes. The internal standards used for this assay were [Proprietary Info] [Proprietary Info] and [Proprietary Info]. The analytes and the internal standards were extracted from K<sub>2</sub> EDTA dog plasma using a methanol protein precipitation followed by LC-MS/MS detection. The finalized analytical method used throughout this validation is described in SRI Test Method 106.201 (Appendix A).

Per the validation plan (Appendix B), the validation included determination of linearity and range, selectivity, intra-batch assay and inter-batch assay accuracy and precision, carryover, matrix effects, matrix effect on ionization, recovery, dilution assessment, room temperature stability, freeze thaw stability, reinjection (autosampler) stability, post-preparative extract stability, whole blood processing stability, effect of hemolysis, effect of concomitant medication, and stock solution stability. Long term matrix storage stability evaluation is ongoing (an interim evaluation has been performed), and the results from this experiment will be provided in an

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amended report. A full description of these parameters and the acceptance criterion for each is detailed in SRI SOP 006.060, *Bioanalytical Method Validation*.

This validation was not within the scope of U.S. Food and Drug Administration (FDA) “Good Laboratory Practice for Nonclinical Laboratory Studies” (GLP) regulations, as described in 21 CFR Part 58. Nevertheless, this validation was planned, performed, recorded, and reported in accordance with standard practices to ensure data quality and integrity. This report presents the methodology and results of the validation, which demonstrate that the method is appropriate for the quantitation of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA dog plasma over the concentration ranges tested.

The freezer storage temperatures stated in this report are nominal. The temperature of the Quality Control (QC) storage freezers did not go above –60°C from the time of initial storage of the QC samples until the final analysis of these samples. Any recorded departure from the manufacturer’s specifications for a particular freezer unit would result in a facility deviation. No deviations were generated as a result of this over the course of this study.

## II. REFERENCE STANDARDS

The certificates of analysis for the analytes and internal standards are provided in Appendix C.

### Reference Standard Description

Reference Standard	Supplier	Lot Number	Correction Factor	Storage Conditions	Expiration
[Proprietary]	U.S. Pharmacopeia	R077R0	0.997	Refrigerated, Protected from light	Current lot
[Proprietary]	U.S. Pharmacopeia	H0M427	0.993	Refrigerated, Protected from light	Current lot
[Proprietary]	U.S. Pharmacopeia	R044C0	0.940	Refrigerated	Current lot
[Proprietary Info]	Medical Isotopes, Inc.	183	0.980 <sup>b</sup>	-20°C	Retest 06-30-24
[Proprietary Info]	Medical Isotopes, Inc.	411	0.970 <sup>b</sup>	-20°C	Retest 04-30-20
[Proprietary Info]	Medical Isotopes, Inc.	091	0.970 <sup>b</sup>	-20°C	Retest 10-31-20

<sup>a</sup> [Proprietary] was supplied as [Proprietary] monohydrate. The final correction factor used during stock solution preparation is based on the amount of [Proprietary] present when weighing.

<sup>b</sup> The purity was assumed as 100% during weighing.



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### **III. VALIDATION RESULTS**

#### **A. Summary of Runs Performed**

Eight analytical runs were analyzed as part of this validation and are summarized in Table 1.

Pooled control K<sub>2</sub> EDTA dog plasma (lot number BGL110446, expiration 04-30-21), which was used in the preparation of calibration curves, QC samples, and assay blanks, was obtained from BioIVT, Westbury, NY. on 04-02-19 and was stored in a -20°C freezer until use. Six unique lots of matrix (lot numbers BGL110440 to BGL110445, expiration 04-30-21) from individual animals used for the assessment of selectivity and matrix effects were also obtained from this supplier on this date. Pooled K<sub>2</sub> EDTA dog whole blood (lot number BGL111230, expiration 05-30-19), used in the assessment of whole blood processing stability, was also obtained from BioIVT on 05-01-19, and was stored refrigerated until use.

#### **B. Assay Acceptance Criterion**

For a calibration curve to be considered acceptable, at least 75% of calibration standards must be accurate to within  $\pm 15\%$  of the nominal concentration ( $\pm 20\%$  at the lower limit of quantitation, or LLOQ), including at least one replicate at the lowest and highest concentrations.

For accuracy and precision experiments, individual QC samples at low, mid and high concentrations were considered acceptable if they were accurate to within  $\pm 15\%$  of the nominal concentration ( $\pm 20\%$  at the LLOQ). The intra- and inter-batch accuracy and precision were acceptable if they were  $\leq 15\%$  ( $\pm 20\%$  at the LLOQ). Within a run, at least 50% of the QC samples at each concentration must have satisfied the acceptance criterion, with at least 67% of the total QC samples in a run satisfying the acceptance criterion.

For QC samples used in batch acceptance, at least 67% of all QC samples must be within  $\pm 15\%$  of the nominal concentration, with at least 50% of the QC samples at each concentration meeting this criterion.

For QC samples not used in accuracy and precision evaluation, including, but not limited to, stability QC samples, effect of hemolysis samples, and dilution QC samples, at least 50% of the individual replicates at each concentration were considered acceptable if they were within  $\pm 15\%$  of the nominal concentration. Overall accuracy and precision at each concentration were considered acceptable if they were within  $\pm 15\%$ . For QC samples prepared at the low concentration for the assessment of matrix effects in 6 unique lots of plasma, at least 50% of individual replicates for each lot were considered acceptable if they were within  $\pm 15\%$  of the nominal concentration, and the overall accuracy and precision of each lot was within  $\pm 15\%$ .

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For whole blood processing stability, the % difference between the mean calculated concentration of the timepoint under evaluation and the mean of the time zero calculated concentration must be within  $\pm 15\%$  to be considered stable.

For solution stability, the % difference between the mean peak area ratio of the fresh solution and the mean peak area ratio of the stored solution must be within  $\pm 10\%$  to be considered stable.

Full details of the acceptance criterion for each validation parameter are detailed in SRI SOP 006.060.

**C. Standard Curve Linearity**

A total of eight calibration standards, extracted in duplicate, were used in the construction of each calibration curve. The correlation coefficient (r) in each run was at least 0.9992 for [Proprietary] and 0.9993 for [Proprietary] using a least-squares linear regression with a  $1/x$  weighting. The correlation coefficient (r) in each run was at least 0.9992 for [Proprietary] using a least-squares linear regression with a  $1/x^2$  weighting (Tables 2-4). The K<sub>2</sub> EDTA dog plasma back-calculated calibration curve concentrations generated from all validation runs are presented in Tables 5-7. The calibration curve ranged from 5.00 ng/ml (LLOQ) to 1000 ng/ml (upper limit of quantitation, ULOQ) for [Proprietary] and [Proprietary] and from 25.0 ng/ml to 5000 ng/ml for [Proprietary]. Calibration standards were prepared fresh on each day of extraction. Representative calibration curves from Run MV1-RI are shown in Figures 1-3.

**D. Accuracy and Precision of QC Samples**

To assess the accuracy and precision of the method, QC samples in K<sub>2</sub> EDTA dog plasma were freshly prepared on the day of analysis at the LLOQ (5.00 ng/ml for [Proprietary] and [Proprietary] and 25.0 ng/ml for [Proprietary]) low (15.0 ng/ml for [Proprietary] and [Proprietary] and 75.0 ng/ml for [Proprietary]) mid (400 ng/ml for [Proprietary] and [Proprietary] and 2000 ng/ml for [Proprietary] and high (800 ng/ml for [Proprietary] and [Proprietary] and 4000 ng/ml for [Proprietary]) concentrations. To determine intra- and inter- batch accuracy and precision, these QC samples were extracted in three analytical runs in replicates of six at each concentration.

The intra-batch precision across all concentrations and analytical runs ranged from 1.1% to 5.5% [Proprietary Info] 1.0% to 5.5% [Proprietary] and 0.6% to 6.0% [Proprietary Info]. The intra-batch accuracy ranged from 91.4% to 105.1% [Proprietary Info] 94.7% to 106.8% [Proprietary] and 94.5% to 109.0% [Proprietary Info]. The inter-batch precision across all concentrations and analytical runs ranged from 2.6% to 7.3% [Proprietary Info] 2.2% to 6.3% [Proprietary] and 2.2% to 6.3% [Proprietary Info]. The inter-batch accuracy ranged from 98.4% to 99.3% [Proprietary Info] 96.5% to 100.4% [Proprietary] and 96.6% to 102.5% [Proprietary Info]. Summaries of the intra- and inter-batch accuracy and precision results are provided in Tables 8-10.



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**E. Maximum Batch Size**

One accuracy and precision batch of sufficient size was analyzed to represent a typical batch when running study samples. The maximum batch size of 93 samples was determined by counting the number of injections made from the first calibration standard to the last calibration standard, in Run MV2.

**F. Selectivity**

To confirm the selectivity of the assay for each analyte, blank K<sub>2</sub> EDTA dog plasma (containing no analyte or internal standard) from six individual animals were analyzed in replicates of n=1. Additionally, a single BI/BI sample in plasma matrix representing 0.5% and 2% hemolysis was also prepared and analyzed. No significant interference (defined as a peak at the retention time of analyte with a peak area of >20% of the mean peak area of the LLOQ calibration standard) was detected in any individual lot, or in the hemolyzed samples. There was no significant interference at the retention time of the internal standard (>5% of the mean internal standard peak area); therefore, lack of selectivity did not appear to be significant. Refer to Tables 11-13 for a summary of the results for each analyte.

Additionally, pooled K<sub>2</sub> EDTA dog plasma was used to prepare double blank samples containing no analytes or internal standards (BI/BI) and blank samples containing only internal standards but no analytes (BI/IS); these samples were extracted in duplicate in each analytical run. There was no significant interference at the retention time of either the analytes or internal standards in the BI/BI samples, as defined above, with the following exceptions. In Run MV1-RI, the second BI/BI sample showed a significant interference at the analyte retention time for both [Proprietary] and [Proprietary]. This was probably due to carryover, as the sample directly preceding this was a concomitant medication sample, which was prepared at low [Proprietary] concentrations but high (2000 ng/ml) [Proprietary] and [Proprietary] concentrations, in order to determine the effect of [Proprietary] and [Proprietary] on [Proprietary] quantitation. No other BI/BI samples for either [Proprietary] or [Proprietary] showed significant interference. The second BI/BI sample from Run MV2-RI showed significant interference for [Proprietary] but again this was probably related to carryover as a high QC sample directly preceded injection of this sample. In all BI/IS samples, there was no significant interference at the retention time of the analytes, as defined above. Refer to Tables 14-16 for a summary of the results for each analyte.

In one analytical run, a sample prepared at the ULOQ but containing no internal standards (ULOQ/BI) was extracted in replicates of n=1. This ULOQ sample contained only [Proprietary] or only [Proprietary] or only [Proprietary] – the analytes were not co-spiked. There was no significant interference originating from the analyte at the retention time of the internal standards (defined as >5% of the mean extracted internal standard peak area). There was no significant interference at the analyte retention time in the [Proprietary] or [Proprietary] ion channels when the ULOQ/BI [Proprietary] sample was injected, and similarly, no interference was observed in the other analyte ion channels when the ULOQ/BI [Proprietary] and [Proprietary] samples were injected.

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**G. Carryover Assessment**

Carryover was assessed in each analytical run by injecting a BI/BI sample immediately after each ULOQ calibration standard. There was no significant interference (defined as a peak at the retention time of [Proprietary] and [Proprietary] with a peak area of >20% of the mean analyte peak area of the LLOQ calibration standards, or a peak at the retention time of the internal standard with a peak area of >5% of the mean internal standard peak area); therefore, carryover does not appear to have a significant impact on this assay for these analytes. Significant carryover was seen in one carryover blank sample in batches MV1-RI, MV2, and MV2-RI for [Proprietary]. Refer to Tables 14-16 for a summary of the results.

**H. Dilution Assessment**

To evaluate the ability to dilute a sample prepared at a concentration higher than the analytical range, a dilution QC sample was prepared at 5000 ng/ml [Proprietary] and [Proprietary] and 25000 ng/ml [Proprietary] fresh on the day of extraction. This QC was diluted 10-fold and 50-fold with control K<sub>2</sub> EDTA dog plasma and extracted in replicates of six. The overall results for both dilution schemes satisfied the acceptance criterion as defined by SRI SOP 006.060. Refer to Tables 17-19 for a summary of the results.

**I. Matrix Effects**

To evaluate matrix effects, K<sub>2</sub> EDTA dog plasma from six individual animals was spiked at the low QC concentration (15.0 ng/ml for [Proprietary] and [Proprietary] and 75.0 ng/ml for [Proprietary] and extracted in replicates of four. The results for all six lots satisfied the acceptance criterion for each analyte as defined by SRI SOP 006.060, demonstrating that matrix effects do not have a significant impact on this assay. Refer to Tables 20-22 for a summary of the results.

**J. Recovery**

The recovery of the method for the analytes and the internal standards was assessed by comparing the peak areas of each analyte and internal standard in extracted QC samples at low, mid and high concentrations with the peak areas obtained from extracted BI/BI plasma samples. The methanol supernatant from the extracted BI/BI samples was diluted with recovery solutions containing all analytes and internal standards to give final concentrations equivalent to the theoretical levels in the extracted samples (post-extracted spiked samples). All samples were evaluated in replicates of six. The recovery solution diluent was water:methanol (90:10, v:v) with 0.1% acetic acid.

The mean recovery of [Proprietary], [Proprietary] and [Proprietary] across concentrations was 95.4%, 94.6%, and 100.1%, respectively. The mean recovery of [Proprietary Info], [Proprietary Info] and [Proprietary Info] was 108.9%, 107.8%, and 105.4%, respectively. There is no defined acceptance criterion for recovery, and these mean recovery values indicate



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that the degree of recovery achieved during this extraction procedure is satisfactory. Refer to Tables 23-28 for a summary of the results.

**K. Matrix Effect on Ionization**

The ability of the matrix to enhance or suppress the analyte or internal standard response was assessed by comparing the peak areas of analytes and internal standards in the post-extracted spiked BI/BI samples described in the Recovery section, above, with the recovery solutions. The recovery solutions were diluted with 0.2% acetic acid in methanol beforehand, which represented the plasma supernatant. All samples were evaluated in replicates of six. A value of >100% indicates enhancement caused by the matrix, and a value <100% indicates suppression.

The matrix effect on ionization of the response for [Proprietary Info] [Proprietary Info] and [Proprietary Info] was 110.0%, 142.2%, and 99.0%, respectively. The matrix effect on ionization of the response for [Proprietary Info] [Proprietary Info] and [Proprietary Info] was 111.9%, 143.7%, and 98.4%, respectively. There is no defined acceptance criterion for the acceptable degree of matrix effect on ionization. These values indicate that the matrix effect on ionization observed during this extraction procedure would not have significant impact on the assay. Refer to Tables 29-34 for a summary of the results.

**L. Room Temperature Matrix Stability**

Room temperature matrix stability of each analyte in K<sub>2</sub> EDTA dog plasma was determined by extracting QC samples at low and high concentrations, in replicates of four, which had been stored at room temperature for 26 hours before extraction. These QC samples were analyzed against a fresh calibration curve and fresh QC samples. The overall results for room temperature stability for up to 26 hours satisfied the acceptance criterion as defined by SRI SOP 006.060. Refer to Tables 35-37 for a summary of the results.

**M. Freeze Thaw Matrix Stability**

Freeze thaw matrix stability of each analyte in K<sub>2</sub> EDTA dog plasma was determined by extracting QC samples at low and high concentrations, in replicates of four, which had been subjected to five freeze thaw cycles before extraction. QC samples were stored frozen at ≤-60°C for a minimum of 24 hours prior to the first thaw and stored frozen for subsequent periods of not less than 12 hours. Samples were thawed for a minimum of 1 hour at room temperature prior to re-freezing. These QC samples were analyzed against a fresh calibration curve and fresh QC samples. The overall results for freeze thaw stability for up to five cycles satisfied the acceptance criterion as defined by SRI SOP 006.060. Refer to Tables 38-40 for a summary of the results.



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**N. Reinjection (Autosampler) Stability**

Reinjection stability of each analyte in K<sub>2</sub> EDTA dog plasma was demonstrated by reinjecting the calibration standards, the LLOQ, low, mid and high accuracy and precision QC samples, and the BI/BI, BI/IS, and carryover samples from Run MV2 after 188 hours of refrigerated storage (set point 5°C). The re-injected run was designated as Run MV2-RI. The overall results for reinjection stability for up to 188 hours under refrigerated conditions satisfied the acceptance criterion as defined by SRI SOP 006.060. Refer to Tables 41-43 for a summary of the results.

**O. Post-Preparative Extract Stability**

Post-preparative extract stability of each analyte in K<sub>2</sub> EDTA dog plasma was determined by extracting QC samples at low and high concentrations, in replicates of four, in Run MV3. These QC extracts were then stored in a refrigerated autosampler (set point 5°C) and analyzed with a fresh calibration curve and fresh QC samples prepared in Run MV4. The overall results for post-preparative extract stability for up to 85 hours under refrigerated conditions satisfied the acceptance criterion as defined by SRI SOP 006.060. Refer to Tables 44-46 for a summary of the results.

**P. Whole Blood Processing Stability**

The stability of each analyte during the processing of spiked K<sub>2</sub> EDTA dog whole blood was determined by spiking whole blood samples at two concentrations and harvesting the plasma at time zero (T0) and 1 hour and 4 hours after spiking. The whole blood samples were stored refrigerated (5°C ± 3°C) during this period of storage. The whole blood was spiked to give a final concentration of 7.50 ng/ml [Proprietary] and [Proprietary] and 37.5 ng/ml [Proprietary] representing the low analyte concentration, (assuming that 100% of the analytes partitions into the plasma) and 400 ng/ml [Proprietary] and [Proprietary] and 2000 ng/ml [Proprietary] representing the high analyte concentration. Following plasma collection, the plasma samples were stored at ≤-60°C prior to extraction. These samples were then extracted in replicates of four at each concentration, in Run MV4. The calculated final (mean) T0 concentrations in plasma at the low concentration were 12.5 ng/ml, 11.6 ng/ml, and 60.1 ng/ml for [Proprietary] [Proprietary] and [Proprietary] respectively. The calculated final (mean) T0 concentrations in plasma at the high concentration were 633 ng/ml, 611 ng/ml, and 3160 ng/ml for [Proprietary] [Proprietary] and [Proprietary] respectively. The overall results for whole blood processing stability for up to 4 hours under refrigerated conditions satisfied the acceptance criterion as defined by SRI SOP 006.060. Refer to Tables 47-49 for a summary of the results.

**Q. Effect of Hemolysis**

The effect of hemolysis on the quantitation of each analyte was determined by preparing low and high QC samples in matrix representing 0% hemolysis, 0.5% hemolysis, and 2% hemolysis, to determine whether the presence of whole blood had an impact on the ability to accurately quantify the analyte. The overall results indicated that

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hemolysis at 0.5% and 2% had no significant impact on the quantitation of each analyte as defined by SRI SOP 006.060. Refer to Tables 50-52 for a summary of the results.

**R. Long Term Matrix Storage Stability**

Long term matrix storage stability of each analyte in K<sub>2</sub> EDTA dog plasma was determined by extracting QC samples at low and high concentrations, in replicates of four, after storage for 21 days at  $\leq -60^{\circ}\text{C}$ . These QC samples were analyzed against a fresh calibration curve. Also included in this batch were freshly prepared assay acceptance QC samples (low, mid and high QC samples in replicates of two), which were extracted to determine the accuracy of the calibration curve preparation and the validity of the stability QC final results. The overall results for long term matrix storage stability for up to 21 days at  $\leq -60^{\circ}\text{C}$  satisfied the acceptance criterion as defined by SRI SOP 006.060. Refer to Tables 53-55 for a summary of these results. Determination of long term matrix storage stability of each analyte in K<sub>2</sub> EDTA dog plasma at  $\leq -60^{\circ}\text{C}$  is currently ongoing; the results will be reported in an amended report.

**S. Concomitant Medication**

The effect of [Proprietary] and [Proprietary] on [Proprietary] quantitation was assessed by preparing a plasma sample containing 15.0 ng/ml [Proprietary] (the low QC concentration), 2000 ng/ml [Proprietary] and 20000 ng/ml [Proprietary]. These concentrations of [Proprietary] and [Proprietary] were selected as they were estimated to be at or above the CMax observed in previous studies at high dose concentrations. These samples satisfied the acceptance criterion for [Proprietary] indicating that addition of [Proprietary] and [Proprietary] at high concentrations had no adverse effect on the ability to quantitate this analyte. Similarly, no impact on [Proprietary] at the low QC concentration was observed when [Proprietary] and [Proprietary] were spiked into plasma at 2000 ng/ml and 20000 ng/ml, respectively. No impact on [Proprietary] at the low QC concentration was observed when [Proprietary] and [Proprietary] were spiked into plasma at 2000 ng/ml. Refer to Tables 56-58 for a summary of these results. The results of the assay acceptance QC samples are provided in Tables 59-61.

**T. Stock Solution Stability**

Analyte stock solutions were prepared in glass vials in duplicate at the start of the validation, to give a final concentration of 1.00 mg/ml for each analyte. Dimethyl sulfoxide (DMSO) was the diluent for [Proprietary] and [Proprietary] stocks, while Milli-Q water was used as the diluent for [Proprietary] stock solutions. These solutions were stored refrigerated ( $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ ) during the validation period.

The duplicate solutions were verified before use to determine their equivalency and accuracy of preparation. By comparing the mean peak area ratio (PAR, defined as analyte peak area / internal standard peak area) of each solution, their equivalency could be determined. The mean PAR of the duplicate solutions should be  $\leq 5\%$  of each other to be considered acceptable, calculated as follows:



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$$\frac{\text{Mean PAR Duplicate 1} - \text{Mean PAR Duplicate 2}}{(\text{Mean PAR Duplicate 1} + \text{Mean PAR Duplicate 2}) / 2} \times 100$$

After 40 days of storage, new duplicate stock solutions were prepared as described above. Per SRI SOP 006.060, stock solution stability was evaluated by comparing the mean peak area ratio of the stored stock solution against the mean peak area ratio of the duplicate newly prepared solutions in replicates of six. All stock solutions were diluted prior to injection to ensure that the detection limit of the mass spectrometer was not reached, and internal standard was added in equal amounts to each solution to compensate for any variability in injection volume. Results indicate that a 1.00 mg/ml solution of each analyte was stable for up to 40 days under refrigerated conditions (Tables 62-64).

To assess room temperature stock solution storage stability, an aliquot of each freshly prepared stock solution, at the concentrations described above, was transferred into a glass vial and stored at room temperature for 23 hours. The original fresh stock solutions were stored refrigerated (set point 5°C ± 3°C) during this period. After storage, all solutions were diluted to a suitable concentration and compared in replicates of six. Internal standard was added to each sample to allow peak area ratio comparison. Results indicate that room temperature stock solution stability of each analyte was achieved for up to 23 hours (Tables 65-67).

Stability testing of the internal standard stock and spiking solutions was not conducted. The internal standard was monitored throughout the validation to determine if any breakdown products were likely to interfere with the ability to quantitate the analyte. There was no evidence of significant internal standard deterioration that could affect the analyte response or chromatography, as determined by injections of extracted BI/IS samples in each analytical batch. A response would be considered significant if, on injection of these samples, an analyte peak area >20% of the mean LLOQ peak area was detected.

#### **IV. DEVIATIONS**

There were no deviations to SRI SOPs that had any impact on the integrity of the study.

#### **V. DATA MANAGEMENT**

The LC-MS/MS data were acquired, peak areas were integrated, the calibration line regression was calculated, and the final concentrations were generated using AB Sciex Analyst software, version 1.6.2 (AB SCIEX, Framingham, MA). Figures 4-18 show the integrations performed by the Analyst software on selected chromatographs. The statistics described in this report were generated with this software, using unrounded values, with the exception of the

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statistics used to calculate recovery and matrix effect on ionization, which were generated using Microsoft Excel 2016 (Microsoft, Corp., Redmond, WA).

## **VI. DATA STORAGE**

The final report, raw data, supporting documents, and records specific to this study will be retained and stored by SRI International. All records will be maintained for at least 1 year. At the end of the retention period, the Sponsor will be contacted regarding further disposition of these records.

## **VII. DISCUSSION AND CONCLUSION**

The validation demonstrated that the method is appropriate for quantitation of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA dog plasma over the concentration range 5.00-1000 ng/ml [Proprietary] and [Proprietary] and 25.0-5000 ng/ml [Proprietary Info]

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**VIII. DEFINITIONS AND ABBREVIATIONS**

Accuracy:	$\frac{\text{Calculated concentration}}{\text{Nominal concentration}} * 100$
Bl/Bl:	Double blank, containing no analyte or internal standard
Bl/IS:	Blank, containing only internal standard
CV:	Coefficient of variation; $\text{SD/mean} * 100$
% Difference (stability evaluations):	$\frac{((\text{New value} - \text{original value})/\text{original value}) * 100}{}$
% Difference (specificity / carryover evaluations):	$\frac{((\text{Peak area (blank)}/\text{Mean peak area (LLOQ)}) * 100}{}$
DMSO:	Dimethyl sulfoxide
K <sub>2</sub> EDTA:	Di-potassium ethylenediaminetetraacetic acid
IS:	Internal standard <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span> <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span> <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span>
LC-MS/MS:	Liquid chromatography-mass spectrometer (tandem or triple-quadrupole mass spectrometer)
LLOQ:	Lower limit of quantitation
Matrix effects on Ionization:	$\frac{\text{Mean peak area (post extracted spiked)}}{\text{Mean peak area (recovery solution)}} * 100$
MV:	Method validation
PAR:	Peak area ratio
QC:	Quality control
% Recovery:	$\frac{\text{Mean peak area (extract)}}{\text{Mean peak area (post extracted spiked)}} * 100$
ULOQ:	Upper limit of quantitation
ULOQ/Bl:	Sample contains analyte at the ULOQ concentration but no internal standard

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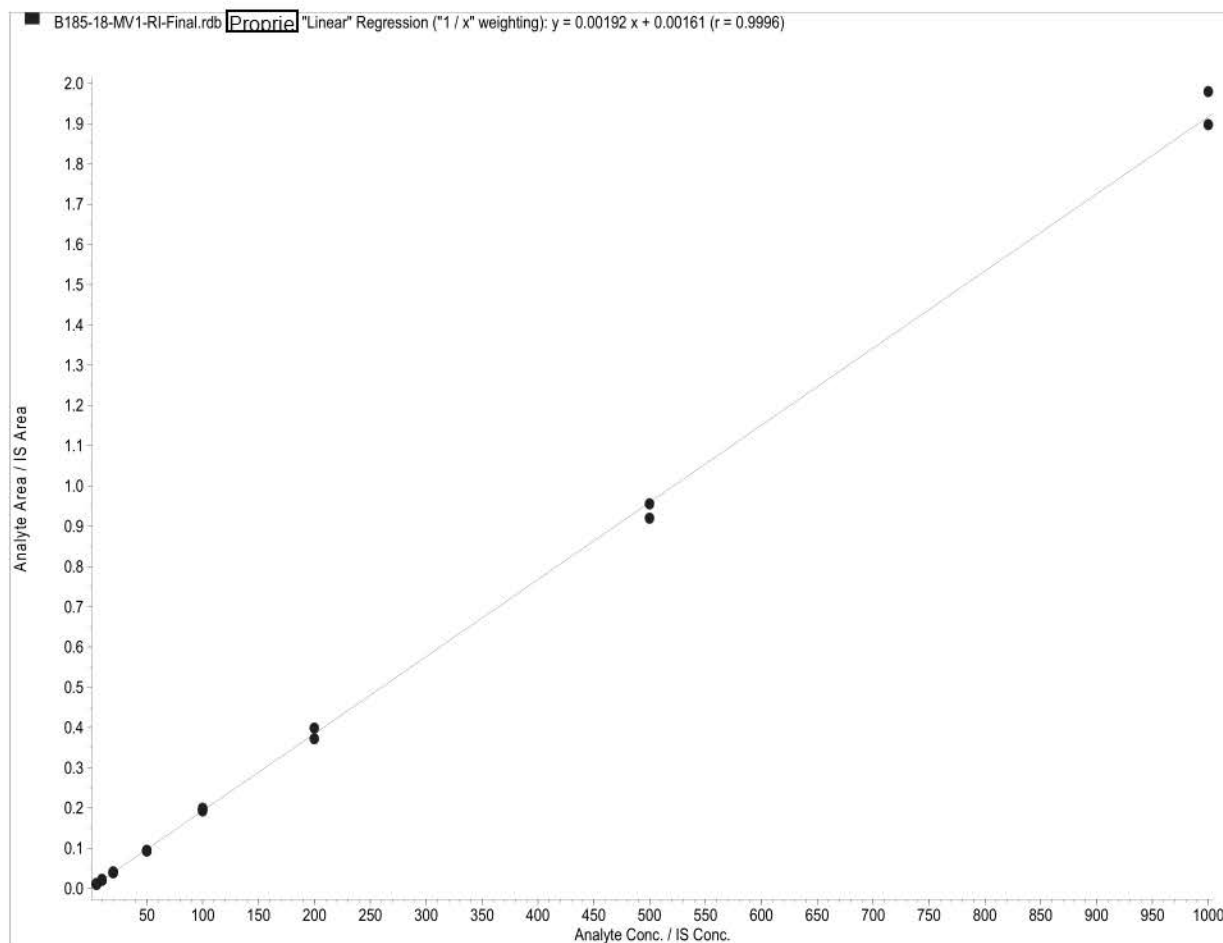
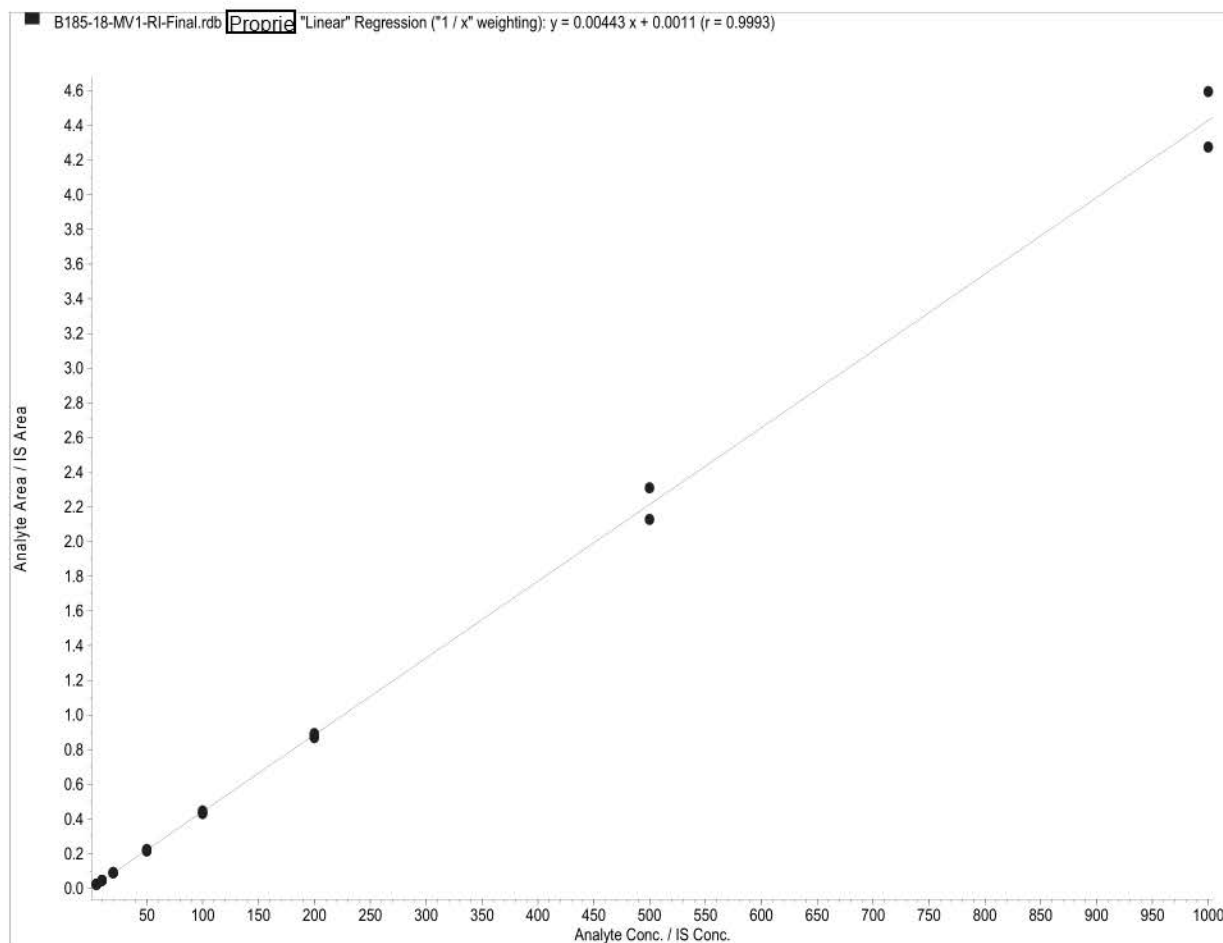


Figure 1. Representative [Proprietary] K<sub>2</sub> EDTA dog plasma calibration curve.

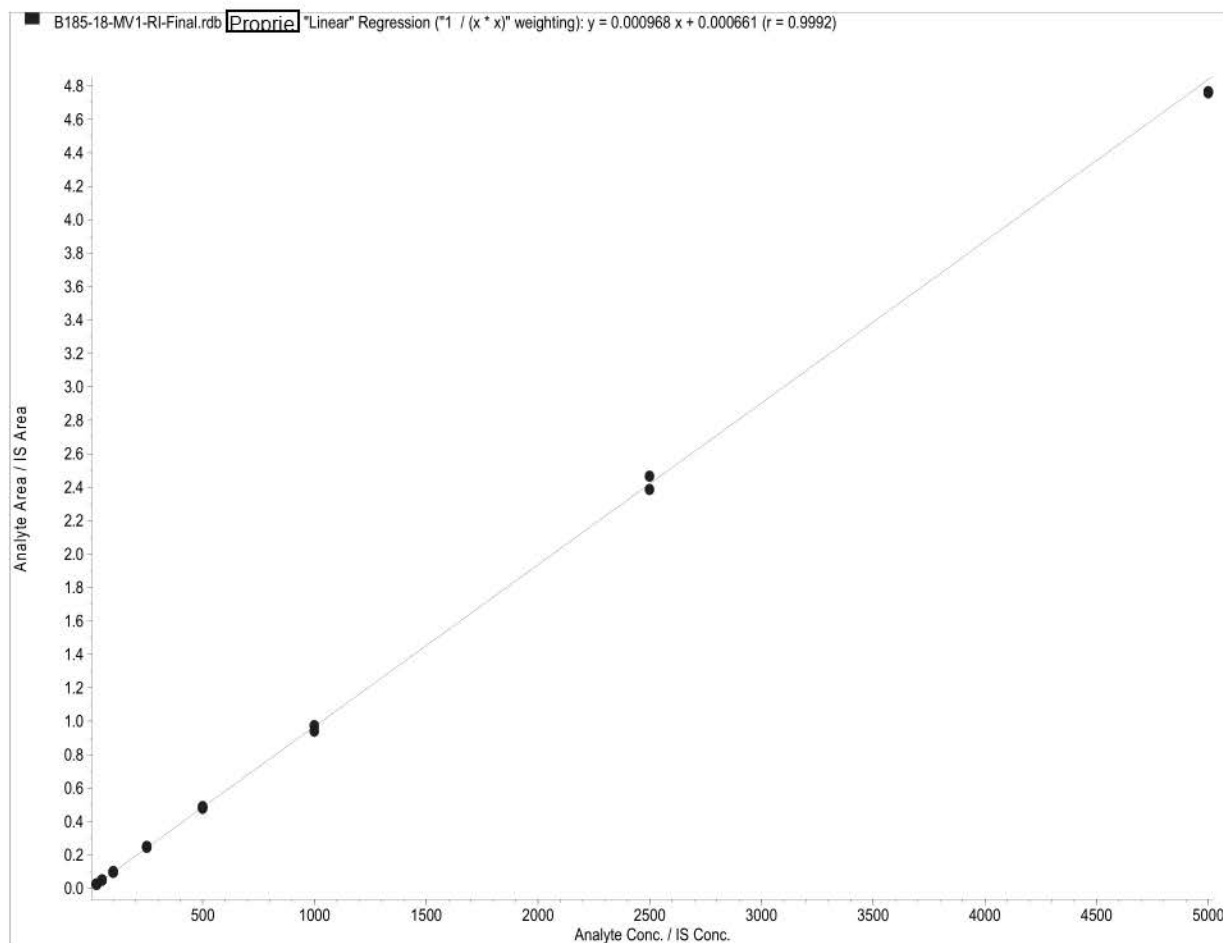


Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma  
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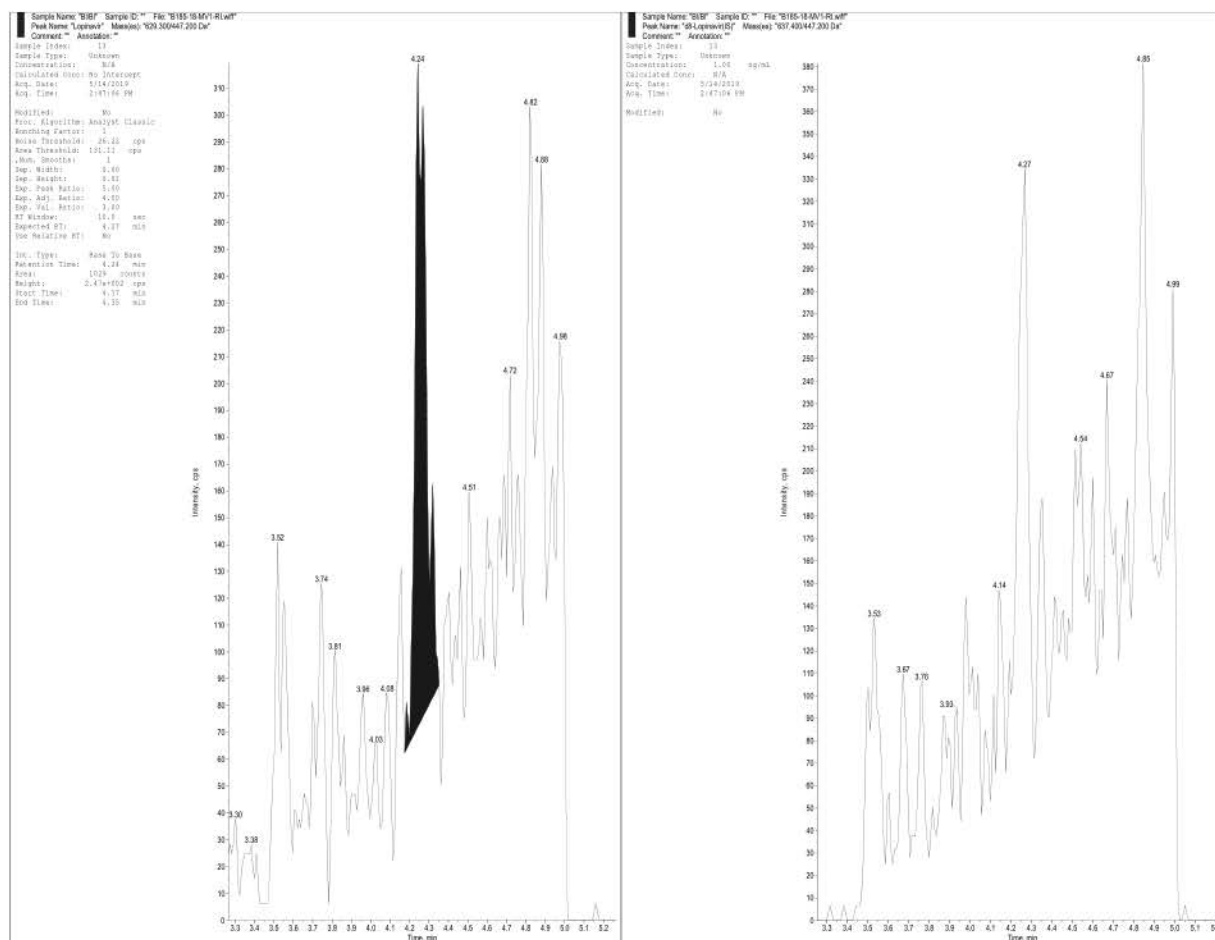
**Figure 2.** Representative [Proprietary] K<sub>2</sub> EDTA dog plasma calibration curve.

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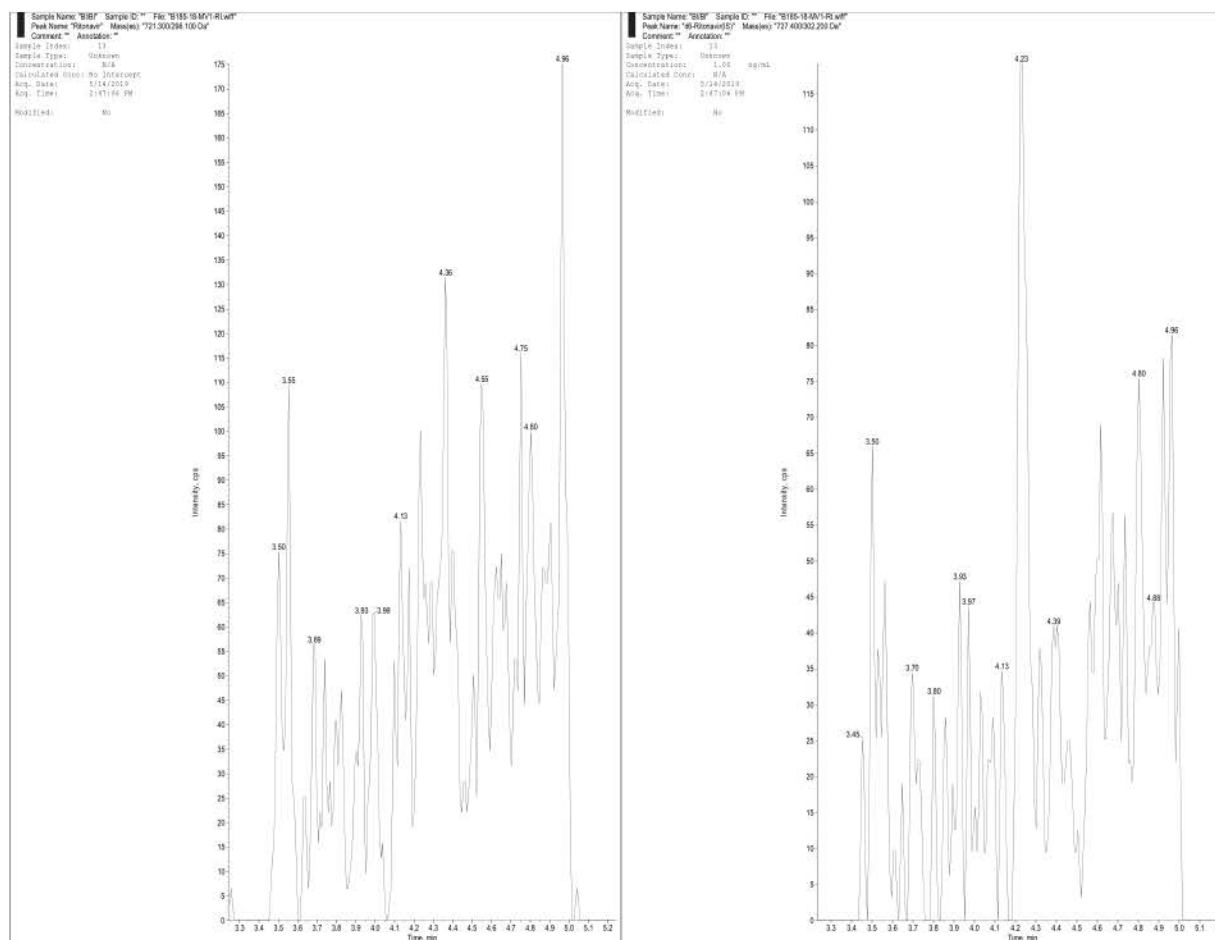
**Figure 3.** Representative [Proprietary] K<sub>2</sub> EDTA dog plasma calibration curve.

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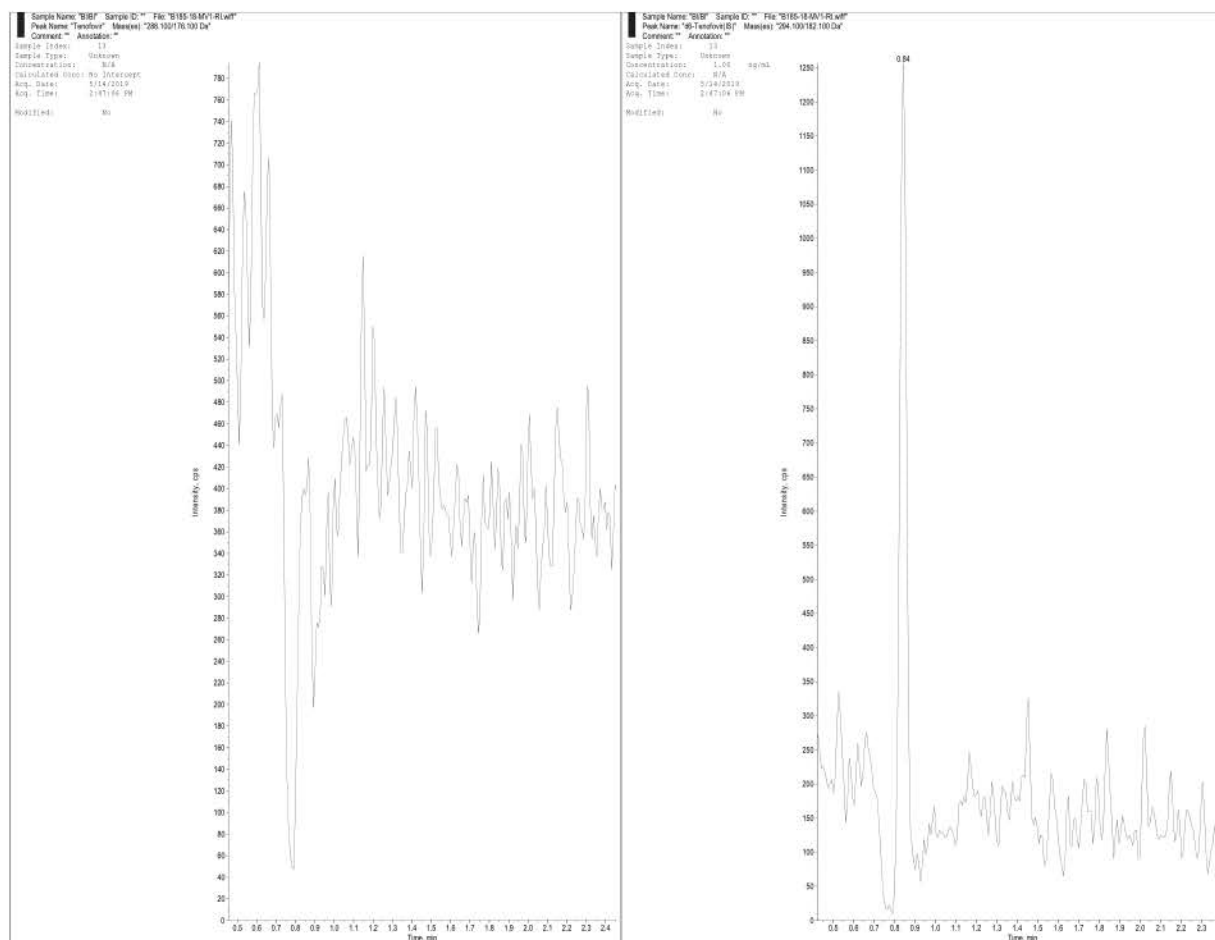
**Figure 4.** Representative chromatogram of a BI/BI K<sub>2</sub> EDTA dog plasma sample (without [Proprietary] or internal standard).

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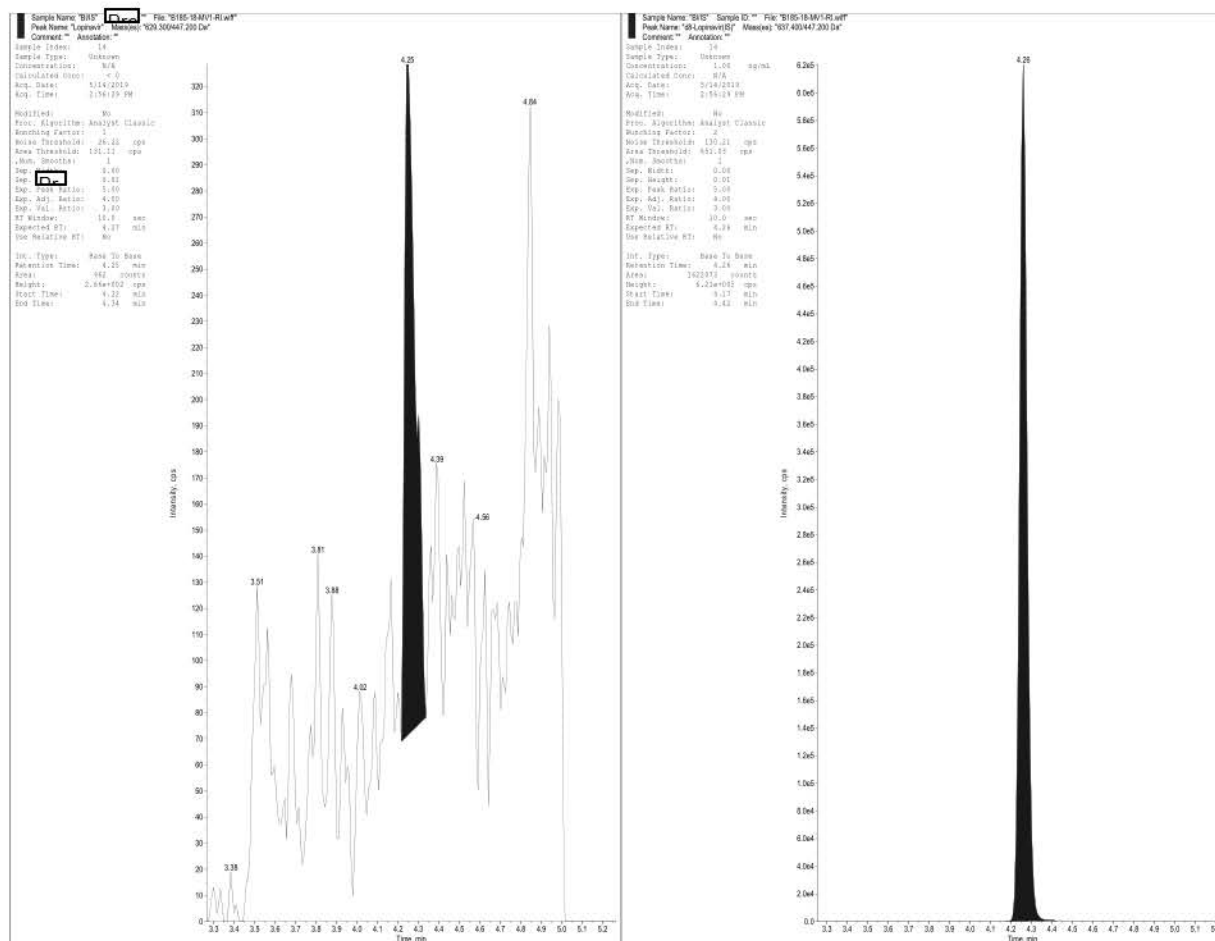
**Figure 5.** Representative chromatogram of a BI/BI K<sub>2</sub> EDTA dog plasma sample (without [Proprietary] or internal standard).

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**Figure 6.** Representative chromatogram of a BI/BI K<sub>2</sub> EDTA dog plasma sample (without [Proprietary] or internal standard).

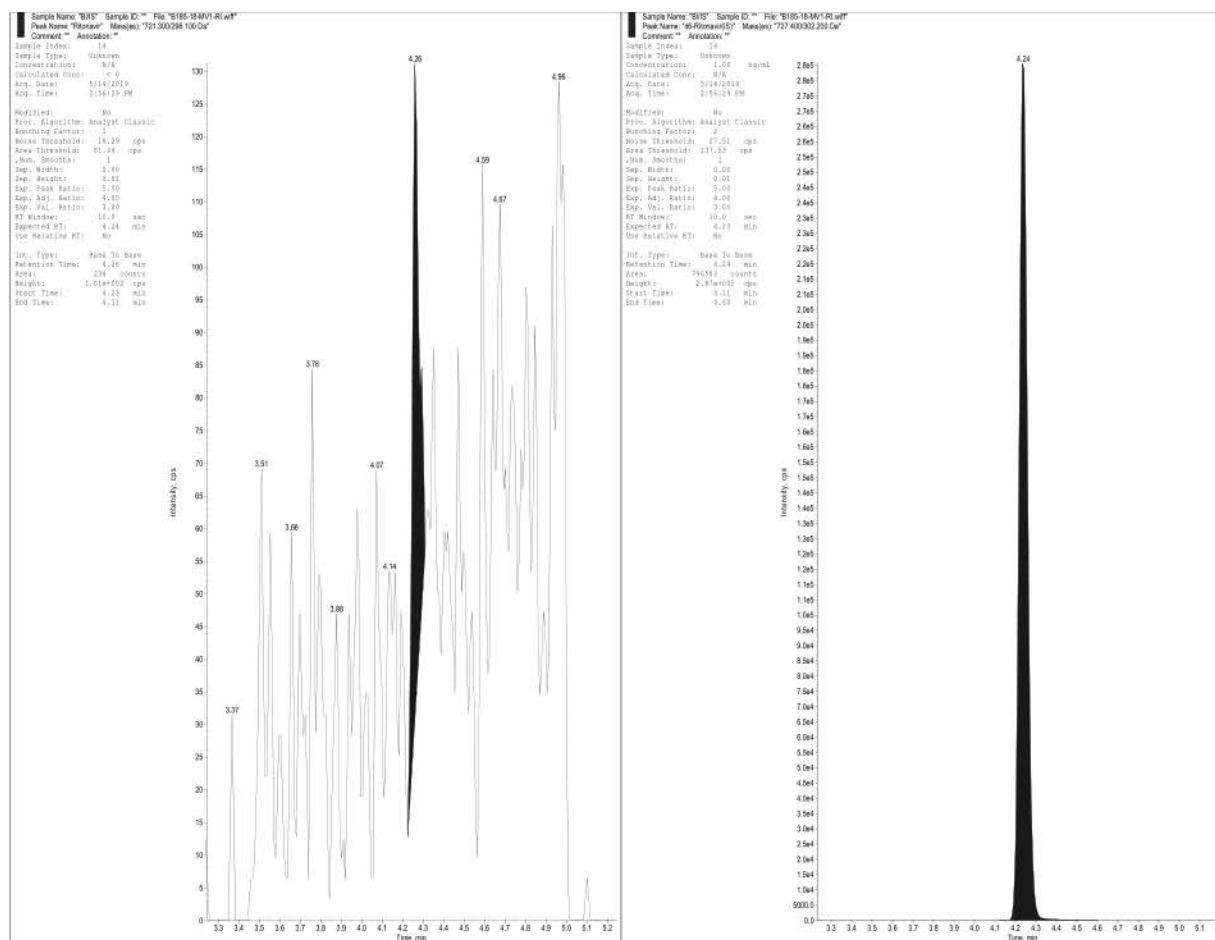
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**Figure 7.** Representative chromatogram of a BI/IS K<sub>2</sub> EDTA dog plasma sample (without [Proprietary] with internal standard).

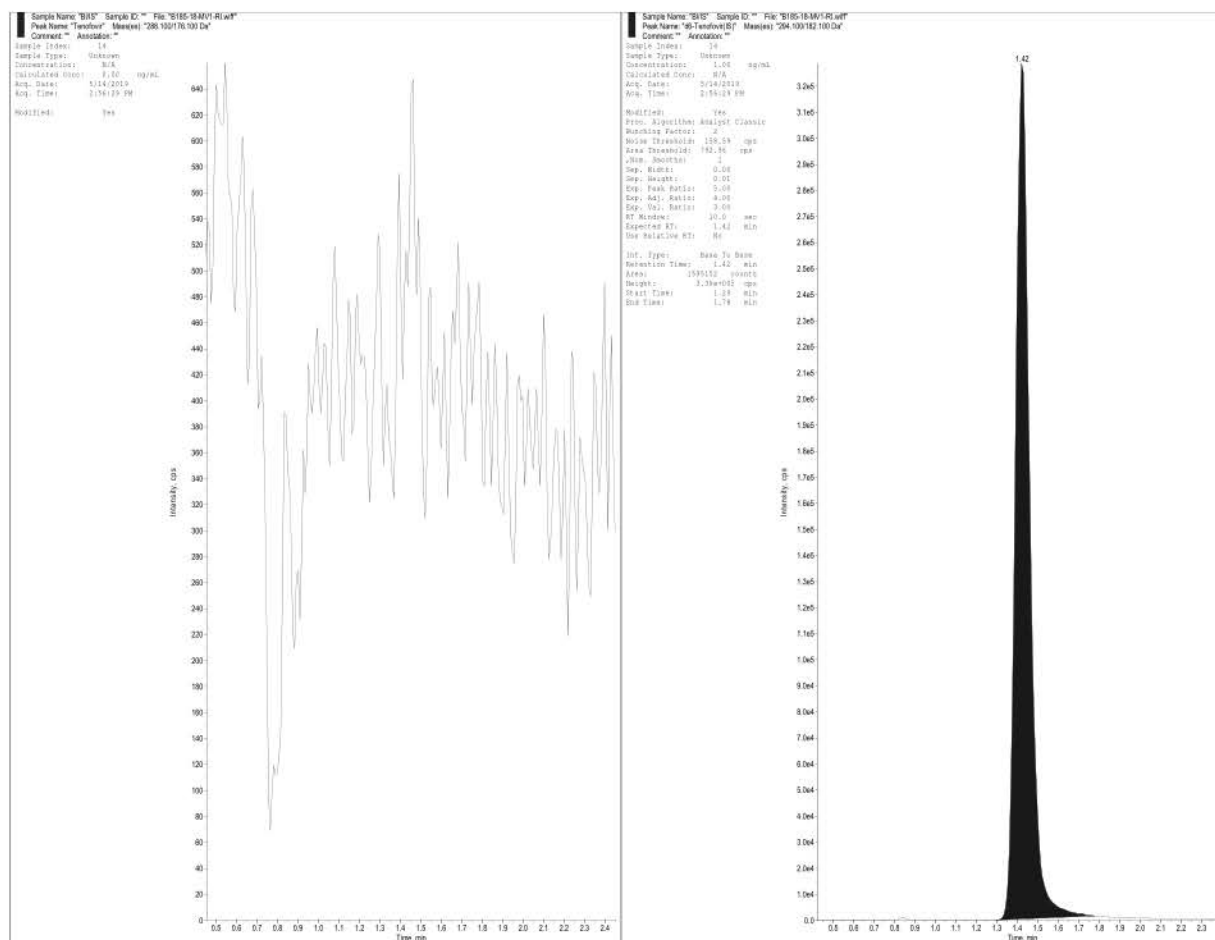


Method Validation Report for the Quantitative Analysis of [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma  
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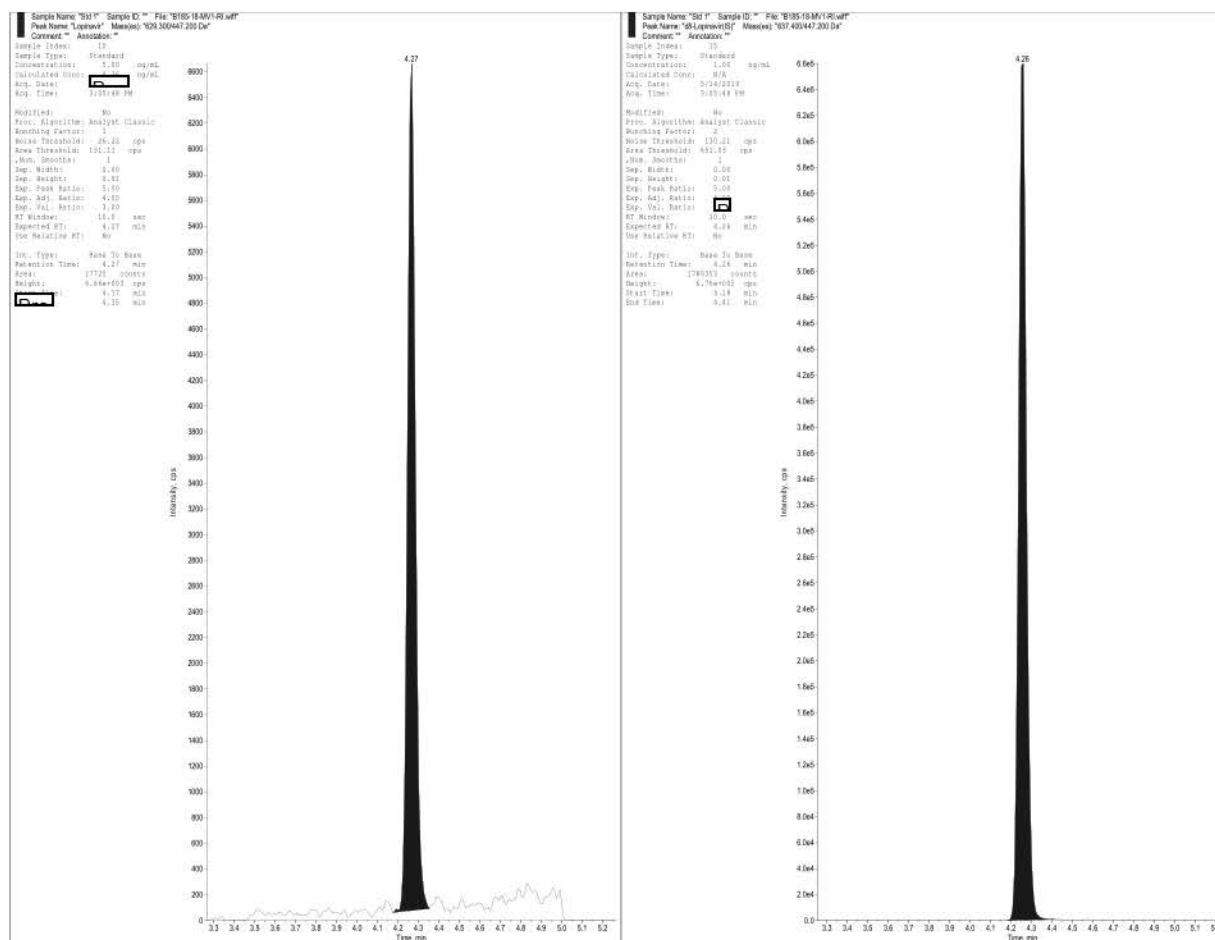
**Figure 8.** Representative chromatogram of a BI/IS K<sub>2</sub> EDTA dog plasma sample (without [Proprietary] with internal standard).

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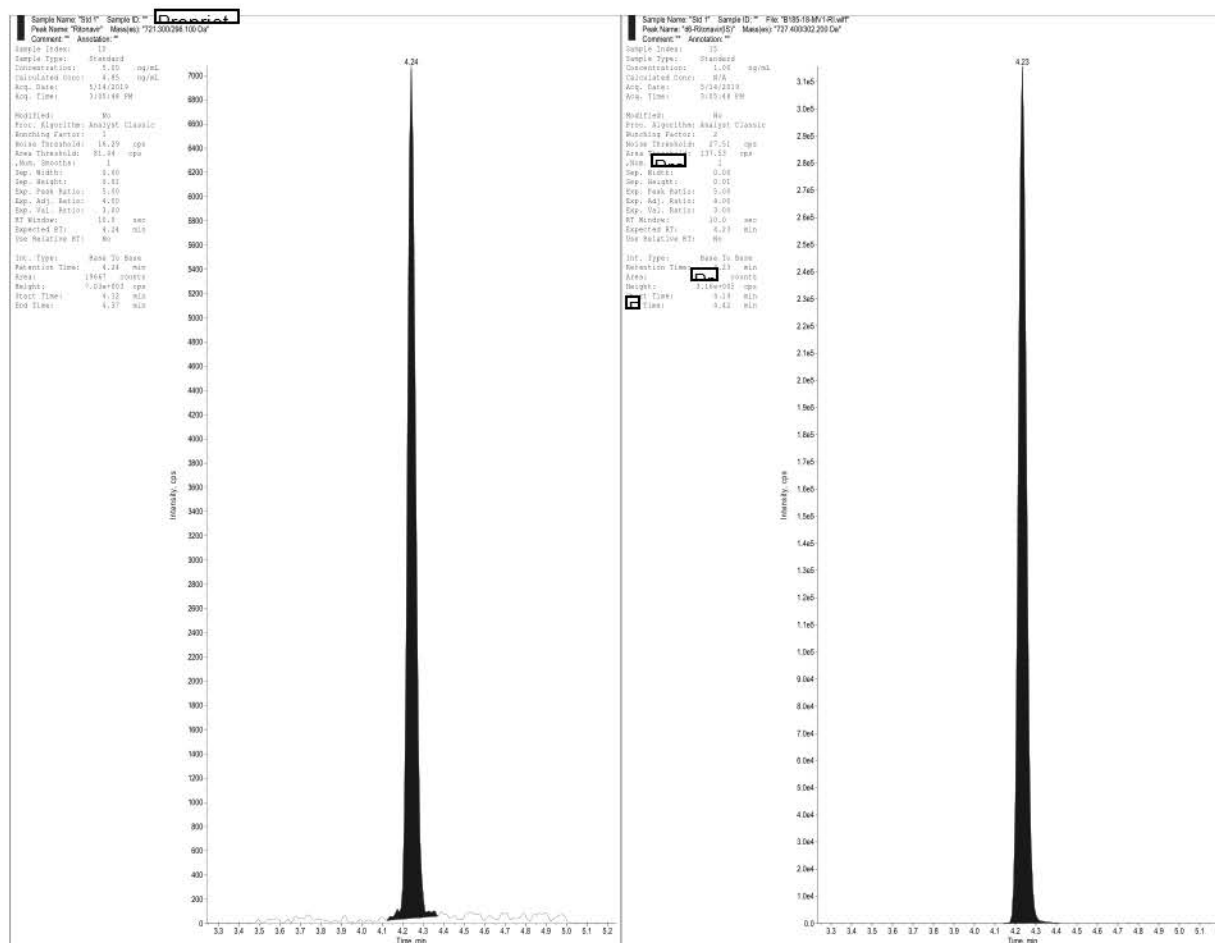
**Figure 9.** Representative chromatogram of a BI/IS K<sub>2</sub> EDTA dog plasma sample (without [Proprietary] with internal standard).

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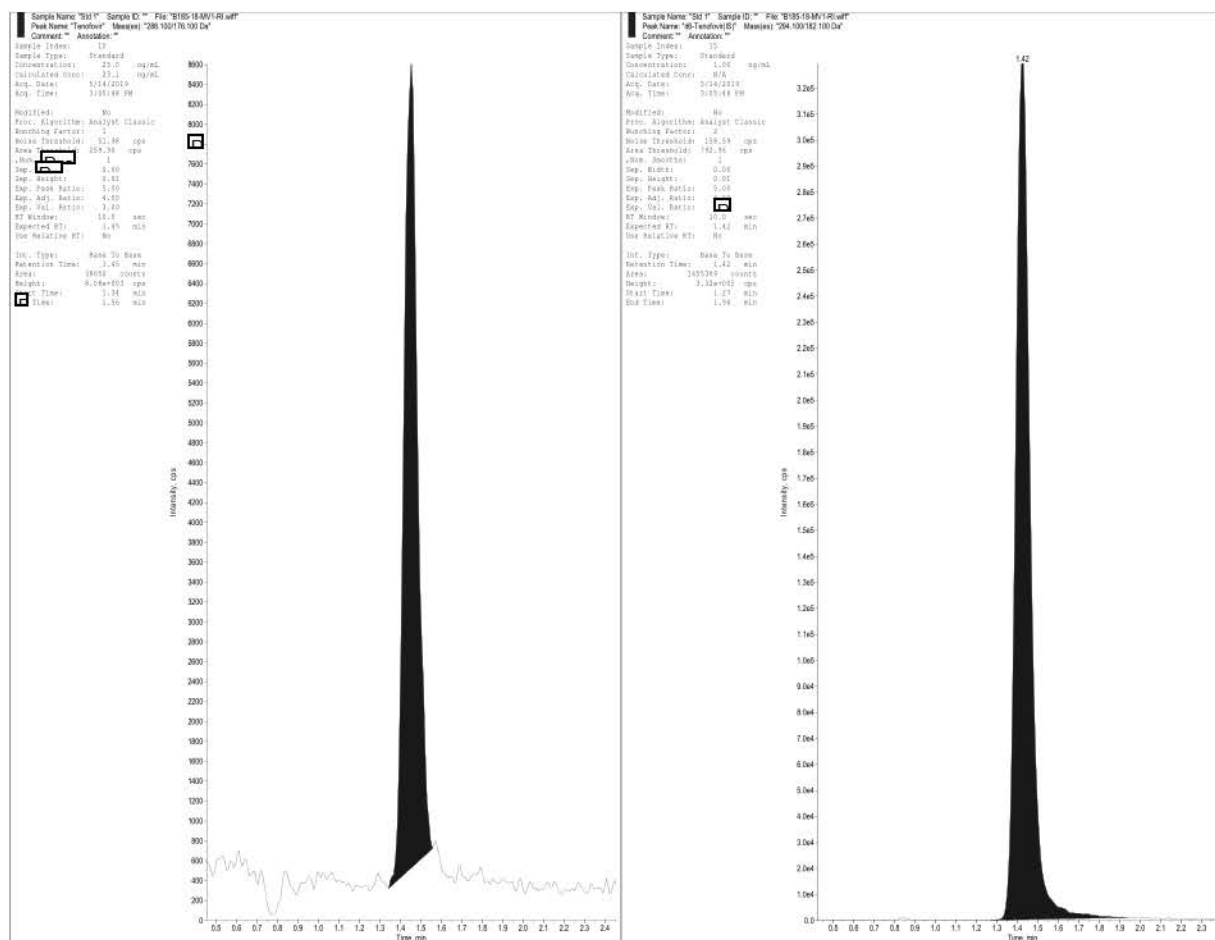
**Figure 10.** Representative chromatogram of a K<sub>2</sub> EDTA dog plasma sample spiked at the [Proprietary] Lower Limit of Quantitation (LLOQ).

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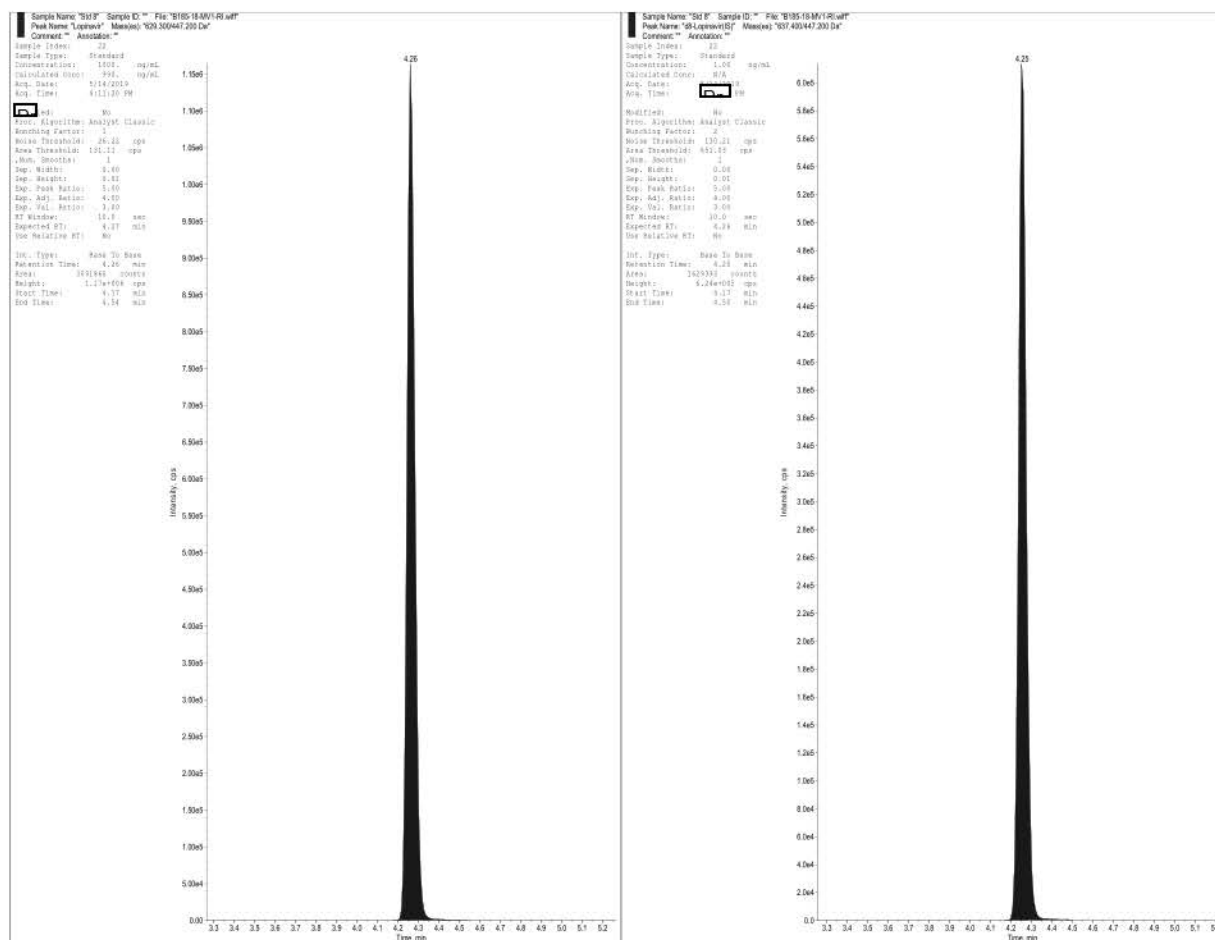
**Figure 11.** Representative chromatogram of a K<sub>2</sub> EDTA dog plasma sample spiked at the [Proprietary] Lower Limit of Quantitation (LLOQ).

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**Figure 12.** Representative chromatogram of a K<sub>2</sub> EDTA dog plasma sample spiked at the [Proprietary] Lower Limit of Quantitation (LLOQ).

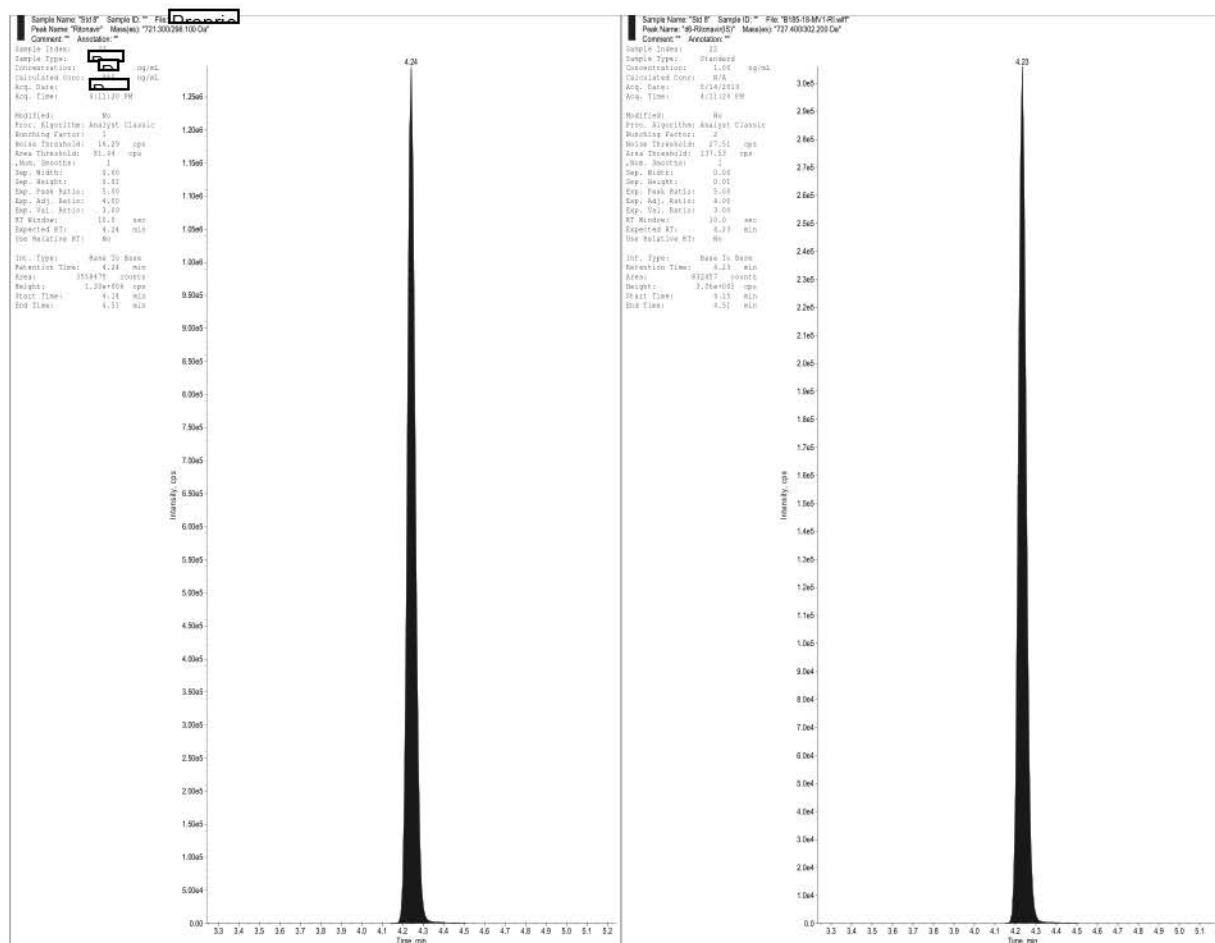
# Method Validation Report for the Quantitative Analysis of Proprietary Proprietary and Proprietary in K<sub>2</sub> EDTA Dog Plasma SRI Study No. B185-18



**Figure 13.** Representative chromatogram of a K<sub>2</sub> EDTA dog plasma sample spiked at the Proprietary Upper Limit of Quantitation (ULQ).

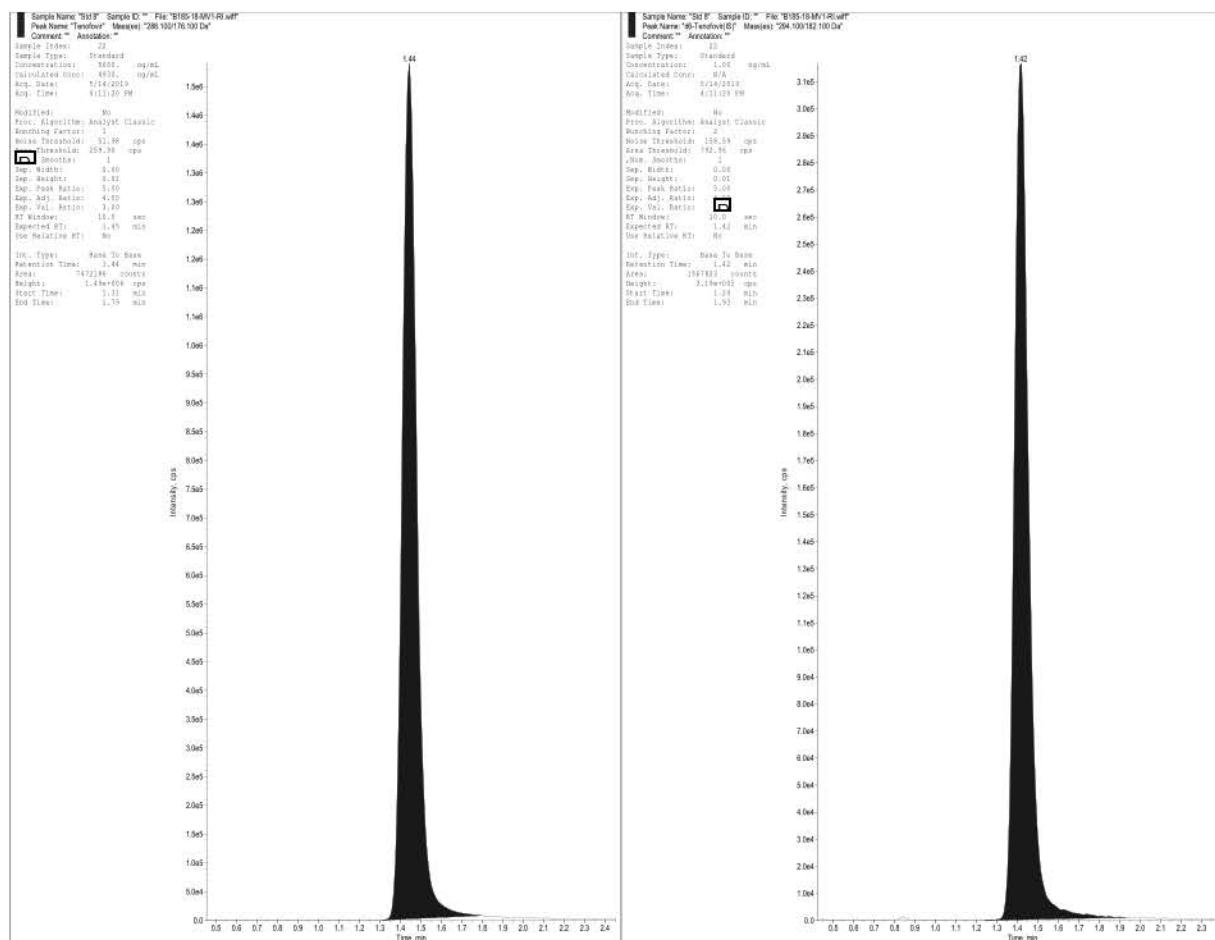


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**Figure 14.** Representative chromatogram of a K<sub>2</sub> EDTA dog plasma sample spiked at the [Proprietary] Upper Limit of Quantitation (ULQ).

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**Figure 15.** Representative chromatogram of a K<sub>2</sub> EDTA dog plasma sample spiked at the [Proprietary] Upper Limit of Quantitation (ULOQ).

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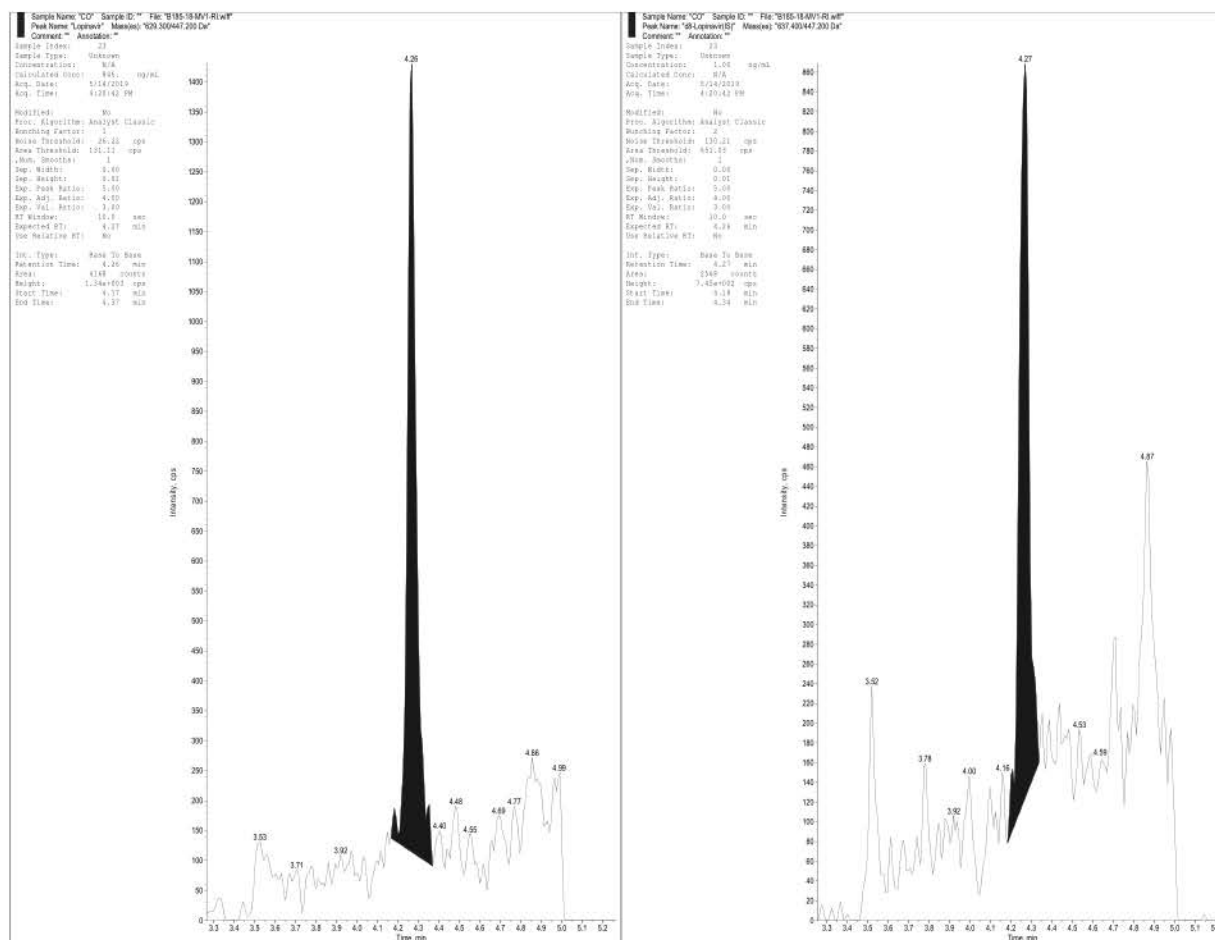


Figure 16. Representative chromatogram of a [Proprietary] carryover blank sample.

## Proprietary



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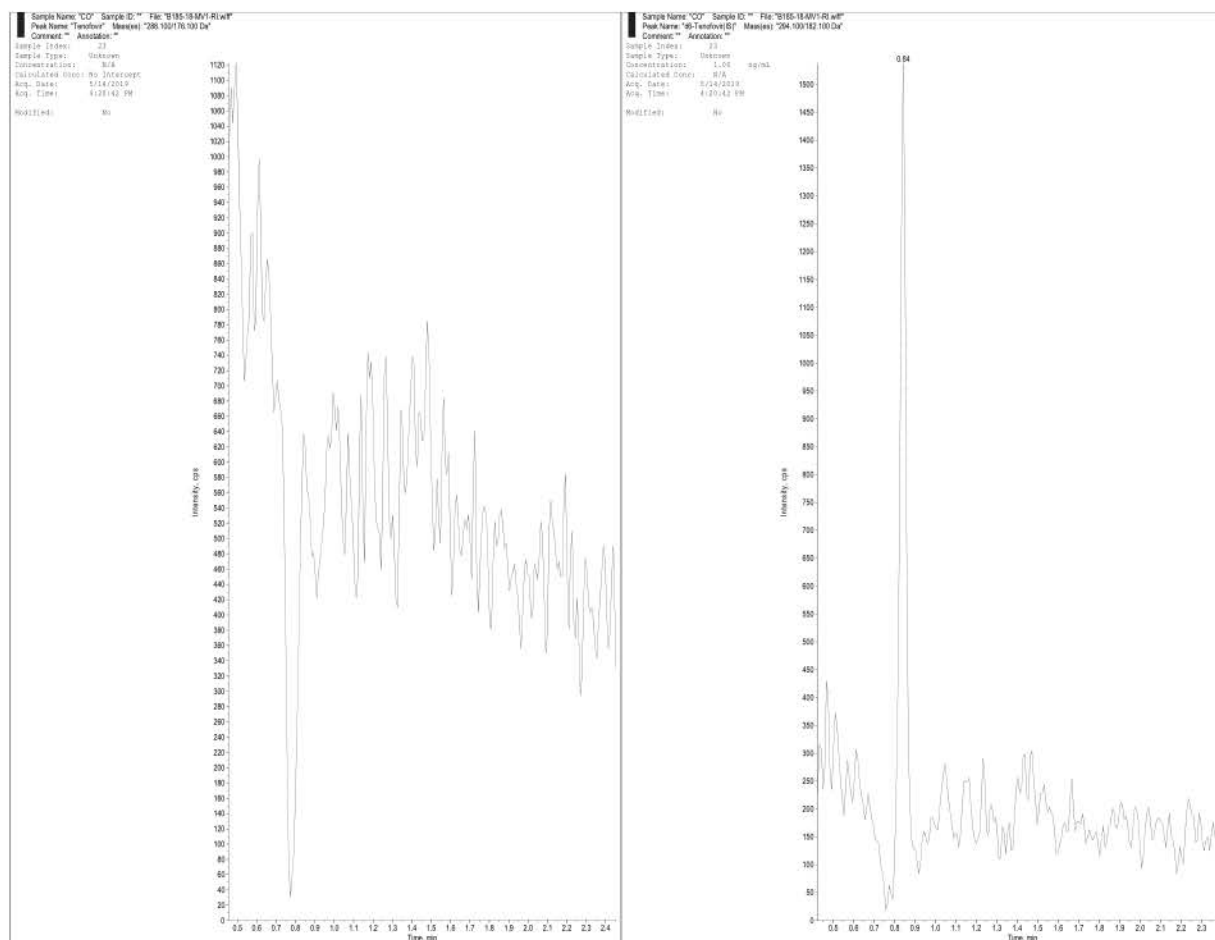


Figure 18. Representative chromatogram of a [Proprietary] carryover blank sample.

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**Table 1**

**Summary of Validation Runs**

Run Number	Date Extracted	Date Analyzed	Outcome (Pass/Fail)	Parameters Investigated
MV1	7-May-19	7-May-19	NA	Accuracy, Precision, Carryover, Effect of Dilution, ULOQ/BL, Concomitant Medication
MV1-RI	7-May-19	14-May-19	Pass	Accuracy, Precision, Carryover, Effect of Dilution, ULOQ/BL, Concomitant Medication
MV2	16-May-19	16-May-19	Pass	Accuracy, Precision, Carryover, Selectivity, Matrix Effects using 6 Unique Lots of Plasma, Maximum Batch Size
MV3	17-May-19	17-May-19	Pass	Accuracy, Precision, Carryover, Room Temperature Stability, Recovery, Matrix Effects on Ionization
MV4	20-May-19	20-May-19	Pass	Carryover, Freeze Thaw Stability, Effect of Hemolysis, Whole Blood Stability, Post-Preparative Extract Stability
MV2-RI	16-May-19	24-May-19	Pass	Reinjection (Autosampler) Stability
MV5	28-May-19	28-May-19	Pass	Interim Long Term Storage Stability in Matrix
B185-061019-Stk-Chk	NA	10-Jun-19	Pass	Refrigerated Stock Solution Storage Stability
B185-061119-Stk-Chk	NA	11-Jun-19	Pass	Room Temperature Stock Solution Stability

NA: Not Applicable. The LC pump failed during analysis and the batch did not inject to completion. The circuit board was replaced and the batch restarted on 05-14-19 as MV1-RI.



**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 2**

**Summary of Calibration Curve Parameters for** Proprietary

Run Number	Slope	Intercept	Correlation Coefficient (r)
MV1-RI	0.00192	0.00161	0.9996
MV2	0.00192	0.000257	0.9996
MV3	0.00191	0.000627	0.9998
MV4	0.00182	0.000191	0.9994
MV2-RI	0.00181	0.00175	0.9992
MV5	0.00178	0.000583	0.9994

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 3**

**Summary of Calibration Curve Parameters for** Proprietary

Run Number	Slope	Intercept	Correlation Coefficient (r)
MV1-RI	0.00443	0.00110	0.9993
MV2	0.00441	0.000877	0.9995
MV3	0.00443	-0.000748	0.9998
MV4	0.00436	-0.00203	0.9998
MV2-RI	0.00439	-0.000607	0.9997
MV5	0.00423	0.000203	0.9998

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 4**

**Summary of Calibration Curve Parameters for** Proprietary

Run Number	Slope	Intercept	Correlation Coefficient (r)
MV1-RI	0.000968	0.000661	0.9992
MV2	0.000947	-0.00117	0.9996
MV3	0.000932	0.000602	0.9997
MV4	0.000931	-0.000986	0.9993
MV2-RI	0.000945	0.0000909	0.9996
MV5	0.000919	-0.000177	0.9995

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 5**

**Back-Calculated Concentrations of [Proprietary] Calibration Standards (ng/ml)**

Run Number	Standard Description															
	5.00	%Acc	10.0	%Acc	20.0	%Acc	50.0	%Acc	100	%Acc	200	%Acc	500	%Acc	1000	%Acc
MV1-RI	4.36	87.2	9.72	97.2	19.4	97.0	48.5	97.1	99.7	99.7	193	96.7	498	99.6	990	99.0
	5.65	113.1	10.9	108.8	20.7	103.5	47.6	95.2	103	103.2	207	103.6	479	95.9	1030	103.3
MV2	4.88	97.7	9.99	99.9	19.2	95.9	48.0	96.0	105	105.2	193	96.3	510	102.0	1010	100.8
	5.16	103.2	10.3	102.8	20.1	100.3	50.5	100.9	102	101.5	205	102.6	470	94.0	1010	100.9
MV3	4.71	94.2	10.1	100.6	18.6	92.8	50.6	101.1	99.4	99.4	200	100.0	516	103.3	984	98.4
	5.80	116.1	9.82	98.2	19.8	99.0	48.9	97.8	100	100.3	195	97.5	506	101.2	1000	100.1
MV4	5.31	106.3	9.68	96.8	21.7	108.3	48.0	96.0	102	101.7	193	96.7	505	100.9	979	97.9
	4.96	99.2	9.07	90.7	19.3	96.6	47.7	95.4	101	101.4	221	110.5	512	102.5	991	99.1
MV2-RI <sup>a</sup>	4.50	90.0	9.55	95.5	20.5	102.6	49.4	98.8	102	102.5	218	108.9	516	103.3	956	95.6
	4.99	99.8	9.83	98.3	20.4	102.1	49.9	99.8	103	102.7	193	96.7	523	104.5	989	98.9
MV5	4.86	97.2	9.64	96.4	18.9	94.4	50.3	100.6	105	105.4	194	96.8	521	104.2	1020	101.9
	4.91	98.2	9.98	99.8	20.4	101.9	50.7	101.5	109	108.7	197	98.6	490	98.0	966	96.6
Mean	5.06		9.91		19.8		49.1		103		200		501		998	
SD	0.434		0.470		0.932		1.30		2.97		9.05		16.4		19.9	
%CV	8.6		4.7		4.7		2.6		2.9		4.5		3.3		2.0	
%Accuracy	101.2		99.1		99.0		98.2		102.6		99.9		100.2		99.8	
n	10		10		10		10		10		10		10		10	

<sup>a</sup> This is a reinjection of Run MV2, and is not included in the statistics.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 6**

**Back-Calculated Concentrations of [Proprietary] Calibration Standards (ng/ml)**

Run Number	Standard Description															
	5.00	%Acc	10.0	%Acc	20.0	%Acc	50.0	%Acc	100	%Acc	200	%Acc	500	%Acc	1000	%Acc
MV1-RI	4.85	97.0	10.0	100.0	20.0	100.0	48.5	96.9	101	100.6	196	98.1	522	104.3	965	96.5
	5.20	104.1	10.2	101.9	20.4	102.0	50.4	100.9	97.1	97.1	201	100.7	480	96.1	1040	103.8
MV2	5.14	102.8	9.94	99.4	20.4	101.9	51.8	103.5	102	101.7	193	96.7	524	104.9	965	96.5
	4.52	90.5	9.95	99.5	20.2	101.0	49.1	98.2	102	101.6	197	98.6	515	103.1	1000	100.0
MV3	4.91	98.3	9.80	98.0	19.3	96.5	51.1	102.2	99.5	99.5	199	99.5	516	103.3	980	98.0
	5.26	105.2	10.0	100.3	20.6	103.0	49.4	98.8	96.9	96.9	198	98.9	507	101.3	1000	100.3
MV4	5.53	110.7	10.5	105.0	18.6	93.0	49.0	98.1	99.2	99.2	196	98.1	505	101.0	1020	101.8
	5.40	107.9	10.3	102.6	18.5	92.6	47.6	95.2	96.0	96.0	199	99.4	502	100.4	989	98.9
MV2-RI <sup>a</sup>	5.04	100.9	10.5	105.4	19.7	98.6	49.4	98.9	103	103.3	202	101.2	525	105.0	999	99.9
	4.86	97.3	10.1	100.7	20.0	99.9	46.4	92.8	100	100.4	199	99.5	488	97.6	987	98.7
MV5	4.99	99.9	10.3	103.2	20.0	99.9	49.3	98.5	100	100.5	199	99.6	521	104.2	990	99.0
	4.86	97.2	10.2	102.3	19.9	99.6	50.2	100.5	98.9	98.9	194	96.9	503	100.6	994	99.4
Mean	5.07		10.1		19.8		49.6		99.2		197		510		994	
SD	0.298		0.213		0.737		1.25		1.98		2.47		13.2		22.3	
%CV	5.9		2.1		3.7		2.5		2.0		1.3		2.6		2.2	
%Accuracy	101.4		101.2		99.0		99.3		99.2		98.7		101.9		99.4	
n	10		10		10		10		10		10		10		10	

<sup>a</sup> This is a reinjection of Run MV2, and is not included in the statistics.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 7**

**Back-Calculated Concentrations of [Proprietary] Calibration Standards (ng/ml)**

Run Number	Standard Description															
	25.0	%Acc	50.0	%Acc	100	%Acc	250	%Acc	500	%Acc	1000	%Acc	2500	%Acc	5000	%Acc
MV1-RI	23.1	92.3	49.4	98.8	98.0	98.0	252	100.8	493	98.7	1010	100.5	2550	101.9	4930	98.5
	26.6	106.5	51.1	102.2	103	103.2	258	103.3	505	101.0	971	97.1	2470	98.6	4920	98.3
MV2	24.5	98.1	51.4	102.9	105	105.0	244	97.7	501	100.3	990	99.0	2520	100.6	4990	99.7
	25.0	99.8	48.9	97.8	104	103.7	248	99.1	493	98.5	983	98.3	2540	101.4	4910	98.1
MV3	25.3	101.3	49.5	99.1	97.2	97.2	246	98.5	514	102.9	1020	101.5	2520	100.6	5060	101.3
	25.1	100.4	50.5	100.9	96.7	96.7	244	97.5	499	99.9	987	98.7	2600	104.1	4970	99.3
MV4	25.4	101.5	47.9	95.7	96.6	96.6	253	101.2	493	98.5	986	98.6	2610	104.5	5150	102.9
	26.2	104.9	47.2	94.4	98.2	98.2	245	98.1	491	98.3	1010	101.3	2530	101.2	5200	104.1
MV2-RI <sup>a</sup>	25.5	102.1	50.1	100.3	100	100.2	247	98.8	496	99.2	995	99.5	2590	103.8	5050	101.0
	24.7	98.9	49.9	99.9	97.8	97.8	240	95.8	502	100.3	957	95.7	2590	103.5	5170	103.3
MV5	24.0	96.1	48.1	96.1	99.7	99.7	248	99.1	493	98.7	997	99.7	2590	103.6	5020	100.4
	26.5	105.8	50.8	101.6	98.1	98.1	246	98.3	509	101.8	981	98.1	2490	99.5	5170	103.3
Mean	25.2		49.5		99.6		248		499		993		2540		5030	
SD	1.11		1.47		3.16		4.63		8.04		14.4		48.3		110	
%CV	4.4		3.0		3.2		1.9		1.6		1.5		1.9		2.2	
%Accuracy	100.7		99.0		99.6		99.4		99.9		99.3		101.6		100.6	
n	10		10		10		10		10		10		10		10	

<sup>a</sup> This is a reinjection of Run MV2, and is not included in the statistics.



**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 8**

**Intra- and Inter-Batch Accuracy and Precision of** Proprietary **QC Samples (ng/ml)**

Run Number	QC Description							
	5.00	%Acc	15.0	%Acc	400	%Acc	800	%Acc
MV1-RI	4.56	91.2	14.5	96.8	402	100.5	803	100.4
	4.37	87.4	15.3	101.8	394	98.6	787	98.3
	4.77	95.3	14.2	94.5	391	97.9	779	97.3
	4.58	91.5	14.8	98.9	389	97.3	741	92.6
	4.86	97.1	14.4	96.2	384	96.1	788	98.6
	4.29	85.8	14.3	95.6	417	104.1	809	101.2
Mean	4.57		14.6		396		784	
SD	0.218		0.398		11.4		24.2	
%CV	4.8		2.7		2.9		3.1	
%Accuracy	91.4		97.3		99.1		98.1	
n	6		6		6		6	
MV2	5.62	112.3	16.2	108.0	404	101.0	794	99.2
	5.14	102.8	15.5	103.4	394	98.5	802	100.2
	5.04	100.9	15.4	102.6	393	98.4	782	97.7
	5.35	106.9	14.4	95.9	398	99.5	792	99.0
	5.45	109.0	14.2	94.8	403	100.8	837	104.7
	4.95	99.0	14.3	95.2	398	99.5	808	101.0
Mean	5.26		15.0		398		803	
SD	0.255		0.820		4.46		19.3	
%CV	4.9		5.5		1.1		2.4	
%Accuracy	105.1		100.0		99.6		100.3	
n	6		6		6		6	
MV3	4.90	98.0	15.0	99.9	420	105.0	787	98.4
	5.35	106.9	14.5	97.0	370	92.5	776	97.0
	5.19	103.7	14.6	97.3	380	95.1	806	100.8
	5.01	100.1	14.6	97.3	385	96.3	824	103.1
	4.83	96.6	14.6	97.6	410	102.5	795	99.4
	5.04	100.8	14.8	98.6	379	94.8	788	98.5
Mean	5.05		14.7		391		796	
SD	0.189		0.166		19.7		17.0	
%CV	3.7		1.1		5.0		2.1	
%Accuracy	101.0		97.9		97.7		99.5	
n	6		6		6		6	

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 8 (concluded)**

**Intra- and Inter-Batch Accuracy and Precision of** Proprietary **QC Samples (ng/ml)**

Run Number	QC Description							
	5.00	%Acc	15.0	%Acc	400	%Acc	800	%Acc
<b>Inter-assay statistics</b>								
Mean	4.96		14.8		395		794	
SD	0.363		0.532		13.0		20.7	
%CV	7.3		3.6		3.3		2.6	
%Accuracy	99.2		98.4		98.8		99.3	
n	18		18		18		18	

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 9**

**Intra- and Inter-Batch Accuracy and Precision of** Proprietary **QC Samples (ng/ml)**

Run Number	QC Description							
	5.00	%Acc	15.0	%Acc	400	%Acc	800	%Acc
MV1-RI	4.91	98.2	14.7	98.3	385	96.3	795	99.3
	4.57	91.5	14.4	96.1	384	96.1	754	94.3
	4.68	93.6	14.4	96.1	390	97.4	798	99.7
	4.89	97.8	13.9	92.7	388	97.0	736	92.0
	4.78	95.7	14.4	95.7	386	96.5	763	95.3
	4.57	91.4	14.3	95.2	396	98.9	782	97.8
Mean	4.73		14.4		388		771	
SD	0.150		0.273		4.11		24.3	
%CV	3.2		1.9		1.1		3.2	
%Accuracy	94.7		95.7		97.0		96.4	
n	6		6		6		6	
MV2	5.05	100.9	14.3	95.1	382	95.4	774	96.7
	5.05	101.0	14.2	94.9	394	98.6	760	95.0
	5.30	106.0	14.6	97.4	415	103.8	803	100.4
	4.47	89.4	14.5	96.9	388	97.0	785	98.1
	5.05	101.1	15.0	99.9	404	100.9	780	97.5
	4.95	99.0	14.5	96.7	396	98.9	782	97.7
Mean	4.98		14.5		396		781	
SD	0.274		0.275		11.9		14.3	
%CV	5.5		1.9		3.0		1.8	
%Accuracy	99.6		96.8		99.1		97.6	
n	6		6		6		6	
MV3	5.35	107.0	14.7	98.0	398	99.4	807	100.8
	5.27	105.4	14.7	98.1	382	95.5	808	101.0
	5.18	103.6	13.8	92.0	398	99.5	817	102.1
	5.47	109.3	14.6	97.2	407	101.7	796	99.5
	5.49	109.9	15.0	99.9	400	100.0	799	99.9
	5.28	105.5	14.4	96.0	399	99.9	815	101.9
Mean	5.34		14.5		397		807	
SD	0.122		0.405		8.22		8.27	
%CV	2.3		2.8		2.1		1.0	
%Accuracy	106.8		96.9		99.3		100.9	
n	6		6		6		6	

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 9 (concluded)**

**Intra- and Inter-Batch Accuracy and Precision of [Proprietary] QC Samples (ng/ml)**

Run Number	QC Description							
	5.00	%Acc	15.0	%Acc	400	%Acc	800	%Acc
<b>Inter-assay statistics</b>								
Mean	5.02		14.5		394		786	
SD	0.314		0.315		9.19		22.3	
%CV	6.3		2.2		2.3		2.8	
%Accuracy	100.4		96.5		98.5		98.3	
n	18		18		18		18	

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 10**

**Intra- and Inter-Batch Accuracy and Precision of** Proprietary **QC Samples (ng/ml)**

Run Number	QC Description							
	25.0	%Acc	75.0	%Acc	2000	%Acc	4000	%Acc
MV1-RI	25.0	100.0	71.6	95.4	1930	96.5	3950	98.7
	25.0	100.1	69.4	92.5	1910	95.3	3940	98.5
	24.7	98.7	71.9	95.9	1930	96.7	3910	97.8
	24.8	99.0	72.5	96.6	1920	96.2	3930	98.3
	22.0	88.1	71.0	94.7	1960	97.9	3960	99.1
	22.2	88.9	68.9	91.9	1960	98.1	3900	97.4
Mean	23.9		70.9		1940		3930	
SD	1.43		1.43		20.8		24.1	
%CV	6.0		2.0		1.1		0.6	
%Accuracy	95.8		94.5		96.8		98.3	
n	6		6		6		6	
MV2	27.5	110.0	75.0	99.9	2000	100.2	3940	98.5
	27.3	109.2	76.5	101.9	2040	102.0	4010	100.4
	27.2	108.9	73.2	97.6	2020	101.0	4160	103.9
	26.5	106.2	76.7	102.3	2000	99.9	4160	104.1
	27.4	109.5	75.2	100.3	1990	99.7	4060	101.4
	27.5	110.0	73.9	98.5	1960	97.9	3950	98.7
Mean	27.2		75.1		2000		4050	
SD	0.360		1.38		27.8		97.6	
%CV	1.3		1.8		1.4		2.4	
%Accuracy	109.0		100.1		100.1		101.1	
n	6		6		6		6	
MV3	25.6	102.6	71.8	95.7	2050	102.7	3980	99.6
	25.9	103.8	74.2	98.9	1940	96.8	4130	103.4
	25.8	103.3	71.6	95.4	2000	100.0	4190	104.7
	26.0	103.9	69.0	92.0	2020	100.8	4120	103.0
	25.7	102.6	71.7	95.6	2030	101.3	4150	103.8
	25.1	100.2	70.4	93.8	2020	101.0	4190	104.8
Mean	25.7		71.4		2010		4130	
SD	0.334		1.73		39.9		75.9	
%CV	1.3		2.4		2.0		1.8	
%Accuracy	102.7		95.2		100.4		103.2	
n	6		6		6		6	

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 10 (concluded)**

**Intra- and Inter-Batch Accuracy and Precision of [Proprietary] QC Samples (ng/ml)**

Run Number	QC Description							
	25.0	%Acc	75.0	%Acc	2000	%Acc	4000	%Acc
<b>Inter-assay statistics</b>								
Mean	25.6		72.5		1980		4040	
SD	1.61		2.39		44.6		107	
%CV	6.3		3.3		2.2		2.7	
%Accuracy	102.5		96.6		99.1		100.9	
n	18		18		18		18	



**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 11**

**Selectivity Determination using Six Unique Lots of K<sub>2</sub> EDTA Dog Plasma and Effect of Hemolysis** Proprietary Info

Selectivity Determination Proprietary using Six Unique Lots of K<sub>2</sub> EDTA Dog Plasma

Run Number	Peak Area					
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
MV2	854	183	787	858	765	523
% Difference from Mean of LLOQ	7.2	1.6	6.7	7.3	6.5	4.4

Peak Area of 1st Lower Limit of Quantitation Calibration Standard: 12849

Peak Area of 2nd Lower Limit of Quantitation Calibration Standard: 10715

Mean: 11782

**Selectivity Determination** Proprietary **with Effect of Hemolysis**

Run Number	Peak Area	
	0.5% hemolysis	2% hemolysis
MV4	1000	517
% Difference from Mean of LLOQ	9.4	4.9

Peak Area of 1st Lower Limit of Quantitation Calibration Standard: 10111

Peak Area of 2nd Lower Limit of Quantitation Calibration Standard: 11079

Mean: 10595

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 12**

**Selectivity Determination using Six Unique Lots of K<sub>2</sub> EDTA Dog Plasma and Effect of Hemolysis** Proprietary Info

Selectivity Determination Proprietary using Six Unique Lots of K<sub>2</sub> EDTA Dog Plasma

Run Number	Peak Area					
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
MV2	728	565	0	246	281	0
% Difference from Mean of LLOQ	5.2	4.0	0.0	1.7	2.0	0.0

Peak Area of 1st Lower Limit of Quantitation Calibration Standard: 16140

Peak Area of 2nd Lower Limit of Quantitation Calibration Standard: 12063

Mean: 14102

**Selectivity Determination** Proprietary **with Effect of Hemolysis**

Run Number	Peak Area	
	0.5% hemolysis	2% hemolysis
MV4	0	0
% Difference from Mean of LLOQ	0.0	0.0

Peak Area of 1st Lower Limit of Quantitation Calibration Standard: 12102

Peak Area of 2nd Lower Limit of Quantitation Calibration Standard: 13122

Mean: 12612

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 13**

**Selectivity Determination using Six Unique Lots of K<sub>2</sub> EDTA Dog Plasma and Effect of Hemolysis** Proprietary Info

Selectivity Determination Proprietary using Six Unique Lots of K<sub>2</sub> EDTA Dog Plasma

Run Number	Peak Area					
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
MV2	0	0	0	174	0	327
% Difference from Mean of LLOQ	0.0	0.0	0.0	0.5	0.0	1.0

Peak Area of 1st Lower Limit of Quantitation Calibration Standard:	35486
Peak Area of 2nd Lower Limit of Quantitation Calibration Standard:	<u>32943</u>
Mean:	34215

**Selectivity Determination** Proprietary Info **with Effect of Hemolysis**

Run Number	Peak Area	
	0.5% hemolysis	2% hemolysis
MV4	0	0
% Difference from Mean of LLOQ	0.0	0.0

Peak Area of 1st Lower Limit of Quantitation Calibration Standard:	29829
Peak Area of 2nd Lower Limit of Quantitation Calibration Standard:	<u>32647</u>
Mean:	31238

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
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**Table 14**

**Selectivity Determination and Carryover of [Proprietary] in Assay BI/BI and BI/IS samples**

Run Number	Peak Area							
	LLOQ	Mean LLOQ	BI/BI	% Difference	BI/IS	% Difference	Carryover	% Difference
MV1-RI	17725	16834	1029	6.1	962	5.7	4168	24.8
	15942		4660	27.7	1935	11.5	2872	17.1
MV2	12849	11782	704	6.0	483	4.1	2543	21.6
	10715		465	3.9	593	5.0	1987	16.9
MV3	12300	12302	515	4.2	377	3.1	2386	19.4
	12304		2425	19.7	1404	11.4	2139	17.4
MV4	10111	10595	880	8.3	745	7.0	2089	19.7
	11079		2007	18.9	1141	10.8	2008	19.0
MV2-RI	10241	10119	414	4.1	380	3.8	2554	25.2
	9997		2398	23.7	816	8.1	2003	19.8
MV5	9011	9160	438	4.8	0	0.0	1685	18.4
	9309		1552	16.9	733	8.0	1803	19.7

% Difference is calculated using the mean of the LLOQ peak areas.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 15**

**Selectivity Determination and Carryover of [Proprietary] in Assay BI/BI and BI/IS samples**

Run Number	Peak Area							
	LLOQ	Mean LLOQ	BI/BI	% Difference	BI/IS	% Difference	Carryover	% Difference
MV1-RI	19667	18030	0	0.0	234	1.3	2276	12.6
	16392		3778	21.0	1579	8.8	1650	9.2
MV2	16140	14102	0	0.0	205	1.5	1556	11.0
	12063		0	0.0	0	0.0	1188	8.4
MV3	13999	13207	152	1.2	130	1.0	1247	9.4
	12415		1999	15.1	872	6.6	1341	10.2
MV4	12102	12612	0	0.0	0	0.0	0	0.0
	13122		1254	9.9	500	4.0	1689	13.4
MV2-RI	12396	11365	0	0.0	0	0.0	1481	13.0
	10334		1378	12.1	550	4.8	1599	14.1
MV5	11779	11873	0	0.0	0	0.0	1696	14.3
	11966		1407	11.9	758	6.4	1808	15.2

% Difference is calculated using the mean of the LLOQ peak areas.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 16**

**Selectivity Determination and Carryover of [Proprietary] in Assay BI/BI and BI/IS samples**

Run Number	Peak Area							
	LLOQ	Mean LLOQ	BI/BI	% Difference	BI/IS	% Difference	Carryover	% Difference
MV1-RI	38050	39930	0	0.0	0	0.0	0	0.0
	41810		1826	4.6	1577	3.9	1996	5.0
MV2	35486	34215	0	0.0	623	1.8	1437	4.2
	32943		0	0.0	0	0.0	0	0.0
MV3	37219	35749	429	1.2	635	1.8	580	1.6
	34278		1070	3.0	2188	6.1	1323	3.7
MV4	29829	31238	0	0.0	341	1.1	757	2.4
	32647		698	2.2	3131	10.0	586	1.9
MV2-RI	31495	30745	0	0.0	1474	4.8	1239	4.0
	29994		1803	5.9	1839	6.0	1833	6.0
MV5	28445	30797	0	0.0	529	1.7	0	0.0
	33148		873	2.8	2373	7.7	0	0.0

% Difference is calculated using the mean of the LLOQ peak areas.



**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 17**

**Dilution Assessment of [Proprietary] (ng/ml)**

Run Number	Dilution QC Concentration			
	5000 <sup>a</sup>	%Accuracy	5000 <sup>b</sup>	%Accuracy
MV1-RI	4910	98.2	5130	102.6
	4840	96.9	4930	98.5
	4940	98.8	4980	99.5
	4620	92.3	5000	100.1
	4970	99.3	5050	101.1
	5170	103.4	5170	103.3
Mean	4910		5040	
SD	180		92.3	
%CV	3.7		1.8	
%Accuracy	98.2		100.9	
n	6		6	

<sup>a</sup> Dilution QC was prepared at 5000 ng/ml and was subsequently diluted (1:10) with control matrix to give a final concentration of 500 ng/ml.

<sup>b</sup> Dilution QC was prepared at 5000 ng/ml and was subsequently diluted (1:50) with control matrix to give a final concentration of 100 ng/ml.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 18**

**Dilution Assessment of [Proprietary] (ng/ml)**

Run Number	Dilution QC Concentration			
	5000 <sup>a</sup>	%Accuracy	5000 <sup>b</sup>	%Accuracy
MV1-RI	5010	100.1	4920	98.5
	4920	98.4	4870	97.5
	4860	97.1	4980	99.6
	4940	98.9	5060	101.3
	4890	97.9	5230	104.7
	4950	98.9	5060	101.2
Mean	4930		5020	
SD	51.0		128	
%CV	1.0		2.5	
%Accuracy	98.6		100.4	
n	6		6	

<sup>a</sup> Dilution QC was prepared at 5000 ng/ml and was subsequently diluted (1:10) with control matrix to give a final concentration of 500 ng/ml.

<sup>b</sup> Dilution QC was prepared at 5000 ng/ml and was subsequently diluted (1:50) with control matrix to give a final concentration of 100 ng/ml.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 19**

**Dilution Assessment of [Proprietary] (ng/ml)**

Run Number	Dilution QC Concentration			
	25000 <sup>a</sup>	%Accuracy	25000 <sup>b</sup>	%Accuracy
MV1-RI	24400	97.8	25200	100.9
	24500	98.1	24800	99.3
	24500	97.8	24300	97.3
	24500	98.0	24900	99.6
	24200	96.6	24700	98.9
	23700	94.7	24800	99.3
Mean	24300		24800	
SD	330		284	
%CV	1.4		1.1	
%Accuracy	97.2		99.2	
n	6		6	

<sup>a</sup> Dilution QC was prepared at 25000 ng/ml and was subsequently diluted (1:10) with control matrix to give a final concentration of 2500 ng/ml.

<sup>b</sup> Dilution QC was prepared at 25000 ng/ml and was subsequently diluted (1:50) with control matrix to give a final concentration of 500 ng/ml.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
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**Table 20**

**Matrix Effects of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Low (15.0 ng/ml)											
	Lot 1	%Acc	Lot 2	%Acc	Lot 3	%Acc	Lot 4	%Acc	Lot 5	%Acc	Lot 6	%Acc
MV2	16.0	106.4	15.3	102.0	16.9	112.9	15.7	104.8	15.7	104.9	16.3	109.0
	15.5	103.3	16.7	111.6	15.4	102.8	16.2	107.7	15.3	102.3	17.4	116.1 <sup>a</sup>
	15.4	102.5	15.9	106.2	16.3	108.7	16.6	110.7	15.1	100.4	15.5	103.4
	15.8	105.2	17.2	114.6	15.8	105.1	16.4	109.6	15.7	104.7	15.8	105.2
Mean	15.7		16.3		16.1		16.2		15.5		16.3	
SD	0.265		0.837		0.663		0.385		0.323		0.838	
%CV	1.7		5.1		4.1		2.4		2.1		5.2	
%Accuracy	104.3		108.6		107.4		108.2		103.1		108.4	
n	4		4		4		4		4		4	

<sup>a</sup> Result is outside acceptance criterion ( $\pm 15\%$ ) and was included in the statistics.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
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**Table 21**

**Matrix Effects of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Low (15.0 ng/ml)											
	Lot 1	%Acc	Lot 2	%Acc	Lot 3	%Acc	Lot 4	%Acc	Lot 5	%Acc	Lot 6	%Acc
MV2	14.9	99.2	15.8	105.0	15.6	104.1	16.2	107.9	15.7	104.7	16.3	108.4
	15.7	104.5	15.1	100.8	15.3	102.0	15.2	101.1	16.1	107.2	15.6	103.7
	16.2	107.9	15.8	105.4	15.7	104.9	15.6	104.0	15.7	104.8	15.5	103.6
	16.8	111.8	16.1	107.6	15.5	103.4	15.5	103.4	15.9	106.2	15.8	105.3
Mean	15.9		15.7		15.5		15.6		15.9		15.8	
SD	0.798		0.429		0.180		0.426		0.182		0.333	
%CV	5.0		2.7		1.2		2.7		1.1		2.1	
%Accuracy	105.9		104.7		103.6		104.1		105.7		105.3	
n	4		4		4		4		4		4	

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
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**Table 22**

**Matrix Effects of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Low (75.0 ng/ml)											
	Lot 1	%Acc	Lot 2	%Acc	Lot 3	%Acc	Lot 4	%Acc	Lot 5	%Acc	Lot 6	%Acc
MV2	78.1	104.2	73.4	97.9	80.2	106.9	79.0	105.4	79.0	105.3	75.2	100.3
	78.3	104.4	80.2	106.9	77.3	103.1	81.8	109.1	81.5	108.6	80.8	107.7
	78.0	103.9	80.8	107.7	82.6	110.2	79.0	105.3	78.2	104.2	83.5	111.4
	79.5	105.9	78.5	104.7	78.5	104.7	78.3	104.4	76.2	101.6	80.4	107.2
Mean	78.5		78.2		79.7		79.5		78.7		80.0	
SD	0.672		3.33		2.31		1.55		2.18		3.46	
%CV	0.9		4.3		2.9		2.0		2.8		4.3	
%Accuracy	104.6		104.3		106.2		106.0		104.9		106.7	
n	4		4		4		4		4		4	



**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
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**Table 23**

**Recovery of [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**

Run Number	Peak Area					
	Low QC extract	Low QC post extraction spiked	Mid QC extract	Mid QC post extraction spiked	High QC extract	High QC post extraction spiked
MV3	33560	38587	937527	901845	1662621	1579916
	33631	40020	925076	893456	1689202	1776022
	33919	41880	893044	859901	1728756	1673070
	35159	38427	875222	950986	1778334	1563443
	34409	38200	911923	950786	1658707	1749384
	32142	35834	856384	904488	1590594	1754449
Mean	33803	38825	899863	910244	1684702	1682714
SD	1007	2016	30802	35280	64417	92934
%CV	3.0	5.2	3.4	3.9	3.8	5.5
%Recovery	87.1	NA	98.9	NA	100.1	NA
n	6	6	6	6	6	6
Mean % Recovery	95.4					

NA: Not Applicable.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 24**

**Recovery of [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**

Run Number	Peak Area					
	Low QC extract	Low QC post extraction spiked	Mid QC extract	Mid QC post extraction spiked	High QC extract	High QC post extraction spiked
MV3	38423	44209	1094909	1125178	2059038	1953449
	39050	46111	1089404	1101886	2022358	2195625
	37289	48930	1082006	1039907	2088966	2018730
	39090	45528	1088419	1118771	2067662	1899046
	40653	42758	1103803	1138698	1957776	2080994
	39113	43165	1059171	1093788	1981617	2156127
Mean	38936	45117	1086285	1103038	2029570	2050662
SD	1095	2276	15159	34876	51678	115338
%CV	2.8	5.0	1.4	3.2	2.5	5.6
%Recovery	86.3	NA	98.5	NA	99.0	NA
n	6	6	6	6	6	6
Mean % Recovery	94.6					

NA: Not Applicable.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 25**

**Recovery of [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**

Run Number	Peak Area					
	Low QC extract	Low QC post extraction spiked	Mid QC extract	Mid QC post extraction spiked	High QC extract	High QC post extraction spiked
MV3	98727	91824	2663814	2857348	5488050	5113841
	101098	93673	2740520	2852082	5459958	5467201
	97927	102952	2738338	2767673	5387882	5262464
	99150	97215	2763588	2803970	5611075	5113943
	101329	92560	2720034	2901059	5125829	5398191
	95732	91624	2760013	2963358	5071866	5646313
Mean	98994	94975	2731051	2857582	5357443	5333659
SD	2086	4412	36558	69379	213552	210435
%CV	2.1	4.6	1.3	2.4	4.0	3.9
%Recovery	104.2	NA	95.6	NA	100.4	NA
n	6	6	6	6	6	6
Mean % Recovery	100.1					

NA: Not Applicable.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 26**

**Recovery of [Proprietary Info] (Internal Standard) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	Peak Area					
	Low QC extract	Low QC post extraction spiked	Mid QC extract	Mid QC post extraction spiked	High QC extract	High QC post extraction spiked
MV3	1144851	1154597	1164476	1046169	1102214	1027318
	1180774	1171310	1304123	1030895	1136246	1089015
	1186537	1176164	1224791	979735	1119475	999326
	1230978	1125896	1185479	1050149	1126001	963480
	1200826	1085607	1160680	1079810	1088717	1094112
	1109974	1036928	1178861	1040641	1053238	1062825
Mean	1175657	1125084	1203068	1037900	1104315	1039346
SD	42665	54677	54526	32908	30253	51999
%CV	3.6	4.9	4.5	3.2	2.7	5.0
%Recovery	104.5	NA	115.9	NA	106.3	NA
n	6	6	6	6	6	6
Mean % Recovery	108.9					

NA: Not Applicable.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 27**

**Recovery of [Proprietary Info] (Internal Standard) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	Peak Area					
	Low QC extract	Low QC post extraction spiked	Mid QC extract	Mid QC post extraction spiked	High QC extract	High QC post extraction spiked
MV3	596663	620635	621267	551103	576033	539979
	605836	601341	643576	542539	564729	580245
	617067	629851	613412	504146	577263	514422
	611584	601979	603764	548015	585939	485448
	618940	570662	622476	550104	552884	556969
	619888	547185	598383	531272	548375	542370
Mean	611663	595276	617146	537863	567537	536572
SD	9042	31109	16056	18057	14800	33084
%CV	1.5	5.2	2.6	3.4	2.6	6.2
%Recovery	102.8	NA	114.7	NA	105.8	NA
n	6	6	6	6	6	6
Mean % Recovery	107.8					

NA: Not Applicable.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 28**

**Recovery of [Proprietary Info] (Internal Standard) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	Peak Area					
	Low QC extract	Low QC post extraction spiked	Mid QC extract	Mid QC post extraction spiked	High QC extract	High QC post extraction spiked
MV3	1461395	1414868	1390782	1343677	1476900	1367988
	1448857	1384211	1518087	1339627	1416094	1388953
	1454657	1467000	1468090	1295088	1380158	1318390
	1527267	1378429	1469743	1384085	1460038	1288082
	1502597	1328498	1439213	1396271	1324294	1359826
	1445807	1326234	1464476	1416961	1297044	1411520
Mean	1473430	1383207	1458399	1362618	1392421	1355793
SD	33506	53454	41863	44708	72329	45506
%CV	2.3	3.9	2.9	3.3	5.2	3.4
%Recovery	106.5	NA	107.0	NA	102.7	NA
n	6	6	6	6	6	6
Mean % Recovery	105.4					

NA: Not Applicable.



**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 29**

**Matrix Effect on Ionization of [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**

Run Number	Peak Area					
	Low QC post extraction spiked	Low QC recovery solution	Mid QC post extraction spiked	Mid QC recovery solution	High QC post extraction spiked	High QC recovery solution
MV3	38587	33974	901845	780818	1579916	1592471
	40020	34837	893456	834592	1776022	1639237
	41880	33790	859901	831228	1673070	1611744
	38427	35767	950986	774619	1563443	1624473
	38200	33837	950786	807293	1749384	1682262
	35834	33959	904488	808192	1754449	1536422
Mean	38825	34361	910244	806124	1682714	1614435
SD	2016	789	35280	24816	92934	48769
%CV	5.2	2.3	3.9	3.1	5.5	3.0
% Matrix Effect	113.0	NA	112.9	NA	104.2	NA
n	6	6	6	6	6	6
Mean % Matrix Effect	110.0					

Values >100% indicate matrix enhancement; values <100% indicate matrix suppression.

NA: Not Applicable.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 30**

**Matrix Effect on Ionization of [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**

Run Number	Peak Area					
	Low QC post extraction spiked	Low QC recovery solution	Mid QC post extraction spiked	Mid QC recovery solution	High QC post extraction spiked	High QC recovery solution
MV3	44209	38128	1125178	688504	1953449	1502286
	46111	36787	1101886	762033	2195625	1546683
	48930	31309	1039907	740644	2018730	1497881
	45528	30827	1118771	717044	1899046	1494877
	42758	28933	1138698	713444	2080994	1558265
	43165	29841	1093788	702385	2156127	1499984
Mean	45117	32638	1103038	720676	2050662	1516663
SD	2276	3846	34876	26624	115338	28086
%CV	5.0	11.8	3.2	3.7	5.6	1.9
% Matrix Effect	138.2	NA	153.1	NA	135.2	NA
n	6	6	6	6	6	6
Mean % Matrix Effect	142.2					

Values >100% indicate matrix enhancement; values <100% indicate matrix suppression.

NA: Not Applicable.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 31**

**Matrix Effect on Ionization of [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**

Run Number	Peak Area					
	Low QC post extraction spiked	Low QC recovery solution	Mid QC post extraction spiked	Mid QC recovery solution	High QC post extraction spiked	High QC recovery solution
MV3	91824	97489	2857348	2899543	5113841	5439214
	93673	95272	2852082	2901866	5467201	5557591
	102952	98383	2767673	2794871	5262464	5592402
	97215	94991	2803970	2860733	5113943	5371035
	92560	96802	2901059	2795457	5398191	5455704
	91624	91745	2963358	2781465	5646313	5567026
Mean	94975	95780	2857582	2838989	5333659	5497162
SD	4412	2362	69379	55215	210435	87850
%CV	4.6	2.5	2.4	1.9	3.9	1.6
% Matrix Effect	99.2	NA	100.7	NA	97.0	NA
n	6	6	6	6	6	6
Mean % Matrix Effect	99.0					

Values >100% indicate matrix enhancement; values <100% indicate matrix suppression.

NA: Not Applicable.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 32**

**Matrix Effect on Ionization of [Proprietary Info] (Internal Standard) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	Peak Area					
	Low QC post extraction spiked	Low QC recovery solution	Mid QC post extraction spiked	Mid QC recovery solution	High QC post extraction spiked	High QC recovery solution
MV3	1154597	972829	1046169	886602	1027318	960234
	1171310	1032605	1030895	931617	1089015	962020
	1176164	984120	979735	936732	999326	934294
	1125896	1007651	1050149	910205	963480	958623
	1085607	995618	1079810	891418	1094112	951323
	1036928	976967	1040641	899671	1062825	982183
Mean	1125084	994965	1037900	909374	1039346	958113
SD	54677	22404	32908	20876	51999	15575
%CV	4.9	2.3	3.2	2.3	5.0	1.6
% Matrix Effect	113.1	NA	114.1	NA	108.5	NA
n	6	6	6	6	6	6
Mean % Matrix Effect	111.9					

Values >100% indicate matrix enhancement; values <100% indicate matrix suppression.

NA: Not Applicable.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 33**

**Matrix Effect on Ionization of [Proprietary Info] (Internal Standard) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	Peak Area					
	Low QC post extraction spiked	Low QC recovery solution	Mid QC post extraction spiked	Mid QC recovery solution	High QC post extraction spiked	High QC recovery solution
MV3	620635	463721	551103	353342	539979	361167
	601341	492819	542539	378059	580245	397940
	629851	392459	504146	367615	514422	373273
	601979	384181	548015	355774	485448	386618
	570662	390015	550104	359706	556969	396560
	547185	381835	531272	354103	542370	386793
Mean	595276	417505	537863	361433	536572	383725
SD	31109	48112	18057	9683	33084	14161
%CV	5.2	11.5	3.4	2.7	6.2	3.7
% Matrix Effect	142.6	NA	148.8	NA	139.8	NA
n	6	6	6	6	6	6
Mean % Matrix Effect	143.7					

Values >100% indicate matrix enhancement; values <100% indicate matrix suppression.

NA: Not Applicable.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 34**

**Matrix Effect on Ionization of [Proprietary Info] (Internal Standard) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	Peak Area					
	Low QC post extraction spiked	Low QC recovery solution	Mid QC post extraction spiked	Mid QC recovery solution	High QC post extraction spiked	High QC recovery solution
MV3	1414868	1419527	1343677	1393262	1367988	1343323
	1384211	1460022	1339627	1404069	1388953	1406595
	1467000	1345674	1295088	1398606	1318390	1398506
	1378429	1380946	1384085	1404674	1288082	1351162
	1328498	1410764	1396271	1371104	1359826	1374271
	1326234	1363681	1416961	1375228	1411520	1393390
Mean	1383207	1396769	1362618	1391157	1355793	1377875
SD	53454	41643	44708	14596	45506	26121
%CV	3.9	3.0	3.3	1.0	3.4	1.9
% Matrix Effect	99.0	NA	97.9	NA	98.4	NA
n	6	6	6	6	6	6
Mean % Matrix Effect	98.4					

Values >100% indicate matrix enhancement; values <100% indicate matrix suppression.

NA: Not Applicable.



**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 35**

**Room Temperature Matrix Stability of Lopinavir (ng/ml) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Description			
	15.0	%Accuracy	800	%Accuracy
MV3 (26 hours)	16.7	111.5	803	100.4
	14.7	97.7	807	100.9
	16.3	108.5	837	104.6
	15.6	104.1	860	107.5
Mean	15.8		827	
SD	0.899		26.7	
%CV	5.7		3.2	
%Accuracy	105.5		103.4	
n	4		4	

Quality Control samples were prepared on 05-07-19 and stored in a ≤-60°C freezer (Unit 4409) prior to thawing and storing at room temperature before analysis.

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 36**

**Room Temperature Matrix Stability of** Proprietary **(ng/ml) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Description			
	15.0	%Accuracy	800	%Accuracy
MV3 (26 hours)	15.7	104.5	766	95.7
	15.0	100.1	813	101.6
	14.8	98.5	797	99.7
	15.2	101.3	780	97.5
Mean	15.2		789	
SD	0.386		20.5	
%CV	2.5		2.6	
%Accuracy	101.1		98.6	
n	4		4	

Quality Control samples were prepared on 05-07-19 and stored in a  $\leq -60^{\circ}\text{C}$  freezer (Unit 4409) prior to thawing and storing at room temperature before analysis.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 37**

**Room Temperature Matrix Stability of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Description			
	75.0	%Accuracy	4000	%Accuracy
MV3 (26 hours)	76.8	102.4	4040	101.0
	73.9	98.5	4060	101.4
	72.5	96.7	3970	99.4
	76.6	102.1	4070	101.8
Mean	74.9		4030	
SD	2.10		41.9	
%CV	2.8		1.0	
%Accuracy	99.9		100.9	
n	4		4	

Quality Control samples were prepared on 05-07-19 and stored in a  $\leq -60^{\circ}\text{C}$  freezer (Unit 4409) prior to thawing and storing at room temperature before analysis.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 38**

**Freeze Thaw Matrix Stability of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Description			
	15.0	%Accuracy	800	%Accuracy
MV4 (5 cycles)	18.2	121.1 <sup>a</sup>	837	104.7
	16.5	110.0	853	106.6
	14.9	99.5	817	102.1
	16.2	108.1	833	104.2
Mean	16.4		835	
SD	1.33		14.9	
%CV	8.1		1.8	
%Accuracy	109.7		104.4	
n	4		4	

Quality Control samples were prepared on 05-07-19 and stored in a  $\leq -60^{\circ}\text{C}$  freezer (Unit 4409) during freeze thaw cycling.

<sup>a</sup> Result is outside acceptance criterion ( $\pm 15\%$ ) and was included in the statistics.

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 39**

**Freeze Thaw Matrix Stability of** Proprietary **(ng/ml) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Description			
	15.0	%Accuracy	800	%Accuracy
MV4 (5 cycles)	16.2	108.2	808	101.0
	15.7	104.9	750	93.7
	15.5	103.5	794	99.2
	15.9	106.2	791	98.9
Mean	15.9		786	
SD	0.299		25.0	
%CV	1.9		3.2	
%Accuracy	105.7		98.2	
n	4		4	

Quality Control samples were prepared on 05-07-19 and stored in a ≤-60°C freezer (Unit 4409) during freeze thaw cycling.

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 40**

**Freeze Thaw Matrix Stability of** Proprietary **(ng/ml) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Description			
	75.0	%Accuracy	4000	%Accuracy
MV4 (5 cycles)	76.6	102.2	4110	102.7
	79.7	106.2	4140	103.5
	77.5	103.3	4190	104.7
	75.6	100.8	4170	104.3
Mean	77.3		4150	
SD	1.73		35.1	
%CV	2.2		0.8	
%Accuracy	103.1		103.8	
n	4		4	

Quality Control samples were prepared on 05-07-19 and stored in a ≤-60°C freezer (Unit 4409) during freeze thaw cycling.



**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 41**

**Reinjection (Autosampler) Stability of** Proprietary **(ng/ml) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Description							
	5.00	%Accuracy	15.0	%Accuracy	400	%Accuracy	800	%Accuracy
MV2-RI (188 hours, Refrigerated)	4.72	94.5	15.9	105.9	406	101.6	823	102.9
	4.58	91.6	14.7	98.0	403	100.8	823	102.8
	4.49	89.8	14.8	98.6	430	107.4	801	100.2
	4.97	99.5	15.5	103.6	425	106.3	830	103.8
	4.00	79.9 <sup>a</sup>	14.0	93.3	404	101.1	774	96.7
	5.09	101.8	15.6	103.8	402	100.6	827	103.4
Mean	4.64		15.1		412		813	
SD	0.390		0.712		12.2		21.7	
%CV	8.4		4.7		3.0		2.7	
%Accuracy	92.8		100.5		103.0		101.6	
n	6		6		6		6	

<sup>a</sup> Result is outside acceptance criterion ( $\pm 20\%$ ) and was included in the statistics.

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 42**

**Reinjection (Autosampler) Stability of** Proprietary **(ng/ml) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Description							
	5.00	%Accuracy	15.0	%Accuracy	400	%Accuracy	800	%Accuracy
MV2-RI (188 hours, Refrigerated)	5.05	101.0	14.8	98.4	395	98.7	781	97.7
	5.55	111.1	15.4	103.0	401	100.2	778	97.3
	5.82	116.3	14.8	98.4	395	98.7	792	99.0
	5.40	108.0	15.1	101.0	396	99.1	817	102.1
	5.37	107.4	15.2	101.0	411	102.7	816	101.9
	5.62	112.4	14.9	99.5	407	101.9	803	100.3
Mean	5.47		15.0		401		798	
SD	0.262		0.268		6.89		16.7	
%CV	4.8		1.8		1.7		2.1	
%Accuracy	109.4		100.2		100.2		99.7	
n	6		6		6		6	

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 43**

**Reinjection (Autosampler) Stability of** Proprietary **(ng/ml) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Description							
	25.0	%Accuracy	75.0	%Accuracy	2000	%Accuracy	4000	%Accuracy
MV2-RI (188 hours, Refrigerated)	26.3	105.2	71.4	95.2	1980	99.0	4060	101.5
	26.8	107.1	73.5	98.0	1930	96.4	3970	99.3
	25.4	101.7	73.2	97.5	2010	100.5	4020	100.5
	24.7	98.8	74.5	99.4	1960	98.1	4090	102.1
	26.1	104.4	75.9	101.2	1960	97.8	4010	100.2
	25.7	102.6	68.5	91.3	1960	98.0	4060	101.4
Mean	25.8		72.8		1970		4030	
SD	0.734		2.59		27.8		41.7	
%CV	2.8		3.6		1.4		1.0	
%Accuracy	103.3		97.1		98.3		100.8	
n	6		6		6		6	

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 44**

**Post-Preparative Extract Stability of Lopinavir (ng/ml) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Description			
	15.0	%Accuracy	800	%Accuracy
MV4 (85 hours, Refrigerated)	16.9	112.5	833	104.1
	15.6	104.0	891	111.4
	16.1	107.0	872	109.0
	14.9	99.5	868	108.6
Mean	15.9		866	
SD	0.815		24.2	
%CV	5.1		2.8	
%Accuracy	105.7		108.3	
n	4		4	

These samples were originally extracted in batch MV3.

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 45**

**Post-Preparative Extract Stability of Ritonavir (ng/ml) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Description			
	15.0	%Accuracy	800	%Accuracy
MV4 (85 hours, Refrigerated)	15.9	106.0	809	101.2
	15.0	100.1	813	101.6
	15.3	102.0	833	104.2
	15.4	102.4	812	101.5
Mean	15.4		817	
SD	0.369		11.1	
%CV	2.4		1.4	
%Accuracy	102.6		102.1	
n	4		4	

These samples were originally extracted in batch MV3.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 46**

**Post-Preparative Extract Stability of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Description			
	75.0	%Accuracy	4000	%Accuracy
MV4 (85 hours, Refrigerated)	78.8	105.1	4180	104.5
	75.7	101.0	4180	104.5
	76.1	101.4	4170	104.3
	77.1	102.8	4180	104.6
Mean	76.9		4180	
SD	1.38		5.46	
%CV	1.8		0.1	
%Accuracy	102.6		104.5	
n	4		4	

These samples were originally extracted in batch MV3.



**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 47**

**Processing Stability of** Proprietary **(ng/ml) in K<sub>2</sub> EDTA Dog Whole Blood**

Run Number	QC Description	
	QC Low	QC High
MV4	12.2	641
Time Zero (T0)	12.8	646
	12.4	630
	12.6	616
Mean	12.5	633
SD	0.243	13.6
%CV	1.9	2.1
n	4	4
MV4	11.5	628
1 hour, refrigerated	12.7	628
	11.1	636
	11.6	633
Mean	11.7	631
SD	0.683	3.88
%CV	5.8	0.6
% Difference from T0	-6.4	-0.3
n	4	4
MV4	12.1	613
4 hours, refrigerated	11.8	614
	12.3	630
	11.5	629
Mean	12.0	622
SD	0.368	9.37
%CV	3.1	1.5
% Difference from T0	-4.0	-1.7
n	4	4

Whole blood stability samples were prepared on 05-06-19 and the resulting plasma stored in a ≤-60°C freezer (Unit 4409) until analysis.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 48**

**Processing Stability of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Dog Whole Blood**

Run Number	QC Description	
	QC Low	QC High
MV4	11.9	611
Time Zero (T0)	11.7	609
	11.4	609
	11.4	614
Mean	11.6	611
SD	0.268	2.41
%CV	2.3	0.4
n	4	4
MV4	11.5	612
1 hour, refrigerated	10.9	600
	11.2	602
	11.5	629
Mean	11.3	611
SD	0.303	13.2
%CV	2.7	2.2
% Difference from T0	-2.6	0.0
n	4	4
MV4	11.2	606
4 hours, refrigerated	12.2	603
	11.5	611
	11.3	623
Mean	11.6	611
SD	0.473	9.00
%CV	4.1	1.5
% Difference from T0	0.0	0.0
n	4	4

Whole blood stability samples were prepared on 05-06-19 and the resulting plasma stored in a  $\leq -60^{\circ}\text{C}$  freezer (Unit 4409) until analysis.

**Method Validation Report for the Quantitative Analysis of** Proprietary Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 49**

**Processing Stability of** Proprietary **(ng/ml) in K<sub>2</sub> EDTA Dog Whole Blood**

Run Number	QC Description	
	QC Low	QC High
MV4	59.9	3210
Time Zero (T0)	60.0	3100
	59.7	3210
	60.8	3110
Mean	60.1	3160
SD	0.477	57.2
%CV	0.8	1.8
n	4	4
MV4	62.3	3240
1 hour, refrigerated	60.7	3220
	57.9	3180
	58.1	3180
Mean	59.8	3200
SD	2.13	30.7
%CV	3.6	1.0
% Difference from T0	-0.5	1.3
n	4	4
MV4	61.2	3220
4 hours, refrigerated	59.6	3170
	60.5	3270
	59.2	3110
Mean	60.1	3190
SD	0.912	68.3
%CV	1.5	2.1
% Difference from T0	0.0	0.9
n	4	4

Whole blood stability samples were prepared on 05-06-19 and the resulting plasma stored in a  $\leq -60^{\circ}\text{C}$  freezer (Unit 4409) until analysis.

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 50**

**Effect of Hemolysis on** Proprietary **Quantitation (ng/ml)**

Run Number	QC Description			
	15.0	%Accuracy	800	%Accuracy
MV4	16.6	110.7	779	97.4
0% hemolysis	16.6	110.5	803	100.4
	16.0	106.7	819	102.3
	15.6	104.1	807	100.9
Mean	16.2		802	
SD	0.481		16.7	
%CV	3.0		2.1	
%Accuracy	108.0		100.3	
n	4		4	
MV4	15.4	102.7	830	103.8
0.5% hemolysis	14.8	98.6	827	103.4
	15.7	104.5	815	101.9
	15.9	106.0	825	103.1
Mean	15.4		824	
SD	0.485		6.52	
%CV	3.1		0.8	
%Accuracy	102.9		103.0	
n	4		4	
MV4	15.6	103.9	773	96.6
2% hemolysis	15.7	104.6	830	103.7
	15.7	104.9	903	112.8
	15.4	103.0	825	103.2
Mean	15.6		833	
SD	0.131		53.2	
%CV	0.8		6.4	
%Accuracy	104.1		104.1	
n	4		4	

Quality Control samples were prepared on 05-06-19 and stored in a  $\leq -60^{\circ}\text{C}$  freezer (Unit 4409) until analysis.

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 51**

**Effect of Hemolysis on** Proprietary **Quantitation (ng/ml)**

Run Number	QC Description			
	15.0	%Accuracy	800	%Accuracy
MV4	15.8	105.4	768	96.0
0% hemolysis	15.6	104.1	779	97.4
	15.6	104.2	771	96.4
	15.4	102.8	760	95.0
Mean	15.6		770	
SD	0.161		8.12	
%CV	1.0		1.1	
%Accuracy	104.1		96.2	
n	4		4	
MV4	15.0	100.3	779	97.3
0.5% hemolysis	15.7	104.6	763	95.4
	15.4	102.9	773	96.6
	14.9	99.0	782	97.8
Mean	15.3		774	
SD	0.376		8.17	
%CV	2.5		1.1	
%Accuracy	101.7		96.8	
n	4		4	
MV4	15.5	103.0	779	97.4
2% hemolysis	15.5	103.3	805	100.6
	16.3	108.4	763	95.4
	15.6	104.2	782	97.8
Mean	15.7		782	
SD	0.372		17.2	
%CV	2.4		2.2	
%Accuracy	104.7		97.8	
n	4		4	

Quality Control samples were prepared on 05-06-19 and stored in a ≤-60°C freezer (Unit 4409) until analysis.

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 52**

**Effect of Hemolysis on** Proprietary **Quantitation (ng/ml)**

Run Number	QC Description			
	75.0	%Accuracy	4000	%Accuracy
MV4	74.1	98.8	4010	100.1
0% hemolysis	75.0	100.0	3970	99.4
	74.6	99.5	4080	101.9
	76.1	101.5	3980	99.6
Mean	75.0		4010	
SD	0.868		45.7	
%CV	1.2		1.1	
%Accuracy	99.9		100.2	
n	4		4	
MV4	76.1	101.5	4040	100.9
0.5% hemolysis	78.0	104.0	4030	100.8
	74.4	99.1	3910	97.8
	77.3	103.1	3990	99.7
Mean	76.5		3990	
SD	1.61		58.4	
%CV	2.1		1.5	
%Accuracy	101.9		99.8	
n	4		4	
MV4	76.8	102.4	3990	99.8
2% hemolysis	77.1	102.8	4160	104.1
	74.3	99.1	4080	102.0
	81.1	108.1	4000	100.0
Mean	77.3		4060	
SD	2.78		79.6	
%CV	3.6		2.0	
%Accuracy	103.1		101.5	
n	4		4	

Quality Control samples were prepared on 05-06-19 and stored in a  $\leq -60^{\circ}\text{C}$  freezer (Unit 4409) until analysis.



**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 53**

**Long Term Storage Stability of** Proprietary **(ng/ml) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Description			
	15.0	%Accuracy	800	%Accuracy
MV5	16.2	108.1	832	104.0
21 days at ≤-60°C	14.8	98.7	839	104.8
	15.6	103.9	862	107.7
	15.3	102.2	806	100.8
Mean	15.5		835	
SD	0.586		22.7	
%CV	3.8		2.7	
%Accuracy	103.2		104.3	
n	4		4	

Quality Control samples were prepared on 05-07-19 and stored in a ≤-60°C freezer (Unit 4409) until analysis.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 54**

**Long Term Storage Stability of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Description			
	15.0	%Accuracy	800	%Accuracy
MV5	15.1	100.5	800	100.0
21 days at ≤-60°C	15.5	103.6	836	104.5
	15.7	104.7	814	101.7
	15.0	99.7	819	102.4
Mean	15.3		817	
SD	0.362		14.8	
%CV	2.4		1.8	
%Accuracy	102.1		102.1	
n	4		4	

Quality Control samples were prepared on 05-07-19 and stored in a ≤-60°C freezer (Unit 4409) until analysis.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 55**

**Long Term Storage Stability of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Description			
	75.0	%Accuracy	4000	%Accuracy
MV5	75.1	100.2	4010	100.3
21 days at $\leq -60^{\circ}\text{C}$	72.9	97.2	3940	98.6
	76.8	102.4	4010	100.2
	72.5	96.6	3940	98.4
Mean	74.3		3980	
SD	2.01		40.3	
%CV	2.7		1.0	
%Accuracy	99.1		99.4	
n	4		4	

Quality Control samples were prepared on 05-07-19 and stored in a  $\leq -60^{\circ}\text{C}$  freezer (Unit 4409) until analysis.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 56**

**Effect of Concomitant Medication [Proprietary] and [Proprietary Info] on [Proprietary] Quantitation (ng/ml) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Description	
	15.0	%Accuracy
MV1-RI	13.7	91.1
	13.9	92.8
	13.2	87.8
	13.5	89.8
Mean	13.6	
SD	0.322	
%CV	2.4	
%Accuracy	90.4	
n	4	

[Proprietary] Low QC samples (15.0 mg/ml) were spiked with [Proprietary] and [Proprietary] at 2000 ng/ml and 20000 ng/ml, respectively.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 57**

**Effect of Concomitant Medication [Proprietary Info] and [Proprietary Info] on [Proprietary] Quantitation (ng/ml) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Description	
	15.0	%Accuracy
MV1-RI	15.2	101.7
	13.8	91.7
	13.8	92.1
	14.7	98.3
Mean	14.4	
SD	0.733	
%CV	5.1	
%Accuracy	95.9	
n	4	

[Proprietary] Low QC samples (15.0 mg/ml) were spiked with [Proprietary] and [Proprietary] at 2000 ng/ml and 20000 ng/ml, respectively.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 58**

**Effect of Concomitant Medication [Proprietary Info] and [Proprietary] on [Proprietary] Quantitation (ng/ml) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Description	
	75.0	%Accuracy
MV1-RI	75.5	100.7
	73.2	97.7
	74.4	99.2
	70.1	93.4
Mean	73.3	
SD	2.37	
%CV	3.2	
%Accuracy	97.8	
n	4	

[Proprietary] Low QC samples (75.0 mg/ml) were spiked with [Proprietary] and [Proprietary] at 2000 ng/ml.



**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 59**

**Batch Acceptance Quality Control (QC) Samples for Analysis of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Description					
	15.0	%Accuracy	400	%Accuracy	800	%Accuracy
MV4	15.8	105.1	410	102.5	840	105.0
	16.0	106.9	391	97.8	831	103.9
MV5	15.2	101.3	411	102.8	820	102.5
	16.4	109.1	408	101.9	780	97.5
Mean	15.8		405		818	
SD	0.494		9.33		26.3	
%CV	3.1		2.3		3.2	
%Accuracy	105.6		101.3		102.2	
n	4		4		4	

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 60**

**Batch Acceptance Quality Control (QC) Samples for Analysis of** Proprietary **(ng/ml) in**  
**K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Description					
	15.0	%Accuracy	400	%Accuracy	800	%Accuracy
MV4	15.3	102.1	409	102.2	804	100.5
	15.5	103.0	407	101.6	793	99.1
MV5	14.9	99.1	386	96.4	816	102.1
	15.0	100.2	395	98.7	805	100.6
Mean	15.2		399		805	
SD	0.263		10.8		9.60	
%CV	1.7		2.7		1.2	
%Accuracy	101.1		99.7		100.6	
n	4		4		4	

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 61**

**Batch Acceptance Quality Control (QC) Samples for Analysis of** Proprietary **(ng/ml) in**  
**K<sub>2</sub> EDTA Dog Plasma**

Run Number	QC Description					
	75.0	%Accuracy	2000	%Accuracy	4000	%Accuracy
MV4	76.0	101.3	2040	101.9	4240	106.0
	75.8	101.1	2110	105.3	4220	105.5
MV5	74.9	99.9	2040	102.2	4240	106.0
	71.1	94.8	1970	98.7	4120	103.1
Mean	74.5		2040		4210	
SD	2.29		54.0		55.5	
%CV	3.1		2.6		1.3	
%Accuracy	99.3		102.1		105.2	
n	4		4		4	

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 62**

**Stock Solution Stability of** Proprietary

Run Number Stock Concentration	Peak Area Ratio		
	Stock Prepared 01-May-19	Stock 1 Prepared 10-June-19	Stock 2 Prepared 10-June-19
B185-061019-Stk-Chk	0.9094	0.9348	0.9295
1.00 mg/ml	0.8545	0.8664	0.8918
	0.9071	0.8509	0.8606
	0.9329	0.8505	0.8761
	0.8564	0.8696	0.8795
	0.8800	0.8948	0.8933
Mean	0.8900	0.8778	0.8885
SD	0.0316	0.0323	0.0234
%CV	3.6	3.7	2.6
n	6	6	6
Mean of new stock solutions 1 and 2	NA	0.8832	
%Difference between old and mean of new	0.8	NA	

All solutions were prepared using DMSO as the diluent.

The solution prepared on 01-May-19 was stored refrigerated (Unit 4206) for 40 days prior to evaluation.

NA: Not Applicable

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 63**

**Stock Solution Stability of** Proprietary

Run Number Stock Concentration	Peak Area Ratio		
	Stock Prepared 01-May-19	Stock 1 Prepared 10-June-19	Stock 2 Prepared 10-June-19
B185-061019-Stk-Chk	2.037	2.050	2.120
1.00 mg/ml	2.020	2.057	2.211
	1.992	2.063	2.149
	2.017	2.035	2.125
	1.989	1.978	2.094
	2.069	2.073	2.132
Mean	2.021	2.043	2.138
SD	0.0299	0.0340	0.0398
%CV	1.5	1.7	1.9
n	6	6	6
Mean of new stock solutions 1 and 2	NA	2.091	
%Difference between old and mean of new	-3.3	NA	

All solutions were prepared using DMSO as the diluent.

The solution prepared on 01-May-19 was stored refrigerated (Unit 4206) for 40 days prior to evaluation.

NA: Not Applicable

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 64**

**Stock Solution Stability of** Proprietary

Run Number Stock Concentration	Peak Area Ratio		
	Stock Prepared 01-May-19	Stock 1 Prepared 10-June-19	Stock 2 Prepared 10-June-19
B185-061019-Stk-Chk	2.217	2.219	2.233
1.00 mg/ml	2.175	2.208	2.209
	2.164	2.138	2.218
	2.148	2.311	2.218
	2.271	2.199	2.242
	2.188	2.213	2.251
Mean	2.194	2.215	2.228
SD	0.0448	0.0556	0.0162
%CV	2.0	2.5	0.7
n	6	6	6
Mean of new stock solutions 1 and 2	NA	2.222	
%Difference between old and mean of new	1.3	NA	

All solutions were prepared using Milli-Q water as the diluent.

The solution prepared on 01-May-19 was stored refrigerated (Unit 4206) for 40 days prior to evaluation.

NA: Not Applicable



**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 65**

**Room Temperature Stock Solution Stability of** Proprietary

Run Number Stock Concentration	Peak Area Ratio	
	Refrigerated Solution	Room Temperature Solution
B185-061119-Stk-Chk 1.00 mg/ml	0.9130	0.8649
	0.8774	0.8546
	0.8631	0.8692
	0.8706	0.8482
	0.8480	0.8697
	0.9134	0.8768
Mean	0.8809	0.8639
SD	0.0268	0.0106
%CV	3.0	1.2
n	6	6
%Difference	NA	-1.9

This solution was prepared using DMSO as the diluent.

The solution was stored at room temperature for 23 hours prior to evaluation.

NA: Not Applicable.

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 66**

**Room Temperature Stock Solution Stability of** Proprietary

Run Number Stock Concentration	Peak Area Ratio	
	Refrigerated Solution	Room Temperature Solution
B185-061119-Stk-Chk 1.00 mg/ml	2.083	2.190
	2.189	2.040
	2.099	1.941
	2.127	2.075
	2.126	2.003
	2.091	1.984
Mean	2.119	2.039
SD	0.0385	0.0870
%CV	1.8	4.3
n	6	6
%Difference	NA	-3.8

This solution was prepared using DMSO as the diluent.

The solution was stored at room temperature for 23 hours prior to evaluation.

NA: Not Applicable.

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Table 67**

**Room Temperature Stock Solution Stability of** Proprietary

Run Number Stock Concentration	Peak Area Ratio	
	Refrigerated Solution	Room Temperature Solution
B185-061119-Stk-Chk 1.00 mg/ml	2.230	2.213
	2.278	2.243
	2.250	2.214
	2.170	2.182
	2.228	2.161
	2.260	2.201
Mean	2.236	2.203
SD	0.0377	0.0284
%CV	1.7	1.3
n	6	6
%Difference	NA	-1.5

This solution was prepared using Milli-Q water as the diluent.

The solution was stored at room temperature for 23 hours prior to evaluation.

NA: Not Applicable.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and  
[Proprietary] in K<sub>2</sub> EDTA Dog Plasma  
SRI Study No. B185-18**

**Appendix A**

**SRI TEST METHOD 106.201; ANALYSIS OF [Proprietary Info] [Proprietary Info]  
AND [Proprietary Info] IN K<sub>2</sub> EDTA DOG PLASMA**

**TEST METHOD**

**Classification:** Project  
**Supersedes:** 106.201 (06/12/19)

**TM No.:** 106.201**Page:** 1 of 26**Effective:** JUL 12 2019

**Subject:** Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma

**A. PURPOSE/SCOPE**

This Test Method describes procedures to be employed for the analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA dog plasma using a protein precipitation extraction procedure and analysis by LC-MS/MS.

During sample analysis, SRI SOPs 006.061 *Bioanalytical Sample Analysis* and 006.062 *Bioanalytical Sample Reanalysis* will also be followed.

**B. BACKGROUND/GENERAL**

This Test Method will fully detail the experimental procedures used in the analysis, detection and quantitation of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA dog plasma. To summarize, [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA dog plasma (0.0200 ml sample size) will be extracted, using [Proprietary Info] [Proprietary Info] and [Proprietary Info] as the internal standards, by a protein precipitation extraction procedure. The supernatant is then diluted prior to injection on the LC-MS/MS system. The range of the assay is 5.00 – 1000 ng/ml [Proprietary] and [Proprietary] and 25.0 – 5000 ng/ml [Proprietary Info]

**SIGNATURES**

Revised by:

Reviewed by:

Management Approval:

QAU Review:

Redacted by agreement

07/11/19

Date

07/11/19


Date

07/11/19

Date

07/12/2019

Date

	<b>TEST METHOD</b> <b>Classification:</b> Project <b>Supersedes:</b> 106.201 (06/12/19)	<b>TM No.:</b> 106.201 <b>Page:</b> 2 of 26 <b>Effective:</b> July 12, 2019
	<b>Subject:</b> Analysis of [Proprietary] [Proprietary] and [Proprietary] in K <sub>2</sub> EDTA Dog Plasma	

## C. HEALTH AND SAFETY

All personnel must observe standard laboratory safety practices. All personnel must wear protective equipment appropriate to the area in which they will work, which may include, but not be limited to: safety glasses, protective clothing and gloves.

## D. TRAINING

All personnel involved in handling chemicals, equipment, and instruments must have attended the pertinent laboratory safety classes from SRI's Environmental Health & Safety Department and must have attended GLP training courses. Training must be documented.

## E. EQUIPMENT AND MATERIALS

Chemicals, consumables or equipment may be substituted provided that equivalent assay performance is obtained.

### E.1 Chemicals


- [Proprietary] [Proprietary] and [Proprietary] USP, Current Lot
- [Proprietary Info] [Proprietary Info] and [Proprietary Info] Medical Isotopes, Inc.
- Ammonium hydroxide, reagent grade
- Acetic acid, LC-MS grade
- Milli-Q water, Millipore
- Formic acid, reagent grade
- Dimethyl Sulfoxide (DMSO), reagent grade
- Methanol, HPLC grade
- Acetonitrile, HPLC grade
- Isopropanol, reagent grade
- K<sub>2</sub> EDTA beagle dog plasma, BioIVT

### E.2 Consumables

- HPLC column: Phenomenex Synergi Polar RP 100 x 2mm, 4µm
- 0.5 µm stainless-steel pre-column frit (Upchurch Scientific)
- Assorted disposable pipette tips

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	<b>TEST METHOD</b>	<b>TM No.:</b> 106.201
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	<b>Subject:</b> Analysis of <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> and <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> in K <sub>2</sub> EDTA Dog Plasma	

- Disposable 1.5 ml and 2.0 ml polypropylene microcentrifuge tubes
- Disposable 15 ml conical polypropylene test tubes and caps
- Disposable glass vials and caps, assorted sizes
- Glass autosampler vials with inserts and caps

### E.3 Equipment

- Air displacement pipettor, Rainin
- Positive displacement pipettor, Gilson
- Repeater pipettor, Eppendorf
- Mettler Toledo AG 285 balance
- VWR Mini Vortexer
- Beckman Coulter Microfuge® 18 Centrifuge, 20 Centrifuge
- Shimadzu Corp. LC-20AD Prominence Pumps (incorporates Shimadzu Corp. CBM-20A Prominence Communications Bus Module and Shimadzu DGU-20A<sub>3R</sub> Prominence degasser
- Shimadzu Corp. CTO-20AC Prominence Column Oven
- CTC Analytics HTS-xt Autosampler
- AB Sciex 5500 Mass Spectrometer

## F. PROCEDURES

Note: SRI Forms 106.201A through 106.201E will be used to assist in raw data recording for the experimental phases of this study. The completed attachments or other documentation must be stored in the study file.


### F.1 Preparation of Reagents

Volumes of these reagents can be adjusted as long as proportionality is maintained and their preparation is documented in the raw data.

#### F.1.1 2% Acetic Acid in Water (Mobile Phase A)

Add 20.0 ml of acetic acid to 1000 ml of Milli-Q water in a glass bottle and store the solution at room temperature. The expiration date for this solution is one month after preparation.

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	<b>TEST METHOD</b>	<b>TM No.:</b> 106.201
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**F.1.2 0.1% Acetic Acid in Acetonitrile (Mobile Phase B)**

Add 1.00 ml of acetic acid to 1000 ml of acetonitrile in a glass bottle and store the solution at room temperature. The expiration date for this solution is one month after preparation.

**F.1.3 Acetonitrile : Isopropanol (80:20 v:v) with 1% Ammonium Hydroxide (Needle Rinse 1)**

Add 400 ml acetonitrile to 100 ml isopropanol and 5.00 ml ammonium hydroxide in a glass bottle and store the solution at room temperature. The expiration date for this solution is one month after preparation.

**F.1.4 Water : Methanol (90:10 v:v) with 1% Formic Acid (Needle Rinse 2)**

Add 450 ml Milli-Q water to 50.0 ml methanol and 5.00 ml formic acid in a glass bottle and store the solution at room temperature. The expiration date for this solution is one month after preparation.

**F.1.5 0.2% Acetic Acid in Methanol (Diluent Solution)**

Add 0.400 ml acetic acid to 200 ml methanol in a glass bottle and store the solution at room temperature. The expiration date for this solution is one month after preparation.


**F.1.6 Water : Methanol (90:10 v:v) with 0.1% Acetic Acid (Reconstitution Solution)**

Add 180 ml Milli-Q water to 20.0 ml methanol and 0.200 ml acetic acid in a glass bottle and store the solution at room temperature. The expiration date for this solution is one month after preparation.

**F.2 Preparation of Stock and Spiking Solutions**

The following standard preparation scheme is a suggested approach. Appropriate modifications to reach the targeted nominal calibrant and quality control (QC) standard concentrations are acceptable. For example, if the targeted nominal concentration is not achieved when the analyte calibration standard primary stock solution is obtained, the volume of this stock solution used in subsequent dilutions can be modified in order to achieve the targeted nominal calibration standard matrix concentrations. The actual volumes of standards used will be documented in the raw data. Volumes of these stock solutions can be adjusted as long as proportionality is maintained and their preparation is documented in the study binder.

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	<b>Subject:</b> Analysis of [Proprietary] [Proprietary] and [Proprietary] in K <sub>2</sub> EDTA Dog Plasma	

Analytical reference standards are corrected for purity, water and salt content, if applicable. Internal standard stocks may not be corrected for purity, water or salt.

Example purity calculation:

$$\text{Purity} = \frac{[\text{HPLC \% purity} \times (100 - \% \text{ water} - \% \text{ residual solvent})]}{100} \times \frac{\text{Free base molecular weight}}{\text{Salt form molecular weight}}$$

#### F.2.1 Preparation of [Proprietary] [Proprietary] and [Proprietary] Stock Solutions (1.00 mg/ml)

Accurately weigh out approximately 5.00 mg of [Proprietary] into a glass vial and dilute to a concentration of 1.00 mg/ml using dimethyl sulfoxide (DMSO). The purity of the compound must be taken into account when preparing this stock (Stock A). Repeat this step to produce a second stock solution at the same concentration (Stock B). Repeat to get duplicate weighings for [Proprietary] at the same concentration.

To prepare [Proprietary] stock solutions, weigh approximately 5.00 mg of [Proprietary] into a glass vial and dilute to a concentration of 1.00 mg/ml using Milli-Q water. Repeat this step to produce a second stock solution at the same concentration.

Store all stock solutions refrigerated (set point 5°C ± 3°C) until use.

#### F.2.2 Preparation of [Proprietary Info] [Proprietary Info] and [Proprietary Info] Stock Solutions (Internal Standard), 1.00 mg/ml

These Internal Standards are supplied by Medical Isotopes, Inc. as 1.00 mg amounts in a glass vial. Add 1.00 ml of DMSO to the [Proprietary Info] and [Proprietary Info] to produce a 1.00 mg/ml stock. Repeat for [Proprietary Info] using Milli-Q water instead of DMSO.

Store all internal standard stock solutions refrigerated (set point 5°C ± 3°C) until use.

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**TEST METHOD****Classification:** Project**Supersedes:** 106.201 (06/12/19)**Subject:** Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**TM No.:** 106.201**Page:** 6 of 26**Effective:** July 12, 2019**F.2.3** Preparation of [Proprietary Info] [Proprietary Info] and [Proprietary Info] Internal Standard Secondary Stock

Accurately add 0.0100 ml of the 1.00 mg/ml [Proprietary Info] and the d<sub>6</sub>-[Proprietary] internal standard stock solutions, and 0.0500 ml of the 1.00 mg/ml [Proprietary Info] internal standard stock solution into a glass vial containing 9.930 ml of 0.2% acetic acid in methanol. The final concentration of the internal standard secondary stock solution will be 1.00 µg/ml [Proprietary Info] and [Proprietary Info] and 5.00 µg/ml [Proprietary Info]. This solution can be stored refrigerated (set point 5°C ± 3°C) until use.

**F.2.4** Preparation of [Proprietary Info] [Proprietary Info] and [Proprietary Info] Internal Standard Spiking Solution

Accurately add 2.50 ml of the internal standard secondary stock solution into a glass bottle containing 97.5 ml of 0.2% acetic acid in methanol. The final concentration of the internal standard spiking solution will be 25.0 ng/ml [Proprietary Info] and [Proprietary Info] and 125 ng/ml [Proprietary Info]. This solution can be stored refrigerated (set point 5°C ± 3°C) until use.

Per SRI SOP 006.063, *Reference Material Receipt and Stock, Spiking Solution and Calibration and Quality Control Sample Preparation / Expiration* internal standard stock and spiking solutions will be given a default expiration date of 6 months after preparation.

**F.2.5** Stock Verification

In order to determine the accuracy of preparation, the duplicate stock solutions will be verified prior to use. A suggested approach for the preparation of stock verification solutions is given here, although alternative final concentrations may be used providing that a suitable analyte and internal standard response is achieved. The duplicate stock solutions prepared in step F.2.1 should be diluted by spiking 0.0100 ml of the 1.00 mg/ml [Proprietary] and [Proprietary] stock solutions and 0.0500 ml of the 1.00 mg/ml [Proprietary] stock solutions into 9.930 ml of Diluent Solution. These duplicate vials are briefly vortexed and 0.100 ml is removed and added to a vial containing 0.900 ml Diluent Solution. Vortex, then remove 0.0325 ml of this solution and place into a separate vial containing 0.0203 ml of internal standard spiking solution and 0.947 ml of Reconstitution Solution. The final concentration of [Proprietary] and [Proprietary] is 3.25 ng/ml and the final concentration of [Proprietary] is 16.3 ng/ml. The final concentration of [Proprietary Info] and [Proprietary Info] is 0.508

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ng/ml and the final concentration of [Proprietary Info] is 2.54 ng/ml. The duplicate samples are injected in replicates of  $n \geq 3$  onto the LC-MS/MS system.

To be considered acceptable for use, the stocks must agree to within 5% of each other, calculated as follows:

% difference =

$$\frac{(\text{Mean of peak area ratio of stock A} - \text{Mean of peak area ratio of stock B})}{(\text{Mean of peak area ratio of stock A} + \text{Mean of peak area ratio of stock B})/2} \times 100$$

If these stocks agree within 5% of each other, one single stock may be used for the preparation of both calibrants and Quality Control (QC) samples. Alternatively, one stock may be used for the preparation of calibrants while the other stock can be used for the preparation of QC samples.

If these stocks do not agree, a third weighing may be performed and the three stocks compared against each other. If two stocks agree with each other these may be used to prepare calibrants and QCs, and the other stock can be discarded.

## F.2.6 Test Mix Preparation

A solution prepared at the LLOQ level (or below Low QC concentration) shall be prepared and injected at the start and the end of each bioanalytical run (system suitability). This solution will contain both analyte and internal standard. To prepare at the LLOQ level, spike 0.0100 ml of the 1.00 mg/ml [Proprietary] and [Proprietary] stock solutions and 0.0500 ml of the 1.00 mg/ml [Proprietary] stock solution into 9.930 ml of Diluent Solution. Vortex remove 0.100 ml and add to a vial containing 0.900 ml of Diluent Solution. Vortex remove 0.0100 ml and add to a vial containing 0.990 ml of Diluent Solution. This solution may be stored refrigerated for up to 3 months from the date of preparation. On the day of use, remove 0.0203 ml of this solution and place into a separate vial containing 0.0203 ml of internal standard spiking solution and 0.959 ml of Reconstitution Solution. The final concentration of [Proprietary] and [Proprietary] is 0.0203 ng/ml and the final concentration of [Proprietary] is 0.102 ng/ml. The final concentration of [Proprietary Info] and [Proprietary Info] is 0.508 ng/ml and the final concentration of [Proprietary Info] is 2.54 ng/ml. The final concentrations mimic the final theoretical concentrations of [Proprietary] [Proprietary] and [Proprietary] and internal standards seen in LLOQ samples post-extraction.

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**F.2.7** Remove approximately 10.0 ml of control K<sub>2</sub> EDTA dog plasma from storage and allow it to equilibrate to room temperature. The matrix may be centrifuged at approximately 3000 RPM for 10 minutes prior to use to remove excess particulates. Prepare QC samples in polypropylene vials as shown in the table below. Note that the Solution Spiking Volumes are combined with the Matrix Volumes. Volumes of these QC samples can be adjusted as long as proportionality is maintained and their preparation is properly documented in the study raw data.

Quality Control Sample Preparation						
QC ID	Spiking Solution ID	Spiking Solution Concentration (µg/ml)	Spiking Volume (ml)	Matrix Volume (ml)	Final Volume (ml)	Nominal Matrix Concentration (ng/ml)
QC- Low	QC-Mid	0.400 / 0.400 / 2.00	0.0375	0.9625	1.00	15.0 / 15.0 / 75.0
QC- Mid	QC-Dil	5.00 / 5.00 / 25.0	0.0800	0.920	1.00	400 / 400 / 2000
QC- High	QC-Dil	5.00 / 5.00 / 25.0	0.160	0.840	1.00	800 / 800 / 4000
QC- Dil	[Proprietary] Stock A/B;	1000	0.0100	1.930	2.00	5000
	[Proprietary] Stock A/B;	1000	0.0100			5000
	[Proprietary] Stock A/B	1000	0.0500			25000

Either Stock A or Stock B may be used, assuming equivalency is achieved.

The values in the "Spiking Solution Concentration" and the "Nominal Matrix Concentration" columns represent the concentrations of [Proprietary] [Proprietary] and [Proprietary] respectively.


These QC samples may be aliquoted into appropriate volumes into polypropylene tubes (suggested 150 µl volumes) and stored in a ≤-60°C freezer until use, providing that sufficient stability in matrix has been successfully validated under these conditions. QC samples may also be freshly prepared on the day of extraction.

**F.3 Extraction Procedure**

Each bioanalytical run will be comprised of bracketing calibration curves (8 points) each with a matrix blank sample (matrix with neither analyte nor internal standard spiked) and a control blank (matrix with only internal standard included). It is recommended that a carryover blank (matrix blank) be injected after each upper limit of quantitation (ULOQ) calibration standard to assess any carryover present. Duplicate System Suitability samples will be injected, one before the first calibration curve and one at the end of the batch. Interspersed between the calibration curves will be  $n \geq 2$  QC samples at low, mid and high concentration.

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The dilution QC, when used, should be extracted in multiples of n=3. It is not required to run the dilution QC with a batch where samples are diluted, provided that the level of sample dilution did not exceed what was previously validated. However, for troubleshooting practices, the dilution QC may be analyzed with each batch, at the discretion of the bioanalytical scientist. If study samples are diluted with multiple dilution factors, then the dilution QC may be similarly diluted (with replicates of n=3 for each dilution factor used), or alternatively, the highest dilution factor used for study samples will be used for the extraction of the dilution QC. It is also acceptable if low dilution factors are applied to study samples to use a high QC in place of the dilution QC, in order that the diluted sample falls within the calibration range. If the level of dilution required for study samples exceeds the dilution factor previously validated, then the dilution QC will need to be revalidated at the dilution factor required, in replicates of 6.

In order to facilitate the equilibration of the instrument, multiple injections of extracts (Conditioning Samples) may be injected before each batch. It is recommended to prepare conditioning samples near the LLOQ level, but providing that internal standard is present in the sample, the actual concentration used may change. Conditioning samples will be pooled, where more than one calibration standard or QC sample, at different concentrations, are combined. Individual study samples, calibration standards, and QC samples will not be used as conditioning samples without pooling. It is not acceptable to condition a batch using an old standard curve from a previous batch. It is acceptable to prepare either multiple pooled conditioning samples, or to re-inject the pooled sample from the same vial, depending on the final extract volume. The conditioning injections will be included as part of the analytical batch and will be printed with the rest of the batch. Approximately 10 conditioning injections will be analyzed prior to the start of each batch. If one batch is analyzed immediately following another, later batches may not require conditioning injections, although duplicate system suitability injections will be included.

Remove the calibration standard spiking solutions, internal standard spiking solution, QC samples and control matrix (approximately 10.0 ml) from storage and allow them to equilibrate to room temperature.

Follow the scheme listed below to prepare the calibration standards. The calibration standards are prepared in polypropylene tubes. Note that the Spiking Solution Volumes are combined with the Matrix Volumes.

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## Preparation of Calibration Standards in Matrix

Calibration Standard ID	Spiking Solution ID	Spiking Solution Concentration (µg/ml)	Spiking Volume (ml)	Matrix Volume (ml)	Final Volume (ml)	Nominal Matrix Concentration (ng/ml)
Std-1	Std-5	0.100 / 0.100 / 0.500	0.0250	0.475	0.500	5.00 / 5.00 / 25.0
Std-2	Std-5	0.100 / 0.100 / 0.500	0.0500	0.450	0.500	10.0 / 10.0 / 50.0
Std-3	Std-5	0.100 / 0.100 / 0.500	0.100	0.400	0.500	20.0 / 20.0 / 100
Std-4	Std-8	1.00 / 1.00 / 5.00	0.0250	0.475	0.500	50.0 / 50.0 / 250
Std-5	Std-8	1.00 / 1.00 / 5.00	0.0500	0.450	0.500	100 / 100 / 500
Std-6	Std-8	1.00 / 1.00 / 5.00	0.100	0.400	0.500	200 / 200 / 1000
Std-7	Std-9	10.0 / 10.0 / 50.0	0.0250	0.475	0.500	500 / 500 / 2500
Std-8	Std-9	10.0 / 10.0 / 50.0	0.0500	0.450	0.500	1000 / 1000 / 5000
Std-9	[Proprietary] Stock A/B;	1000	0.0100	0.930	1.00	10000
	[Proprietary] Stock A/B;	1000	0.0100			10000
	[Proprietary] Stock A/B	1000	0.0500			50000

Std-9 is only used to prepared the standard curve, and is not extracted.

Either Stock A or Stock B may be used, assuming equivalency is achieved.

The values in the “Spiking Solution Concentration” and the “Nominal Matrix Concentration” columns represent the concentrations of [Proprietary] [Proprietary] and [Proprietary] respectively.

These calibration standards may be aliquoted into appropriate volumes into polypropylene tubes (suggested 150 µl volumes) and stored in a ≤-60°C freezer until use, providing that sufficient stability in matrix has been successfully validated under these conditions. Calibration standards may also be freshly prepared on the day of extraction.

- F.3.1** Transfer 0.0200 ml of each calibration standard, QC sample, study sample and blank into separate 1.50 ml microcentrifuge tubes. If needed, extra samples may be extracted in order to be used as Conditioning Samples. These should be pooled before use.
- F.3.2** Add 0.100 ml of 0.2% acetic acid in methanol to the matrix blanks. Cap tubes and vortex for approximately 5 seconds.
- F.3.3** Add 0.100 ml of the Internal Standard Spiking Solution to each calibration standard, QC standard, study sample and control blank. Cap tubes and vortex for approximately 5 seconds.
- F.3.4** Centrifuge tubes at approximately 18000g for approximately 10 minutes.
- F.3.5** Transfer 0.0250 ml of the supernatant into a 2.00 ml HPLC vial containing 1.00 ml Reconstitution Solution. Cap and vortex briefly to mix.

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**F.3.6** Store on the autosampler (set point 5°C ± 3°C) or refrigerated (set point 5°C ± 3°C).

**F.4 Analytical Conditions**

Equipment can be substituted provided that equivalent assay performance is obtained.

Refer to Figures 1-3 in this Test Method for an example of a representative chromatogram for each analyte at the LLOQ level.

**F.4.1 HPLC Conditions**

Autosampler:	CTC Analytics HTS-xt
Pumps:	Shimadzu LC-20AD Prominence. Incorporates Shimadzu CBM-20A Prominence communications bus module and Shimadzu DGU-20A <sub>3R</sub> Prominence degasser
Column Oven:	Shimadzu CTO-20AC Prominence
Autosampler Temp:	Set point 5°C
Column Oven Temp:	Set point 25°C
Column:	Phenomenex Synergi Polar RP 100 x 2 mm, 4μ
Pre-column Frit:	0.5 μm stainless-steel Precolumn Frit (Upchurch Scientific)
Flow Rate:	0.350 ml/min
Run Time:	8.0 minutes
Injection Volume:	10 μl*
Mobile Phase A:	2% acetic acid in water
Mobile Phase B:	0.1% acetic acid in acetonitrile
Needle Rinse 1:	Acetonitrile:isopropanol (80:20 v:v) with 1% ammonium hydroxide
Needle Rinse 2:	Water:methanol (90:10 v:v) with 1% formic acid
Pre clean with Needle	1*, 1*
Rinses 1 and 2:	
Post clean with Needle	2*, 2*
Rinse 1 and 2:	
Valve clean with	2*, 2*
Needle Rinse 1 and 2:	
Retention Time:	4.3 minutes [Proprietary] and IS)
	4.2 minutes [Proprietary] and IS)
	1.4 minutes [Proprietary] and IS)

\* may be modified to improve performance

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Time (min)	% A	% B
0.01	98	2
2.00	98	2
2.10	50	50
4.00	2	98
5.50	2	98
5.51	98	2
8.00	98	2

## Switching Valve program

Total Time (minutes)	Position
0.0 to 0.2*	Divert to Waste
0.2 to 5.0*	Divert to MS
5.0 to 8.0*	Divert to Waste

\* may be modified to improve performance

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Mass Spectrometer: AB Sciex 5500 Mass Spectrometer  
Interface: Turbo IonSpray positive-ion mode  
Scan Mode: Multiple Reaction Monitoring (MRM)  
IS: 5500V\*  
EP: 10V\*  
DP: 71V\* [Proprietary Info] 81V\* [Proprietary Info]  
121V\* [Proprietary Info] 76V\* [Proprietary Info]  
76V\* [Proprietary Info] 56V\* [Proprietary Info]  
CE: 21V\* [Proprietary Info] 19V\* [Proprietary Info]  
25V\* [Proprietary Info] 25V\* [Proprietary Info]  
33V\* [Proprietary Info] 35V\* [Proprietary Info]  
  
CXP: 38V\* [Proprietary Info] 20V\* [Proprietary Info]  
26V\* [Proprietary Info] 24V\* [Proprietary Info]  
14V\* [Proprietary Info] 14V\* [Proprietary Info]  
  
Resolution Q1, Q3: Unit, Unit  
CUR Gas: 20\*  
CAD Gas: 8\*  
GS1: 60\*  
GS2: 60\*  
Source Temp: 650°C\*  
Dwell: 80\* ms

Nominal [Proprietary] m/z 629.3\* → 447.2\*  
Transitions: [Proprietary] m/z 721.3\* → 296.1\*  
[Proprietary] m/z 288.1\* → 176.1\*  
[Proprietary Info] m/z 637.4\* → 447.2\*  
[Proprietary Info] m/z 727.4\* → 302.2\*  
[Proprietary Info] m/z 294.1\* → 182.1\*

May be modified to improve performance. The eventual m/z ratios used must be within ± 0.3 amu from the masses quoted above.)

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## F.5 Calculations

**F.5.1** Chromatograms will be automatically integrated using AB Sciex Analyst software (Version 1.6.2) or equivalent and visually inspected for an acceptable integration.

**F.5.2** Compute the  $1/x$  weighted least-squares linear regression [Proprietary] and [Proprietary] and the  $1/x^2$  weighted least-squares linear regression [Proprietary Info] using Analyst software, relating the peak area ratios (relative to internal standard) of the calibration standards to their respective nominal concentrations (ng/ml in plasma) for [Proprietary] [Proprietary] and [Proprietary]

**F.5.3** Using the peak area ratios (relative to the internal standard) of the standards and the regression equation constants, concentrations for analyte in the QC samples and study samples can be interpolated.

**F.5.4** Compute the correlation coefficient for the standard data.

## F.6 Acceptance Criteria

### F.6.1 System Suitability Standard

There are no formal acceptance criteria for the System Suitability samples. The system suitability sample will be injected at the beginning and at the end of a run and inspected to ensure signal-to-noise ratio and peak shape are adequate for quantitation. Any chromatographic change between these injections which may have an impact on the ability to accurately quantitate the samples will be noted, however there is no formal acceptance criteria for this. The system suitability injections will be printed with the other chromatograms in the analytical batch.

### F.6.2 Calibration Standard Acceptance Criteria


**F.6.2.1** The lower limit of quantitation (LLOQ) standard back-calculated concentration must be within  $\pm 20\%$  of theoretical nominal concentration.

**F.6.2.2** To meet acceptance criteria, the back-calculated concentration of a calibration standards (excluding at the LLOQ level) must be within 15% of their nominal theoretical concentrations.

**F.6.2.3** A minimum of three-quarters of calibration standards must meet these criteria.

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**F.6.2.4** Any standards failing to meet the acceptance criteria will be excluded from the regression, starting with the calibration standard which is furthest away from the nominal concentration.

**F.6.3** Quality Control (QC) Sample Acceptance Criteria.

**F.6.3.1** To meet acceptance criteria, the back-calculated concentration of a QC sample must be within 15% of their nominal theoretical concentrations.

**F.6.3.2** At least two-thirds of all assay QCs (low, mid and high) must meet the acceptance criteria.

**F.6.3.3** At least 50% of the QCs at each level must meet the acceptance criteria.

**F.6.3.4** For dilution QCs, which are generally assayed using multiples of n=3 replicates, at least 67% (rounded) of the QCs must be within 15% of their nominal theoretical concentrations. Failure of a dilution QC does not mean that the batch itself has failed if the low, mid and high QCs meet acceptance criteria as defined above. However, any samples diluted in a batch with a failed dilution QC should be repeated and the value from this batch discarded. If more than one dilution scheme was followed in a batch of samples, with corresponding dilution QCs prepared using different dilution factors, only the dilution QC which failed acceptance criteria will be rejected and the associated samples repeated.


**F.6.4** Blank Acceptance Criteria

At least 50% of matrix blanks (including carryover blanks, BI/BI) and 50% of control blanks (BI/IS) must have a response (peak area) less than or equal to 20% of the mean accepted LLOQ calibration standards. Carryover blanks should be positioned in the run in a manner capable of determining assay carryover, for example, after each ULOQ calibration standard injection.

**F.7** **Data Reporting**

Concentrations found below the lowest calibration standard concentration, will be reported as below the quantitation limit (<LLOQ). Where no peak is detected (ND), the result will be flagged as (<LLOQ). Over-diluted samples falling below

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the calibration range (assuming insufficient to reassay) will be reported as <LLOQ (LLOQ value x dilution factor).

## G. STABILITY/METHOD PARAMETERS

### G.1 Solutions

<u>Parameter Evaluated</u>	<u>Validated Result</u>	<u>Study Reference</u>
Analyte Stability in Stock Solutions at 5±3°C:	40 days	B185-18

### G.2 Matrix, K<sub>2</sub> EDTA Dog Plasma

<u>Parameter Evaluated</u>	<u>Validated Result</u>	<u>Study Reference</u>
Room Temperature Stability in Matrix:	26 hours	B185-18
Freeze/Thaw Stability in Matrix:	5 cycles	B185-18
Re-injection Stability:	188 hours, refrigerated	B185-18
Post Preparative Extract Stability:	85 hours, refrigerated	B185-18
Validated Dilution Factor:	10-fold, 50-fold	B185-18
Long-Term Stability in Matrix at ≤-60°C:	21 days	B185-18
Effect of 2% Hemolysis:	No impact	B185-18
Whole Blood Stability:	4 hours, refrigerated	B185-18
Maximum Batch Size:	93 samples	B185-18
Incurred Sample Reanalysis:	Successful	M397-18

## H. REFERENCES

- H.1** B185-18: "Method Validation Report for the Quantitative Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma"
- H.2** M397-18: GLP-Multiple (5 weekly) Repeat Subcutaneous Toxicity and Toxicokinetics Study with [Proprietary] [Pro] in Male and Female Beagle Dogs
- H.3** SRI SOP 006.061, *Bioanalytical Sample Analysis*
- H.4** SRI SOP 006.062, *Bioanalytical Sample Reanalysis*
- H.5** SRI SOP 006.063, *Reference Material Receipt and Stock, Spiking Solution and Calibration and Quality Control Sample Preparation / Expiration*

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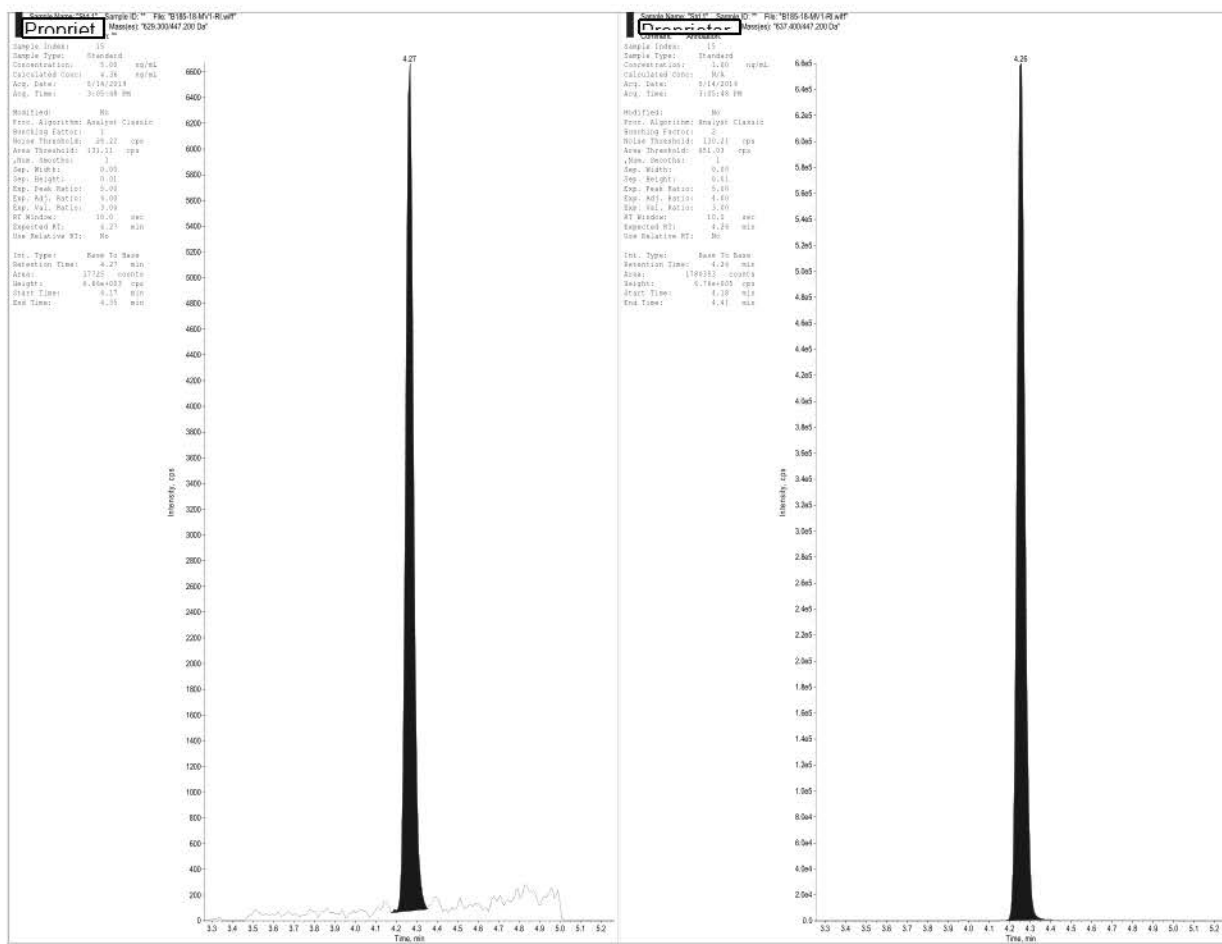
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## I. FIGURES

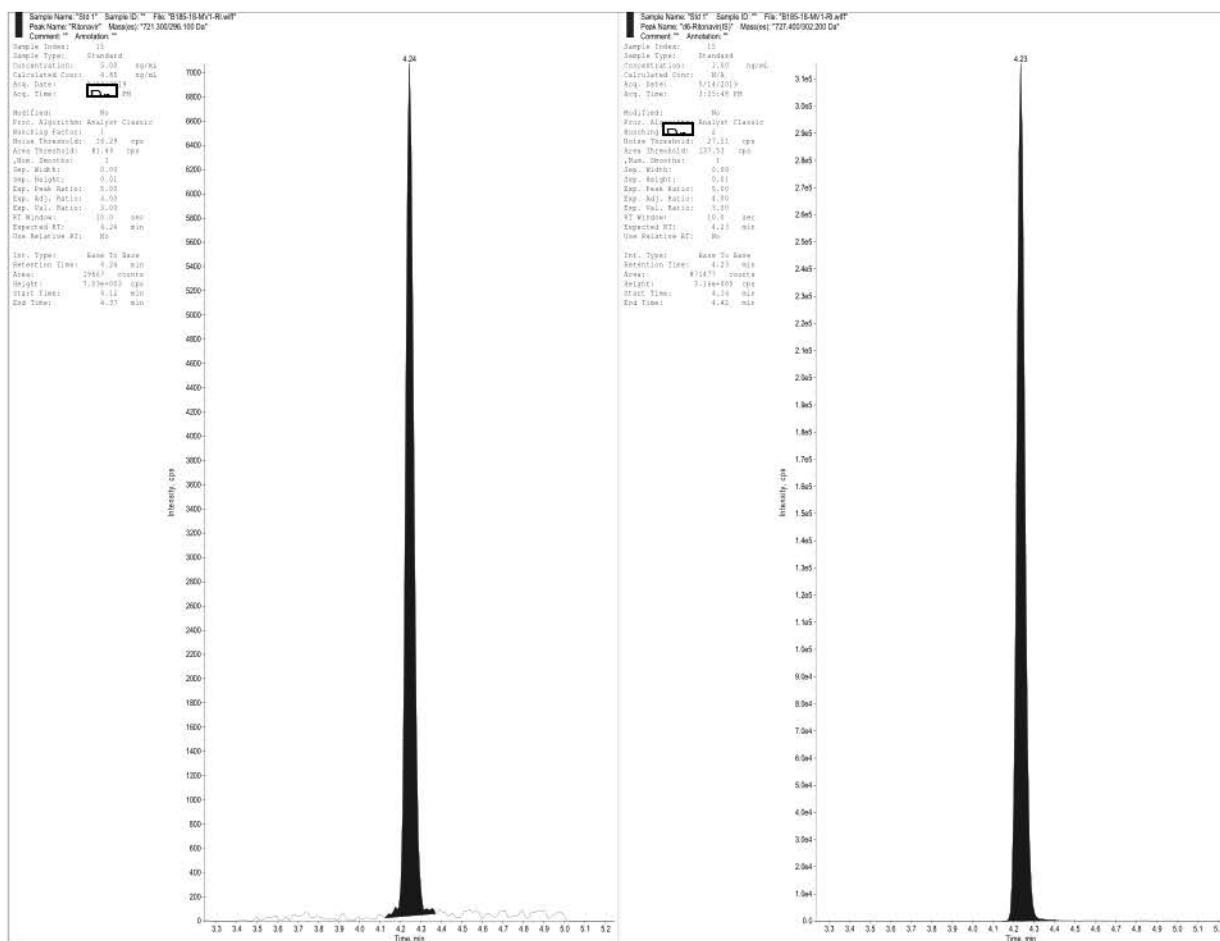
**I.1** Figure 1. Representative [Proprietary] Chromatogram of a K<sub>2</sub> EDTA Dog Plasma Sample Spiked at the Lower Limit of Quantitation (LLOQ)



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**I.2** Figure 2. Representative Proprietary Chromatogram of a K<sub>2</sub> EDTA Dog Plasma Sample Spiked at the Lower Limit of Quantitation (LLOQ)







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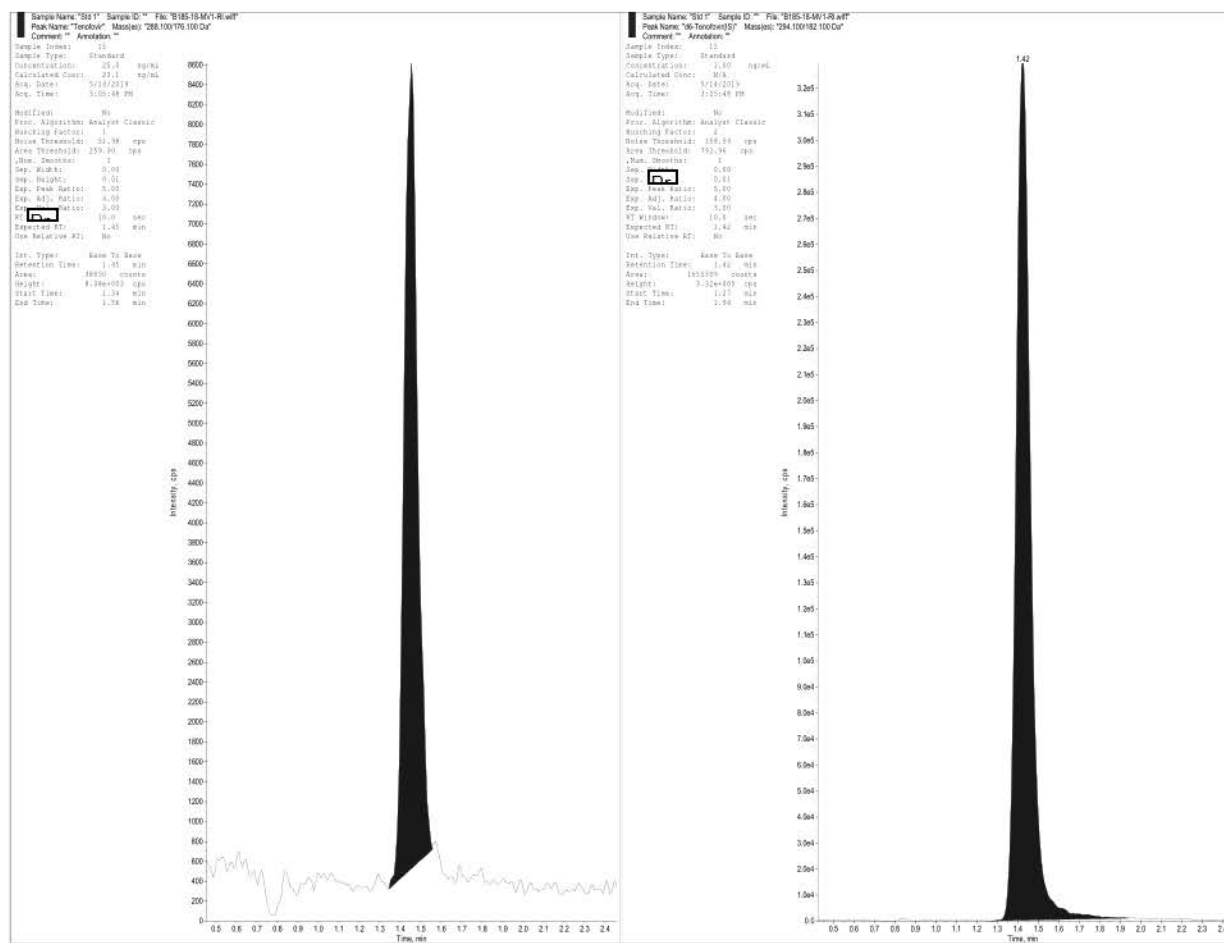
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**I.3** Figure 3. Representative [Proprietary] Chromatogram of a K<sub>2</sub> EDTA Dog Plasma Sample Spiked at the Lower Limit of Quantitation (LLOQ)



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## J. ATTACHMENTS

- J.1** Extraction Form (SRI Form 106.201A)
- J.2** Methodology and Reagent List (SRI Form 106.201B)
- J.3** Instrument Analytical Conditions and Reagents (SRI Form 106.201C)
- J.4** Instrument Reagent Preparation (SRI Form 106.201D)
- J.5** Extraction Reagent Preparation (SRI Form 106.201E)

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Study ID:

Batch ID:

**EXTRACTION FORM**

Preparation of Calibration Standards in Matrix						
Calibration Standard ID	Spiking Solution ID	Spiking Solution Concentration (µg/ml)	Spiking Volume (ml)	Matrix Volume (ml)	Final Volume (ml)	Nominal Matrix Concentration (ng/ml)
Std-1	Std-5	0.100 / 0.100 / 0.500	0.0250	0.475	0.500	5.00 / 5.00 / 25.0
Std-2	Std-5	0.100 / 0.100 / 0.500	0.0500	0.450	0.500	10.0 / 10.0 / 50.0
Std-3	Std-5	0.100 / 0.100 / 0.500	0.100	0.400	0.500	20.0 / 20.0 / 100
Std-4	Std-8	1.00 / 1.00 / 5.00	0.0250	0.475	0.500	50.0 / 50.0 / 250
Std-5	Std-8	1.00 / 1.00 / 5.00	0.0500	0.450	0.500	100 / 100 / 500
Std-6	Std-8	1.00 / 1.00 / 5.00	0.100	0.400	0.500	200 / 200 / 1000
Std-7	Std-9	10.0 / 10.0 / 50.0	0.0250	0.475	0.500	500 / 500 / 2500
Std-8	Std-9	10.0 / 10.0 / 50.0	0.0500	0.450	0.500	1000 / 1000 / 5000
Std-9	Proprietary Stock A/B;	1000	0.0100	0.930	1.00	10000
	Proprietary Stock A/B;	1000	0.0100			10000
	Proprietary Stock A/B	1000	0.0500			50000

Preparation of Quality Control Samples in Matrix						
Quality Control Sample ID	Spiking Solution ID	Spiking Solution Concentration (µg/ml)	Spiking Volume (ml)	Matrix Volume (ml)	Final Volume (ml)	Nominal Matrix Concentration (ng/ml)
QC- Low	QC-Mid	0.400 / 0.400 / 2.00	0.0375	0.9625	1.00	15.0 / 15.0 / 75.0
QC- Mid	QC-Dil	5.00 / 5.00 / 25.0	0.0800	0.920	1.00	400 / 400 / 2000
QC- High	QC-Dil	5.00 / 5.00 / 25.0	0.160	0.840	1.00	800 / 800 / 4000
QC- Dil	Proprietary Stock A/B;	1000	0.0100	1.930	2.00	5000
	Proprietary Stock A/B;	1000	0.0100			5000
	Proprietary Stock A/B	1000	0.0500			25000

Species \_\_\_\_\_ Anticoagulant/Matrix: \_\_\_\_\_ Matrix Supplier: \_\_\_\_\_

Lot: \_\_\_\_\_ Matrix Expiration Date \_\_\_\_\_

Pipette IDs: \_\_\_\_\_

Calibration Spiking Solution: \_\_\_\_\_ Expiration Date: \_\_\_\_\_

QC Sample Spiking Solution: \_\_\_\_\_ Expiration Date: \_\_\_\_\_

Prepared by: \_\_\_\_\_ Date: \_\_\_\_\_

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Study ID:	Batch ID:
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### METHODOLOGY AND REAGENT LIST

Step	Description	Equipment or Pipettes used	Step completed (check)
1	Transfer 0.0200 ml of each calibration standard, QC sample, study sample and blank into separate 1.50 ml microcentrifuge tubes		
2	Add 0.100 ml of 0.2% acetic acid in methanol to the matrix blanks. Cap and vortex for approximately 5 seconds.		
3	Add 0.100 ml of the Internal Standard Spiking Solution to each calibration standard, QC sample, study sample and control blank. Cap and vortex for approximately 5 seconds.		
4	Centrifuge tubes at approximately 18000 g for approximately 10 minutes.		
5	Transfer 0.0250 ml of the supernatant into a 2.00 ml HPLC vial containing 1.00 ml Reconstitution Solution. Cap and vortex briefly to mix.		
6	Store on the autosampler (set point 5°C ± 3°C) or refrigerated (5°C ± 3°C).	End of extraction (time):	

Ⓐ Eppendorf Repeater Plus / M4 (circle one) Equipment ID: \_\_\_\_\_ Exp: \_\_\_\_\_

Dilution Scheme (1: _____)	Add _____ ul sample to _____ ul control matrix and vortex.	Pipettes:
Dilution Scheme (1: _____)	Add _____ ul sample to _____ ul control matrix and vortex.	
Dilution Scheme (1: _____)	Add _____ ul sample to _____ ul control matrix and vortex.	

Procedure Performed by: \_\_\_\_\_ Date: \_\_\_\_\_

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Study ID:	Batch ID:
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### METHODOLOGY AND REAGENT LIST (cont.)

Additional information, if required:

--

Test Mix Dilution: Remove the Test Mix in 2% acetic acid in methanol from storage (ID: \_\_\_\_\_ Exp: \_\_\_\_\_) and remove 0.0203 ml of this solution and place into a separate HPLC vial containing 0.0203 ml of internal standard spiking solution and 0.959 ml of Reconstitution Solution. Store with batch.  
Pipettes: \_\_\_\_\_

### REAGENT LIST

Reagent Description	Assigned ID	Supplier	Lot #	Grade	Exp.
K <sub>2</sub> EDTA Dog Plasma	NA			NA	
Internal Standard		NA	NA	NA	
0.2% Acetic Acid in Methanol (Diluent)		NA	NA	NA	
Water:Methanol (90:10 v:v) with 0.1% Acetic Acid (Reconstitution Solution)		NA	NA	NA	
Initial/Date:					

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Study ID:	Batch ID:
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### INSTRUMENT ANALYTICAL CONDITIONS AND REAGENTS

<b>HPLC Column ID.</b>	<b>Vendor:</b>	<b>Calibration due date</b>
Phenomenex Synergi Polar RP 100 x 2 mm, 4µm	<b>Description:</b>	NA
	<b>Dimension:</b>	
	<b>S/N:</b>	
<b>Column Heater</b>	Equipment Tracking #-	
<b>Column Temp (Set Point 25°C)</b>	Set Point _____ °C	NA
<b>Pump ID</b>	Equipment Tracking #-	
<b>Pump Pressures at Start</b>	_____ psi	NA
<b>Autosampler ID</b>	Equipment Tracking #-	
<b>Autosampler Temp (Set Point 5°C ± 3°C)</b>	Set Point _____ °C	NA
<b>Mass Spectrometer</b>	Equipment Tracking #-	
		<b>Exp. Date</b>
<b>Mobile Phase A</b>		
<b>Mobile Phase B</b>		
<b>Needle Rinse 1</b>		
<b>Needle Rinse 2</b>		
<b>Number of Conditioning Injections</b>		NA

NA: Not Applicable

Initial: \_\_\_\_\_ Date: \_\_\_\_\_

Additional Comments / Incidents during analysis:

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## INSTRUMENT REAGENT PREPARATION

**Study Number:** \_\_\_\_\_

### Mobile Phase A:

Assigned ID: \_\_\_\_\_

2% Acetic Acid in Water

Add \_\_\_\_\_ ml (nominal 20.0 ml) of acetic acid to \_\_\_\_\_ ml (nominal 1000 ml) of Milli-Q Water. This solution should be stored at room temperature for up to one month after preparation.

Expiration Date: \_\_\_\_\_

### Mobile Phase B:

Assigned ID: \_\_\_\_\_

0.1% Acetic Acid in Acetonitrile

Add \_\_\_\_\_ ml (nominal 1.00 ml) of acetic acid to \_\_\_\_\_ ml (nominal 1000 ml) of acetonitrile. This solution should be stored at room temperature for up to one month after preparation.

Expiration Date: \_\_\_\_\_

### Needle Rinse 1:

Assigned ID: \_\_\_\_\_

Acetonitrile:Isopropanol (80:20, v:v) with 1% Ammonium Hydroxide

Add \_\_\_\_\_ ml (nominal 400 ml) of acetonitrile to \_\_\_\_\_ ml (nominal 100 ml) isopropanol and add \_\_\_\_\_ ml (nominal 5.00 ml) ammonium hydroxide in a glass bottle. This solution should be stored at room temperature for up to one month after preparation.

Expiration Date: \_\_\_\_\_

### Needle Rinse 2:

Assigned ID: \_\_\_\_\_

Water:Methanol (90:10, v:v) with 1% Formic Acid

Add \_\_\_\_\_ ml (nominal 450 ml) of Milli-Q water to \_\_\_\_\_ ml (nominal 50.0 ml) methanol and add \_\_\_\_\_ ml (nominal 5.00 ml) formic acid in a glass bottle. This solution should be stored at room temperature for up to one month after preparation.

Expiration Date: \_\_\_\_\_

**Milli-Q water:** Decanted from Milli-Q unit on the day of use. Unit ID: \_\_\_\_\_ Exp: \_\_\_\_\_  
Is resistivity  $\geq 18.0 \text{ M}\Omega\text{-cm}$ ? Y / N (circle) Is TOC  $< 50.0 \text{ ppb}$ ? Y / N (circle)

### Ammonium Hydroxide:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

### Acetic Acid:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

### Formic Acid:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

### Methanol:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

### Acetonitrile:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

### Isopropanol:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

Prepared by: \_\_\_\_\_ Date: \_\_\_\_\_

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## EXTRACTION / STOCK REAGENT PREPARATION

Study Number: \_\_\_\_\_

### 0.2% Acetic Acid in Methanol (Diluent):

Assigned ID: \_\_\_\_\_

Add \_\_\_\_\_ ml (nominal 0.400 ml) of acetic acid to \_\_\_\_\_ ml (nominal 200 ml) of methanol. This solution should be stored at room temperature for up to one month after preparation.

Expiration Date: \_\_\_\_\_

### Water:Methanol (90:10 v:v) with 0.1% acetic acid (Reconstitution Solution):

Assigned ID: \_\_\_\_\_

Add \_\_\_\_\_ ml (nominal 0.200 ml) of acetic acid to \_\_\_\_\_ ml (nominal 180 ml) Milli-Q water and \_\_\_\_\_ ml (nominal 20.0 ml) of methanol. This solution should be stored at room temperature for up to one month after preparation.

Expiration Date: \_\_\_\_\_

### Test Mix (System Suitability):

Assigned ID: \_\_\_\_\_

To prepare at the LLOQ level, spike 0.0100 ml of the 1.00 mg/ml **Proprietary** and **Proprietary** stock solutions and 0.0500 ml of the 1.00 mg/ml **Proprietary** stock solution into 9.930 ml of Diluent Solution. Vortex, remove 0.100 ml, and add to a vial containing 0.900 ml of Diluent Solution. Vortex, remove 0.0100 ml, and add to a vial containing 0.990 ml of Diluent Solution. This solution may be stored refrigerated for up to 3 months from the date of preparation. On the day of use, remove 0.0203 ml of this solution and place into a separate vial containing 0.0203 ml of internal standard spiking solution and 0.959 ml of Reconstitution Solution. Expiration Date: \_\_\_\_\_

Storage Unit / Temperature: \_\_\_\_\_

**Milli-Q water:** Decanted from Milli-Q unit on the day of use. Unit ID: \_\_\_\_\_ Exp: \_\_\_\_\_  
Is resistivity  $\geq 18.0 \text{ M}\Omega\text{-cm}$ ? Y / N (circle) Is TOC  $< 50.0 \text{ ppb}$ ? Y / N (circle)

### Acetic Acid:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

### Methanol:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

**Proprietary** Stock Solution (1.00 mg/ml) ID: \_\_\_\_\_ Expire: \_\_\_\_\_

**Proprietary** Stock Solution (1.00 mg/ml) ID: \_\_\_\_\_ Expire: \_\_\_\_\_

**Proprietary** Stock Solution (1.00 mg/ml) ID: \_\_\_\_\_ Expire: \_\_\_\_\_

**Internal Standard Spiking Solution** ID: \_\_\_\_\_ Expire: \_\_\_\_\_

**Diluent** ID: \_\_\_\_\_ Expire: \_\_\_\_\_

**Reconstitution Solution** ID: \_\_\_\_\_ Expire: \_\_\_\_\_

Pipettes: \_\_\_\_\_

Prepared by: \_\_\_\_\_ Date: \_\_\_\_\_

SRI Form 106.201E

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**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary] and  
[Proprietary] in K<sub>2</sub> EDTA Dog Plasma  
SRI Study No. B185-18**

**Appendix B**

**VALIDATION PLAN OF THE BIOANALYTICAL METHOD FOR ANALYSIS OF  
[Proprietary Info] [Proprietary Info] AND [Proprietary Info] IN K<sub>2</sub> EDTA DOG PLASMA**

**Amended Validation Plan for the Bioanalytical Method for  
Analysis of [Proprietary Info] [Proprietary Info] and [Proprietary Info] in K<sub>2</sub>  
EDTA Dog Plasma**

**SRI International Study Number: B185-18**

Redacted by agreement

## SCOPE

This validation will be limited to the analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA dog plasma using 0.0200 ml sample volumes, using a protein precipitation extraction procedure followed by LC-MS/MS detection. The internal standards used in this assay are [Proprietary Info] [Proprietary Info] and d<sub>6</sub>-[Proprietary]

This plan is based on SRI SOP 006.060, *Bioanalytical Method Validation*. Further details on the conduct of a typical validation study are described in this SOP.

This validation plan was amended to correct an error in the Stock Solution Stability section. The original plan referred to a 5.00 mg/ml [Proprietary] stock solution. However, a 1.00 mg/ml solution was prepared, as per the Stock Solution Preparation section of the original plan.

## METHOD DEVELOPMENT

Based on the outcome of method development, it is determined that the validation of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA dog plasma may proceed. Method development experiments consisted of three days of accuracy and precision, selectivity, recovery, matrix effects on ionization, matrix effects using 6 unique lots of matrix, freeze thaw stability, room temperature matrix stability, re-injection stability, and post-preparative extract stability. Full details of the method development experiments performed, and the corresponding results, can be found in the study method development raw data.

## OBJECTIVE

The objective of this validation plan is to describe the experimental procedures, the validation parameters, the statistical analyses to be performed, and the corresponding acceptance criteria, including the basis for acceptance or rejection of the validity of the method for its intended use. The validation investigates the following parameters: method linearity and range, maximum batch size, selectivity, carryover, accuracy (intra- and inter-batch), precision (intra- and inter-batch), recovery, matrix effects on ionization, effect of dilution, matrix effects using 6 unique lots of matrix, freeze thaw stability, room temperature matrix stability, re-injection stability, post-preparative extract stability, long term matrix storage stability, whole blood processing stability, effect of hemolysis, effect of concomitant medications, and analyte stock solution stability. Following completion of the validation, a final validation report will be written that will include a brief description of the method development outcome, a summary of the method used to validate this assay, any changes made over the course of the validation, including revalidation if applicable, the results obtained, and the validation conclusions.

## SUMMARY OF METHOD

Full details of the methodology used during the conduct of this validation study will be detailed in SRI Test Method *Analysis of* [Proprietary] [Proprietary] and [Proprietary] *in K<sub>2</sub> EDTA Dog Plasma*. This Test Method will be issued as a draft Test Method during the conduct of the validation, and will be formally issued and approved by department management and Quality Assurance (QA) prior to the start of sample analysis. Until an SRI Test Method number can be formally assigned, following completion of the validation, the initial draft Test Method number used during validation will be B185-18-TM-Draft 1, with subsequent revisions numbered -Draft 2, -Draft 3, etc.

During validation, this draft Test Method may be modified, if necessary, by the validation scientist providing that the integrity of the validation is not compromised, and the modifications are fully documented. Any modifications to the method occurring after the first accepted validation run may require revalidation of the method.

All details pertaining to equipment used, materials, reagents etc will be included in the raw data and detailed in the resulting final bioanalytical Test Method.

All temperatures quoted in this validation outline are nominal.

## COMPOUND INFORMATION

The actual details of the [Proprietary] [Proprietary] and [Proprietary] and the stable label internal standards used will be noted in the raw data. The final report will include the supplier of the test articles and internal standards, the purities, and the expiration dates.

## Stock Solution Preparation

Duplicate weighings of [Proprietary] [Proprietary] and [Proprietary] will be performed and 1.00 mg/ml stock solutions of each analyte (adjusting for water or solvent present and purity, as appropriate) will be prepared. Dimethyl sulfoxide (DMSO) will be used as the diluent for the [Proprietary] and [Proprietary] stocks, while Milli-Q water will be the diluent for the [Proprietary] stock solutions. These stock solutions will be stored in a refrigerator (5°C ± 3°C) until use. Per SRI SOP 006.063 *Reference Material Receipt and Stock, Spiking Solution and Calibration and Quality Control Sample Preparation / Expiration*, a comparison of these solutions will be performed, prior to use in the validation, to ensure equivalency and accuracy of preparation.

The internal standards [Proprietary Info] [Proprietary Info] and [Proprietary Info] will be weighed and a 1.00 mg/ml stock solution of each prepared. The diluent for the [Proprietary Info] and [Proprietary Info] stocks will be DMSO and the diluent for the [Proprietary Info] will be



Milli-Q water. It is not necessary to apply a correction factor to the internal standards following weighing.

The preparation of working solutions from the analyte and internal standard stocks, if applicable, will be detailed in the raw data.

## METHOD VALIDATION PARAMETERS

### Method Linearity and Range

On each day of method validation, a freshly prepared calibration standard curve (5.00 to 1000 ng/ml for [Proprietary] and [Proprietary] and 25.0 to 5000 ng/ml for [Proprietary] in K<sub>2</sub> EDTA dog plasma will be extracted and the peak area ratios (PAR) of analyte to internal standard in the calibration standards will be fitted to a weighted regression analysis. The eventual weighting used will be documented in the raw data and will be consistently applied across all batches.

The calibration standards (n=2 per analytical batch) will contain the following concentrations of [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA dog plasma: 5.00, 10.0, 20.0, 50.0, 100, 200, 500 and 1000 ng/ml. These standards will also contain [Proprietary] at 25.0, 50.0, 100, 250, 500, 1000, 2500, and 5000 ng/ml. Also included with each batch will be at least n=2 blank samples containing no analyte or internal standard (BI/BI) and at least n=2 blank samples containing no analyte but containing internal standard (BI/IS).

The linearity of the assay will be assessed by the correlation coefficient (r) obtained from the regression analysis of the peak area ratios, with perfect fit of the data yielding an r value of 1.000. The minimum value for r for an assay to be acceptable is 0.990.

On each day of accuracy and precision assessment, the peak response vs. standard concentration in the calibration standards will be fitted to a regression analysis. The simplest weighting should be used wherever possible, however, other weightings may be used if the fit is more appropriate. The best fit will be determined using the sum of residual squares, where the lowest value will be determined to be the most suitable. This will be performed using linear 1/x or 1/x<sup>2</sup> at a minimum, with the inclusion of quadratic regression if appropriate. Quality Control (QC) sample results from all accuracy and precision batches, across all concentrations, will be used in the calculations to determine the sum of residual squares. The specific standard curve model fit used, including weighting, will be documented in the raw data and will be consistently applied across all batches.

In order for the calibration curve to be considered acceptable there will not be more than a 15% difference between the nominal and observed concentrations, except at the lower limit of quantitation (LLOQ) where a 20% deviation is permitted. At least 75% of the calibration standards, including at least one

replicate at the lowest and highest concentrations, will fulfill this criterion. Individual calibration standards which do not fulfill the criterion will be excluded from the regression.

As this validation has not yet initiated, the analytical range stated above, and the resulting QC concentrations selected, may change from stated here depending on experimental findings. This will be recorded in the raw data.

### **Maximum Batch Size**

One accuracy and precision batch of sufficient size should be analyzed to represent a typical batch when running study samples. In order to get the desired number of samples, additional blanks (BI/BI or BI/IS) may be analyzed with this batch, and no acceptance criteria will be attached to these additional samples. The maximum batch size will be counted from the first calibration standard to the last calibration standard.

### **Selectivity**

To confirm the selectivity of the assay for the analyte and the internal standard, plasma samples containing no analyte or internal standard (BI/BI) from at least 6 unique lots of K<sub>2</sub> EDTA dog plasma will be extracted in at least one analytical batch in replicates of n=1. Additionally, a BI/BI sample at 0.5% and 2% hemolysis will be extracted and analyzed in replicates of n=1 to determine if there is a significant impact on either the analyte or internal standard responses due to the presence of whole blood. Selectivity for the analyte will be indicated by the absence of an apparent chromatographic peak at the retention time for the analyte that shows a peak area greater than 20% of the mean peak area observed for the extracted calibration standards at the LLOQ. Selectivity for the internal standard in these samples will be indicated by the absence of an apparent chromatographic peak at the retention time for the internal standard that shows a peak area greater than 5% of the mean peak area observed for the extracted samples containing the internal standard.

In all analytical batches, at least n=2 samples containing no analytes or internal standards (BI/BI) will be extracted. Any peak detected at the retention time of the analytes should be less than 20% of the analyte mean peak area observed for the extracted calibration standards at the LLOQ. Any peak detected at the retention time of the internal standards should be less than 5% of the mean peak area observed for the extracted samples containing the internal standard. The same plasma lot used to prepare the fresh calibration curve should be used for this evaluation.

In all analytical batches, at least n=2 samples containing no analytes but with internal standards included (BI/IS) will be extracted to confirm the suitability of the internal standard for use in the assay at that concentration. Any peak detected



at the retention time of the analytes as a result of the internal standard addition should be less than 20% of the analyte mean peak area observed for the extracted calibration standards at the LLOQ. The same plasma lot used to prepare the fresh calibration curve should be used for this evaluation.

In at least one analytical batch, at least n=1 sample containing only [Proprietary] at the upper limit of quantitation but containing no internal standard (ULOQ/BI), will be extracted. Any peak detected at the retention time of the internal standard as a result of the analyte addition at the ULOQ should be less than 5% of the internal standard mean peak area observed for the extracted samples containing internal standard. This will also be performed with at least n=1 sample containing only [Proprietary] and at least n=1 sample containing only [Proprietary] at the ULOQ concentration.

### Carryover

Carryover of the analytes will be determined during all validation batches by injecting at least one BI/BI sample immediately following injection of each highest calibration standard. Any peak detected at the retention time of the analyte in the first injected BI/BI sample should be less than 20% of the analyte mean peak area observed for the extracted samples at the LLOQ. Any peak detected at the retention time of the internal standards should be less than 5% of the internal standard mean peak area observed for the extracted samples containing internal standard.

### Method Accuracy and Precision

Quality Control (QC) samples at four concentrations will be freshly prepared in K<sub>2</sub> EDTA dog plasma on each day of analysis for use in accuracy and precision batches. These samples will contain both [Proprietary] and [Proprietary] at 5.00 ng/ml (LLOQ), 15.0 ng/ml (low), 400 ng/ml (mid), and 800 ng/ml (high) concentrations, and also [Proprietary] at 25.0 ng/ml, 75.0 ng/ml, 2000 ng/ml, and 4000 ng/ml respectively.

To assess the accuracy and precision of the assay for these analytes, these QCs will be processed in n=6 replicates on at least three separate days of analysis. For intra-batch (single run) and inter-batch (minimum of n=3 runs) accuracy (% accuracy), the results will be acceptable if the mean of the replicates at each concentration is within  $\pm 15\%$  of the nominal concentration ( $\pm 20\%$  at the LLOQ), and precision (as determined by %CV) at each concentration is  $\pm 15\%$  ( $\pm 20\%$  at the LLOQ). Additionally, for a single batch to be considered acceptable, at least 50% of the individual replicates at each concentration must be within  $\pm 15\%$  of the nominal concentration ( $\pm 20\%$  at the LLOQ), and at least 67% of the QCs in a batch must meet this acceptance criterion. A QC sample may be excluded from the overall statistics only for analytical reasons (poor chromatography, etc), or if determined that it is a statistical outlier by means of a Grubbs test. Results will be

shown both with and without the inclusion of a statistical outlier. The LLOQ QC samples will be independently prepared from the LLOQ calibration standards used to construct the calibration curve on the day of analysis.

The validation is considered to start on acceptance of the first intra-batch accuracy and precision run. If the calibration curve for a batch satisfied acceptance criteria, the accuracy and precision run will be reported and included in the inter-batch statistics, including those where the QC samples failed to meet acceptance criteria for which an assignable cause for failure (eg. documented preparation error, instrument breakdown, poor chromatography, etc) could not be determined.

### **Recovery**

The recovery of the method for the analytes and internal standards will be assessed by comparing the mean peak areas of the analytes and the internal standards in the QC samples (n=6) at low, mid and high concentrations after extraction to the mean peak areas obtained from extracted BI/BI (n=6) samples which were spiked post extraction with solutions containing all analytes and internal standards, to give final concentrations equivalent to the expected concentrations in the final extracts, assuming the sample extraction efficiency is 100%. Determination of recovery is to characterize the assay, and there is no acceptance criterion for recovery.

Recovery will be determined by the following:

$$\frac{\text{Mean peak area of extracted samples}}{\text{Mean peak area of post-extracted spiked standards}} \times 100$$

### **Matrix Effects on Ionization**

The extent of the matrix effects on ionization of the analytes and internal standards will be assessed. A comparison of the mean peak areas of analytes and internal standards in extracted BI/BI samples spiked post-extraction with solutions containing all analytes and internal standards (at concentrations equivalent to low, mid and high QCs in the final extracts, assuming 100% recovery) to injections of these neat solutions (n=6) will be performed. Determination of matrix effects is to characterize the assay, and there is no acceptance criterion for this.

Matrix effects on ionization will be determined by the following:

$$\frac{\text{Mean peak area of post extracted spiked samples}}{\text{Mean peak area of solution standards}} \times 100$$

Values over 100% indicate signal enhancement caused by the matrix while values less than 100% indicate matrix induced signal suppression.



### Effect of Dilution

The ability to effectively dilute a high concentration study sample will be assessed by preparing a dilution QC at a concentration of 5000 ng/ml [Proprietary] and [Proprietary] and 25000 ng/ml [Proprietary Info]. This QC will be diluted 1:10 and 1:50 on the day of extraction using blank K<sub>2</sub> EDTA dog plasma, and will be extracted using n= 6 replicates. Effect of dilution will be considered successful if at least 50% of the individual replicates are within  $\pm 15\%$  of the nominal concentration and that overall accuracy and precision is  $\pm 15\%$ .

### Matrix Effects Using 6 Unique Lots of Matrix

Matrix effects on ionization will be assessed at the low QC concentrations by spiking 6 unique lots of K<sub>2</sub> EDTA dog plasma (minimum of n=4 replicates per lot) with a solution of analytes to give a final plasma concentration of 15.0 ng/ml [Proprietary] and [Proprietary] and 75.0 ng/ml [Proprietary Info]. These samples will then be extracted as per the Test Method. Matrix effects on ionization are not considered significant if at least 50% of the individual replicates are within  $\pm 15\%$  of the nominal concentration and that overall accuracy and precision of each lot is  $\pm 15\%$ . In the event that two or more lots fail acceptance criteria, another (different) 6 lots should be evaluated in a similar manner, and at least 9/12 lots must meet acceptance criteria.

### Matrix Stability

Plasma QC samples will be prepared at low and high concentrations, subdivided into suitable volume aliquots, and stored in polypropylene vials in an ultra-low temperature freezer ( $\leq -60^{\circ}\text{C}$ ) for at least 24 hours prior to analysis. Matrix stability assessments will be conducted against a fresh calibration curve and fresh QC samples. Stability (freeze thaw stability, room temperature matrix stability, post-preparative extract stability, and long term storage stability in matrix) will be considered successful if at least 50% of the individual replicates at each concentration are within  $\pm 15\%$  of the nominal concentration and that overall accuracy and precision at each concentration is  $\pm 15\%$ . The acceptance criteria for re-injection stability and whole blood processing stability are detailed below.

#### Freeze Thaw Stability

Plasma QC samples at low and high QC concentrations in at least n=4 replicates will be removed from the  $\leq -60^{\circ}\text{C}$  freezer after storage for a minimum of 24 hours after initial preparation and allowed to thaw unassisted at room temperature for a minimum period of 1 hour. The samples will then be placed back in the  $\leq -60^{\circ}\text{C}$  freezer for a minimum of 12 hours. This process will then be repeated at least twice more prior to analysis. In the event that stability cannot be established or is unlikely to be successful, a smaller number of freeze thaw cycles may be examined,

or the samples may be thawed on ice instead of at room temperature. This will be noted in the raw data.

### **Room Temperature Matrix Stability**

Plasma QC samples at low and high QC concentrations in at least  $n=4$  replicates will be removed from the  $\leq -60^{\circ}\text{C}$  freezer after storage and allowed to thaw unassisted at room temperature for a defined period. In the event that stability cannot be established over the original room temperature storage period, room temperature stability may be conducted over a shorter time period, or the samples may be stored on ice or in a refrigerator for a defined period of time instead of at room temperature. The actual storage times will be noted in the raw data.

### **Re-injection Stability**

To determine if it is appropriate to re-inject an entire batch, all calibration standards, QCs ( $n=6$  at LLOQ, low, mid and high concentrations) and all assay BI/BI and BI/IS samples (excluding individual specificity blanks) will be re-injected on the instrument following a defined period of storage. It is not necessary to re-inject other samples from additional experiments (matrix effects, effect of dilution, etc) which may have been originally extracted and analyzed with the original batch. The sample extracts after initial injection (prior to re-injection) will be stored in the autosampler (temperature set point  $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ ) or in a refrigerator, assuming equivalency between these two locations. Re-injection stability will be considered successful if the mean (intra-batch) accuracy and precision of the QCs is  $\pm 15\%$  ( $\pm 20\%$  at the LLOQ). At least 50% of the individual replicates at each concentration must be within  $\pm 15\%$  of the nominal concentration ( $\pm 20\%$  at the LLOQ), and at least 67% of the QCs in a batch must meet this acceptance criterion. The period of re-injection stability will be defined as the time from the end of extraction to the time of injection of the first calibration standard from the re-injected batch.

### **Post Preparative Extract Stability**

To determine the stability of the analyte after extraction from the plasma and while in the autosampler (temperature set point  $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ ) or refrigerator prior to injection, plasma QC samples at low and high concentrations in replicates of  $n=4$  at each concentration, will be extracted and stored in the autosampler for a defined period of time. These QCs will then be injected alongside a freshly extracted calibration curve and QCs. The period of stability will be determined by the time between placement in storage to the time of injection of the first stored QC.



### Long Term Matrix Storage Stability

Plasma QC samples at low and high QC concentrations in at least n=4 replicates will be removed from the  $\leq -60^{\circ}\text{C}$  freezer after storage for a defined period of time and extracted and analyzed against a fresh calibration curve alongside fresh batch acceptance QC samples. Stability evaluations will be conducted at a minimum of one time-point and will at least cover the expected duration of sample storage from future sample analysis studies.

### Whole Blood Processing Stability

To determine the stability of the analyte in whole blood, prior to processing the blood sample to plasma, QC samples at two concentrations (representing low and high analyte levels, assuming 100% of analyte partitions into plasma) will be prepared in a pool of whole blood, which will then be subdivided after mixing. Plasma will be collected immediately after spiking (time zero) and at defined intervals (eg. 1 hour and 4 hours, stored refrigerated) after spiking. The plasma samples will be extracted in replicates of n=4 at each concentration. The mean of the values from each stability timepoint will be compared to the mean of the value obtained from the time zero assessment. Stability will be achieved if the % difference is  $\pm 15\%$ . Full details on the conduct of this experiment are provided in SRI SOP 006.060 *Bioanalytical Method Validation*.

### Effect of Hemolysis

Plasma QC samples at low and high QC concentrations will be prepared in plasma matrix containing red blood cells to represent 0%, 0.5%, and 2% hemolysis, and extracted in at least n=4 replicates. Matrix effects of hemolysis will not be considered significant if at least 50% of the individual replicates at each concentration are within  $\pm 15\%$  of the nominal concentration and that overall accuracy and precision at each concentration is  $\pm 15\%$ .

### Concomitant Medications

A QC sample will be prepared containing 15.0 ng/ml [Proprietary] 2000 ng/ml [Proprietary] and 20000 ng/ml [Proprietary] and the impact of [Proprietary] and [Proprietary] on [Proprietary] quantitation will be assessed. Similarly, the effect of [Proprietary] and [Proprietary] on the quantitation of [Proprietary] will be assessed by preparing a QC sample at 2000 ng/ml [Proprietary] 15.0 ng/ml [Proprietary] and 20000 ng/ml [Proprietary]. To determine the effect of [Proprietary] and [Proprietary] on [Proprietary] quantitation, a QC sample containing 2000 ng/ml [Proprietary] and [Proprietary] and 75.0 ng/ml [Proprietary] will be prepared. The values selected for the concomitant medication concentrations were higher than the CMax values from SRI Study Number M355-17, where the animals were dosed at similar concentrations. These samples will

be extracted in at least n=4 replicates. The effect of concomitant medications will not be considered significant if at least 50% of the individual replicates at each concentration are within  $\pm 15\%$  of the nominal concentration and that overall accuracy and precision at each concentration is  $\pm 15\%$ .

### Stock Solution Stability

The stability of the analyte stock solution will be determined by placing a 1.00 mg/ml stock solution of the analyte in DMSO [Proprietary] and [Proprietary] into a refrigerator for a defined period of time. After storage, the solution will be removed, diluted appropriately for injection onto the LC-MS/MS system and compared against similarly diluted preparations derived from duplicate fresh (prepared from new weighings) stock solutions. Internal standard will be added to both the old and the new stock solution dilutions in order to allow comparison by peak area ratio. This will be conducted with a minimum of n=4 replicates. The mean of the duplicates will be used as the fresh comparator, assuming these fresh stocks agree within 5% of each other. This will also be performed using a 1.00 mg/ml stock solution of [Proprietary] in Milli-Q water.

The stability of the analyte stock solutions stored at room temperature will be determined by placing an aliquot of fresh stock solutions into a suitable location at room temperature for a defined period of time. After storage, the solutions will be removed, diluted appropriately for injection onto the LC-MS/MS system, and compared against similarly diluted preparations derived from the same fresh stock solutions which had been stored refrigerated. Internal standards will be added to both stock solution dilutions in order to allow comparison by peak area ratio. This will be conducted with a minimum of n=4 replicates.

Per SOP 006.063, stability of the internal standard stock and spiking solutions will be nominally assigned a 6 month expiration date from the date of preparation. During the validation, the internal standard will be monitored to determine if any chromatographic interferences occur, which may be an indicator of lack of stability. If this occurs, the stability of the internal standard will be evaluated at room temperature and under regular storage conditions.

In all cases, the stock solutions will be considered to be stable if the mean of the replicates of each stability sample is within  $\pm 10\%$  of the mean of the replicates of the freshly prepared solution.

Stock solution stability is calculated as follows (using mean peak area ratios):

$$\frac{(\text{stored stock} - \text{fresh stock})}{(\text{fresh stock})} \times 100$$



## DATA GENERATION AND RESULTS

The results from this study will be generated using AB Sciex Analyst software, version 1.6.2. This software will generate the peak area ratios for all samples, which will then be used to generate the calibration curve data and resulting QC concentrations, using unrounded values. The Analyst software will then use these values to calculate the overall precision and accuracy statistics. Microsoft Excel may be used in the calculation of some statistics.

## CHANGES TO THE VALIDATION PLAN

In the event that it becomes necessary to make significant changes to the procedures outlined in this validation plan, these changes will be fully documented in the study binder and communicated to the Sponsor, with a new validation plan generated if necessary.

Any deviations to any SRI SOP will be maintained with the raw data. If a deviation is judged to have an impact on the integrity of the study, a copy of the deviation will be sent to the Sponsor and will be discussed in the final report. Deviations with no impact on the integrity of the study will be included in the final report at the discretion of the bioanalytical scientist responsible for the conduct of the validation.

## REFERENCES

SRI- Test Method B185-18: *Analysis of* [Proprietary] [Proprietary] *and* [Proprietary] *in K<sub>2</sub> EDTA Dog Plasma – (DRAFT).*

SRI SOP 006.060: *Bioanalytical Method Validation*

SRI SOP 006.063: *Reference Material Receipt and Stock, Spiking Solution and Calibration and Quality Control Sample Preparation / Expiration.*

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary **and**  
Proprietary **in K<sub>2</sub> EDTA Dog Plasma**  
**SRI Study No. B185-18**

**Appendix C**

**CERTIFICATES OF ANALYSIS (REFERENCE STANDARDS AND INTERNAL  
STANDARDS)**

# Certificate

Proprietary Info

**LABEL TEXT**

For use with specified USP compendial tests.  
Not for use as a drug. See SDS prior to use  
at [www.usp.org/sds](http://www.usp.org/sds).

**USP REFERENCE STANDARD**

Proprietary Info

**350 mg**

For quantitative applications, determine the water content  
titrimetrically at the time of use. Use as is material and correct  
weight for water content. Use a value of 0.997 mg of Proprietary per  
mg of material on the anhydrous basis. Keep container tightly  
closed. Protect from light. Store in a refrigerator.

USP, 12601 Twinbrook Pkwy, Rockville, MD, +1-301-881-0666  
Cat. No. 1370101 Material mfd. in India

LOT: R077R0



Redacted by agreement

*Quality Assurance*

**Calculation Value**

If a value is not provided on the label or accompanying documentation and the Reference Standard has a quantitative USP compendial application, a value of 100.0% is used. The purity value is not applicable for qualitative uses. Please refer to the specific Reference Standard label for further information.

**Expiration**

Current lots are identified in the current USP Catalog. In some cases, the previous lot may still be considered valid for use. If so, it is identified in the column marked "Previous Lot/Valid Use Date."

It is the responsibility of each user to determine that this lot is current or valid when used. For the most up-to-date information, please refer to the USP Store at [www.usp.org](http://www.usp.org).

**Instructions for Use**

Follow the instructions on the label of the USP Reference Standard and in the appropriate USP documentary standard(s).

**Non-Monograph Use**

The suitability of this Reference Standard for use in non-compendial applications is solely the responsibility of the user.

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# Certificate

Proprietary Info



**LABEL TEXT**



**USP REFERENCE STANDARD**  
**Proprietary 200 mg**  
Warning! Harmful if swallowed. Causes skin irritation.  
Causes serious eye irritation.  
Do not dry. For quantitative applications, use a value of 0.993 mg of **Proprietary** per mg of material on the as is basis. Keep container tightly closed. Protect from light.  
USP, 12801 Twinbrook Pkwy, Rockville, MD, +1-301-881-0666  
CAT No. 1604803 Material mfd. in Italy

Wash thoroughly after handling. Wear protective gloves. Wear eye/face protection. If swallowed: Call a poison center/doctor if you feel unwell. Rinse mouth. If on skin: Wash with plenty of water. If skin irritation occurs: Get medical advice/attention. Take off contaminated clothing and wash before reuse. If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical advice/attention. Dispose of contents/container in accordance with local/regional/national/international regulations.

Redacted by agreement

*Quality Assurance*

**Calculation Value**

If a value is not provided on the label or accompanying documentation and the Reference Standard has a quantitative USP compendial application, a value of 100.0% is used. The purity value is not applicable for qualitative uses. Please refer to the specific Reference Standard label for further information.

**Expiration**

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# Certificate

Proprietary Info

**LABEL TEXT**

For use with specified USP compendial tests.  
Not for use as a drug. See SDS prior to use at  
[www.usp.org/sds](http://www.usp.org/sds).



**REFERENCE STANDARD**

Proprietary Info

**15 mg**

This is the monohydrate form of Proprietary Info Do not dry.  
Keep container tightly closed. Store in the refrigerator.

USP, 12601 Twinbrook Pkwy, Rockville, MD, +1-301-881-0666  
Cat. No. 1643601 Material mfd. in China

LOT: R044C0



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*Quality Assurance*

**Calculation Value**

If a value is not provided on the label or accompanying documentation and the Reference Standard has a quantitative USP compendial application, a value of 100.0% is used. The purity value is not applicable for qualitative uses. Please refer to the specific Reference Standard label for further information.

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**MEDICAL ISOTOPES, INC.**  
100 Bridge Street  
Pelham, NH 03076 USA  
Tel: 603 635-2255, Toll Free: 800 374-9513  
Fax: 603 635-2448  
E-Mail: [info@medicalisotopes.com](mailto:info@medicalisotopes.com)  
URL: [www.medicalisotopes.com](http://www.medicalisotopes.com)

R120718-1 p.34  
LWF 12/17/18

## CERTIFICATE OF ANALYSIS

**Product Name:**

Proprietary Info

**Catalog No:**

Proprietary Info

**Lot No:**

**Date:**

June 2017

**Re Test Date:**

June 2024

**Method of Analysis:**

<sup>1</sup>H-NMR and Mass Spec

**Purity:**

Chemical purity: 98%

Isotopic purity: 98%

**Molecular Formula:**

**Molecular Weight:**

Proprietary Info

**Appearance of Product:**

Pale Beige Solid

**Stability:**

N/A

**Melting Point:**

N/A

**Boiling Point:**

N/A

**Solubility:**

N/A

**Storage:**

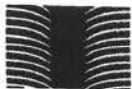
-20°C in freezer. Under inert atmosphere

**Additional Information:**

NMR and MS conforms to structure

Personal Info

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**MEDICAL ISOTOPES, INC.**  
100 Bridge Street  
Pelham, NH 03076 USA  
Tel: 603 635-2255, Toll Free: 800 374-9513  
Fax: 603 635-2448  
E-Mail: [info@medicalisotopes.com](mailto:info@medicalisotopes.com)  
URL: [www.medicalisotopes.com](http://www.medicalisotopes.com)

R120718-2 p. 3

7  
CW 12/08/18

city of  
CW 12/07/18

## CERTIFICATE OF ANALYSIS

**Product Name:** Proprietary Info

**Catalog No:** Proprietary Info

**Lot No:**

**Date:** April 2016

**Retest Date:** April 2020

**Method of Analysis:**

<sup>1</sup>H NMR and Mass Spec

**Purity:**

Chemical purity: 97%

Isotopic purity: 99%

**Molecular Formula:**

**Molecular Weight:**

Proprietary Info

**Appearance of Product:**

Pale Yellow Solid

**Stability:**

N/A

**Melting Point:**

N/A

**Boiling Point:**

N/A

**Solubility:**

N/A

**Storage:**

-20°C in freezer, Under Inert Atmosphere

**Additional Information:**

<sup>1</sup>H NMR and mass spectra conform to structure.

TLC: Single Spot

Personal Info

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MEDICAL ISOTOPES, INC.  
100 Bridge Street  
Pelham, NH 03076 USA  
Tel: 603 635-2255, Toll Free: 800 374-9513  
Fax: 603 635-2448  
E-Mail: [info@medicalisotopes.com](mailto:info@medicalisotopes.com) URL: [www.medicalisotopes.com](http://www.medicalisotopes.com)

R120718-3 p.3  
LW12/07/18

## CERTIFICATE OF ANALYSIS

**Product Name:**

Proprietary Info

**Catalog No:**

**Lot No:**

**Date:**

October 2016

**Re Test Date:**

October 2020

**Method of Analysis:**

<sup>1</sup>H-NMR, HPLC, and Mass Spec

**Purity:**

Chemical purity: 97%

Isotopic Purity: 99%

**Molecular Formula:**

Proprietary Info

**Molecular Weight:**

**Appearance of Product:**

White Solid

**Stability:**

N/A

**Melting Point:**

N/A

**Boiling Point:**

N/A

**Solubility:**

N/A

**Storage:**

-20°C in freezer

**Additional Information:**

NMR and Mass Spec conforms to structure

Personal Info

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Final Report • November 12, 2019

# METHOD VALIDATION REPORT FOR THE QUANTITATIVE ANALYSIS OF [Proprietary Info] [Proprietary Info] AND [Proprietary Info] IN K<sub>2</sub> EDTA RAT PLASMA

**Validation Scientist:**

Anush Harutyunyan, MS

**Testing Facility:**SRI International  
Biosciences Division  
333 Ravenswood Avenue  
Menlo Park, CA 94025**SRI Study Number:**

B181-18

**SRI Project Number:**

P25035.412

**Sponsor:**National Institute of Allergy and Infectious Diseases  
Division of AIDS  
5601 Fishers Lane [Redacted]  
Bethesda, MD 20892-9830**Sponsor's Representative:**

[Redacted by agreement]

**NIAID Contract No.:**

HHSN272201400006I/TO- HHSN27200008

Obtained via FOIA by White Coat Waste Project



Method Validation Report for the Quantitative Analysis of Proprietary Proprietary  
and Proprietary in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18

APPROVAL SIGNATURES

Validation Scientist:

Redacted by agreement

11/12/19  
Date

Approved by:

11/07/19  
Date

11/7/2019  
Date

11-7-19  
Date

SRI International  
Biosciences Division  
333 Ravenswood Avenue  
Menlo Park, CA 94025

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary  
**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
**SRI Study No. B181-18**

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**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary  
**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
**SRI Study No. B181-18**

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**SUMMARY**

A bioanalytical method was validated for the quantitative analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA rat plasma. Full details of the validated analytical method are provided in SRI Test Method 106.202 (Appendix A). The validation demonstrated that the method is appropriate for quantitation of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA rat plasma.

This validation was initiated following method development, which included determination of the assay range, selectivity, intra-batch accuracy and precision, sensitivity, recovery, matrix effect on ionization, matrix effects using 6 unique lots of matrix, room temperature stability in matrix, and freeze thaw stability in matrix, whole blood processing stability, effect of hemolysis, and effect of concomitant medications. The parameters investigated during this validation were based on the results obtained from these method development experiments. There were no significant changes made to the methodology between method development and validation. Results from the method development experiments will not be reported here.



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**QUALITY ASSURANCE UNIT**

**Final Report and  
Conflict of Interest Statement**

SRI's Quality Assurance Unit assures that the non-GLP study - Method Validation Report for the Quantitative Analysis of Proprietary Proprietary and Proprietary in K<sub>2</sub> EDTA Rat Plasma, SRI Study No. B181-18-- has been reviewed for consistency with the U.S. Food and Drug Administration Good Laboratory Practice Regulations (21 CFR Part 58).

The following inspections were conducted during this study:

<u>Phase Inspected</u>	<u>Date of Inspection</u>	<u>Date Findings Reported to Management/Study Director</u>
Bioanalytical Method Validation	06-18-19	06-18-19
Raw Data	09-05-19	09-05-19
Draft Final Report	09-05-19	09-05-19
Final Report Verification	11-12-19	11-12-19

This statement certifies that the personnel listed below participated in the inspections and audit of this study. These personnel have not been involved in the generation or evaluation of the data. Participation by the individuals listed below poses no conflict of interest.

Redacted by agreement

Redacted by agreement

11/12/19  
Date

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**KEY PERSONNEL**

**Name**

**Functional Role**

Redacted by agreement

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**SUMMARY OF VALIDATION PARAMETERS**

Analytes	<span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span>
Internal standards	<span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span> <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span> <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span>
Matrix	K <sub>2</sub> EDTA rat plasma
Quantitation range	5.00-1000 ng/ml <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> and <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> 25.0-5000 ng/ml <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span>
Sample volume	0.0200 ml
Extraction procedure	Methanol protein precipitation
Analytical method	LC-MS/MS
Regression type, weighting	Linear, 1/x <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> and <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> Linear, 1/x <sup>2</sup> <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span>
Maximum batch size	<span style="border: 1px solid black; padding: 0 5px;">Pro</span> samples
Correlation Coefficient	≥0.9986 <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span> ≥0.9995 <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> and <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span>
Intra- and inter-assay QC levels	
LLOQ	5.00 ng/ml <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> and <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> 25.0 ng/ml <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span>
Low	15.0 ng/ml <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> and <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> 75.0 ng/ml <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span>
Mid	400 ng/ml <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> and <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> 2000 ng/ml <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span>
High	800 ng/ml <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> and <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> 4000 ng/ml <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span>
Dilution QC level	5000 ng/ml <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> and <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> 25000 ng/ml <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span> diluted 10-fold, 50-fold
Intra-batch precision	1.3% to 5.6% <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span> 1.4% to 5.4% <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> 1.9% to 2.8% <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span>
Intra-batch accuracy	98.8% to 102.5% <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span> 99.0% to <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> 98.8% to 100.2% <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span>
Mean recovery of Analyte	96.9% <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span> 95.3% <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> 105.9% <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span>
Mean recovery of Internal Standard	96.9% <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span> 94.5% <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span> 98.8% <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span>
Mean matrix effect on ionization Analyte	111.2% <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span> 162.8% <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> 103.2% <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span>
Mean matrix effect on ionization Internal Standard	111.3% <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span> 164.7% <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span> 102.3% <span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span>

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Selectivity	6 out of 6 lots satisfied acceptance criteria
Matrix effects	6 out of 6 lots satisfied acceptance criteria
Analyte carryover	<20% of mean LLOQ peak area for [Proprietary] Some BI/BI and Carryover BI/BI samples showed carryover for [Proprietary] and [Proprietary] Refer to the Carryover section of this report for additional information.
Internal Standard carryover	<5% of mean internal standard peak area
Room temperature stability	25 hr established (all analytes)
Freeze thaw stability	5 cycles established (all analytes)
Reinjection (autosampler) stability	94 hr (refrigerated) established (all analytes)
Post-preparative extract stability	99 hr (refrigerated) established (all analytes)
Whole blood processing stability	4 hr (refrigerated) established (all analytes)
Effect of hemolysis	0.5% and 2% hemolysis; no impact (all analytes)
Long term matrix storage stability	Interim 25 days established ( $\leq -60^{\circ}\text{C}$ ) (all analytes). Stability is ongoing.

## I. INTRODUCTION

A bioanalytical method was validated for the quantitative analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA rat plasma, over the concentration ranges 5.00-1000 ng/ml [Proprietary] and [Proprietary] and 25.0 ng/ml-5000 ng/ml [Proprietary Info] with 0.0200 ml sample volumes. The internal standards used for this assay were [Proprietary Info] [Proprietary Info] and [Proprietary Info]. The analytes and the internal standards were extracted from K<sub>2</sub> EDTA rat plasma using a methanol protein precipitation followed by LC-MS/MS detection. The finalized analytical method used throughout this validation is described in SRI Test Method 106.202 (Appendix A).

This method was fully validated in dog in SRI Study No. B185-18. As there was no change in the analytical range used or the methodology, this was done as a partial validation in rat with one accuracy and precision batch.

Per the partial validation plan (Appendix B), the partial validation included determination of linearity and range, selectivity, intra-batch assay accuracy and precision, carryover, matrix effects, matrix effect on ionization, recovery, dilution assessment, room temperature stability, freeze thaw stability, reinjection (autosampler) stability, post-preparative extract stability, whole blood processing stability, effect of hemolysis, and effect of concomitant medication. Analyte stock solution stability for [Proprietary] [Proprietary] and [Proprietary] was established in SRI Study No. B185-18 and, therefore, was not performed in this study. Long term matrix storage stability evaluation is ongoing (an interim evaluation has been performed), and the results from this experiment will be provided in an amended report. A full description of these parameters and the acceptance criterion for each is detailed in SRI SOP 006.060, *Bioanalytical Method Validation*.



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This validation was not within the scope of U.S. Food and Drug Administration (FDA) “Good Laboratory Practice for Nonclinical Laboratory Studies” (GLP) regulations, as described in 21 CFR Part 58. Nevertheless, this validation was planned, performed, recorded, and reported in accordance with standard practices to ensure data quality and integrity. This report presents the methodology and results of the validation, which demonstrate that the method is appropriate for the quantitation of Proprietary Proprietary and Proprietary in K<sub>2</sub> EDTA rat plasma over the concentration ranges tested.

The freezer storage temperatures stated in this report are nominal. The temperature of the Quality Control (QC) storage freezers did not go above –60°C from the time of initial storage of the QC samples until the final analysis of these samples. Any recorded departure from the manufacturer’s specifications for a particular freezer unit would result in a facility deviation. No deviations were generated as a result of this over the course of this study.

## II. REFERENCE STANDARDS

The certificates of analysis for the analytes and internal standards are provided in Appendix C.

### Reference Standard Description

Reference Standard	Supplier	Lot Number	Correction Factor	Storage Conditions	Expiration
<span style="border: 1px solid black; padding: 0 5px;">Proprietary</span>	U.S. Pharmacopeia	R077R0	0.997	Refrigerated, Protected from light	Current lot
<span style="border: 1px solid black; padding: 0 5px;">Proprietary</span>	U.S. Pharmacopeia	H0M427	0.993	Refrigerated, Protected from light	Current lot
<span style="border: 1px solid black; padding: 0 5px;">Proprietary</span>	U.S. Pharmacopeia	R044C0	0.940	Refrigerated	Current lot
<span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span>	Medical Isotopes, Inc.	183	0.980 <sup>b</sup>	-20°C	Retest 06-30-24
<span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span>	Medical Isotopes, Inc.	411	0.970 <sup>b</sup>	-20°C	Retest 04-30-20
<span style="border: 1px solid black; padding: 0 5px;">Proprietary Info</span>	Medical Isotopes, Inc.	091	0.970 <sup>b</sup>	-20°C	Retest 10-31-20

<sup>a</sup> Proprietary was supplied as Proprietary monohydrate. The final correction factor used during stock solution preparation is based on the amount of Proprietary present when weighing.

<sup>b</sup> The purity was assumed as 100% during weighing.

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### **III. VALIDATION RESULTS**

#### **A. Summary of Runs Performed**

Six analytical runs were analyzed as part of this validation and are summarized in Table 1.

Pooled control K<sub>2</sub> EDTA rat plasma (lot number RAT397850, expiration 05-31-21), which was used in the preparation of calibration curves, QC samples, and assay blanks, was obtained from BioIVT, Westbury, NY. on 05-21-19 and was stored in a -20°C freezer until use. Six unique lots of matrix (lot numbers RAT397851 to RAT397856, expiration 05-31-21) from individual animals used for the assessment of selectivity and matrix effects were also obtained from this supplier on this date. Pooled K<sub>2</sub> EDTA rat whole blood (lot number RAT399689, expiration 07-18-19), used in the assessment of whole blood processing stability, was also obtained from BioIVT on 06-19-19, and was stored, refrigerated, until use.

#### **B. Assay Acceptance Criterion**

For a calibration curve to be considered acceptable, at least 75% of calibration standards must be accurate to within  $\pm 15\%$  of the nominal concentration ( $\pm 20\%$  at the lower limit of quantitation, or LLOQ), including at least one replicate at the lowest and highest concentrations.

For accuracy and precision experiments, individual QC samples at low, mid and high concentrations were considered acceptable if they were accurate to within  $\pm 15\%$  of the nominal concentration ( $\pm 20\%$  at the LLOQ). The intra-batch accuracy and precision were acceptable if they were  $\leq 15\%$  ( $\pm 20\%$  at the LLOQ). Within a run, at least 50% of the QC samples at each concentration must have satisfied the acceptance criterion, with at least 67% of the total QC samples in a run satisfying the acceptance criterion.

For QC samples used in batch acceptance, at least 67% of all QC samples must be within  $\pm 15\%$  of the nominal concentration, with at least 50% of the QC samples at each concentration meeting this criterion.

For QC samples not used in accuracy and precision evaluation, including, but not limited to, stability QC samples, effect of hemolysis samples, and dilution QC samples, at least 50% of the individual replicates at each concentration were considered acceptable if they were within  $\pm 15\%$  of the nominal concentration. Overall accuracy and precision at each concentration were considered acceptable if they were within  $\pm 15\%$ . For QC samples prepared at the low concentration for the assessment of matrix effects in 6 unique lots of plasma, at least 50% of individual replicates for each lot were considered acceptable if they were within  $\pm 15\%$  of the nominal concentration, and the overall accuracy and precision of each lot was within  $\pm 15\%$ .



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For whole blood processing stability, the % difference between the mean calculated concentration of the timepoint under evaluation and the mean of the time zero calculated concentration must be within  $\pm 15\%$  to be considered stable.

Full details of the acceptance criterion for each validation parameter are detailed in SRI SOP 006.060.

**C. Standard Curve Linearity**

A total of eight calibration standards, extracted in duplicate, were used in the construction of each calibration curve. The correlation coefficient (r) in each run was at least 0.9995 for [Proprietary] and [Proprietary] using a least-squares linear regression with a  $1/x$  weighting. The correlation coefficient (r) in each run was at least 0.9986 for [Proprietary] using a least-squares linear regression with a  $1/x^2$  weighting (Tables 2-4). The K<sub>2</sub> EDTA rat plasma back-calculated calibration curve concentrations generated from all validation runs are presented in Tables 5-7. The calibration curve ranged from 5.00 ng/ml (LLOQ) to 1000 ng/ml (upper limit of quantitation, ULOQ) for [Proprietary] and [Proprietary] and from 25.0 ng/ml to 5000 ng/ml for [Proprietary]. Calibration standards were prepared fresh on each day of extraction. Representative calibration curves from Run MV1 are shown in Figures 1-3.

**D. Accuracy and Precision of QC Samples**

To assess the accuracy and precision of the method, QC samples in K<sub>2</sub> EDTA rat plasma were freshly prepared on the day of analysis at the LLOQ (5.00 ng/ml for [Proprietary] and [Proprietary] and 25.0 ng/ml for [Proprietary] low (15.0 ng/ml for [Proprietary] and [Proprietary] and 75.0 ng/ml for [Proprietary] mid (400 ng/ml for [Proprietary] and [Proprietary] and 2000 ng/ml for [Proprietary] and high (800 ng/ml for [Proprietary] and [Proprietary] and 4000 ng/ml for [Proprietary] concentrations. To determine intra-batch accuracy and precision, these QC samples were extracted in one analytical run in replicates of six at each concentration.

The intra-batch precision across all concentrations in this analytical run ranged from 1.3% to 5.6% [Proprietary Info] 1.4% to 5.4% [Proprietary] and 1.9% to 2.8% [Proprietary Info]. The intra-batch accuracy ranged from 98.8% to 102.5% [Proprietary Info] 99.0% to [Proprietary] [Proprietary] and 98.8% to 100.2% [Proprietary Info]. Summaries of the intra-batch accuracy and precision results are provided in Tables 8-10.

**E. Maximum Batch Size**

One accuracy and precision batch of sufficient size was analyzed to represent a typical batch when running study samples. The maximum batch size of 101 samples was determined by counting the number of injections made from the first calibration standard to the last calibration standard, in Run MV1.

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**F. Selectivity**

To confirm the selectivity of the assay for each analyte, blank K<sub>2</sub> EDTA rat plasma (containing no analyte or internal standard) from six individual animals were analyzed in replicates of n=1. Additionally, a single BI/BI sample in plasma matrix representing 0.5% and 2% hemolysis was also prepared and analyzed. No significant interference (defined as a peak at the retention time of analyte with a peak area of >20% of the mean peak area of the LLOQ calibration standard) was detected in any individual lot, or in the hemolyzed samples. There was no significant interference at the retention time of the internal standard (>5% of the mean internal standard peak area); therefore, lack of selectivity did not appear to be significant. Refer to Tables 11-13 for a summary of the results for each analyte.

Additionally, pooled K<sub>2</sub> EDTA rat plasma was used to prepare double blank samples containing no analytes or internal standards (BI/BI) and blank samples containing only internal standards but no analytes (BI/IS); these samples were extracted in duplicate in each analytical run. There was no significant interference at the retention time of either the analytes or internal standards in the BI/BI samples, as defined above, with the following exceptions. In Runs MV1-RI, MV2, MV4 and MV5 the second BI/BI sample showed a significant interference at the analyte retention time for [Proprietary]. Also, a significant interference was observed in the second BI/BI sample in Run MV1-RI for [Proprietary]. This was probably due to carryover, as a high QC sample directly preceded injection of these samples. No other BI/BI samples for either [Proprietary] [Proprietary] or [Proprietary] showed significant interference. In all BI/IS samples, there was no significant interference at the retention time of the analytes, as defined above. Refer to Tables 14-16 for a summary of the results for each analyte.

In one analytical run, a sample prepared at the ULOQ but containing no internal standards (ULOQ/BI) was extracted in replicates of n=1. This ULOQ sample contained only [Proprietary] or only [Proprietary] or only [Proprietary] – the analytes were not co-spiked. There was no significant interference originating from the analyte at the retention time of the internal standards (defined as >5% of the mean extracted internal standard peak area). There was no significant interference at the analyte retention time in the [Proprietary] or [Proprietary] ion channels when the ULOQ/BI [Proprietary] sample was injected, and similarly, no interference was observed in the other analyte ion channels when the ULOQ/BI [Proprietary Info] and [Proprietary] samples were injected.

**G. Carryover Assessment**

Carryover was assessed in each analytical run by injecting a BI/BI sample immediately after each ULOQ calibration standard. There was no significant interference (defined as a peak at the retention time of [Proprietary] with a peak area of >20% of the mean analyte peak area of the LLOQ calibration standards, or a peak at the retention time of the internal standard with a peak area of >5% of the mean internal standard peak area); therefore, carryover does not appear to have a significant impact on this assay for this analyte. Significant carryover was seen in both carryover blank samples in Runs MV1, MV1-RI, MV3, MV4 and MV5 for [Proprietary] with one carryover blank sample affected in Run MV2. Also, carryover was seen in



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one carryover blank sample in Runs MV1, MV1-RI and MV2 for [Proprietary] Refer to Tables 14-16 for a summary of the results. Carryover will be monitored during sample analysis.

#### **H. Dilution Assessment**

To evaluate the ability to dilute a sample prepared at a concentration higher than the analytical range, a dilution QC sample was prepared at 5000 ng/ml [Proprietary] and [Proprietary] and 25000 ng/ml [Proprietary] fresh on the day of extraction. This QC was diluted 10-fold and 50-fold with control K<sub>2</sub> EDTA rat plasma and extracted in replicates of six. The overall results for both dilution schemes satisfied the acceptance criterion as defined by SRI SOP 006.060. Refer to Tables 17-19 for a summary of the results.

#### **I. Matrix Effects**

To evaluate matrix effects, K<sub>2</sub> EDTA rat plasma from six individual animals was spiked at the low QC concentration (15.0 ng/ml for [Proprietary] and [Proprietary] and 75.0 ng/ml for [Proprietary] and extracted in replicates of four. The results for all six lots satisfied the acceptance criterion for each analyte as defined by SRI SOP 006.060, demonstrating that matrix effects do not have a significant impact on this assay. Refer to Tables 20-22 for a summary of the results.

#### **J. Recovery**

The recovery of the method for the analytes and the internal standards was assessed by comparing the peak areas of each analyte and internal standard in extracted QC samples at low, mid and high concentrations with the peak areas obtained from extracted BI/BI plasma samples. The methanol supernatant from the extracted BI/BI samples was diluted with recovery solutions containing all analytes and internal standards to give final concentrations equivalent to the theoretical levels in the extracted samples (post-extracted spiked samples). All samples were evaluated in replicates of six. The recovery solution diluent was water:methanol (90:10, v:v) with 0.1% acetic acid.

The mean recovery of [Proprietary] [Proprietary] and [Proprietary] across concentrations was 96.9%, 95.3%, and 105.9%, respectively. The mean recovery of [Proprietary Info] [Proprietary Info] and [Proprietary Info] was 96.9%, 94.5%, and 98.8%, respectively. There is no defined acceptance criterion for recovery, and these mean recovery values indicate that the degree of recovery achieved during this extraction procedure is satisfactory. Refer to Tables 23-28 for a summary of the results.

#### **K. Matrix Effect on Ionization**

The ability of the matrix to enhance or suppress the analyte or internal standard response was assessed by comparing the peak areas of analytes and internal standards in the post-extracted spiked BI/BI samples described in the Recovery section, above, with the recovery solutions. The recovery solutions were diluted with 0.2% acetic acid in methanol beforehand, which represented the plasma supernatant. All samples were evaluated in

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replicates of six. The first injection of the low recovery solution for [Proprietary] and [Proprietary Info] was determined to be a Grubbs outlier, and was excluded from the summary statistics. The other two analytes in this sample were not statistical outliers. A value of >100% indicates enhancement caused by the matrix, and a value <100% indicates suppression.

The matrix effect on ionization of the response for [Proprietary] [Proprietary] and [Proprietary] was 111.2%, 162.8%, and 103.2%, respectively. The matrix effect on ionization of the response for [Proprietary Info] [Proprietary Info] and [Proprietary Info] was 111.3%, 164.7%, and 102.3%, respectively. There is no defined acceptance criterion for the acceptable degree of matrix effect on ionization. These values indicate that the matrix effect on ionization observed during this extraction procedure would not have significant impact on the assay. Refer to Tables 29-34 for a summary of the results.

**L. Room Temperature Matrix Stability**

Room temperature matrix stability of each analyte in K<sub>2</sub> EDTA rat plasma was determined by extracting QC samples at low and high concentrations, in replicates of four, which had been stored at room temperature for 25 hr before extraction. These QC samples were analyzed against a fresh calibration curve and fresh QC samples. The overall results for room temperature stability for up to 25 hr satisfied the acceptance criterion as defined by SRI SOP 006.060. Refer to Tables 35-37 for a summary of the results.

**M. Freeze Thaw Matrix Stability**

Freeze thaw matrix stability of each analyte in K<sub>2</sub> EDTA rat plasma was determined by extracting QC samples at low and high concentrations, in replicates of four, which had been subjected to five freeze thaw cycles before extraction. QC samples were stored frozen at ≤-60°C for a minimum of 24 hr prior to the first thaw and stored frozen for subsequent periods of not less than 12 hr. Samples were thawed for a minimum of 1 hr at room temperature prior to re-freezing. These QC samples were analyzed against a fresh calibration curve and fresh QC samples. The overall results for freeze thaw stability for up to five cycles satisfied the acceptance criterion as defined by SRI SOP 006.060. Refer to Tables 38-40 for a summary of the results.

**N. Reinjection (Autosampler) Stability**

Reinjection stability of each analyte in K<sub>2</sub> EDTA rat plasma was demonstrated by reinjecting the calibration standards, the LLOQ, low, mid and high accuracy and precision QC samples, and the BI/BI, BI/IS, and carryover samples from Run MV1 after 94 hr of refrigerated storage (set point 5°C). The re-injected run was designated as Run MV1-RI. The overall results for reinjection stability for up to 94 hr under refrigerated conditions satisfied the acceptance criterion as defined by SRI SOP 006.060. Refer to Tables 41-43 for a summary of the results.



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**O. Post-Preparative Extract Stability**

Post-preparative extract stability of each analyte in K<sub>2</sub> EDTA rat plasma was determined by extracting QC samples at low and high concentrations, in replicates of four, in Run MV2. These QC extracts were then stored in a refrigerated autosampler (set point 5°C) and analyzed with a fresh calibration curve and fresh QC samples prepared in Run MV3. The overall results for post-preparative extract stability for up to 99 hr under refrigerated conditions satisfied the acceptance criterion as defined by SRI SOP 006.060. Refer to Tables 44-46 for a summary of the results.

**P. Whole Blood Processing Stability**

The stability of each analyte during the processing of spiked K<sub>2</sub> EDTA rat whole blood was determined by spiking whole blood samples at two concentrations and harvesting the plasma at time zero (T0) and 1 hr and 4 hr after spiking. The whole blood samples were stored refrigerated (5°C ± 3°C) during this period of storage. The whole blood was spiked to give a final concentration of 7.50 ng/ml [Proprietary] and [Proprietary] and 37.5 ng/ml [Proprietary] representing the low analyte concentration, and 400 ng/ml [Proprietary] and [Proprietary] and 2000 ng/ml [Proprietary] representing the high analyte concentration (assuming that 100% of the analytes partitions into the plasma). Following plasma collection, the plasma samples were stored at ≤-60°C prior to extraction. These samples were then extracted in replicates of four at each concentration, in Run MV4. The calculated final (mean) T0 concentrations in plasma at the low concentration were 11.1 ng/ml, 9.36 ng/ml, and 53.7 ng/ml for [Proprietary] [Proprietary] and [Proprietary] respectively. The calculated final (mean) T0 concentrations in plasma at the high concentration were 611 ng/ml, 533 ng/ml, and 3010 ng/ml for [Proprietary] [Proprietary] and [Proprietary] respectively. The overall results for whole blood processing stability for up to 4 hr under refrigerated conditions satisfied the acceptance criterion as defined by SRI SOP 006.060. Refer to Tables 47-49 for a summary of the results.

**Q. Effect of Hemolysis**

The effect of hemolysis on the quantitation of each analyte was determined by preparing low and high QC samples in matrix representing 0% hemolysis, 0.5% hemolysis, and 2% hemolysis, to determine whether the presence of whole blood had an impact on the ability to accurately quantify the analyte. The overall results indicated that hemolysis at 0.5% and 2% had no significant impact on the quantitation of each analyte as defined by SRI SOP 006.060. Refer to Tables 50-52 for a summary of the results.

**R. Long Term Matrix Storage Stability**

Long term matrix storage stability of each analyte in K<sub>2</sub> EDTA rat plasma was determined by extracting QC samples at low and high concentrations, in replicates of four, after storage for 25 days at ≤-60°C. These QC samples were analyzed against a fresh calibration curve. Also included in this batch were freshly prepared assay acceptance QC samples (low, mid and high QC samples in replicates of two), which were extracted to determine the accuracy of the calibration curve preparation and the validity of the stability QC



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final results. The overall results for long term matrix storage stability for up to 25 days at  $\leq -60^{\circ}\text{C}$  satisfied the acceptance criterion as defined by SRI SOP 006.060. Refer to Tables 53-55 for a summary of these results. Determination of long term matrix storage stability of each analyte in K<sub>2</sub> EDTA rat plasma at  $\leq -60^{\circ}\text{C}$  is currently ongoing; the results will be reported in an amended report.

**S. Concomitant Medication**

The effect of [Proprietary] and [Proprietary] on [Proprietary] quantitation was assessed by preparing a plasma sample containing 15.0 ng/ml [Proprietary] (the low QC concentration), 2000 ng/ml [Proprietary] and 20000 ng/ml [Proprietary]. These concentrations of [Proprietary] and [Proprietary] were selected as they were estimated to be at or above the C<sub>max</sub> observed in previous studies at high dose concentrations. These samples satisfied the acceptance criterion for [Proprietary] indicating that addition of [Proprietary] and [Proprietary] at high concentrations had no adverse effect on the ability to quantitate this analyte. Similarly, no impact on [Proprietary] at the low QC concentration was observed when [Proprietary] and [Proprietary] were spiked into plasma at 2000 ng/ml and 20000 ng/ml, respectively. No impact on [Proprietary] at the low QC concentration was observed when [Proprietary] and [Proprietary] were spiked into plasma at 2000 ng/ml. Refer to Tables 56-58 for a summary of these results. The results of the assay acceptance QC samples are provided in Tables 59-61.

**IV. DEVIATIONS**

There were no deviations to SRI SOPs that had any impact on the integrity of the study.

**V. DATA MANAGEMENT**

The LC-MS/MS data were acquired, peak areas were integrated, the calibration line regression was calculated, and the final concentrations were generated using AB Sciex Analyst software, version 1.6.2 (AB SCIEX, Framingham, MA). Figures 4-18 show the integrations performed by the Analyst software on selected chromatographs. The statistics described in this report were generated with this software, using unrounded values, with the exception of the statistics used to calculate recovery and matrix effect on ionization, which were generated using Microsoft Excel 2016 (Microsoft, Corp., Redmond, WA).

**VI. DATA STORAGE**

The final report, raw data, supporting documents, and records specific to this study will be retained and stored by SRI International. All records will be maintained for at least 1 year. At the end of the retention period, the Sponsor will be contacted regarding further disposition of these records.

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## **VII. DISCUSSION AND CONCLUSION**

The validation demonstrated that the method is appropriate for quantitation of [Proprietary Info] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA rat plasma over the concentration range 5.00-1000 ng/ml [Proprietary Info] and [Proprietary] and 25.0-5000 ng/ml [Proprietary Info]

## **VIII. DEFINITIONS AND ABBREVIATIONS**

Accuracy:	$\frac{\text{Calculated concentration}}{\text{Nominal concentration}} * 100$
Bl/Bl:	Double blank, containing no analyte or internal standard
Bl/IS:	Blank, containing only internal standard
CV:	Coefficient of variation; $\text{SD/mean} * 100$
% Difference (stability evaluations):	$((\text{New value} - \text{original value}) / \text{original value}) * 100$
% Difference (specificity / carryover evaluations):	$((\text{Peak area (blank)} / \text{Mean peak area (LLOQ)}) * 100$
DMSO:	Dimethyl sulfoxide
K <sub>2</sub> EDTA:	Di-potassium ethylenediaminetetraacetic acid
IS:	Internal standard [Proprietary Info] [Proprietary Info] [Proprietary Info]
LC-MS/MS:	Liquid chromatography-mass spectrometer (tandem or triple-quadrupole mass spectrometer)
LLOQ:	Lower limit of quantitation
Matrix effects on Ionization:	$\frac{\text{Mean peak area (post extracted spiked)}}{\text{Mean peak area (recovery solution)}} * 100$
MV:	Method validation
PAR:	Peak area ratio
QC:	Quality control
% Recovery:	$\frac{\text{Mean peak area (extract)}}{\text{Mean peak area (post extracted spiked)}} * 100$
ULOQ:	Upper limit of quantitation
ULOQ/Bl:	Sample contains analyte at the ULOQ concentration but no internal standard

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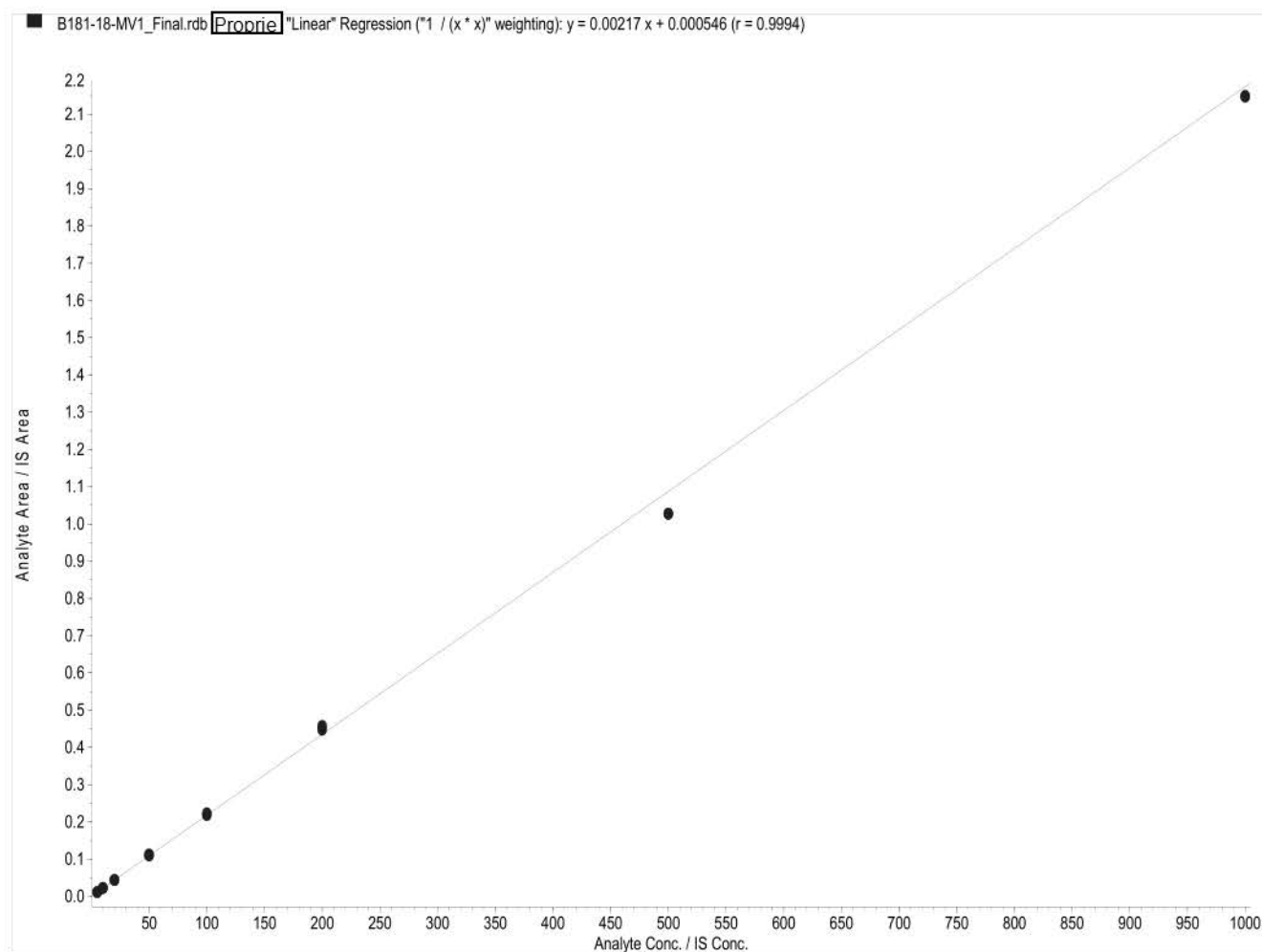
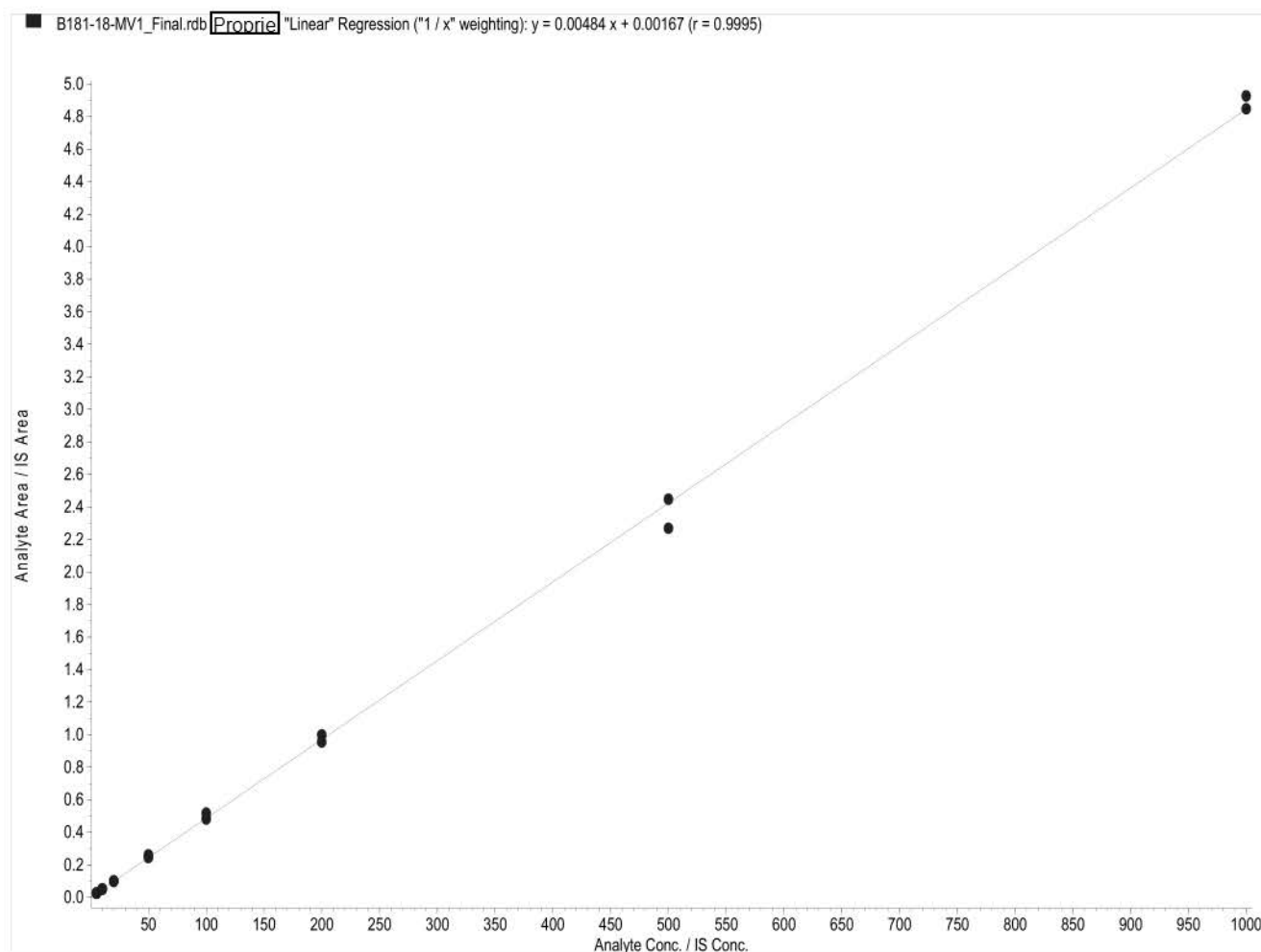


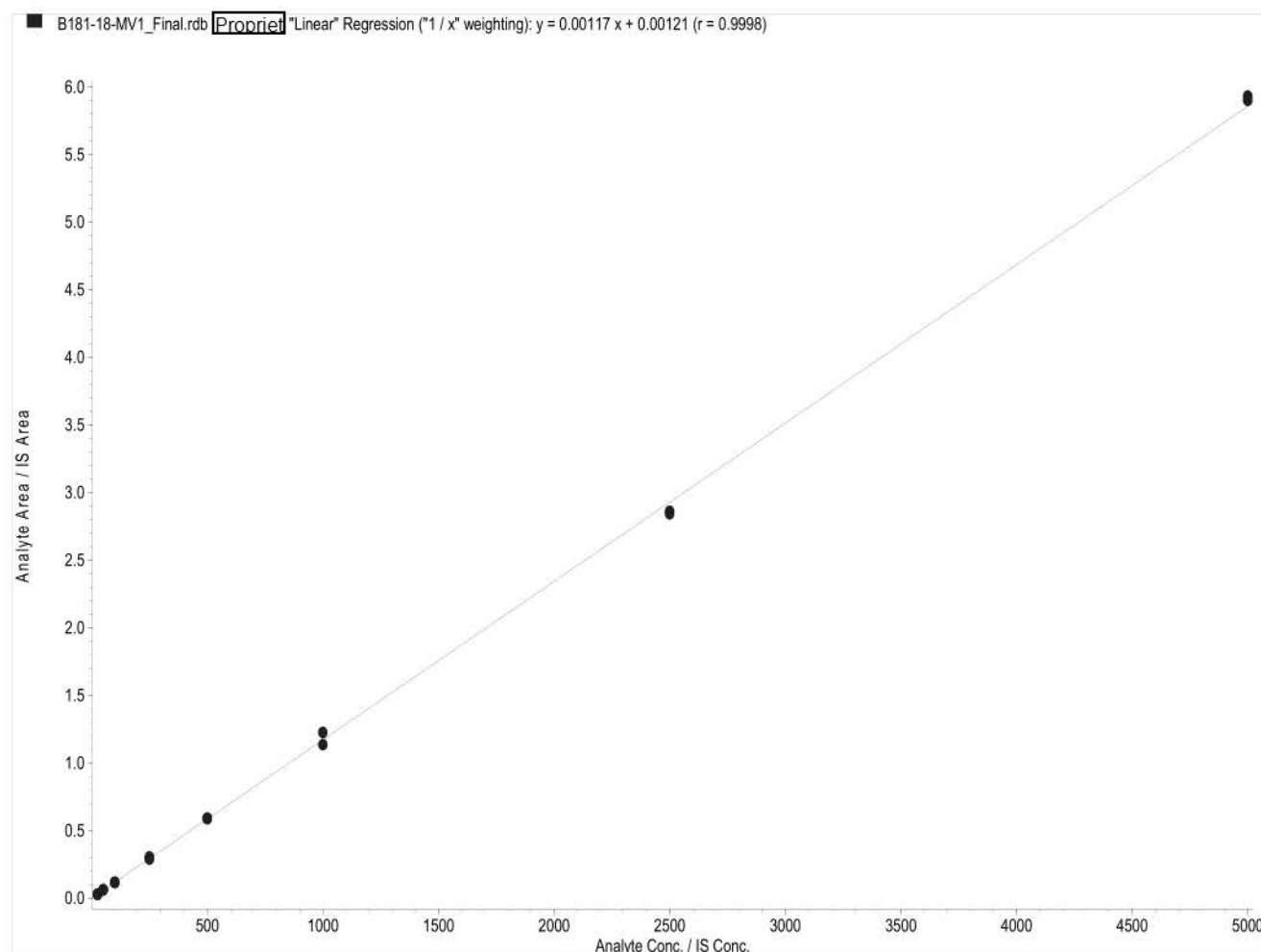
Figure 1. Representative [Proprietary] K<sub>2</sub> EDTA rat plasma calibration curve.

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**Figure 2.** Representative Proprietary K<sub>2</sub> EDTA rat plasma calibration curve.

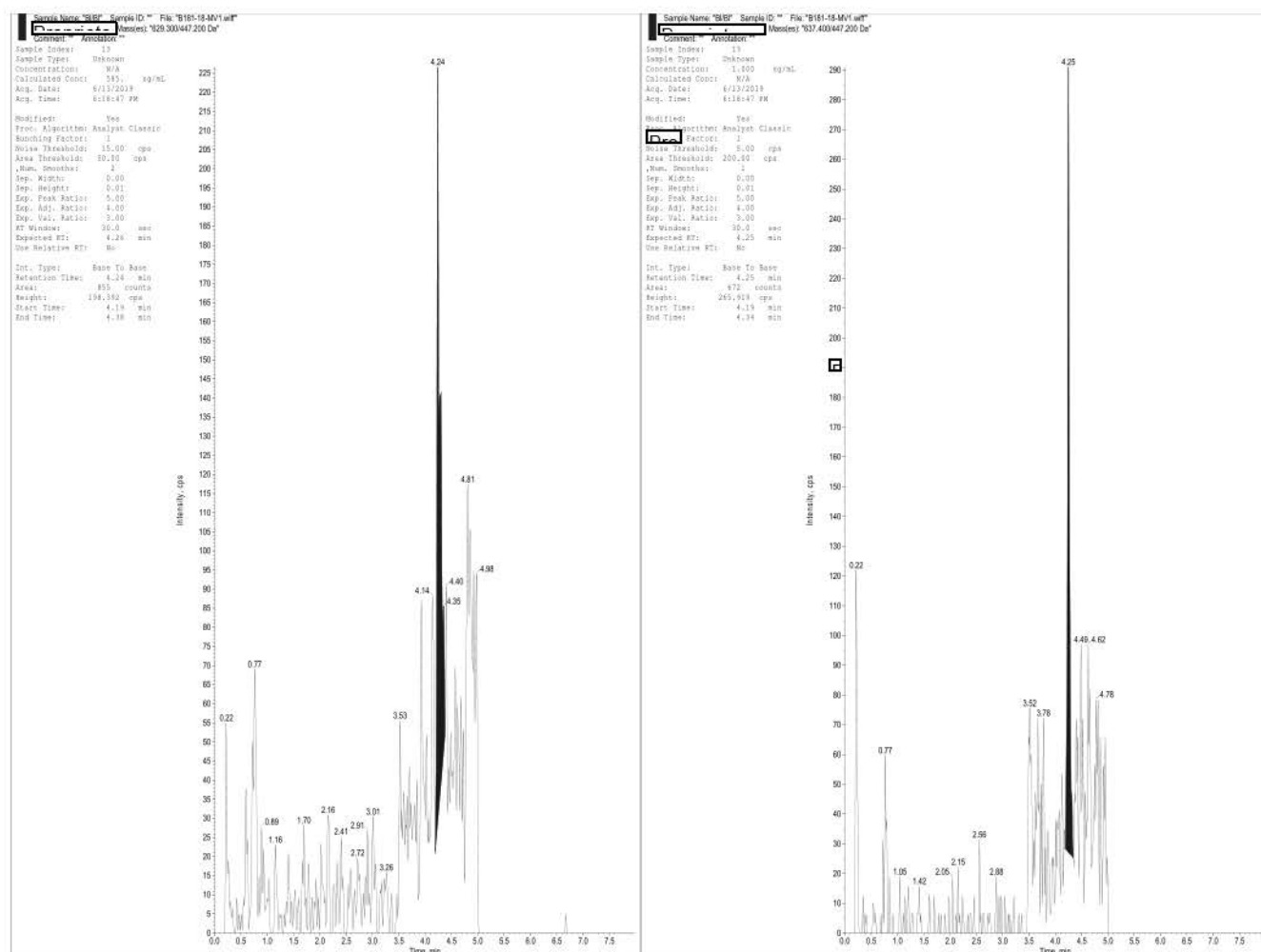
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**Figure 3.** Representative [Proprietary] K<sub>2</sub> EDTA rat plasma calibration curve.

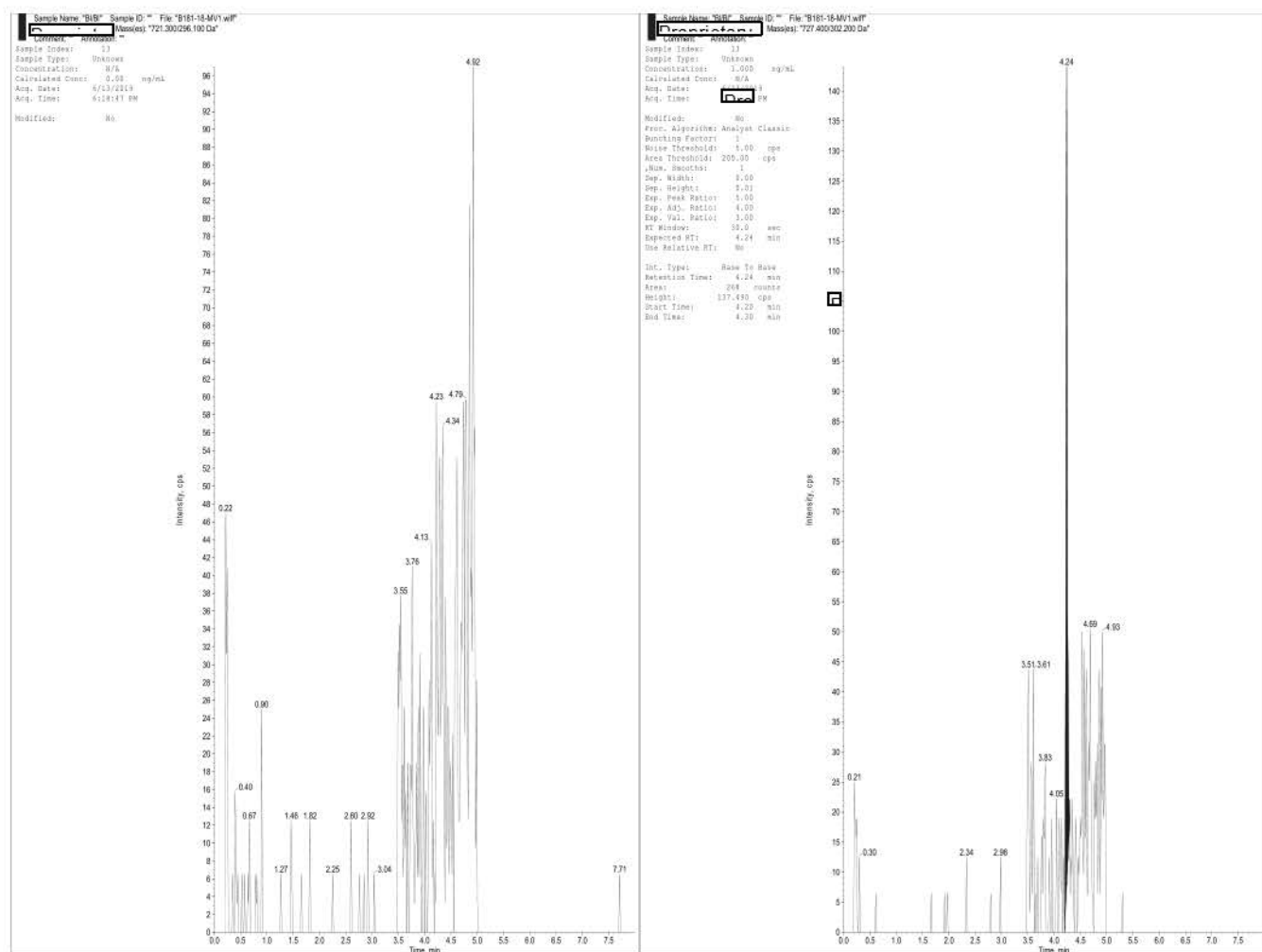


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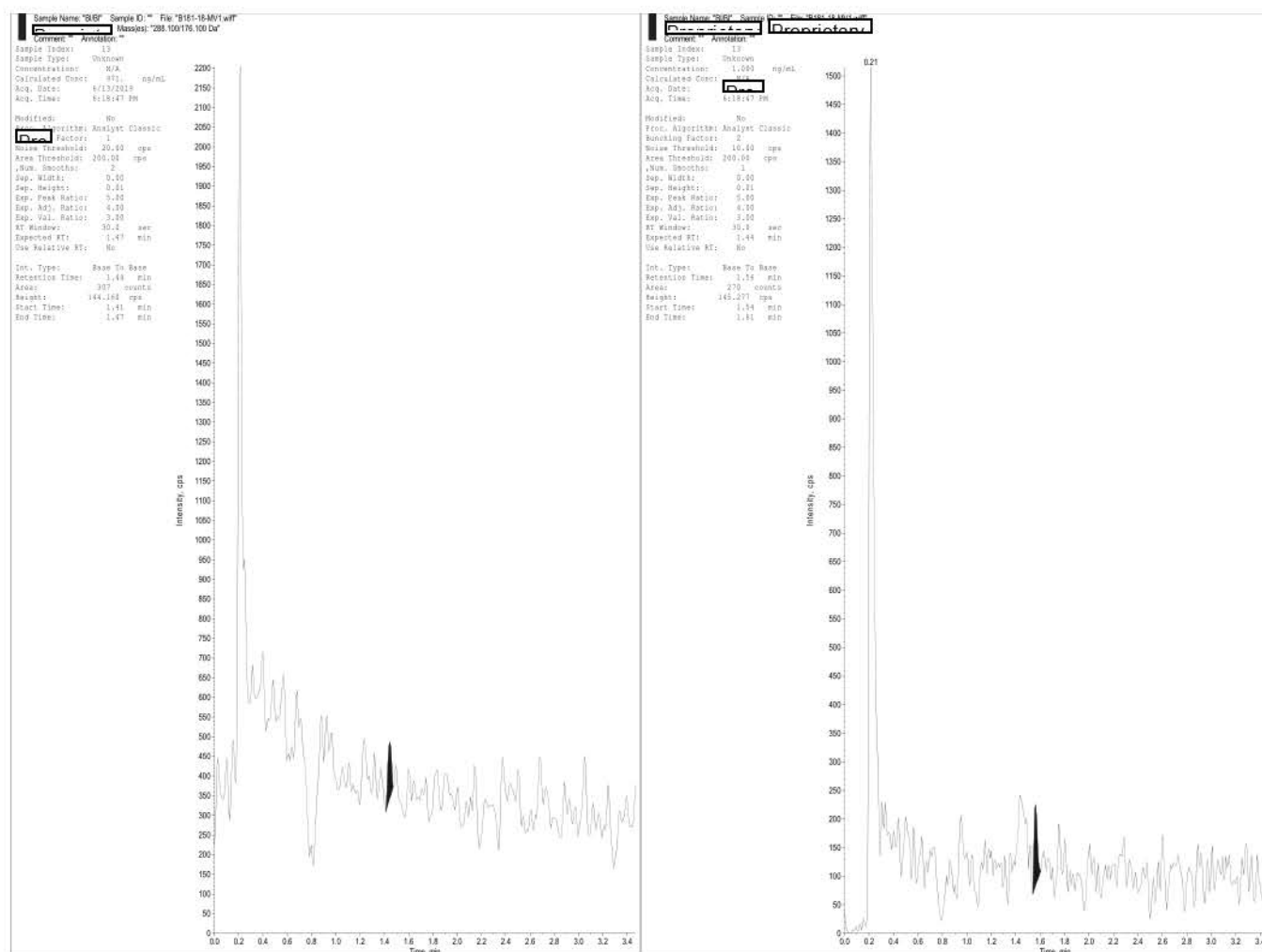
**Figure 4.** Representative chromatogram of a BI/BI K<sub>2</sub> EDTA rat plasma sample (without Proprietary or internal standard).

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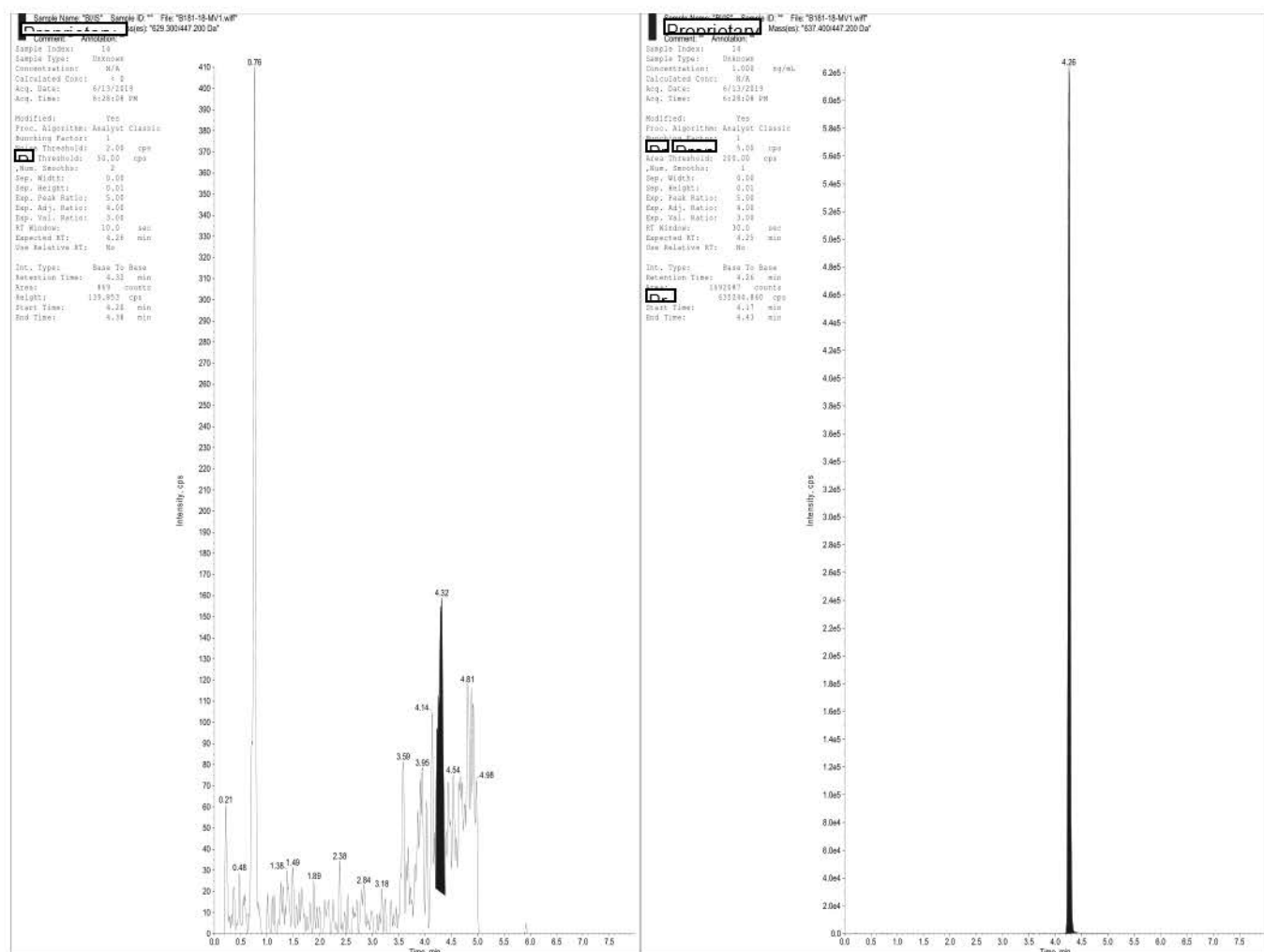
**Figure 5.** Representative chromatogram of a BI/BI K<sub>2</sub> EDTA rat plasma sample (without Proprietary or internal standard).

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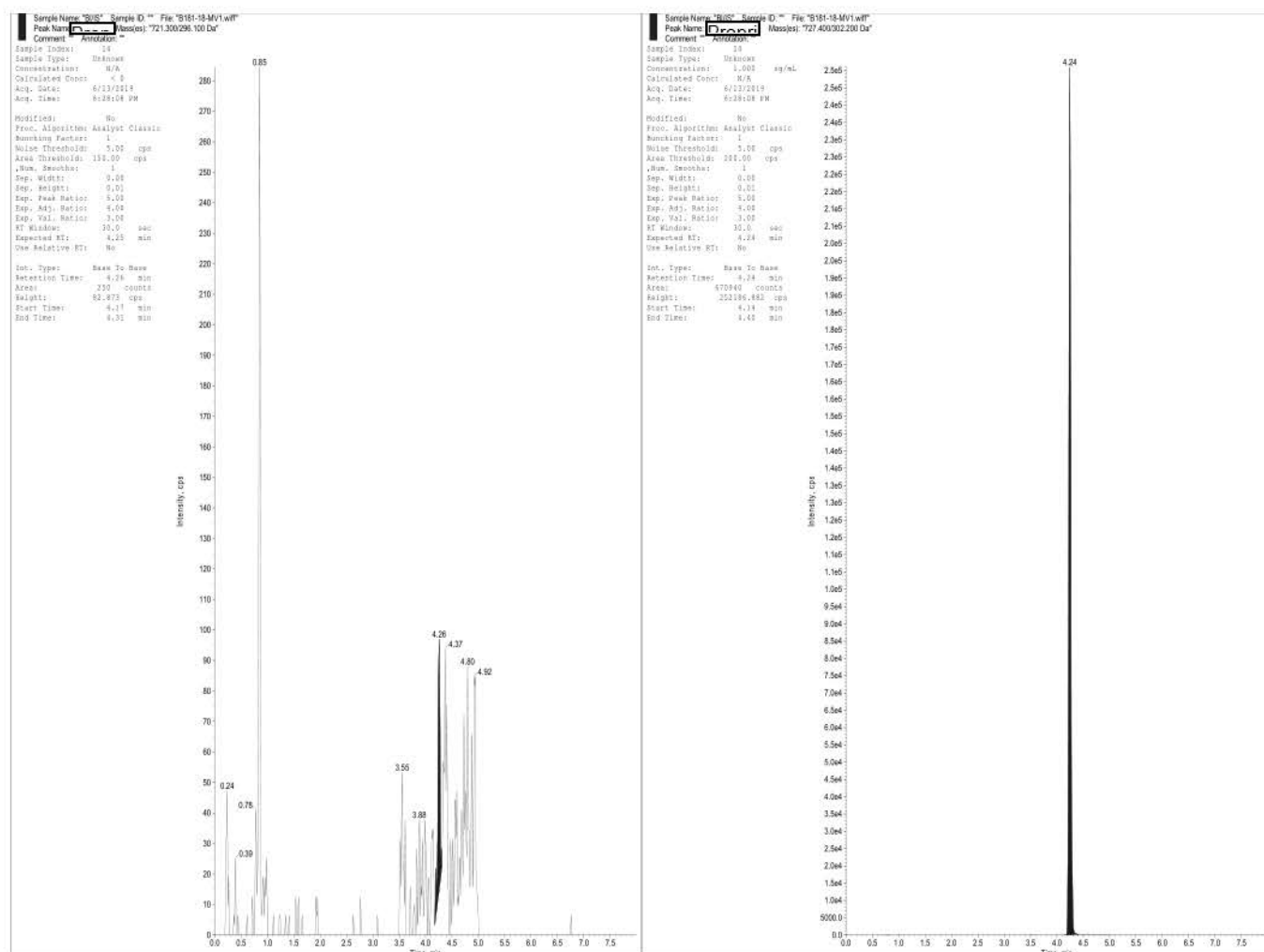
**Figure 6.** Representative chromatogram of a BI/BI K<sub>2</sub> EDTA rat plasma sample (without Proprietary or internal standard).

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**Figure 7.** Representative chromatogram of a BI/IS K<sub>2</sub> EDTA rat plasma sample (without Proprietary with internal standard).

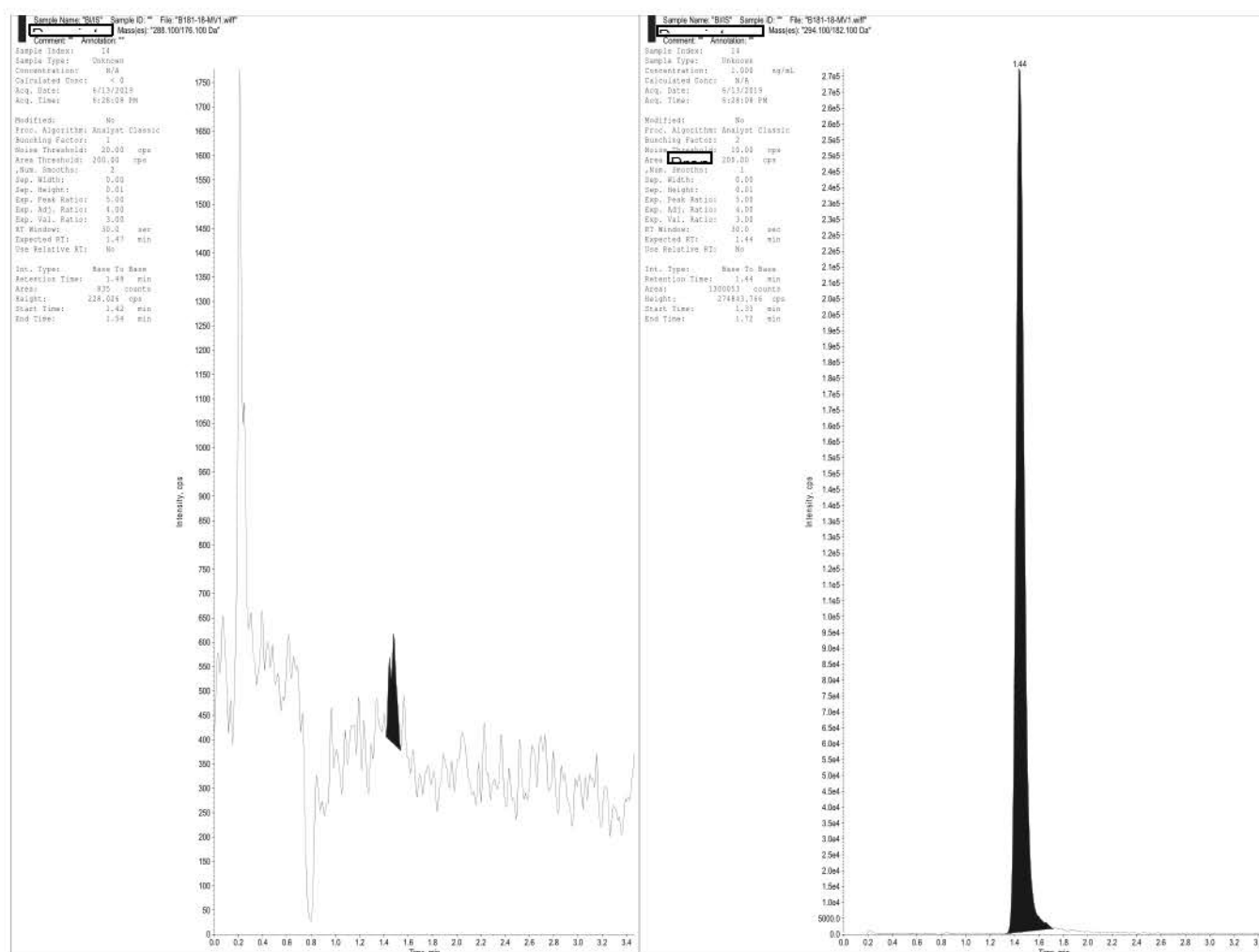
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**Figure 8.** Representative chromatogram of a BI/IS K<sub>2</sub> EDTA rat plasma sample (without Proprietary with internal standard).

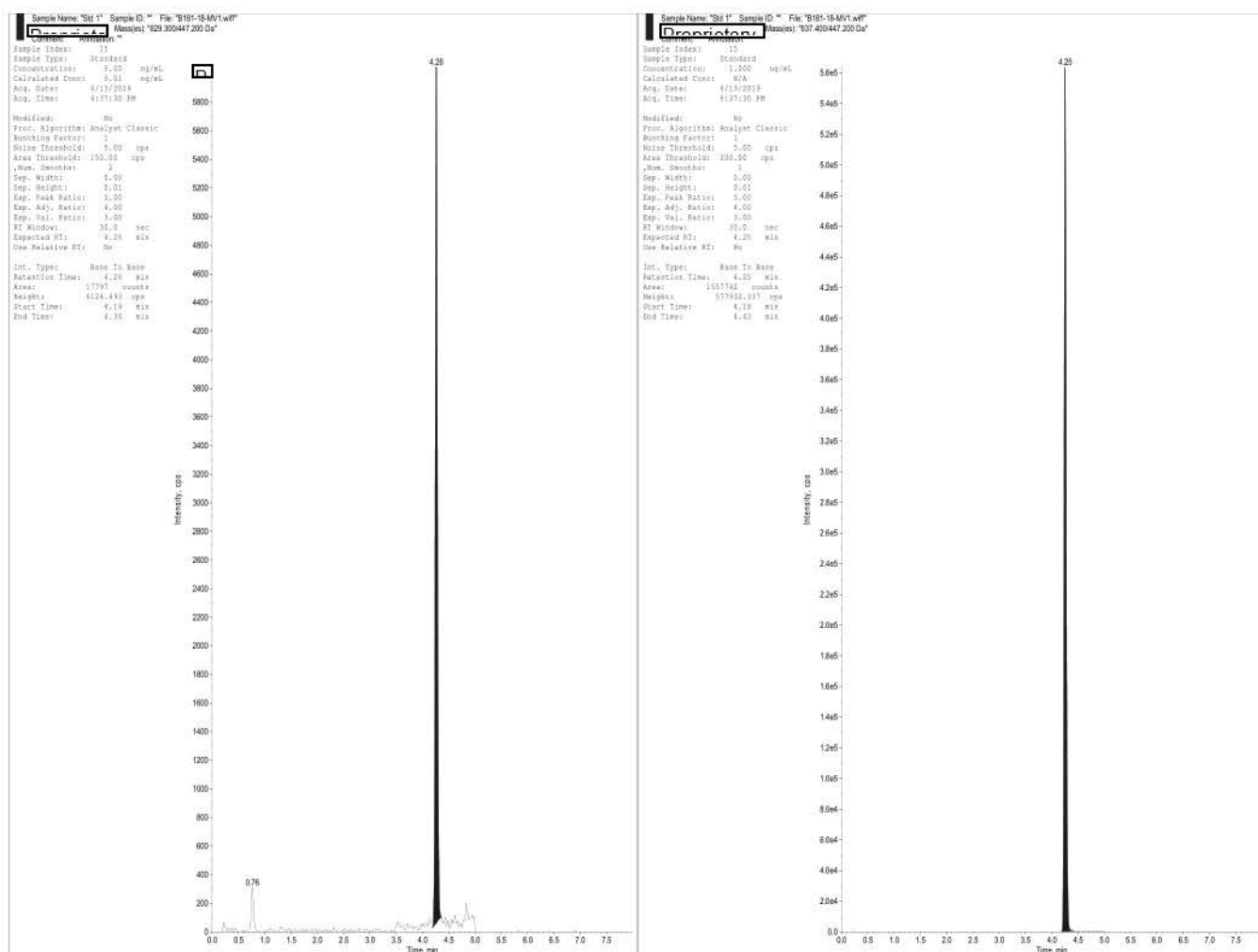


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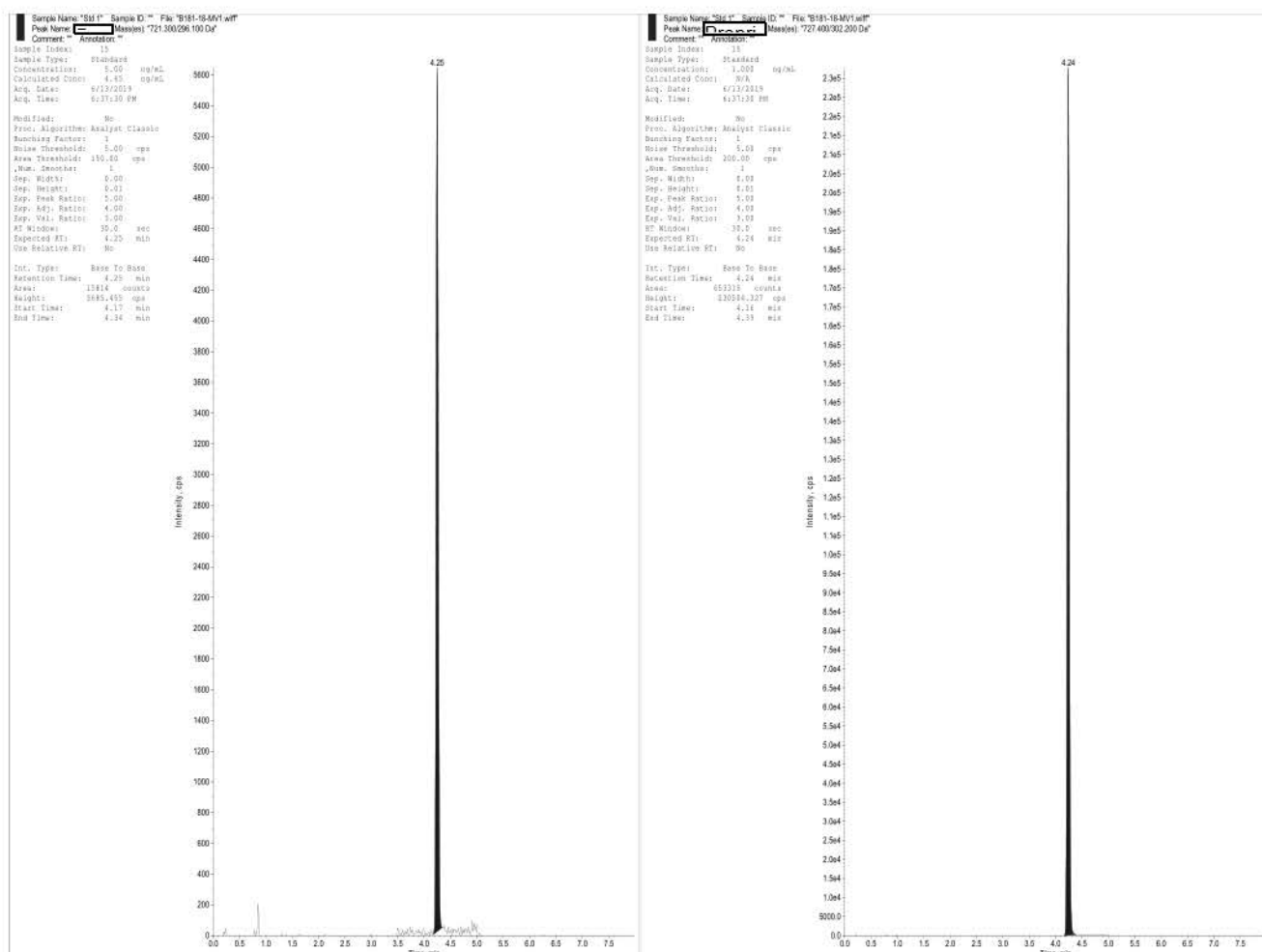
**Figure 9.** Representative chromatogram of a B1/IS K<sub>2</sub> EDTA rat plasma sample (without Proprietary with internal standard).

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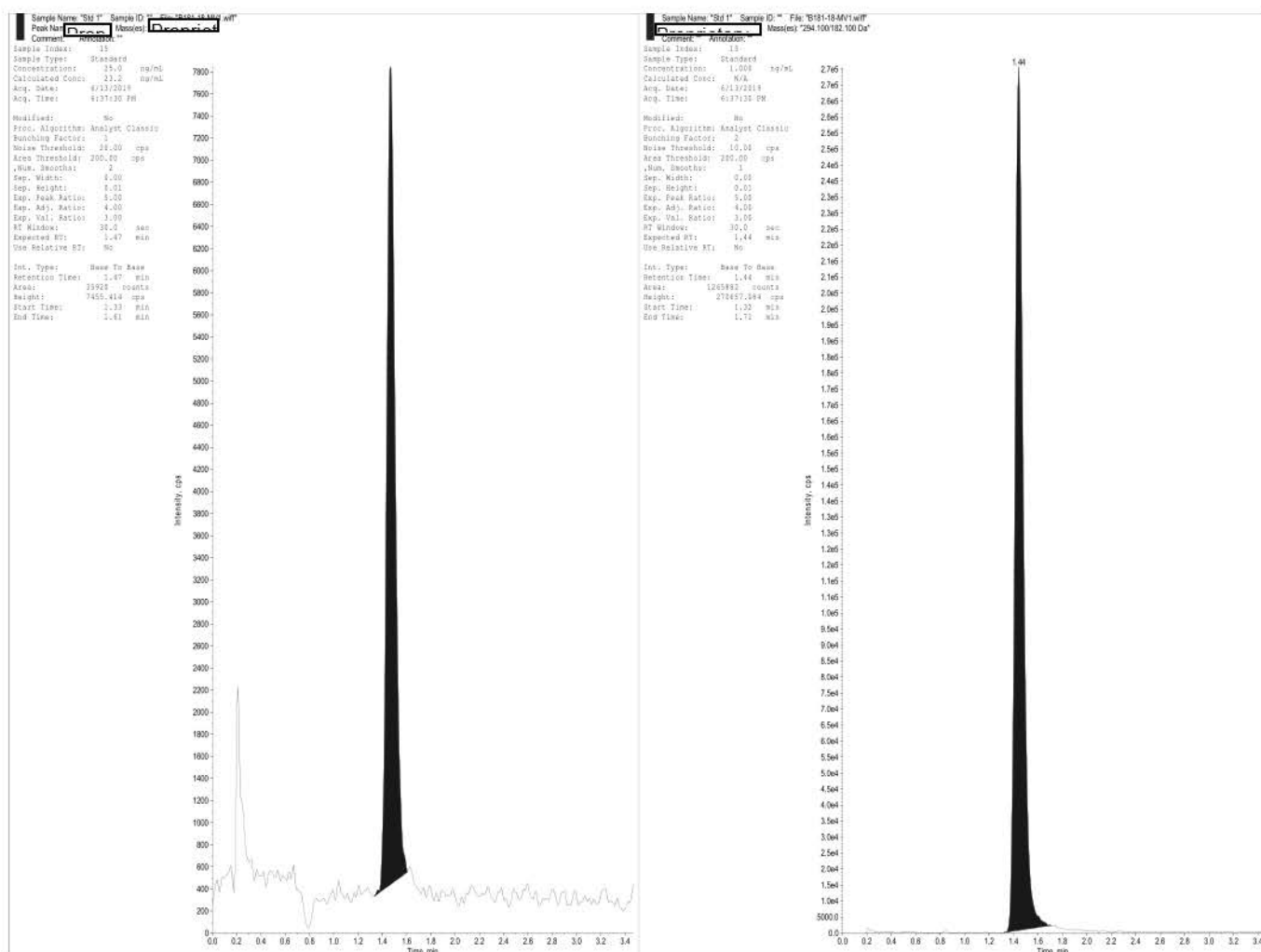
**Figure 10.** Representative chromatogram of a K<sub>2</sub> EDTA rat plasma sample spiked at the Proprietary lower limit of quantitation (LLOQ).

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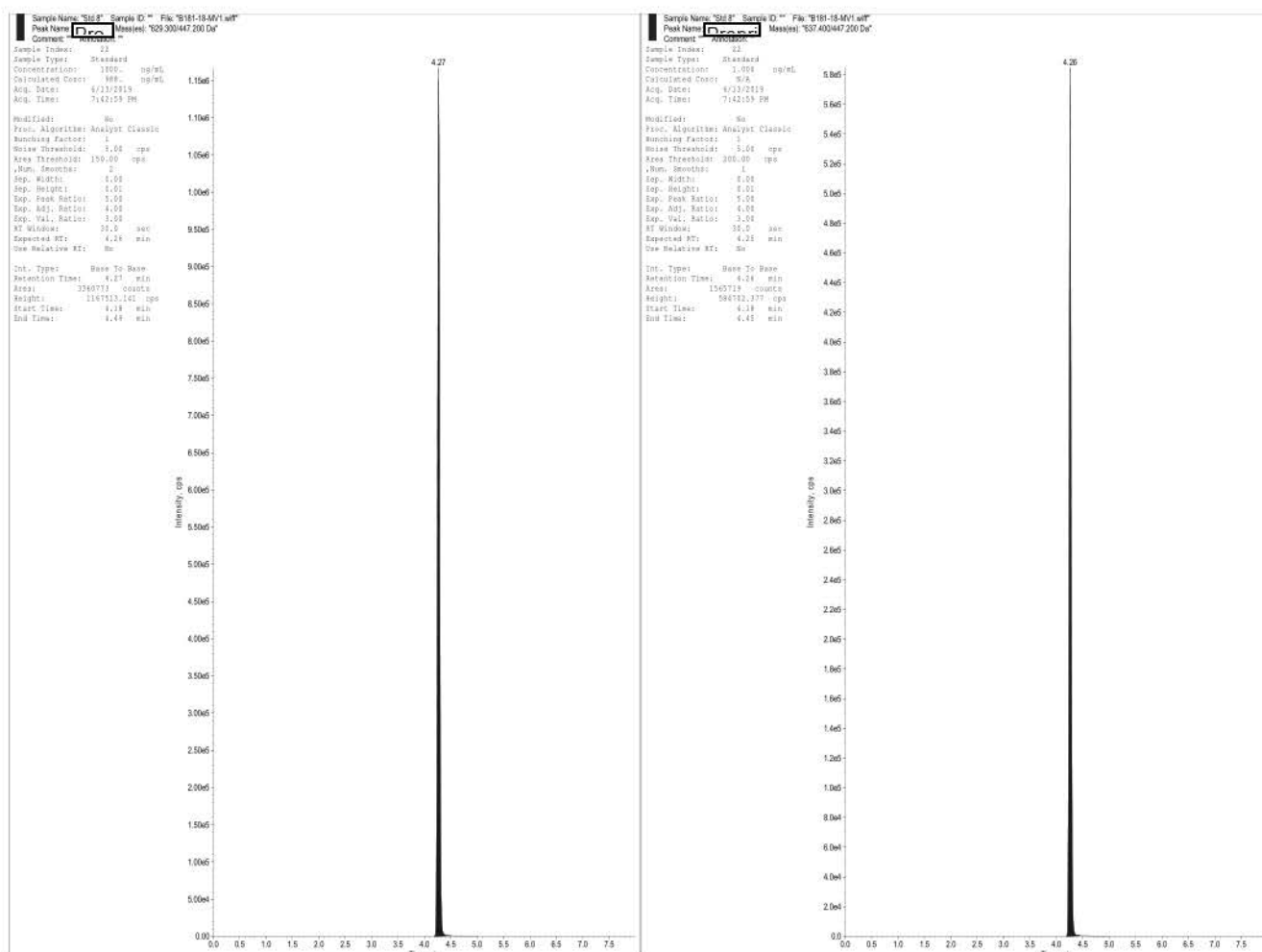
**Figure 11.** Representative chromatogram of a K<sub>2</sub> EDTA rat plasma sample spiked at the Proprietary lower limit of quantitation (LLOQ).

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**Figure 12.** Representative chromatogram of a K<sub>2</sub> EDTA rat plasma sample spiked at the Proprietary lower limit of quantitation (LLOQ).

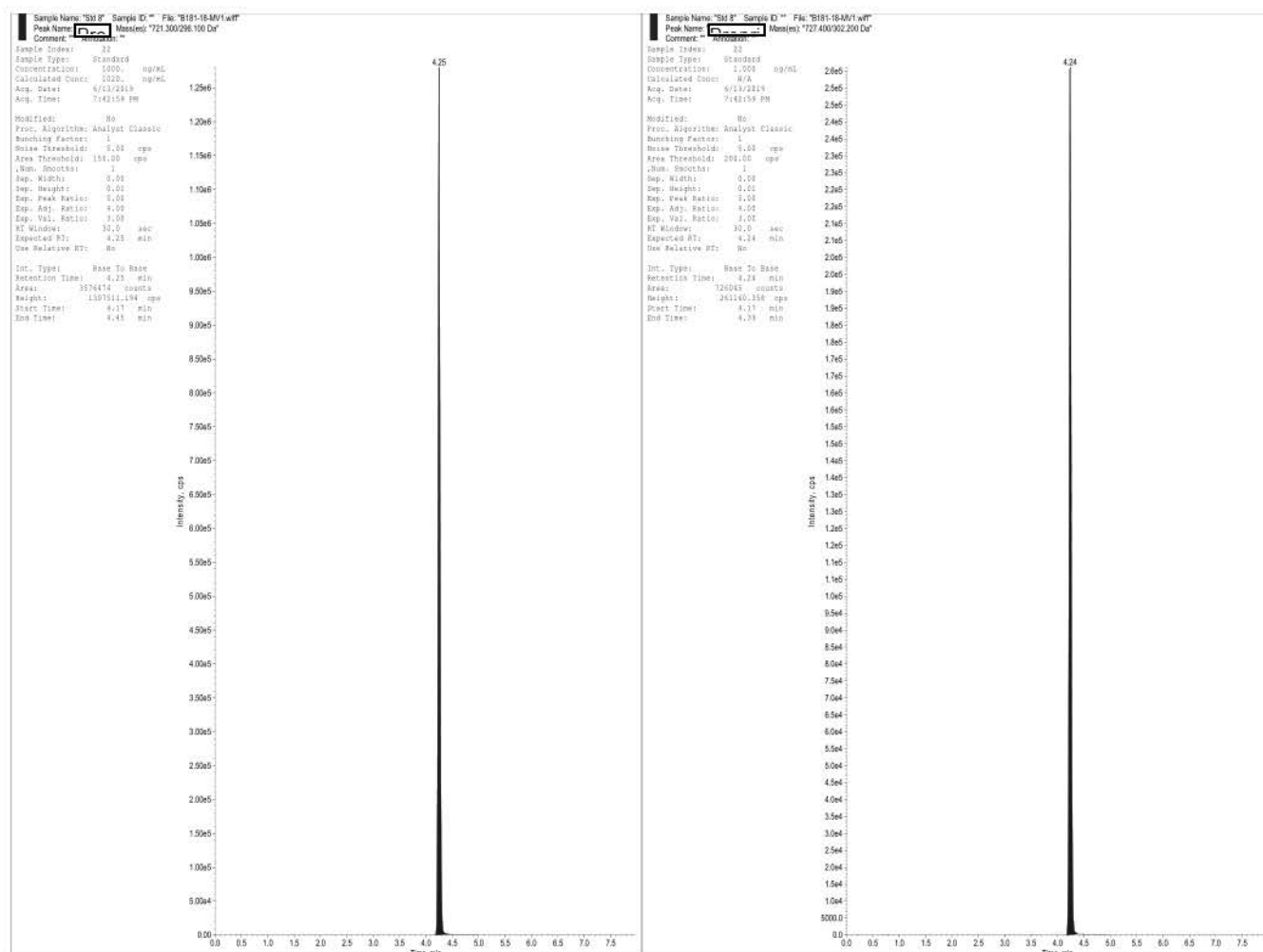
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**Figure 13.** Representative chromatogram of a K<sub>2</sub> EDTA rat plasma sample spiked at the Proprietary upper limit of quantitation (ULOQ).

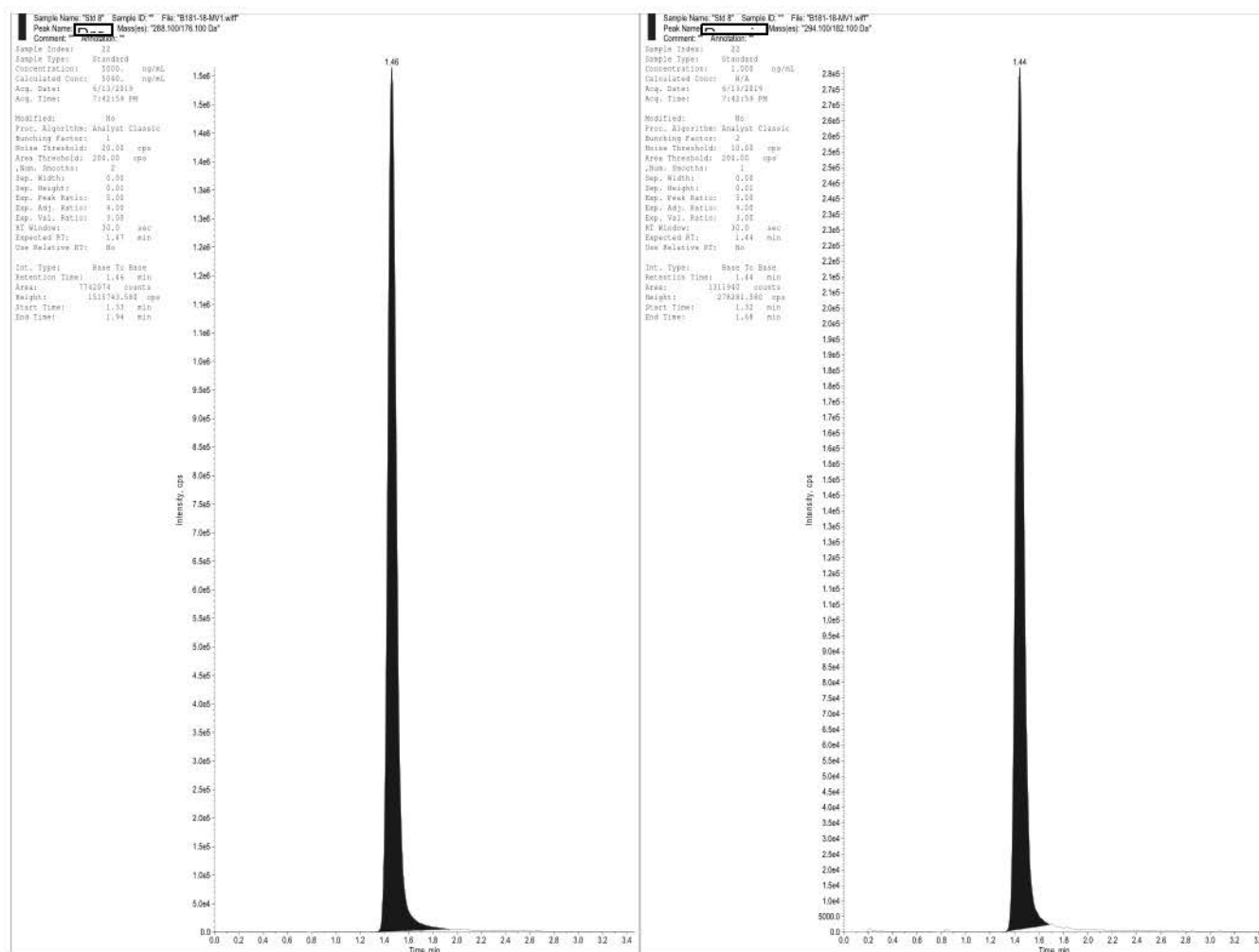


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**Figure 14.** Proprietary Representative chromatogram of a K<sub>2</sub> EDTA rat plasma sample spiked at the Proprietary upper limit of quantitation (ULOQ).

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**Figure 15.** Representative chromatogram of a K<sub>2</sub> EDTA rat plasma sample spiked at the Proprietary upper limit of quantitation (ULOQ).

Proprietary Info    Proprietary



Proprietary

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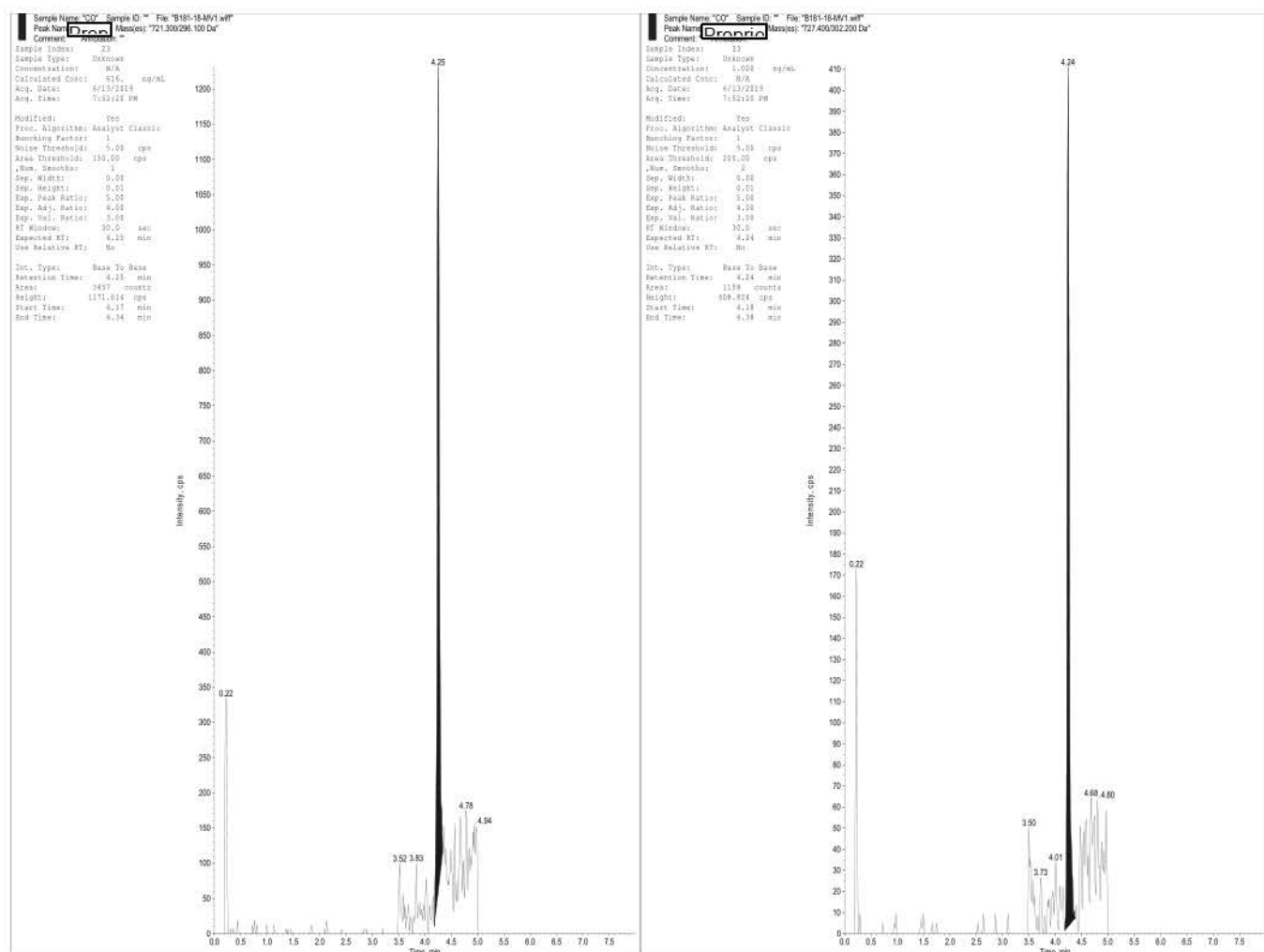
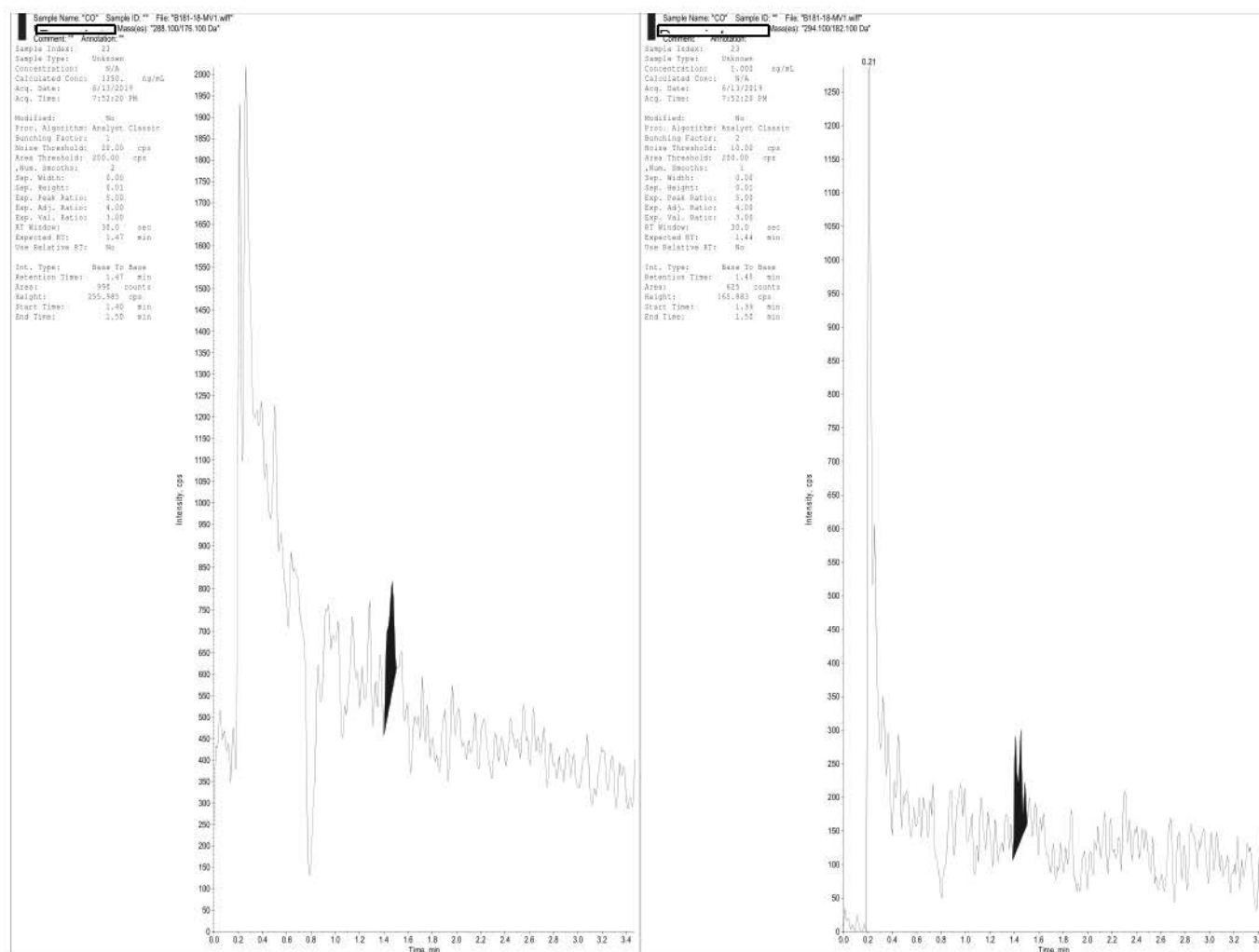


Figure 17. Representative chromatogram of a Proprietary carryover blank sample.

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**Figure 18.** Representative chromatogram of a Proprietary carryover blank sample.



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**Table 1**

**Summary of Validation Runs**

Run Number	Date Extracted	Date Analyzed	Outcome (Pass/Fail)	Parameters Investigated
MV1	13-Jun-19	13-Jun-19	Pass	Accuracy, Precision, Carryover, Recovery, Matrix Effect on Ionization, Effect of Dilution, Maximum Batch Size
MV1-RI	13-Jun-19	17-Jun-19	Pass	Reinjection (Autosampler) Stability
MV2	14-Jun-19	14-Jun-19	Pass	Selectivity, Matrix Effects using 6 Unique Lots of Plasma, ULOQ/BL, Carryover, Concomitant Medication
MV3	18-Jun-19	18-Jun-19	Pass	Room Temperature Stability, Post-Preparative Extract Stability, Carryover
MV4	20-Jun-19	20-Jun-19	Pass	Carryover, Whole Blood Stability, Effect of Hemolysis, Freeze-Thaw Stability
MV5	8-Jul-19	8-Jul-19	Pass	Interim Long Term Storage Stability in Matrix

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 2**

**Summary of Calibration Curve Parameters for Lopinavir**

Run Number	Slope	Intercept	Correlation Coefficient (r)
MV1	0.00217	0.000546	0.9994
MV1-RI	0.00213	0.00155	0.9993
MV2	0.00221	0.00126	0.9986
MV3	0.00211	0.00119	0.9994
MV4	0.00214	0.00151	0.9996
MV5	0.00210	0.00131	0.9992

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 3**

**Summary of Calibration Curve Parameters for [Proprietary]**

Run Number	Slope	Intercept	Correlation Coefficient (r)
MV1	0.00484	0.00167	0.9995
MV1-RI	0.00486	0.00183	0.9998
MV2	0.00489	0.00282	0.9998
MV3	0.00466	0.00287	0.9995
MV4	0.00483	0.00160	0.9997
MV5	0.00484	0.00311	0.9996

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 4**

**Summary of Calibration Curve Parameters for [Proprietary]**

Run Number	Slope	Intercept	Correlation Coefficient (r)
MV1	0.00117	0.00121	0.9998
MV1-RI	0.00115	0.00305	0.9998
MV2	0.00120	0.00506	0.9997
MV3	0.00115	0.00154	0.9999
MV4	0.00118	0.000994	0.9999
MV5	0.00117	0.00289	0.9995

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary  
**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
**SRI Study No. B181-18**

**Table 5**

**Back-Calculated Concentrations of** Proprietary **Calibration Standards (ng/ml)**

Run Number	Standard Description															
	5.00	%Acc	10.0	%Acc	20.0	%Acc	50.0	%Acc	100	%Acc	200	%Acc	500	%Acc	1000	%Acc
MV1	5.01	100.1	9.88	98.8	19.8	98.8	51.5	103.1	99.9	99.9	205	102.7	472	94.5	988	98.8
	4.93	98.6	10.3	103.0	20.2	100.8	49.9	99.8	102	102.5	210	105.2	473	94.5	989	98.9
MV1-RI <sup>a</sup>	4.95	98.9	10.4	104.3	20.5	102.3	50.7	101.4	100	100.3	200	99.8	471	94.2	1010	101.1
	4.86	97.2	9.90	99.0	21.0	105.2	51.0	102.0	103	102.5	199	99.3	472	94.5	980	98.0
MV2	4.90	98.0	9.45	94.5	22.0	110.1	48.5	97.0	102	102.3	203	101.7	482	96.3	994	99.4
	4.97	99.3	10.2	102.3	21.5	107.3	51.7	103.5	98.8	98.8	192	95.9	492	98.5	952	95.2
MV3	4.80	96.0	9.82	98.2	19.6	98.2	50.0	100.0	105	104.7	194	97.0	504	100.7	1030	102.6
	5.17	103.4	10.2	102.3	20.5	102.5	51.6	103.1	95.3	95.3	199	99.3	500	100.0	967	96.7
MV4	4.78	95.5	9.85	98.5	20.2	100.8	51.6	103.3	101	101.3	196	98.0	492	98.5	1000	100.4
	5.24	104.9	10.0	100.1	20.0	100.1	49.0	97.9	100	100.4	199	99.5	492	98.4	1030	102.5
MV5	4.87	97.4	9.76	97.6	20.6	103.1	53.1	106.2	100	100.4	209	104.4	483	96.6	999	99.9
	5.18	103.6	9.63	96.3	20.9	104.3	48.0	95.9	97.5	97.5	197	98.7	494	98.8	993	99.3
Mean	4.98		9.92		20.5		50.5		100		200		488		994	
SD	0.164		0.278		0.755		1.68		2.70		6.27		10.7		22.8	
%CV	3.3		2.8		3.7		3.3		2.7		3.1		2.2		2.3	
%Accuracy	99.7		99.2		102.6		101.0		100.3		100.2		97.7		99.4	
n	10		10		10		10		10		10		10		10	

<sup>a</sup> This is a reinjection of run MV1, and is not included in the statistics.



**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary  
**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
**SRI Study No. B181-18**

**Table 6**

**Back-Calculated Concentrations of** Proprietary **Calibration Standards (ng/ml)**

Run Number	Standard Description															
	5.00	%Acc	10.0	%Acc	20.0	%Acc	50.0	%Acc	100	%Acc	200	%Acc	500	%Acc	1000	%Acc
MV1	4.65	93.0	10.3	103.3	19.7	98.4	53.3	106.6	99.0	99.0	206	102.8	468	93.6	1020	101.7
	4.96	99.2	9.48	94.8	20.4	102.0	49.9	99.8	106	106.4	197	98.4	505	101.0	1000	100.0
MV1-RI <sup>a</sup>	4.67	93.5	9.86	98.6	20.6	103.0	51.1	102.2	97.9	97.9	209	104.4	495	99.0	1010	101.5
	4.91	98.1	10.3	102.7	21.2	106.2	48.6	97.2	100	100.3	195	97.4	493	98.7	994	99.4
MV2	4.90	98.0	10.2	101.6	20.2	101.1	50.6	101.2	98.6	98.6	203	101.3	488	97.5	993	99.3
	4.59	91.8	10.0	100.4	20.9	104.5	50.8	101.6	100	100.4	207	103.4	486	97.1	1020	102.3
MV3	4.77	95.4	9.82	98.2	19.5	97.5	51.1	102.2	104	103.9	203	101.7	524	104.9	995	99.5
	4.55	91.0	10.3	102.7	20.8	104.0	49.6	99.1	104	103.5	196	98.2	509	101.8	964	96.4
MV4	4.95	99.0	9.49	94.9	19.8	99.1	48.6	97.2	102	102.1	207	103.4	483	96.6	1020	102.2
	4.88	97.5	10.3	103.5	20.9	104.6	49.9	99.7	104	103.8	198	99.2	486	97.3	999	99.9
MV5	4.77	95.4	10.0	100.0	21.0	105.0	49.9	99.9	99.7	99.7	209	104.6	490	98.0	1010	100.9
	4.64	92.8	10.2	102.0	20.1	100.7	49.8	99.7	100	100.0	212	105.9	476	95.2	1000	100.4
Mean	4.77		10.0		20.3		50.3		102		204		491		1000	
SD	0.152		0.322		0.554		1.24		2.59		5.23		16.7		17.3	
%CV	3.2		3.2		2.7		2.5		2.5		2.6		3.4		1.7	
%Accuracy	95.3		100.1		101.7		100.7		101.7		101.9		98.3		100.3	
n	10		10		10		10		10		10		10		10	

<sup>a</sup> This is a reinjection of run MV1, and is not included in the statistics.

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary  
**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
**SRI Study No. B181-18**

**Table 7**

**Back-Calculated Concentrations of** Proprietary **Calibration Standards (ng/ml)**

Run Number	Standard Description															
	25.0	%Acc	50.0	%Acc	100	%Acc	250	%Acc	500	%Acc	1000	%Acc	2500	%Acc	5000	%Acc
MV1	23.2	92.8	52.6	105.3	99.8	99.8	246	98.5	505	101.1	1050	104.6	2440	97.7	5040	100.8
	24.3	97.2	52.2	104.5	98.5	98.5	260	104.0	501	100.2	969	96.9	2430	97.1	5060	101.3
MV1-RI <sup>a</sup>	24.8	99.1	51.5	103.0	95.1	95.1	252	100.9	501	100.1	1060	105.7	2450	97.9	5040	100.7
	23.2	92.9	50.1	100.2	101	101.0	250	99.8	520	104.0	1010	101.4	2480	99.0	4950	99.0
MV2	23.4	93.4	49.0	97.9	102	102.1	256	102.2	512	102.4	1040	103.6	2510	100.5	4970	99.4
	22.7	90.8	49.3	98.7	107	106.8	250	100.1	510	102.1	1040	103.9	2390	95.5	5020	100.4
MV3	23.5	93.9	49.4	98.7	99.8	99.8	255	102.0	506	101.2	1010	100.8	2560	102.4	4960	99.2
	24.4	97.7	52.8	105.6	101	101.4	248	99.0	496	99.2	990	99.0	2530	101.3	4940	98.9
MV4	25.7	102.7	49.4	98.7	99.6	99.6	243	97.2	508	101.6	1010	100.8	2510	100.2	5000	99.9
	24.2	96.8	49.1	98.2	101	100.9	253	101.1	517	103.4	1020	101.6	2410	96.5	5040	100.9
MV5	23.0	91.8	53.1	106.1	103	102.9	250	99.9	489	97.8	1060	106.2	2480	99.0	5010	100.2
	23.9	95.4	47.9	95.8	102	102.1	242	96.8	526	105.1	1060	106.0	2350	94.2	5030	100.6
Mean	23.8		50.5		101		250		507		1020		2460		5010	
SD	0.874		1.95		2.34		5.71		10.4		30.5		66.8		39.1	
%CV	3.7		3.9		2.3		2.3		2.0		3.0		2.7		0.8	
%Accuracy	95.2		100.9		101.4		100.1		101.4		102.3		98.4		100.2	
n	10		10		10		10		10		10		10		10	

<sup>a</sup> This is a reinjection of run MV1, and is not included in the statistics.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 8**

**Intra-Batch Accuracy and Precision of [Proprietary] QC Samples (ng/ml)**

Run Number	QC Description							
	5.00	%Acc	15.0	%Acc	400	%Acc	800	%Acc
MV1	5.19	103.8	15.3	102.0	423	105.8	766	95.7
	5.52	110.5	15.3	101.9	390	97.4	786	98.3
	4.95	99.1	15.4	102.6	374	93.5	775	96.9
	5.17	103.5	15.3	101.7	395	98.6	806	100.7
	4.96	99.2	15.8	105.0	395	98.7	820	102.5
	4.68	93.6	15.3	101.8	413	103.2	791	98.8
Mean	5.08		15.4		398		791	
SD	0.285		0.193		17.4		19.9	
%CV	5.6		1.3		4.4		2.5	
%Accuracy	101.6		102.5		99.5		98.8	
n	6		6		6		6	

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 9**

**Intra-Batch Accuracy and Precision of [Proprietary] QC Samples (ng/ml)**

Run Number	QC Description							
	5.00	%Acc	15.0	%Acc	400	%Acc	800	%Acc
MV1	5.46	109.3	15.2	101.5	414	103.6	782	97.7
	4.87	97.5	15.6	104.3	392	98.0	794	99.3
	5.06	101.2	15.0	100.0	396	98.9	747	93.4
	4.96	99.1	15.2	101.2	416	104.1	824	103.0
	5.33	106.6	15.4	102.7	395	98.8	786	98.3
	4.74	94.9	15.3	101.8	408	101.9	818	102.3
Mean	5.07		15.3		404		792	
SD	0.276		0.218		10.6		27.7	
%CV	5.4		1.4		2.6		3.5	
%Accuracy	101.4		101.9		100.9		99.0	
n	6		6		6		6	

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 10**

**Intra-Batch Accuracy and Precision of [Proprietary] QC Samples (ng/ml)**

Run Number	QC Description							
	25.0	%Acc	75.0	%Acc	2000	%Acc	4000	%Acc
MV1	24.9	99.5	73.4	97.9	2070	103.6	4010	100.2
	25.0	99.8	76.2	101.6	1920	96.0	4020	100.5
	25.1	100.4	74.7	99.5	1980	98.9	3880	97.0
	25.2	100.8	73.7	98.2	1970	98.5	4050	101.3
	23.4	93.6	77.1	102.8	1980	99.1	3970	99.1
	24.6	98.4	75.6	100.8	2050	102.7	4130	103.3
Mean	24.7		75.1		2000		4010	
SD	0.667		1.45		56.8		84.8	
%CV	2.7		1.9		2.8		2.1	
%Accuracy	98.8		100.2		99.8		100.2	
n	6		6		6		6	



**Method Validation Report for the Quantitative Analysis of** Proprietary Info **Ritonavir,**  
**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
**SRI Study No. B181-18**

**Table 11**

**Selectivity Determination using Six Unique Lots of K<sub>2</sub> EDTA Rat Plasma and  
Effect of Hemolysis** Proprietary Info

**Selectivity Determination using Six Unique Lots of K<sub>2</sub> EDTA Rat Plasma**

Run Number	Peak Area					
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
MV2	1846	1277	885	808	482	350
% Difference from Mean of LLOQ	9.8	6.8	4.7	4.3	2.6	1.9

Peak Area of 1st Lower Limit of Quantitation Calibration Standard:	19377
Peak Area of 2nd Lower Limit of Quantitation Calibration Standard:	<u>18226</u>
Mean:	18802

**Selectivity Determination with Effect of Hemolysis**

Run Number	Peak Area	
	0.5% hemolysis	2% hemolysis
MV4	1462	866
% Difference from Mean of LLOQ	7.1	4.2

Peak Area of 1st Lower Limit of Quantitation Calibration Standard:	19756
Peak Area of 2nd Lower Limit of Quantitation Calibration Standard:	<u>21646</u>
Mean:	20701

**Method Validation Report for the Quantitative Analysis of Proprietary Info Ritonavir,  
and Proprietary in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 12**

**Selectivity Determination using Six Unique Lots of K<sub>2</sub> EDTA Rat Plasma and  
Effect of Hemolysis Proprietary Info**

**Selectivity Determination using Six Unique Lots of K<sub>2</sub> EDTA Rat Plasma**

Run Number	Peak Area					
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
MV2	1229	517	335	197	0	203
% Difference from Mean of LLOQ	7.1	3.0	1.9	1.1	0.0	1.2

Peak Area of 1st Lower Limit of Quantitation Calibration Standard: 18525

Peak Area of 2nd Lower Limit of Quantitation Calibration Standard: 16243

Mean: 17384

**Selectivity Determination with Effect of Hemolysis**

Run Number	Peak Area	
	0.5% hemolysis	2% hemolysis
MV4	708	0
% Difference from Mean of LLOQ	3.8	0.0

Peak Area of 1st Lower Limit of Quantitation Calibration Standard: 18396

Peak Area of 2nd Lower Limit of Quantitation Calibration Standard: 19135

Mean: 18766

**Method Validation Report for the Quantitative Analysis of** Proprietary Info **Ritonavir,**  
**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
**SRI Study No. B181-18**

**Table 13**

**Selectivity Determination using Six Unique Lots of K<sub>2</sub> EDTA Rat Plasma and  
Effect of Hemolysis** Proprietary Info

**Selectivity Determination using Six Unique Lots of K<sub>2</sub> EDTA Rat Plasma**

Run Number	Peak Area					
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
MV2	887	756	762	0	0	1285
% Difference from Mean of LLOQ	2.3	1.9	2.0	0.0	0.0	3.3

Peak Area of 1st Lower Limit of Quantitation Calibration Standard: 41017

Peak Area of 2nd Lower Limit of Quantitation Calibration Standard: 36810

Mean: 38914

**Selectivity Determination with Effect of Hemolysis**

Run Number	Peak Area	
	0.5% hemolysis	2% hemolysis
MV4	310	236
% Difference from Mean of LLOQ	0.9	0.7

Peak Area of 1st Lower Limit of Quantitation Calibration Standard: 35655

Peak Area of 2nd Lower Limit of Quantitation Calibration Standard: 33646

Mean: 34651

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary  
**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
**SRI Study No. B181-18**

**Table 14**

**Selectivity Determination and Carryover of** Proprietary **in Assay BI/BI and BI/IS Samples**

Run Number	Peak Area							
	LLOQ	Mean LLOQ	BI/BI	% Difference	BI/IS	% Difference	Carryover	% Difference
MV1	17797	18120	855	4.7	869	4.8	4460	24.6
	18442		473	2.6	712	3.9	4161	23.0
MV1-RI	19305	18719	1181	6.3	995	5.3	4923	26.3
	18132		5297	28.3	2010	10.7	4806	25.7
MV2	19377	18802	969	5.2	708	3.8	5042	26.8
	18226		3953	21.0	1512	8.0	3660	19.5
MV3	17897	18422	347	1.9	191	1.0	4341	23.6
	18947		3279	17.8	1745	9.5	4260	23.1
MV4	19756	20701	1231	5.9	1715	8.3	5949	28.7
	21646		4941	23.9	1831	8.8	5545	26.8
MV5	19347	19123	519	2.7	413	2.2	5764	30.1
	18899		3966	20.7	1521	8.0	4715	24.7

% Difference is calculated using the mean of the LLOQ peak areas.

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary  
**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
**SRI Study No. B181-18**

**Table 15**

**Selectivity Determination and Carryover of** Proprietary **in Assay BI/BI and BI/IS Samples**

Run Number	Peak Area							
	LLOQ	Mean LLOQ	BI/BI	% Difference	BI/IS	% Difference	Carryover	% Difference
MV1	15814	16862	0	0.0	250	1.5	3457	20.5
	17910		185	1.1	488	2.9	3059	18.1
MV1-RI	17037	17200	0	0.0	248	1.4	3319	19.3
	17362		3802	22.1	1877	10.9	3693	21.5
MV2	18525	17384	0	0.0	348	2.0	3615	20.8
	16243		2520	14.5	1236	7.1	2995	17.2
MV3	17588	17023	0	0.0	437	2.6	3225	18.9
	16458		2750	16.2	1338	7.9	3086	18.1
MV4	18396	18766	0	0.0	345	1.8	3528	18.8
	19135		3630	19.3	1215	6.5	3619	19.3
MV5	18962	18324	0	0.0	224	1.2	3511	19.2
	17685		2943	16.1	1335	7.3	3614	19.7

% Difference is calculated using the mean of the LLOQ peak areas.



**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary  
**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
**SRI Study No. B181-18**

**Table 16**

**Selectivity Determination and Carryover of** Proprietary **in Assay BI/BI and BI/IS Samples**

Run Number	Peak Area							
	LLOQ	Mean LLOQ	BI/BI	% Difference	BI/IS	% Difference	Carryover	% Difference
MV1	35920	37222	307	0.8	835	2.2	990	2.7
	38524		228	0.6	950	2.6	2805	7.5
MV1-RI	38736	37204	230	0.6	362	1.0	1650	4.4
	35671		2259	6.1	1475	4.0	2760	7.4
MV2	41017	38914	423	1.1	1722	4.4	1501	3.9
	36810		2632	6.8	1877	4.8	1194	3.1
MV3	34106	34531	549	1.6	889	2.6	1671	4.8
	34956		2126	6.2	1315	3.8	891	2.6
MV4	35655	34651	845	2.4	146	0.4	508	1.5
	33646		888	2.6	2382	6.9	2385	6.9
MV5	38351	37141	0	0.0	0	0.0	1833	4.9
	35931		1503	4.0	1862	5.0	1715	4.6

% Difference is calculated using the mean of the LLOQ peak areas.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 17**

**Dilution Assessment of [Proprietary] (ng/ml)**

Run Number	Dilution QC Concentration			
	5000 <sup>a</sup>	%Accuracy	5000 <sup>b</sup>	%Accuracy
MV1	4970	99.3	5140	102.7
	4860	97.2	5020	100.4
	4850	97.0	4990	99.9
	4780	95.5	4900	98.0
	4850	97.0	5010	100.2
	4590	91.8	4930	98.7
Mean	4810		5000	
SD	127		81.5	
%CV	2.6		1.6	
%Accuracy	96.3		100.0	
n	6		6	

<sup>a</sup> Dilution QC was prepared at 5000 ng/ml and was subsequently diluted (1:10) with control matrix to give a final concentration of 500 ng/ml.

<sup>b</sup> Dilution QC was prepared at 5000 ng/ml and was subsequently diluted (1:50) with control matrix to give a final concentration of 100 ng/ml.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
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**Table 18**

**Dilution Assessment of [Proprietary] (ng/ml)**

Run Number	Dilution QC Concentration			
	5000 <sup>a</sup>	%Accuracy	5000 <sup>b</sup>	%Accuracy
MV1	4840	96.8	5090	101.7
	4930	98.5	5160	103.1
	4900	97.9	4980	99.7
	4690	93.7	4990	99.8
	4970	99.4	4880	97.6
	4910	98.2	5070	101.4
Mean	4870		5030	
SD	99.7		97.3	
%CV	2.0		1.9	
%Accuracy	97.4		100.5	
n	6		6	

<sup>a</sup> Dilution QC was prepared at 5000 ng/ml and was subsequently diluted (1:10) with control matrix to give a final concentration of 500 ng/ml.

<sup>b</sup> Dilution QC was prepared at 5000 ng/ml and was subsequently diluted (1:50) with control matrix to give a final concentration of 100 ng/ml.

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**Table 19**

**Dilution Assessment of [Proprietary] (ng/ml)**

Run Number	Dilution QC Concentration			
	25000 <sup>a</sup>	%Accuracy	25000 <sup>b</sup>	%Accuracy
MV1	23900	95.4	25000	100.1
	24400	97.7	24100	96.5
	25600	102.4	24700	98.7
	23800	95.1	24200	96.8
	24900	99.6	24900	99.7
	24000	95.9	24900	99.6
Mean	24400		24600	
SD	709		384	
%CV	2.9		1.6	
%Accuracy	97.7		98.6	
n	6		6	

<sup>a</sup> Dilution QC was prepared at 25000 ng/ml and was subsequently diluted (1:10) with control matrix to give a final concentration of 2500 ng/ml.

<sup>b</sup> Dilution QC was prepared at 25000 ng/ml and was subsequently diluted (1:50) with control matrix to give a final concentration of 500 ng/ml.

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**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
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**Table 20**

**Matrix Effects of** Proprietary **(ng/ml) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Low (15.0 ng/ml)											
	Lot 1	%Acc	Lot 2	%Acc	Lot 3	%Acc	Lot 4	%Acc	Lot 5	%Acc	Lot 6	%Acc
MV2	15.9	106.1	13.4	89.3	14.1	94.1	14.1	94.3	13.1	87.1	14.5	96.9
	14.7	98.0	14.1	93.8	14.5	96.7	14.7	98.1	13.0	87.0	13.9	92.6
	13.5	90.2	14.1	94.1	14.3	95.2	14.1	93.8	13.8	92.1	15.4	102.4
	14.6	97.0	14.5	96.8	14.3	95.3	13.5	90.2	14.2	94.4	14.4	96.3
Mean	14.7		14.0		14.3		14.1		13.5		14.6	
SD	0.975		0.468		0.160		0.488		0.556		0.606	
%CV	6.6		3.3		1.1		3.5		4.1		4.2	
%Accuracy	97.8		93.5		95.3		94.1		90.1		97.1	
n	4		4		4		4		4		4	



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**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
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**Table 21**

**Matrix Effects of** Proprietary **(ng/ml) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Low (15.0 ng/ml)											
	Lot 1	%Acc	Lot 2	%Acc	Lot 3	%Acc	Lot 4	%Acc	Lot 5	%Acc	Lot 6	%Acc
MV2	17.1	113.8	14.0	93.4	14.2	94.9	14.1	94.2	14.7	98.1	13.7	91.3
	15.1	100.5	14.1	94.3	13.7	91.2	13.9	92.7	14.2	94.7	14.4	96.0
	14.7	98.0	13.7	91.1	14.2	94.9	15.0	100.1	13.6	90.7	14.3	95.1
	14.4	95.7	14.3	95.4	13.6	90.4	14.9	99.3	14.0	93.6	14.2	95.0
Mean	15.3		14.0		13.9		14.5		14.1		14.2	
SD	1.21		0.276		0.358		0.548		0.453		0.312	
%CV	7.9		2.0		2.6		3.8		3.2		2.2	
%Accuracy	102.0		93.6		92.8		96.6		94.3		94.4	
n	4		4		4		4		4		4	

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**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
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**Table 22**

**Matrix Effects of** Proprietary **(ng/ml) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Low (75.0 ng/ml)											
	Lot 1	%Acc	Lot 2	%Acc	Lot 3	%Acc	Lot 4	%Acc	Lot 5	%Acc	Lot 6	%Acc
MV2	69.0	92.0	69.3	92.4	67.2	89.6	71.2	94.9	71.4	95.3	67.4	89.8
	69.7	92.9	69.9	93.2	69.9	93.2	72.3	96.4	66.7	89.0	69.0	92.0
	67.1	89.5	70.3	93.7	68.0	90.7	70.8	94.5	67.7	90.3	69.7	92.9
	66.6	88.7	69.9	93.1	73.7	98.3	71.4	95.2	68.9	91.9	70.8	94.3
Mean	68.1		69.8		69.7		71.4		68.7		69.2	
SD	1.49		0.407		2.90		0.635		2.04		1.42	
%CV	2.2		0.6		4.2		0.9		3.0		2.1	
%Accuracy	90.8		93.1		93.0		95.3		91.6		92.3	
n	4		4		4		4		4		4	

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary  
**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
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**Table 23**

**Recovery of** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**

Run Number	Peak Area					
	Low QC extract	Low QC post extraction spiked	Mid QC extract	Mid QC post extraction spiked	High QC extract	High QC post extraction spiked
MV1	53845	53641	1379406	1312738	2522319	2717466
	53137	52948	1255348	1231084	2804629	2729669
	51077	52688	1263283	1347451	2490972	2789947
	47911	53892	1311973	1359127	2587012	2754808
	54035	54902	1355466	1354431	2627070	2693284
	51734	56257	1392203	1356775	2524504	2736188
Mean	51957	54055	1326280	1326934	2592751	2736894
SD	2301	1331	58694	49985	114961	33068
%CV	4.4	2.5	4.4	3.8	4.4	1.2
%Recovery	96.1	NA	100.0	NA	94.7	NA
n	6	6	6	6	6	6
Mean % Recovery	96.9					

NA: Not Applicable.

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary  
**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
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**Table 24**

**Recovery of** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**

Run Number	Peak Area					
	Low QC extract	Low QC post extraction spiked	Mid QC extract	Mid QC post extraction spiked	High QC extract	High QC post extraction spiked
MV1	55497	57299	1482614	1438319	2711974	2859331
	57868	57403	1342190	1402299	3066155	2929551
	54323	61060	1427042	1494283	2694348	3025907
	50681	61363	1433258	1417238	2770408	3087390
	54462	58057	1489878	1433629	2760175	2976746
	53565	58413	1455134	1449884	2709739	2933969
Mean	54399	58933	1438353	1439275	2785467	2968816
SD	2357	1815	53473	31699	140777	80156
%CV	4.3	3.1	3.7	2.2	5.1	2.7
%Recovery	92.3	NA	99.9	NA	93.8	NA
n	6	6	6	6	6	6
Mean % Recovery	95.3					

NA: Not Applicable.

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary  
**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
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**Table 25**

**Recovery of** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**

Run Number	Peak Area					
	Low QC extract	Low QC post extraction spiked	Mid QC extract	Mid QC post extraction spiked	High QC extract	High QC post extraction spiked
MV1	114405	101497	3133853	2984007	5954256	5780115
	117741	97545	2850410	2958582	6453923	5973886
	113256	99635	3008367	2950847	5817222	5987415
	108614	101950	2966484	2928664	5947200	5836982
	117743	101940	3075688	3003579	5879210	5915215
	115334	99354	3098207	3000366	6062874	5990154
Mean	114516	100320	3022168	2971008	6019114	5913961
SD	3403	1776	103838	29818	228335	87902
%CV	3.0	1.8	3.4	1.0	3.8	1.5
%Recovery	114.2	NA	101.7	NA	101.8	NA
n	6	6	6	6	6	6
Mean % Recovery	105.9					

NA: Not Applicable.



**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary  
**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
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**Table 26**

**Recovery of** Proprietary Info **(Internal Standard) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	Peak Area					
	Low QC extract	Low QC post extraction spiked	Mid QC extract	Mid QC post extraction spiked	High QC extract	High QC post extraction spiked
MV1	1593567	1569079	1499054	1525938	1515583	1461479
	1573535	1575166	1481499	1477614	1641254	1548664
	1502801	1648663	1552557	1541938	1478179	1551602
	1422159	1655182	1529348	1588156	1476606	1630031
	1553319	1624533	1578892	1541895	1473695	1571140
	1534131	1638128	1551259	1572056	1468826	1586158
Mean	1529919	1618459	1532102	1541266	1509024	1558179
SD	61427	37409	36428	38559	66924	55854
%CV	4.0	2.3	2.4	2.5	4.4	3.6
%Recovery	94.5	NA	99.4	NA	96.8	NA
n	6	6	6	6	6	6
Mean % Recovery	96.9					

NA: Not Applicable.

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary  
**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
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**Table 27**

**Recovery of** Proprietary Info **(Internal Standard) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	Peak Area					
	Low QC extract	Low QC post extraction spiked	Mid QC extract	Mid QC post extraction spiked	High QC extract	High QC post extraction spiked
MV1	735570	787420	737944	761876	715885	732125
	747281	780455	706087	739942	796351	766222
	730772	810677	744225	768154	743835	769141
	673943	811230	710027	749702	694025	774559
	713621	808064	777326	743312	724276	756099
	707885	799544	736516	750023	683357	745981
Mean	718179	799565	735354	752168	726288	757355
SD	26044	12996	25871	10848	40529	16006
%CV	3.6	1.6	3.5	1.4	5.6	2.1
%Recovery	89.8	NA	97.8	NA	95.9	NA
n	6	6	6	6	6	6
Mean % Recovery	94.5					

NA: Not Applicable.

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary  
**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
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**Table 28**

**Recovery of** Proprietary Info **(Internal Standard) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	Peak Area					
	Low QC extract	Low QC post extraction spiked	Mid QC extract	Mid QC post extraction spiked	High QC extract	High QC post extraction spiked
MV1	1311699	1307041	1290555	1264872	1267808	1279313
	1301761	1332823	1266714	1272894	1370012	1304128
	1277643	1333312	1298353	1306930	1280357	1287020
	1241225	1305884	1285101	1289230	1252747	1301792
	1286389	1326607	1324271	1302363	1265677	1305087
	1284497	1302509	1287215	1298364	1252740	1312739
Mean	1283869	1318029	1292035	1289109	1281557	1298347
SD	24326	14388	18941	16907	44559	12557
%CV	1.9	1.1	1.5	1.3	3.5	1.0
%Recovery	97.4	NA	100.2	NA	98.7	NA
n	6	6	6	6	6	6
Mean % Recovery	98.8					

NA: Not Applicable.

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary  
**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
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**Table 29**

**Matrix Effect on Ionization of** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**

Run Number	Peak Area					
	Low QC post extraction spiked	Low QC recovery solution	Mid QC post extraction spiked	Mid QC recovery solution	High QC post extraction spiked	High QC recovery solution
MV1	53641	52413	1312738	1198150	2717466	2427403
	52948	49261	1231084	1193425	2729669	2398602
	52688	49638	1347451	1204472	2789947	2440410
	53892	49571	1359127	1221350	2754808	2370876
	54902	47821	1354431	1213693	2693284	2456840
	56257	45495	1356775	1201340	2736188	2385616
Mean	54055	49033	1326934	1205405	2736894	2413291
SD	1331	2286	49985	10358	33068	33502
%CV	2.5	4.7	3.8	0.9	1.2	1.4
% Matrix Effect	110.2	NA	110.1	NA	113.4	NA
n	6	6	6	6	6	6
Mean % Matrix Effect	111.2					

Values >100% indicate matrix enhancement; values <100% indicate matrix suppression.

NA: Not Applicable.

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**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
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**Table 30**

**Matrix Effect on Ionization of** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**

Run Number	Peak Area					
	Low QC post extraction spiked	Low QC recovery solution	Mid QC post extraction spiked	Mid QC recovery solution	High QC post extraction spiked	High QC recovery solution
MV1	57299	50197 <sup>a</sup>	1438319	875402	2859331	1776654
	57403	39527	1402299	871397	2929551	1798964
	61060	35722	1494283	890693	3025907	1826463
	61363	36066	1417238	866496	3087390	1900654
	58057	35438	1433629	891766	2976746	1853302
	58413	35153	1449884	877802	2933969	1805252
Mean	58933	36381	1439275	878926	2968816	1826882
SD	1815	1791	31699	10278	80156	44521
%CV	3.1	4.9	2.2	1.2	2.7	2.4
% Matrix Effect	162.0	NA	163.8	NA	162.5	NA
n	6	5	6	6	6	6
Mean % Matrix Effect	162.8					

Values >100% indicate matrix enhancement; values <100% indicate matrix suppression.

NA: Not Applicable.

<sup>a</sup> Result is a statistical outlier according to Grubbs test, and therefore this value was excluded from the statistics.



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**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
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**Table 31**

**Matrix Effect on Ionization of** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**

Run Number	Peak Area					
	Low QC post extraction spiked	Low QC recovery solution	Mid QC post extraction spiked	Mid QC recovery solution	High QC post extraction spiked	High QC recovery solution
MV1	101497	94519	2984007	2888905	5780115	5749659
	97545	95337	2958582	2946402	5973886	5802451
	99635	95924	2950847	2920929	5987415	5828005
	101950	94862	2928664	2948847	5836982	5645156
	101940	97025	3003579	2942551	5915215	5804011
	99354	94250	3000366	2913606	5990154	5704277
Mean	100320	95320	2971008	2926873	5913961	5755593
SD	1776	1026	29818	23507	87902	70212
%CV	1.8	1.1	1.0	0.8	1.5	1.2
% Matrix Effect	105.2	NA	101.5	NA	102.8	NA
n	6	6	6	6	6	6
Mean % Matrix Effect	103.2					

Values >100% indicate matrix enhancement; values <100% indicate matrix suppression.

NA: Not Applicable.

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**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
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**Table 32**

**Matrix Effect on Ionization of** Proprietary Info **(Internal Standard) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	Peak Area					
	Low QC post extraction spiked	Low QC recovery solution	Mid QC post extraction spiked	Mid QC recovery solution	High QC post extraction spiked	High QC recovery solution
MV1	1569079	1515727	1525938	1392185	1461479	1363933
	1575166	1468464	1477614	1390439	1548664	1427395
	1648663	1468658	1541938	1379940	1551602	1415136
	1655182	1471485	1588156	1376250	1630031	1430975
	1624533	1473382	1541895	1362403	1571140	1361271
	1638128	1417120	1572056	1354048	1586158	1363624
Mean	1618459	1469139	1541266	1375878	1558179	1393722
SD	37409	31307	38559	15184	55854	34137
%CV	2.3	2.1	2.5	1.1	3.6	2.4
% Matrix Effect	110.2	NA	112.0	NA	111.8	NA
n	6	6	6	6	6	6
Mean % Matrix Effect	111.3					

Values >100% indicate matrix enhancement; values <100% indicate matrix suppression.

NA: Not Applicable.

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary  
**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
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**Table 33**

**Matrix Effect on Ionization of** Proprietary Info **(Internal Standard) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	Peak Area					
	Low QC post extraction spiked	Low QC recovery solution	Mid QC post extraction spiked	Mid QC recovery solution	High QC post extraction spiked	High QC recovery solution
MV1	787420	650299 <sup>a</sup>	761876	452502	732125	452975
	780455	526383	739942	440449	766222	476573
	810677	490975	768154	431090	769141	474164
	811230	479730	749702	445194	774559	472062
	808064	470927	743312	442800	756099	478903
	799544	480378	750023	445204	745981	466294
Mean	799565	489679	752168	442873	757355	470162
SD	12996	21714	10848	7046	16006	9460
%CV	1.6	4.4	1.4	1.6	2.1	2.0
% Matrix Effect	163.3	NA	169.8	NA	161.1	NA
n	6	5	6	6	6	6
Mean % Matrix Effect	164.7					

Values >100% indicate matrix enhancement; values <100% indicate matrix suppression.

NA: Not Applicable.

<sup>a</sup> Result is a statistical outlier according to Grubbs test, and therefore this value was excluded from the statistics.

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**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
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**Table 34**

**Matrix Effect on Ionization of** Proprietary Info **(Internal Standard) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	Peak Area					
	Low QC post extraction spiked	Low QC recovery solution	Mid QC post extraction spiked	Mid QC recovery solution	High QC post extraction spiked	High QC recovery solution
MV1	1307041	1298117	1264872	1275818	1279313	1245723
	1332823	1324914	1272894	1225841	1304128	1250800
	1333312	1276158	1306930	1274427	1287020	1266744
	1305884	1307892	1289230	1270852	1301792	1224063
	1326607	1305619	1302363	1292046	1305087	1258476
	1302509	1291080	1298364	1273589	1312739	1244397
Mean	1318029	1300630	1289109	1268762	1298347	1248367
SD	14388	16515	16907	22333	12557	14555
%CV	1.1	1.3	1.3	1.8	1.0	1.2
% Matrix Effect	101.3	NA	101.6	NA	104.0	NA
n	6	6	6	6	6	6
Mean % Matrix Effect	102.3					

Values >100% indicate matrix enhancement; values <100% indicate matrix suppression.

NA: Not Applicable.

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and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
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**Table 35**

**Room Temperature Matrix Stability of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Description			
	15.0	%Accuracy	800	%Accuracy
MV3 (25 hours )	15.4	102.7	822	102.8
	15.3	102.1	780	97.5
	15.6	103.9	776	97.0
	15.2	101.3	752	94.1
Mean	15.4		783	
SD	0.165		29.1	
%CV	1.1		3.7	
%Accuracy	102.5		97.8	
n	4		4	

Quality Control samples were prepared on 06-13-19 and stored in a  $\leq -60^{\circ}\text{C}$  freezer (Unit 4409) prior to thawing and storing at room temperature before analysis.



**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
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**Table 36**

**Room Temperature Matrix Stability of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Description			
	15.0	%Accuracy	800	%Accuracy
MV3 (25 hours )	14.8	99.0	796	99.5
	15.5	103.2	760	95.1
	14.7	98.0	799	99.9
	14.2	94.6	767	95.8
Mean	14.8		781	
SD	0.534		19.8	
%CV	3.6		2.5	
%Accuracy	98.7		97.6	
n	4		4	

Quality Control samples were prepared on 06-13-19 and stored in a  $\leq -60^{\circ}\text{C}$  freezer (Unit 4409) prior to thawing and storing at room temperature before analysis.

**Method Validation Report for the Quantitative Analysis of** Proprietary Info Proprietary  
**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
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**Table 37**

**Room Temperature Matrix Stability of** Proprietary **(ng/ml) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Description			
	75.0	%Accuracy	4000	%Accuracy
MV3 (25 hours )	74.0	98.6	3960	99.1
	78.8	105.1	4000	100.0
	76.7	102.2	3990	99.7
	71.9	95.9	3970	99.2
Mean	75.3		3980	
SD	3.03		16.9	
%CV	4.0		0.4	
%Accuracy	100.5		99.5	
n	4		4	

Quality Control samples were prepared on 06-13-19 and stored in a  $\leq -60^{\circ}\text{C}$  freezer (Unit 4409) prior to thawing and storing at room temperature before analysis.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 38**

**Freeze Thaw Matrix Stability of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Description			
	15.0	%Accuracy	800	%Accuracy
MV4	15.4	102.9	795	99.4
(5 cycles)	15.0	99.7	747	93.3
	14.0	93.5	737	92.1
	13.8	92.3	761	95.1
Mean	14.6		760	
SD	0.760		25.5	
%CV	5.2		3.4	
%Accuracy	97.1		95.0	
n	4		4	

Quality Control samples were prepared on 06-13-19 and stored in a  $\leq -60^{\circ}\text{C}$  freezer (Unit 4409) during freeze-thaw cycling.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 39**

**Freeze Thaw Matrix Stability of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Description			
	15.0	%Accuracy	800	%Accuracy
MV4	15.7	104.6	755	94.4
(5 cycles)	15.1	100.9	776	97.0
	14.3	95.2	754	94.3
	15.0	100.2	795	99.4
Mean	15.0		770	
SD	0.580		19.5	
%CV	3.9		2.5	
%Accuracy	100.2		96.3	
n	4		4	

Quality Control samples were prepared on 06-13-19 and stored in a  $\leq -60^{\circ}\text{C}$  freezer (Unit 4409) during freeze-thaw cycling.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 40**

**Freeze Thaw Matrix Stability of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Description			
	75.0	%Accuracy	4000	%Accuracy
MV4 (5 cycles)	71.5	95.3	3890	97.2
	75.8	101.0	3820	95.5
	71.3	95.1	3770	94.2
	73.8	98.4	3770	94.4
Mean	73.1		3810	
SD	2.11		55.1	
%CV	2.9		1.4	
%Accuracy	97.5		95.3	
n	4		4	

Quality Control samples were prepared on 06-13-19 and stored in a  $\leq -60^{\circ}\text{C}$  freezer (Unit 4409) during freeze-thaw cycling.



**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 41**

**Reinjection (Autosampler) Stability of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Description							
	5.00	%Accuracy	15.0	%Accuracy	400	%Accuracy	800	%Accuracy
MV1-RI (94 hours, Refrigerated)	4.86	97.2	15.2	101.0	413	103.2	790	98.7
	4.51	90.2	14.9	99.2	394	98.4	806	100.7
	4.62	92.4	15.6	104.0	398	99.5	797	99.7
	4.65	93.1	14.9	99.3	390	97.5	816	102.0
	4.67	93.3	15.7	104.6	394	98.4	751	93.9
	4.69	93.7	14.5	96.9	397	99.2	806	100.8
Mean	4.67		15.1		397		794	
SD	0.113		0.444		8.07		22.9	
%CV	2.4		2.9		2.0		2.9	
%Accuracy	93.3		100.8		99.4		99.3	
n	6		6		6		6	

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 42**

**Reinjection (Autosampler) Stability of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Description							
	5.00	%Accuracy	15.0	%Accuracy	400	%Accuracy	800	%Accuracy
MV1-RI (94 hours, Refrigerated)	5.30	105.9	15.5	103.2	407	101.9	820	102.5
	5.04	100.7	15.2	101.1	379	94.7	786	98.2
	4.89	97.9	16.3	108.7	399	99.7	804	100.5
	4.79	95.8	15.1	100.7	412	102.9	789	98.7
	4.90	98.0	15.2	101.5	399	99.9	825	103.2
	4.88	97.6	15.2	101.6	389	97.1	796	99.5
Mean	4.97		15.4		397		803	
SD	0.179		0.456		12.2		16.3	
%CV	3.6		3.0		3.1		2.0	
%Accuracy	99.3		102.8		99.4		100.4	
n	6		6		6		6	

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 43**

**Reinjection (Autosampler) Stability of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Description							
	25.0	%Accuracy	75.0	%Accuracy	2000	%Accuracy	4000	%Accuracy
MV1-RI (94 hours, Refrigerated)	24.2	96.8	69.6	92.8	1990	99.4	3970	99.3
	22.5	90.0	74.1	98.9	2010	100.4	4010	100.2
	23.4	93.4	73.9	98.5	1990	99.7	4010	100.3
	23.5	94.0	71.9	95.9	2010	100.3	4220	105.4
	23.0	92.1	72.8	97.1	1920	95.9	3950	98.8
	22.5	89.9	72.8	97.1	2040	101.8	4030	100.7
Mean	23.2		72.5		1990		4030	
SD	0.656		1.65		39.9		96.1	
%CV	2.8		2.3		2.0		2.4	
%Accuracy	92.7		96.7		99.6		100.8	
n	6		6		6		6	

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 44**

**Post-Preparative Extract Stability of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Description			
	15.0	%Accuracy	800	%Accuracy
MV3 (99 hours, Refrigerated)	16.2	108.2	782	97.7
	15.3	102.0	822	102.8
	15.7	104.7	863	107.9
	14.3	95.4	791	98.9
Mean	15.4		815	
SD	0.809		36.9	
%CV	5.3		4.5	
%Accuracy	102.6		101.8	
n	4		4	

These samples were originally extracted in batch MV2.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 45**

**Post-Preparative Extract Stability of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Description			
	15.0	%Accuracy	800	%Accuracy
MV3 (99 hours, Refrigerated)	15.9	105.7	830	103.8
	14.6	97.3	830	103.7
	15.6	104.1	834	104.3
	14.6	97.4	807	100.9
Mean	15.2		825	
SD	0.658		12.5	
%CV	4.3		1.5	
%Accuracy	101.1		103.2	
n	4		4	

These samples were originally extracted in batch MV2.



**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 46**

**Post-Preparative Extract Stability of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Description			
	75.0	%Accuracy	4000	%Accuracy
MV3 (99 hours, Refrigerated)	73.3	97.8	4110	102.8
	79.3	105.8	4210	105.4
	76.0	101.3	4140	103.5
	79.8	106.4	4030	100.8
Mean	77.1		4120	
SD	3.04		76.1	
%CV	3.9		1.8	
%Accuracy	102.8		103.1	
n	4		4	

These samples were originally extracted in batch MV2.

**Method Validation Report for the Quantitative Analysis of [Proprietary] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 47**

**Processing Stability of Lopinavir (ng/ml) in K<sub>2</sub> EDTA Rat Whole Blood**

Run Number	QC Description	
	QC Low	QC High
MV4	12.3	595
Time Zero (T0)	10.6	592
	10.9	623
	10.7	635
Mean	11.1	611
SD	0.806	21.1
%CV	7.2	3.4
n	4	4
MV4	10.9	592
1 hour, refrigerated	10.7	591
	11.4	627
	10.9	578
Mean	11.0	597
SD	0.282	21.0
%CV	2.6	3.5
% Difference from T0	-0.9	-2.3
n	4	4
MV4	10.6	624
4 hours, refrigerated	11.2	584
	11.1	615
	10.9	582
Mean	10.9	601
SD	0.224	21.6
%CV	2.0	3.6
% Difference from T0	-1.8	-1.6
n	4	4

Whole blood stability samples were prepared on 06-19-19 and the resulting plasma stored in a  $\leq -60^{\circ}\text{C}$  freezer (Unit 4409) until analysis.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 48**

**Processing Stability of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Rat Whole Blood**

Run Number	QC Description	
	QC Low	QC High
MV4	9.27	521
Time Zero (T0)	9.31	544
	9.71	516
	9.15	551
Mean	9.36	533
SD	0.242	16.9
%CV	2.6	3.2
n	4	4
MV4	10.1	554
1 hour, refrigerated	9.56	541
	9.08	587
	9.68	545
Mean	9.61	557
SD	0.434	21.0
%CV	4.5	3.8
% Difference from T0	2.7	4.5
n	4	4
MV4	10.0	580
4 hours, refrigerated	9.64	573
	9.47	577
	9.89	554
Mean	9.76	571
SD	0.250	12.0
%CV	2.6	2.1
% Difference from T0	4.3	7.1
n	4	4

Whole blood stability samples were prepared on 06-19-19 and the resulting plasma stored in a  $\leq -60^{\circ}\text{C}$  freezer (Unit 4409) until analysis.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 49**

**Processing Stability of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Rat Whole Blood**

Run Number	QC Description	
	QC Low	QC High
MV4	52.3	2900
Time Zero (T0)	53.4	3040
	54.8	3020
	54.5	3080
Mean	53.7	3010
SD	1.15	78.5
%CV	2.1	2.6
n	4	4
MV4	54.2	2940
1 hour, refrigerated	54.3	2900
	53.1	3000
	54.9	2950
Mean	54.1	2950
SD	0.733	41.6
%CV	1.4	1.4
% Difference from T0	0.7	-2.0
n	4	4
MV4	55.7	3110
4 hours, refrigerated	51.8	2880
	54.9	3030
	55.5	2940
Mean	54.5	2990
SD	1.82	99.3
%CV	3.3	3.3
% Difference from T0	1.5	-0.7
n	4	4

Whole blood stability samples were prepared on 06-19-19 and the resulting plasma stored in a  $\leq -60^{\circ}\text{C}$  freezer (Unit 4409) until analysis.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 50**

**Effect of Hemolysis on [Proprietary] Quantitation (ng/ml)**

Run Number	QC Description			
	15.0	%Accuracy	800	%Accuracy
MV4	15.4	102.8	762	95.2
0% hemolysis	14.6	97.0	786	98.3
	14.9	99.2	790	98.8
	14.0	93.2	762	95.3
Mean	14.7		775	
SD	0.606		15.1	
%CV	4.1		2.0	
%Accuracy	98.1		96.9	
n	4		4	
MV4	14.8	99.0	734	91.8
0.5% hemolysis	14.2	94.6	773	96.6
	14.0	93.5	786	98.3
	14.4	96.2	773	96.7
Mean	14.4		767	
SD	0.359		22.5	
%CV	2.5		2.9	
%Accuracy	95.8		95.8	
n	4		4	
MV4	14.4	96.1	799	99.8
2% hemolysis	14.2	94.4	756	94.5
	13.8	92.1	794	99.2
	14.4	95.9	789	98.6
Mean	14.2		784	
SD	0.273		19.3	
%CV	1.9		2.5	
%Accuracy	94.6		98.0	
n	4		4	

Quality Control samples were prepared on 06-19-19 and stored in a  $\leq -60^{\circ}\text{C}$  freezer (Unit 4409) until analysis.



**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 51**

**Effect of Hemolysis on [Proprietary] Quantitation (ng/ml)**

Run Number	QC Description			
	15.0	%Accuracy	800	%Accuracy
MV4	16.4	109.4	795	99.4
0% hemolysis	14.5	96.4	780	97.6
	14.9	99.5	776	97.0
	14.6	97.2	763	95.3
Mean	15.1		778	
SD	0.899		13.3	
%CV	6.0		1.7	
%Accuracy	100.6		97.3	
n	4		4	
MV4	14.1	94.1	790	98.8
0.5% hemolysis	14.6	97.6	783	97.9
	14.7	97.7	787	98.4
	15.1	100.4	763	95.3
Mean	14.6		781	
SD	0.390		12.5	
%CV	2.7		1.6	
%Accuracy	97.5		97.6	
n	4		4	
MV4	14.6	97.2	791	98.9
2% hemolysis	14.8	98.6	771	96.4
	14.5	96.8	719	89.9
	14.7	97.9	742	92.8
Mean	14.6		756	
SD	0.118		31.8	
%CV	0.8		4.2	
%Accuracy	97.6		94.5	
n	4		4	

Quality Control samples were prepared on 06-19-19 and stored in a  $\leq -60^{\circ}\text{C}$  freezer (Unit 4409) until analysis.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 52**

**Effect of Hemolysis on [Proprietary] Quantitation (ng/ml)**

Run Number	QC Description			
	75.0	%Accuracy	4000	%Accuracy
MV4	74.4	99.2	3990	99.7
0% hemolysis	74.1	98.8	3910	97.8
	73.7	98.2	3900	97.4
	73.6	98.1	3880	96.9
Mean	73.9		3920	
SD	0.403		48.7	
%CV	0.5		1.2	
%Accuracy	98.6		98.0	
n	4		4	
MV4	73.1	97.5	3840	96.0
0.5% hemolysis	73.5	97.9	3910	97.7
	71.9	95.8	3900	97.5
	71.4	95.1	3850	96.3
Mean	72.5		3880	
SD	1.00		34.1	
%CV	1.4		0.9	
%Accuracy	96.6		96.9	
n	4		4	
MV4	72.8	97.1	3800	95.1
2% hemolysis	70.7	94.2	3960	98.9
	75.0	100.0	3770	94.2
	73.3	97.7	3740	93.4
Mean	72.9		3820	
SD	1.78		97.9	
%CV	2.4		2.6	
%Accuracy	97.2		95.4	
n	4		4	

Quality Control samples were prepared on 06-19-19 and stored in a  $\leq -60^{\circ}\text{C}$  freezer (Unit 4409) until analysis.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 53**

**Long Term Storage Stability of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Description			
	15.0	%Accuracy	800	%Accuracy
MV5	14.5	96.7	788	98.5
25 days at ≤-60°C	15.4	102.7	769	96.2
	14.7	97.7	787	98.4
	14.5	96.4	791	98.8
Mean	14.8		784	
SD	0.442		9.76	
%CV	3.0		1.2	
%Accuracy	98.4		98.0	
n	4		4	

Quality Control samples were prepared on 06-13-19 and stored in a ≤-60°C freezer (Unit 4409) until analysis.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 54**

**Long Term Storage Stability of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Description			
	15.0	%Accuracy	800	%Accuracy
MV5 25 days at ≤-60°C	14.7	98.0	769	96.1
	15.7	105.0	793	99.1
	14.9	99.2	798	99.8
	14.8	98.4	776	96.9
Mean	15.0		784	
SD	0.487		14.0	
%CV	3.2		1.8	
%Accuracy	100.1		98.0	
n	4		4	

Quality Control samples were prepared on 06-13-19 and stored in a ≤-60°C freezer (Unit 4409) until analysis.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 55**

**Long Term Storage Stability of [Proprietary] (ng/ml) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Description			
	75.0	%Accuracy	4000	%Accuracy
MV5 25 days at ≤-60°C	71.3	95.1	3880	97.1
	75.2	100.3	3920	97.9
	70.6	94.2	3920	97.9
	71.3	95.0	3710	92.8
Mean	72.1		3860	
SD	2.10		98.4	
%CV	2.9		2.6	
%Accuracy	96.1		96.4	
n	4		4	

Quality Control samples were prepared on 06-13-19 and stored in a ≤-60°C freezer (Unit 4409) until analysis.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary]  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 56**

**Effect of Concomitant Medication [Proprietary] and [Proprietary Info] on [Proprietary]  
Quantitation (ng/ml) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Description	
	15.0	%Accuracy
MV2	15.3	101.8
	14.6	97.6
	14.8	98.4
	15.1	100.4
Mean	14.9	
SD	0.285	
%CV	1.9	
%Accuracy	99.5	
n	4	

[Proprietary] Low QC samples (15.0 mg/ml) were spiked with [Proprietary] and [Proprietary] at 2000 ng/ml and 20000 ng/ml, respectively.



**Method Validation Report for the Quantitative Analysis of [Proprietary Info] [Proprietary]  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 57**

**Effect of Concomitant Medication [Proprietary Info] and [Proprietary Info] on [Proprietary]  
Quantitation (ng/ml) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Description	
	15.0	%Accuracy
MV2	15.5	103.0
	15.7	104.3
	14.8	99.0
	14.6	97.2
Mean	15.1	
SD	0.504	
%CV	3.3	
%Accuracy	100.9	
n	4	

[Proprietary] Low QC samples (15.0 mg/ml) were spiked with [Proprietary Info] and [Proprietary] at 2000 ng/ml and 20000 ng/ml, respectively.

**Method Validation Report for the Quantitative Analysis of** [Proprietary Info] [Proprietary]  
**and** [Proprietary] **in K<sub>2</sub> EDTA Rat Plasma**  
**SRI Study No. B181-18**

**Table 58**

**Effect of Concomitant Medication** [Proprietary Info] **and** [Proprietary] **on** [Proprietary]  
**Quantitation (ng/ml) in K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Description	
	75.0	%Accuracy
MV2	75.0	100.1
	71.6	95.5
	72.4	96.5
	74.5	99.4
Mean	73.4	
SD	1.65	
%CV	2.2	
%Accuracy	97.9	
n	4	

[Proprietary] Low QC samples (75.0 mg/ml) were spiked with [Proprietary] and [Proprietary] at 2000 ng/ml.

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 59**

**Batch Acceptance Quality Control (QC) Samples for Analysis of [Proprietary] (ng/ml) in  
K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Description					
	15.0	%Accuracy	400	%Accuracy	800	%Accuracy
MV2	14.3	95.4	398	99.4	789	98.6
	15.1	100.5	388	97.1	788	98.5
MV3	13.8	91.8	376	94.1	758	94.8
	16.0	106.4	387	96.8	799	99.9
MV4	13.8	92.0	387	96.9	758	94.8
	15.2	101.4	364	91.1	775	96.8
MV5	14.8	98.4	387	96.8	779	97.4
	16.1	107.2	390	97.5	736	92.0
Mean	14.9		385		773	
SD	0.891		10.1		20.7	
%CV	6.0		2.6		2.7	
%Accuracy	99.1		96.2		96.6	
n	8		8		8	

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 60**

**Batch Acceptance Quality Control (QC) Samples for Analysis of [Proprietary] (ng/ml) in  
K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Description					
	15.0	%Accuracy	400	%Accuracy	800	%Accuracy
MV2	15.1	100.3	411	102.7	825	103.2
	14.8	98.3	385	96.3	794	99.2
MV3	14.3	95.4	387	96.8	760	95.0
	15.4	102.9	384	95.9	778	97.3
MV4	14.6	97.5	380	94.9	774	96.7
	15.4	102.9	382	95.6	777	97.1
MV5	15.0	99.8	385	96.3	759	94.9
	15.2	101.3	388	97.0	773	96.6
Mean	15.0		388		780	
SD	0.396		9.63		21.3	
%CV	2.6		2.5		2.7	
%Accuracy	99.8		96.9		97.5	
n	8		8		8	

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Table 61**

**Batch Acceptance Quality Control (QC) Samples for Analysis of [Proprietary] (ng/ml) in  
K<sub>2</sub> EDTA Rat Plasma**

Run Number	QC Description					
	75.0	%Accuracy	2000	%Accuracy	4000	%Accuracy
MV2	68.6	91.4	2020	101.1	4050	101.3
	68.2	91.0	1970	98.5	3990	99.8
MV3	73.6	98.2	2060	103.0	4010	100.2
	77.5	103.3	1960	98.2	3970	99.2
MV4	73.2	97.6	1970	98.3	3970	99.2
	73.1	97.5	1910	95.3	3850	96.1
MV5	76.0	101.4	1900	95.2	3870	96.8
	74.5	99.4	1950	97.4	3790	94.8
Mean	73.1		1970		3940	
SD	3.26		53.0		89.6	
%CV	4.5		2.7		2.3	
%Accuracy	97.5		98.4		98.4	
n	8		8		8	

**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Appendix A**

**SRI TEST METHOD 106.202; ANALYSIS OF [Proprietary Info] [Proprietary Info] AND  
[Proprietary Info] IN K<sub>2</sub> EDTA RAT PLASMA**



**TEST METHOD****Classification:** Project**Supersedes:** 106.202 (07/15/19)**Subject:** Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma**TM No.:** 106.202**Page:** 1 of 26**Effective:** AUG 21 2019**A. PURPOSE/SCOPE**

This Test Method describes procedures to be employed for the analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA rat plasma using a protein precipitation extraction procedure and analysis by LC-MS/MS.

During sample analysis, SRI SOPs 006.061 *Bioanalytical Sample Analysis* and 006.062 *Bioanalytical Sample Reanalysis* will also be followed.

**B. BACKGROUND/GENERAL**

This Test Method will fully detail the experimental procedures used in the analysis, detection and quantitation of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA rat plasma. To summarize, [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA rat plasma (0.0200 ml sample size) will be extracted, using [Proprietary Info] [Proprietary Info] and [Proprietary Info] as the internal standard, by a protein precipitation extraction procedure. The supernatant is then diluted prior to injection on the LC-MS/MS system. The range of the assay is 5.00 – 1000 ng/ml [Proprietary Info] and [Proprietary Info] and 25.0 – 5000 ng/ml [Proprietary Info]

**SIGNATURES**

Revised by:

Reviewed by:


Management Approval

QAU Review:

Redacted by agreement

08/20/19  
Date08/20/19  
Date8/20/2019  
Date08/21/2019  
Date

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## C. HEALTH AND SAFETY

All personnel must observe standard laboratory safety practices. All personnel must wear protective equipment appropriate to the area in which they will work, which may include, but not be limited to: safety glasses, protective clothing and gloves.

## D. TRAINING

All personnel involved in handling chemicals, equipment, and instruments must have attended the pertinent laboratory safety classes from SRI's Environmental Health & Safety Department and must have attended GLP training courses. Training must be documented.

## E. EQUIPMENT AND MATERIALS

Chemicals, consumables or equipment may be substituted provided that equivalent assay performance is obtained.


### E.1 Chemicals

- [Proprietary] [Proprietary] and [Proprietary] USP, Current Lot
- [Proprietary Info] [Proprietary Info] and [Proprietary Info] Medical Isotopes, Inc.
- Ammonium hydroxide, reagent grade
- Acetic acid, LC-MS grade
- Milli-Q water, Millipore
- Formic acid, reagent grade
- Dimethyl Sulfoxide (DMSO), reagent grade
- Methanol, HPLC grade
- Acetonitrile, HPLC grade
- Isopropanol, reagent grade
- K<sub>2</sub> EDTA rat plasma, BioIVT

### E.2 Consumables

- HPLC column: Phenomenex Synergi Polar RP 100 x 2mm, 4µm
- 0.5 µm stainless-steel pre-column frit (Upchurch Scientific)
- Assorted disposable pipette tips
- Disposable 1.5 ml and 2.0 ml polypropylene microcentrifuge tubes

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	<b>Subject:</b> Analysis of <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> and <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> in K <sub>2</sub> EDTA Rat Plasma	

- Disposable 15 ml conical polypropylene test tubes and caps
- Disposable glass vials and caps, assorted sizes
- Glass autosampler vials with inserts and caps

### E.3 Equipment

- Air displacement pipettor, Rainin
- Positive displacement pipettor, Gilson
- Repeater pipettor, Eppendorf
- Mettler Toledo AG 285 balance
- VWR Mini Vortexer
- Beckman Coulter Microfuge® 18 Centrifuge, 20 Centrifuge
- Shimadzu Corp. LC-20AD Prominence Pumps (incorporates Shimadzu Corp. CBM-20A Prominence Communications Bus Module and Shimadzu DGU-20A<sub>3R</sub> Prominence degasser
- Shimadzu Corp. CTO-20AC Prominence Column Oven
- CTC Analytics HTS-xt Autosampler
- AB Sciex 5500 Mass Spectrometer

## F. PROCEDURES

Note: SRI Forms 106.202A through 106.202E will be used to assist in raw data recording for the experimental phases of this study. The completed attachments or other documentation must be stored in the study file.

### F.1 Preparation of Reagents


Volumes of these reagents can be adjusted as long as proportionality is maintained and their preparation is documented in the raw data.

#### F.1.1 2% Acetic Acid in Water (Mobile Phase A)

Add 20.0 ml of acetic acid to 1000 ml of Milli-Q water in a glass bottle and store the solution at room temperature. The expiration date for this solution is one month after preparation.

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**F.1.2 0.1% Acetic Acid in Acetonitrile (Mobile Phase B)**

Add 1.00 ml of acetic acid to 1000 ml of acetonitrile in a glass bottle and store the solution at room temperature. The expiration date for this solution is one month after preparation.

**F.1.3 Acetonitrile : Isopropanol (80:20, v:v) with 1% Ammonium Hydroxide (Needle Rinse 1)**

Add 400 ml acetonitrile to 100 ml isopropanol and 5.00 ml ammonium hydroxide in a glass bottle and store the solution at room temperature. The expiration date for this solution is one month after preparation.

**F.1.4 Water : Methanol (90:10, v:v) with 1% Formic Acid (Needle Rinse 2)**

Add 450 ml Milli-Q water to 50.0 ml methanol and 5.00 ml formic acid in a glass bottle and store the solution at room temperature. The expiration date for this solution is one month after preparation.

**F.1.5 0.2% Acetic Acid in Methanol (Diluent Solution)**

Add 0.400 ml acetic acid to 200 ml methanol in a glass bottle and store the solution at room temperature. The expiration date for this solution is one month after preparation.


**F.1.6 Water : Methanol (90:10, v:v) with 0.1% Acetic Acid (Reconstitution Solution)**

Add 180 ml Milli-Q water to 20.0 ml methanol and 0.200 ml acetic acid in a glass bottle and store the solution at room temperature. The expiration date for this solution is one month after preparation.

**F.2 Preparation of Stock and Spiking Solutions**

The following standard preparation scheme is a suggested approach. Appropriate modifications to reach the targeted nominal calibrant and quality control (QC) standard concentrations are acceptable. For example, if the targeted nominal concentration is not achieved when the analyte calibration standard primary stock solution is obtained, the volume of this stock solution used in subsequent dilutions can be modified in order to achieve the targeted nominal calibration standard matrix concentrations. The actual volumes of standards used will be documented in the raw data. Volumes of these stock solutions can be adjusted as long as proportionality is maintained and their preparation is documented in the study binder.

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Analytical reference standards are corrected for purity, water and salt content, if applicable. Internal standard stocks may not be corrected for purity, water or salt.

Example purity calculation:

$$\text{Purity} = \frac{[\text{HPLC \% purity} \times (100 - \% \text{ water} - \% \text{ residual solvent})]}{100} \times \frac{\text{Free base molecular weight}}{\text{Salt form molecular weight}}$$

#### F.2.1 Preparation of [Proprietary] [Proprietary] and [Proprietary] Stock Solutions (1.00 mg/ml)

Accurately weigh out approximately 5.00 mg of [Proprietary] into a glass vial and dilute to a concentration of 1.00 mg/ml using dimethyl sulfoxide (DMSO). The purity of the compound must be taken into account when preparing this stock (Stock A). Repeat this step to produce a second stock solution at the same concentration (Stock B). Repeat to get duplicate weighings for [Proprietary] at the same concentration.

To prepare [Proprietary] stock solutions, weigh approximately 5.00 mg of [Proprietary] into a glass vial and dilute to a concentration of 1.00 mg/ml using Milli-Q water. Repeat this step to produce a second stock solution at the same concentration.

Store all stock solutions refrigerated (set point 5 °C ± 3 °C) until use.

#### F.2.2 Preparation of [Proprietary Info] [Proprietary Info] and [Proprietary Info] Stock Solutions (Internal Standard), 1.00 mg/ml

These Internal Standards are supplied by Medical Isotopes, Inc. as 1.00 mg amounts in a glass vial. Add 1.00 ml of DMSO to the [Proprietary Info] and [Proprietary Info] to produce a 1.00 mg/ml stock. Repeat for [Proprietary Info] using Milli-Q water instead of DMSO.

Store all internal standard stock solutions refrigerated (set point 5 °C ± 3 °C) until use.

#### F.2.3 Preparation of [Proprietary Info] [Proprietary Info] and [Proprietary Info] Internal Standard Secondary Stock Solutions

Accurately add 0.0100 ml of the 1.00 mg/ml [Proprietary Info] and [Proprietary Info] internal standard stock solutions, and 0.0500 ml of the 1.00 mg/ml [Proprietary Info] internal standard stock solution into a glass vial containing 9.930 ml of 0.2%

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acetic acid in methanol. The final concentration of the internal standard secondary stock solution will be 1.00 µg/ml [Proprietary Info] and [Proprietary Info] and 5.00 µg/ml [Proprietary Info]. This solution can be stored refrigerated (set point 5 °C ± 3 °C) until use.

**F.2.4** Preparation of [Proprietary Info] [Proprietary Info] and [Proprietary Info] Internal Standard Spiking Solution

Accurately add 2.50 ml of the internal standard secondary stock solution into a glass bottle containing 97.5 ml of 0.2% acetic acid in methanol. The final concentration of the internal standard spiking solution will be 25.0 ng/ml [Proprietary Info] and [Proprietary Info] and 125 ng/ml [Proprietary Info]. This solution can be stored refrigerated (set point 5 °C ± 3 °C) until use.

Per SRI SOP 006.063, *Reference Material Receipt and Stock, Spiking Solution and Calibration and Quality Control Sample Preparation / Expiration* internal standard stock and spiking solutions will be given a default expiration date of 6 months after preparation.

**F.2.5** Stock Verification

In order to determine the accuracy of preparation, the duplicate stock solutions will be verified prior to use. A suggested approach for the preparation of stock verification solutions is given here, although alternative final concentrations may be used providing that a suitable analyte and internal standard response is achieved. The duplicate stock solutions prepared in step F.2.1 should be diluted by spiking 0.0100 ml of the 1.00 mg/ml [Proprietary] and [Proprietary] stock solutions and 0.0500 ml of the 1.00 mg/ml [Proprietary] stock solution into 9.930 ml of Diluent Solution. These duplicate vials are briefly vortexed and 0.100 ml is removed and added to a vial containing 0.900 ml Diluent Solution. Vortex, then remove 0.0325 ml of this solution and place into a separate vial containing 0.0203 ml of internal standard spiking solution and 0.947 ml of Reconstitution Solution. The final concentration of [Proprietary] and [Proprietary] is 3.25 ng/ml and the final concentration of [Proprietary] is 16.3 ng/ml. The final concentration of [Proprietary Info] and [Proprietary Info] is 0.508 ng/ml and the final concentration of [Proprietary Info] is 2.54 ng/ml. The duplicate samples are injected in replicates of  $n \geq 3$  onto the LC-MS/MS system.

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To be considered acceptable for use, the stocks must agree to within 5% of each other, calculated as follows:

% difference =

$$\frac{(\text{Mean of peak area ratio of stock A} - \text{Mean of peak area ratio of stock B})}{(\text{Mean of peak area ratio of stock A} + \text{Mean of peak area ratio of stock B})/2} \times 100$$

If these stocks agree within 5% of each other, one single stock may be used for the preparation of both calibrants and Quality Control (QC) samples.

Alternatively, one stock may be used for the preparation of calibrants while the other stock can be used for the preparation of QC samples.

If these stocks do not agree, a third weighing may be performed and the three stocks compared against each other. If two stocks agree with each other these may be used to prepare calibrants and QCs, and the other stock can be discarded.

**F.2.6 Test Mix Preparation**

A solution prepared at the LLOQ level (or below Low QC concentration) shall be prepared and injected at the start and the end of each bioanalytical run (system suitability). This solution will contain both analyte and internal standard. To prepare at the LLOQ level, spike 0.0100 ml of the 1.00 mg/ml [Proprietary] and [Proprietary] stock solutions and 0.0500 ml of the 1.00 mg/ml [Proprietary] stock solution into 9.930 ml of Diluent Solution. Vortex, remove 0.100 ml, and add to a vial containing 0.900 ml of Diluent Solution. Vortex, remove 0.0100 ml, and add to a vial containing 0.990 ml of Diluent Solution. This solution may be stored refrigerated for up to 3 months from the date of preparation. On the day of use, remove 0.0203 ml of this solution and place into a separate vial containing 0.0203 ml of internal standard spiking solution and 0.959 ml of Reconstitution Solution. The final concentration of [Proprietary] and [Proprietary] is 0.0203 ng/ml and the final concentration of [Proprietary] is 0.102 ng/ml. The final concentration of [Proprietary Info] and [Proprietary Info] is 0.508 ng/ml and the final concentration of [Proprietary Info] is 2.54 ng/ml. The final concentrations mimic the final theoretical concentrations of [Proprietary] [Proprietary] and [Proprietary] and internal standards seen in LLOQ samples post-extraction.

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**F.2.7** Remove approximately 40.0 ml of control K<sub>2</sub> EDTA rat plasma from storage and allow it to equilibrate to room temperature. The matrix may be centrifuged at approximately 3000 RPM for 10 minutes prior to use to remove excess particulates. Prepare QC samples in polypropylene vials as shown in the table below. Note that the Solution Spiking Volumes are combined with the Matrix Volumes. Volumes of these QC samples can be adjusted as long as proportionality is maintained and their preparation is properly documented in the study raw data.

Quality Control Sample Preparation						
QC ID	Spiking Solution ID	Spiking Solution Concentration (µg/ml)	Spiking Volume (ml)	Matrix Volume (ml)	Final Volume (ml)	Nominal Matrix Concentration (ng/ml)
QC- Low	QC-Mid	0.400 / 0.400 / 2.00	0.0375	0.9625	1.00	15.0 / 15.0 / 75.0
QC- Mid	QC-Dil	5.00 / 5.00 / 25.0	0.0800	0.920	1.00	400 / 400 / 2000
QC- High	QC-Dil	5.00 / 5.00 / 25.0	0.160	0.840	1.00	800 / 800 / 4000
QC- Dil	[Proprietary] Stock A/B;	1000	0.0100	1.930	2.00	5000
	[Proprietary] Stock A/B;	1000	0.0100			5000
	[Proprietary] Stock A/B.	1000	0.0500			25000

Either Stock A or Stock B may be used, assuming equivalency is achieved.

The values in the "Spiking Solution Concentration" and the "Nominal Matrix Concentration" columns represent the concentrations of [Proprietary] [Proprietary] and [Proprietary] respectively.


These QC samples may be aliquoted into appropriate volumes into polypropylene tubes (suggested 150 µl volumes) and stored in a ≤-60 °C freezer until use, providing that sufficient stability in matrix has been successfully validated under these conditions. QC samples may also be freshly prepared on the day of extraction.

**F.3 Extraction Procedure**

Each bioanalytical run will be comprised of bracketing calibration curves (8 points) each with a matrix blank sample (matrix with neither analyte nor internal standard spiked) and a control blank (matrix with only internal standard included). It is recommended that a carryover blank (matrix blank) be injected after each upper limit of quantitation (ULOQ) calibration standard to assess any carryover present. Duplicate System Suitability samples will be injected, one before the first calibration curve and one at the end of the batch. Interspersed between the calibration curves will be  $n \geq 2$  QC samples at low, mid and high concentration.

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The dilution QC, when used, should be extracted in multiples of  $n=3$ . It is not required to run the dilution QC with a batch where samples are diluted, provided that the level of sample dilution did not exceed what was previously validated. However, for troubleshooting practices, the dilution QC may be analyzed with each batch, at the discretion of the bioanalytical scientist. If study samples are diluted with multiple dilution factors, then the dilution QC may be similarly diluted (with replicates of  $n=3$  for each dilution factor used), or alternatively, the highest dilution factor used for study samples will be used for the extraction of the dilution QC. It is also acceptable if low dilution factors are applied to study samples to use a high QC in place of the dilution QC, in order that the diluted sample falls within the calibration range. If the level of dilution required for study samples exceeds the dilution factor previously validated, then the dilution QC will need to be revalidated at the dilution factor required, in replicates of 6.

In order to facilitate the equilibration of the instrument, multiple injections of extracts (Conditioning Samples) may be injected before each batch. It is recommended to prepare conditioning samples near the LLOQ level, but providing that internal standard is present in the sample, the actual concentration used may change. Conditioning samples will be pooled, where more than one calibration standard or QC sample, at different concentrations, are combined. Individual study samples, calibration standards, and QC samples will not be used as conditioning samples without pooling. It is not acceptable to condition a batch using an old standard curve from a previous batch. It is acceptable to prepare either multiple pooled conditioning samples, or to re-inject the pooled sample from the same vial, depending on the final extract volume. The conditioning injections will be included as part of the analytical batch and will be printed with the rest of the batch. Approximately 10 conditioning injections will be analyzed prior to the start of each batch. If one batch is analyzed immediately following another, later batches may not require conditioning injections, although duplicate system suitability injections will be included.

Remove the calibration standard spiking solutions, internal standard spiking solution, QC samples and control matrix (approximately 10.0 ml) from storage and allow them to equilibrate to room temperature.

Follow the scheme listed below to prepare the calibration standards. The calibration standards are prepared in polypropylene tubes. Calibration standards may be discarded after use. Note that the Spiking Solution Volumes are combined with the Matrix Volumes.

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Preparation of Calibration Standards in Matrix						
Calibration Standard ID	Spiking Solution ID	Spiking Solution Concentration (µg/ml)	Spiking Volume (ml)	Matrix Volume (ml)	Final Volume (ml)	Nominal Matrix Concentration (ng/ml)
Std-1	Std-5	0.100 / 0.100 / 0.500	0.0250	0.475	0.500	5.00 / 5.00 / 25.0
Std-2	Std-5	0.100 / 0.100 / 0.500	0.0500	0.450	0.500	10.0 / 10.0 / 50.0
Std-3	Std-5	0.100 / 0.100 / 0.500	0.100	0.400	0.500	20.0 / 20.0 / 100
Std-4	Std-8	1.00 / 1.00 / 5.00	0.0250	0.475	0.500	50.0 / 50.0 / 250
Std-5	Std-8	1.00 / 1.00 / 5.00	0.0500	0.450	0.500	100 / 100 / 500
Std-6	Std-8	1.00 / 1.00 / 5.00	0.100	0.400	0.500	200 / 200 / 1000
Std-7	Std-9	10.0 / 10.0 / 50.0	0.0250	0.475	0.500	500 / 500 / 2500
Std-8	Std-9	10.0 / 10.0 / 50.0	0.0500	0.450	0.500	1000 / 1000 / 5000
Std-9	[Proprietary] Stock A/B;	1000	0.0100	0.930	1.00	10000
	[Proprietary] Stock A/B;	1000	0.0100			10000
	[Proprietary] Stock A/B	1000	0.0500			50000

Either Stock A or Stock B may be used, assuming equivalency is achieved.

The values in the "Spiking Solution Concentration" and the "Nominal Matrix Concentration" columns represent the concentrations of [Proprietary] [Proprietary] and [Proprietary] respectively.

These calibration standards may be aliquoted into appropriate volumes into polypropylene tubes (suggested 150 µl volumes) and stored in a ≤-60 °C freezer until use, providing that sufficient stability in matrix has been successfully validated under these conditions. Calibration standards may also be freshly prepared on the day of extraction.

- F.3.1** Transfer 0.0200 ml of each calibration standard, QC sample, study sample and blank into separate 1.50 ml microcentrifuge tubes. If needed, extra samples may be extracted in order to be used as Conditioning Samples. These should be pooled before use.
- F.3.2** Add 0.100 ml of 0.2% acetic acid in methanol to the matrix blanks. Cap tubes and vortex for approximately 5 seconds.
- F.3.3** Add 0.100 ml of the Internal Standard Spiking Solution to each calibration standard, QC standard, study sample and control blank. Cap tubes and vortex for approximately 5 seconds.
- F.3.4** Centrifuge tubes at approximately 18000g for approximately 10 minutes.
- F.3.5** Transfer 0.0250 ml of the supernatant into a 2.00 ml HPLC vial containing 1.00 ml Reconstitution Solution. Cap and vortex briefly to mix.

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**F.3.6** Store on the autosampler (set point 5 °C ± 3 °C) or refrigerated (set point 5 °C ± 3 °C).

**F.4 Analytical Conditions**

Equipment can be substituted provided that equivalent assay performance is obtained.

Refer to Figures 1-3 in this Test Method for an example of representative chromatograms of each analyte at the LLOQ level.

**F.4.1 HPLC Conditions**

Autosampler:	CTC Analytics HTS-xt
Pumps:	Shimadzu LC-20AD Prominence. Incorporates Shimadzu CBM-20A Prominence communications bus module and Shimadzu DGU-20A <sub>3R</sub> Prominence degasser
Column Oven:	Shimadzu CTO-20AC Prominence
Autosampler Temp:	Set point 5 °C
Column Oven Temp:	Set point 25 °C
Column:	Phenomenex Synergi Polar RP 100 x 2 mm, 4µm
Pre-column Frit:	0.5 µm stainless-steel Precolumn Frit (Upchurch Scientific)
Flow Rate:	0.350 ml/min
Run Time:	8.0 minutes
Injection Volume:	10 µl*
Mobile Phase A:	2% acetic acid in water
Mobile Phase B:	0.1% acetic acid in acetonitrile
Needle Rinse 1:	Acetonitrile:isopropanol (80:20, v:v) with 1% ammonium hydroxide
Needle Rinse 2:	Water:methanol (90:10, v:v) with 1% formic acid
Pre clean with Needle	1*, 1*
Rinses 1 and 2:	
Post clean with Needle	2*, 2*
Rinse 1 and 2:	
Valve clean with Needle	2*, 2*
Rinse 1 and 2:	
Retention Time:	4.3 minutes [Proprietary] and IS)
	4.2 minutes [Proprietary] and IS)
	1.4 minutes [Proprietary] and IS)

\* may be modified to improve performance

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**TEST METHOD****Classification:** Project**Supersedes:** 106.202 (07/15/19)**Subject:** Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma**TM No.:** 106.202**Page:** 12 of 26**Effective:** August 21, 2019**F.4.2 LC Program**

Time (min)	% A	% B
0.01	98	2
2.00	98	2
2.10	50	50
4.00	2	98
5.50	2	98
5.51	98	2
8.00	98	2

## Switching Valve program

Total Time (minutes)	Position
0.0 to 0.2*	Divert to Waste
0.2 to 5.0*	Divert to MS
5.0 to 8.0*	Divert to Waste

\* may be modified to improve performance

SRI PROPRIETARY / CONFIDENTIAL



**TEST METHOD****Classification:** Project**Supersedes:** 106.202 (07/15/19)**Subject:** Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma**TM No.:** 106.202**Page:** 13 of 26**Effective:** August 21, 2019**F.4.3 MS/MRM Conditions**

Mass Spectrometer:	AB Sciex 5500 Mass Spectrometer		
Interface:	Turbo IonSpray positive-ion mode		
Scan Mode:	Multiple Reaction Monitoring (MRM)		
IS:	5500V*		
EP:	10V*		
DP:	71V*	[Proprietary Info]	81V*
	121V*	[Proprietary Info]	76V*
	76V*	[Proprietary Info]	56V*
	21V*	[Proprietary Info]	19V*
	25V*	[Proprietary Info]	25V*
	33V*	[Proprietary Info]	35V*
CE:	21V*	[Proprietary Info]	19V*
	25V*	[Proprietary Info]	25V*
	33V*	[Proprietary Info]	35V*
	38V*	[Proprietary Info]	20V*
CXP:	26V*	[Proprietary Info]	24V*
	14V*	[Proprietary Info]	14V*
Resolution Q1, Q3:	Unit, Unit		
CUR Gas:	20*		
CAD Gas:	8*		
GS1:	60*		
GS2:	60*		
Source Temp:	650 °C*		
Dwell:	80* ms		
Nominal Transitions:	[Proprietary Info]	m/z 629.3* → 447.2*	
	[Proprietary Info]	m/z 721.3* → 296.1*	
	[Proprietary Info]	m/z 288.1* → 176.1*	
	[Proprietary Info]	m/z 637.4* → 447.2*	
	[Proprietary Info]	m/z 727.4* → 302.2*	
	[Proprietary Info]	m/z 294.1* → 182.1*	

\* May be modified to improve performance. The eventual m/z ratios used must be within ± 0.3 amu from the masses quoted above.)

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**TEST METHOD****Classification:** Project**Supersedes:** 106.202 (07/15/19)**Subject:** Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma**TM No.:** 106.202**Page:** 14 of 26**Effective:** August 21, 2019**F.5 Calculations**

**F.5.1** Chromatograms will be automatically integrated using AB Sciex Analyst software (Version 1.6.2) or equivalent and visually inspected for an acceptable integration.

**F.5.2** Compute the 1/x weighted least-squares linear regression [Proprietary] and [Proprietary] and the 1/x<sup>2</sup> weighted least-squares linear regression [Proprietary Info] using Analyst software, relating the peak area ratios (relative to internal standard) of the calibration standards to their respective nominal concentrations (ng/ml in plasma) for [Proprietary] [Proprietary] and [Proprietary]

**F.5.3** Using the peak area ratios (relative to the internal standard) of the standards and the regression equation constants, concentrations for analyte in the QC samples and study samples can be interpolated.

**F.5.4** Compute the correlation coefficient for the standard data.

**F.6. Acceptance Criteria****F.6.1 System Suitability Standard**

There are no formal acceptance criteria for the System Suitability samples. The system suitability sample will be injected at the beginning and at the end of a run and inspected to ensure signal-to-noise ratio and peak shape are adequate for quantitation. Any chromatographic change between these injections which may have an impact on the ability to accurately quantitate the samples will be noted, however there is no formal acceptance criteria for this. The system suitability injections will be printed with the other chromatograms in the analytical batch.


**F.6.2 Calibration Standard Acceptance Criteria**

**F.6.2.1** The lower limit of quantitation (LLOQ) standard back-calculated concentration must be within  $\pm 20\%$  of theoretical nominal concentration.

**F.6.2.2** To meet acceptance criteria, the back-calculated concentration of a calibration standards (excluding at the LLOQ level) must be within 15% of their nominal theoretical concentrations.

**F.6.2.3** A minimum of three-quarters of calibration standards must meet these criteria.

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	<b>TEST METHOD</b> <b>Classification:</b> Project <b>Supersedes:</b> 106.202 (07/15/19) <b>Subject:</b> Analysis of [Proprietary] [Proprietary] and [Proprietary] in K <sub>2</sub> EDTA Rat Plasma	<b>TM No.:</b> 106.202 <b>Page:</b> 15 of 26 <b>Effective:</b> August 21, 2019
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**F.6.2.4** Any standards failing to meet the acceptance criteria will be excluded from the regression, starting with the calibration standard which is furthest away from the nominal concentration.

**F.6.3** Quality Control (QC) Sample Acceptance Criteria.

**F.6.3.1** To meet acceptance criteria, the back-calculated concentration of a QC sample must be within 15% of their nominal theoretical concentrations.

**F.6.3.2** At least two-thirds of all assay QCs (low, mid and high) must meet the acceptance criteria.

**F.6.3.3** At least 50% of the QCs at each level must meet the acceptance criteria.

**F.6.3.4** For dilution QCs, which are generally assayed using multiples of n=3 replicates, at least 67% (rounded) of the QCs must be within 15% of their nominal theoretical concentrations. Failure of a dilution QC does not mean that the batch itself has failed if the low, mid and high QCs meet acceptance criteria as defined above. However, any samples diluted in a batch with a failed dilution QC should be repeated and the value from this batch discarded. If more than one dilution scheme was followed in a batch of samples, with corresponding dilution QCs prepared using different dilution factors, only the dilution QC which failed acceptance criteria will be rejected and the associated samples repeated.

**F.6.4** Blank Acceptance Criteria


At least 50% of matrix blanks (including carryover blanks, BI/BI) and 50% of control blanks (BI/IS) must have a response (peak area) less than or equal to 20% of the mean accepted LLOQ calibration standards. Carryover blanks should be positioned in the run in a manner capable of determining assay carryover, for example, after each ULOQ calibration standard injection.

**F.7** **Data Reporting**

Concentrations found below the lowest calibration standard concentration, will be reported as below the quantitation limit. Where no peak is detected (ND), the result will be flagged as (<LLOQ). Over-diluted samples falling below the calibration range (assuming insufficient to reassay) will be reported as <LLOQ (LLOQ value x dilution factor).

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	<b>Subject:</b> Analysis of [Proprietary] [Proprietary] and [Proprietary] in K <sub>2</sub> EDTA Rat Plasma	

## G. STABILITY/METHOD PARAMETERS

### G.1 Solutions

<u>Parameter Evaluated</u>	<u>Validated Result</u>	<u>Study Reference</u>
Analyte Stability in Stock Solutions at 5±3 °C:	40 days	B185-18

### G.2 Matrix, K<sub>2</sub> EDTA Rat Plasma

<u>Parameter Evaluated</u>	<u>Validated Result</u>	<u>Study Reference</u>
Room Temperature Stability in Matrix:	25 hours	B181-18
Freeze/Thaw Stability in Matrix:	5 cycles	B181-18
Re-injection Stability:	94 hours, refrigerated	B181-18
Post Preparative Extract Stability:	99 hours, refrigerated	B181-18
Validated Dilution Factor:	10-fold, 50-fold	B181-18
Long-Term Stability in Matrix at ≤-60°C:	25 days	B181-18
Effect of 2% Hemolysis:	No impact	B181-18
Whole Blood Stability:	4 hours, refrigerated	B181-18
Maximum Batch Size:	101 samples	B181-18
Incurred Sample Reanalysis:	Successful	M398-18

## H. REFERENCES

- H.1** B181-18: "Method Validation Report for the Quantitative Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma"
- H.2** B185-18: "Method Validation Report for the Quantitative Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma".
- H.3** M398-18: "GLP-Multiple (5-Weekly) Repeat Subcutaneous Dose Toxicity Study with
- H.4** [Proprietary] [Pro] in Sprague Dawley Rats".
- H.5** SRI SOP 006.061, *Bioanalytical Sample Analysis*
- H.6** SRI SOP 006.062, *Bioanalytical Sample Reanalysis*
- H.7** SRI SOP 006.063, *Reference Material Receipt and Stock, Spiking Solution and Calibration and Quality Control Sample Preparation / Expiration*

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## TEST METHOD

**Classification:** Project

**Supersedes:** 106.202 (07/15/19)

**Subject:** Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma

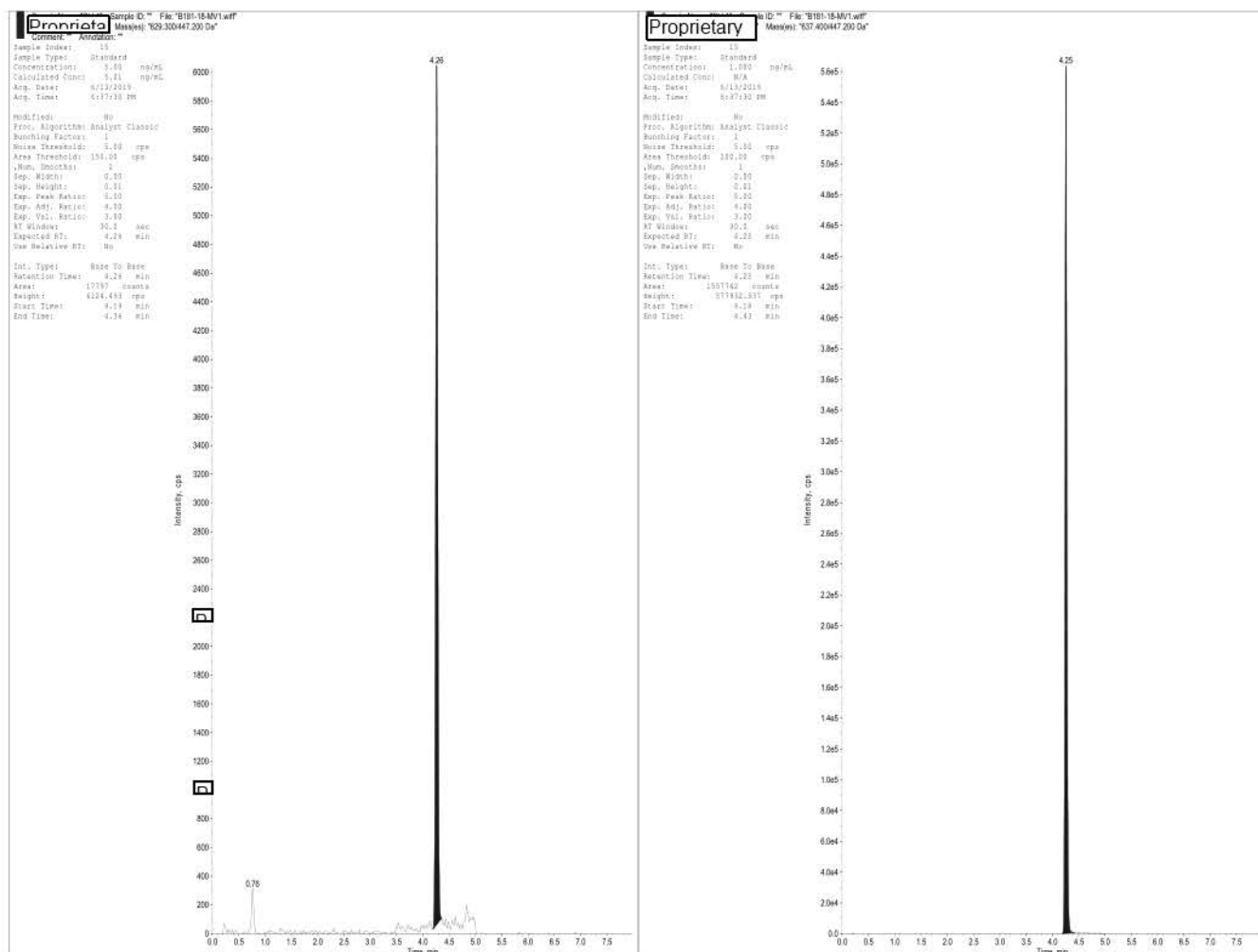
**TM No.:** 106.202

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## I. FIGURES

**I.1** Figure 1. Representative [Proprietary] Chromatogram of a K<sub>2</sub> EDTA Rat Plasma Sample Spiked at the Lower Limit of Quantitation (LLOQ)



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## TEST METHOD

**Classification:** Project

**Supersedes:** 106.202 (07/15/19)

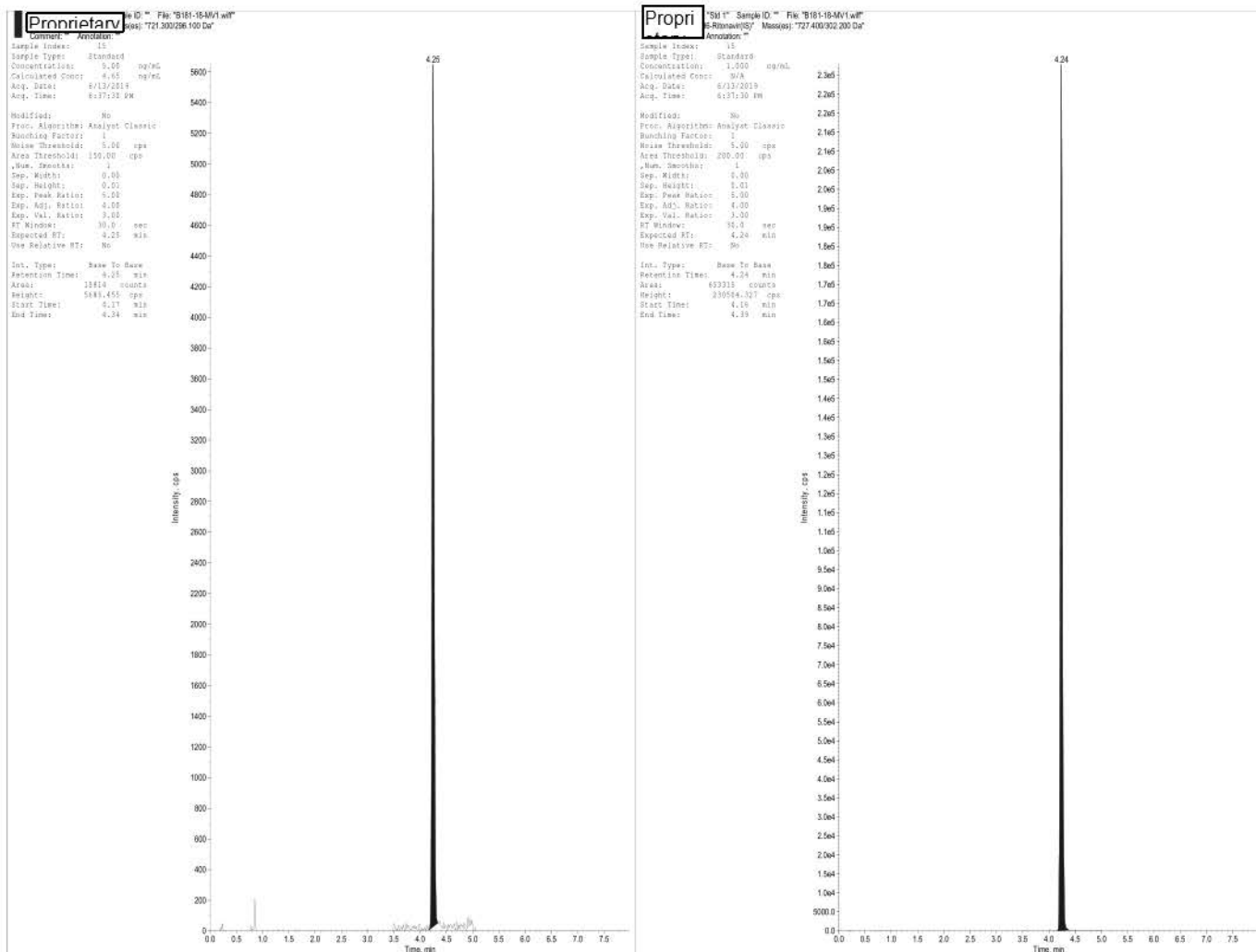
**Subject:** Analysis of Proprietary Proprietary and Proprietary in K<sub>2</sub> EDTA Rat Plasma

**TM No.:** 106.202

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**I.2** Figure 2. Representative Proprietary Chromatogram of a K<sub>2</sub> EDTA Rat Plasma Sample Spiked at the Lower Limit of Quantitation (LLOQ)



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## TEST METHOD

**Classification:** Project

**Supersedes:** 106.202 (07/15/19)

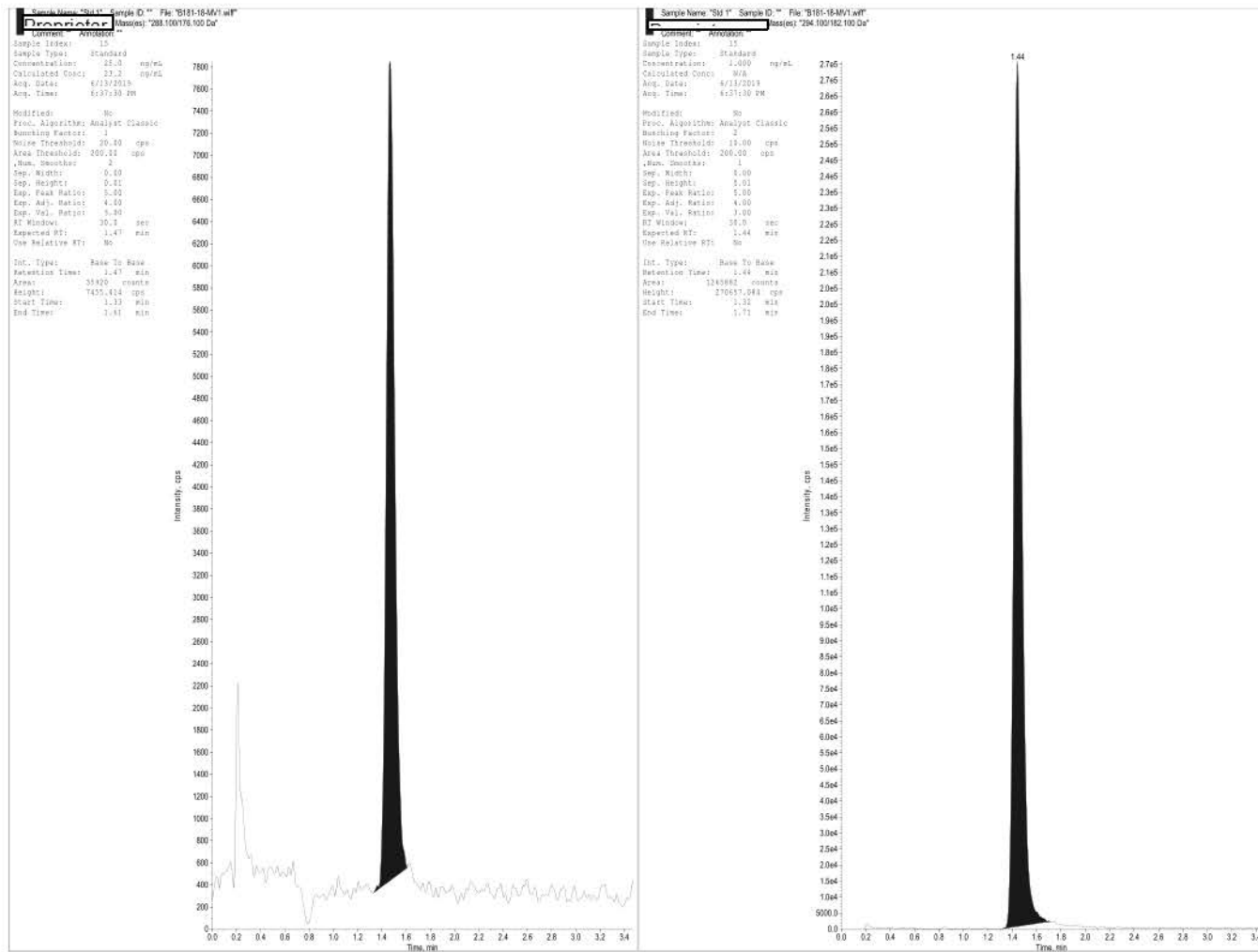
**Subject:** Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma

**TM No.:** 106.202


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**I.3** Figure 3. Representative [Proprietary] Chromatogram of a K<sub>2</sub> EDTA Rat Plasma Sample Spiked at the Lower Limit of Quantitation (LLOQ)



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	<b>TEST METHOD</b> <b>Classification:</b> Project <b>Supersedes:</b> 106.202 (07/15/19)	<b>TM No.:</b> 106.202 <b>Page:</b> 20 of 26 <b>Effective:</b> August 21, 2019
	<b>Subject:</b> Analysis of <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> and <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> in K <sub>2</sub> EDTA Rat Plasma	

## J. ATTACHMENTS

- J.1** Extraction Form (SRI Form 106.202A)
- J.2** Methodology and Reagent List (SRI Form 106.202B)
- J.3** Instrument Analytical Conditions and Reagents (SRI Form 106.202C)
- J.4** Instrument Reagent Preparation (SRI Form 106.202D)
- J.5** Extraction Reagent Preparation (SRI Form 106.202E)

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Study ID:

Batch ID:

**EXTRACTION FORM**

Preparation of Calibration Standards in Matrix						
Calibration Standard ID	Spiking Solution ID	Spiking Solution Concentration (µg/ml)	Spiking Volume (ml)	Matrix Volume (ml)	Final Volume (ml)	Nominal Matrix Concentration (ng/ml)
Std-1	Std-5	0.100 / 0.100 / 0.500	0.0250	0.475	0.500	5.00 / 5.00 / 25.0
Std-2	Std-5	0.100 / 0.100 / 0.500	0.0500	0.450	0.500	10.0 / 10.0 / 50.0
Std-3	Std-5	0.100 / 0.100 / 0.500	0.100	0.400	0.500	20.0 / 20.0 / 100
Std-4	Std-8	1.00 / 1.00 / 5.00	0.0250	0.475	0.500	50.0 / 50.0 / 250
Std-5	Std-8	1.00 / 1.00 / 5.00	0.0500	0.450	0.500	100 / 100 / 500
Std-6	Std-8	1.00 / 1.00 / 5.00	0.100	0.400	0.500	200 / 200 / 1000
Std-7	Std-9	10.0 / 10.0 / 50.0	0.0250	0.475	0.500	500 / 500 / 2500
Std-8	Std-9	10.0 / 10.0 / 50.0	0.0500	0.450	0.500	1000 / 1000 / 5000
Std-9	Proprietary Stock A/B;	1000	0.0100	0.930	1.00	10000
	Proprietary Stock A/B;	1000	0.0100			10000
	Proprietary Stock A/B	1000	0.0500			50000

Preparation of Quality Control Samples in Matrix						
QC ID	Spiking Solution ID	Spiking Solution Concentration (µg/ml)	Spiking Volume (ml)	Matrix Volume (ml)	Final Volume (ml)	Nominal Matrix Concentration (ng/ml)
QC-Low	QC-Mid	0.400 / 0.400 / 2.00	0.0375	0.9625	1.00	15.0 / 15.0 / 75.0
QC-Mid	QC-Dil	5.00 / 5.00 / 25.0	0.0800	0.920	1.00	400 / 400 / 2000
QC-High	QC-Dil	5.00 / 5.00 / 25.0	0.160	0.840	1.00	800 / 800 / 4000
QC-Dil	Proprietary Stock A/B;	1000	0.0100	1.930	2.00	5000
	Proprietary Stock A/B;	1000	0.0100			5000
	Proprietary Stock A/B	1000	0.0500			25000

Species: \_\_\_\_\_ Anticoagulant/Matrix: \_\_\_\_\_ Matrix Supplier: \_\_\_\_\_

Lot: \_\_\_\_\_ Matrix Expiration Date: \_\_\_\_\_

Pipette IDs: \_\_\_\_\_

Calibration Spiking Solution: \_\_\_\_\_ Expiration Date: \_\_\_\_\_

QC Sample Spiking Solution: \_\_\_\_\_ Expiration Date: \_\_\_\_\_

Prepared by: \_\_\_\_\_ Date: \_\_\_\_\_

SRI Form 106.202A  
08/21/19**SRI PROPRIETARY / CONFIDENTIAL**

Obtained via FOIA by White Coat Waste Project

Study ID:

Batch ID:

**METHODOLOGY AND REAGENT LIST**

Step	Description	Equipment or Pipettes used	Step completed (check)
1	Transfer 0.0200 ml of each calibration standard, QC sample, study sample and blank into separate 1.50 ml microcentrifuge tubes.		
2	Add 0.100 ml of 0.2% acetic acid in methanol to the matrix blanks. Cap and vortex for approximately 5 seconds.		
3	Add 0.100 ml of the Internal Standard Spiking Solution to each calibration standard, QC sample, study sample and control blank. Cap and vortex for approximately 5 seconds.		
4	Centrifuge tubes at approximately 18000 g for approximately 10 minutes.		
5	Transfer 0.0250 ml of the supernatant into a 2.00 ml HPLC vial containing 1.00 ml Reconstitution Solution. Cap and vortex briefly to mix.		
6	Store on the autosampler (set point 5°C ± 3°C) or refrigerated (5°C ± 3°C).	End of extraction (time):	

Ⓐ Eppendorf Repeater Plus / M4 (circle one) Equipment ID: \_\_\_\_\_ Exp: \_\_\_\_\_

<b>Dilution Scheme</b> (1:_____)	Add _____ ul sample to _____ ul control matrix and vortex.	Pipettes:
<b>Dilution Scheme</b> (1:_____)	Add _____ ul sample to _____ ul control matrix and vortex.	
<b>Dilution Scheme</b> (1:_____)	Add _____ ul sample to _____ ul control matrix and vortex.	

Procedure Performed by: \_\_\_\_\_ Date: \_\_\_\_\_

Study ID:

Batch ID:

**METHODOLOGY AND REAGENT LIST (cont.)****Additional information, if required:**

Test Mix Dilution: Remove the Test Mix in 2% acetic acid in methanol from storage (ID: \_\_\_\_\_ Exp: \_\_\_\_\_) and remove 0.0203 ml of this solution and place into a separate HPLC vial containing 0.0203 ml of internal standard spiking solution and 0.959 ml of Reconstitution Solution. Store with batch.

Pipettes: \_\_\_\_\_

**REAGENT LIST**

Reagent Description	Assigned ID	Supplier	Lot #	Grade	Exp.
K <sub>2</sub> EDTA Rat Plasma	NA			NA	
Internal Standard		NA	NA	NA	
0.2% Acetic Acid in Methanol (Diluent)		NA	NA	NA	
Water:Methanol (90:10, v:v) with 0.1% Acetic Acid (Reconstitution Solution)		NA	NA	NA	
Initial/Date:					

Study ID:	Batch ID:
-----------	-----------

### INSTRUMENT ANALYTICAL CONDITIONS AND REAGENTS

HPLC Column ID.	Vendor:	Calibration due date
Phenomenex Synergi Polar RP 100 x 2 mm, 4µm	Description:	NA
	Dimension:	
	S/N:	
Column Heater	Equipment Tracking #-_____	
Column Temp (Set Point 25°C)	Set Point _____ °C	NA
Pump ID	Equipment Tracking #-_____	
Pump Pressures at Start	_____ psi	NA
Autosampler ID	Equipment Tracking #-_____	
Autosampler Temp (Set Point 5°C ± 3°C)	Set Point _____ °C	NA
Mass Spectrometer	Equipment Tracking #-_____	
		<b>Exp. Date</b>
Mobile Phase A		
Mobile Phase B		
Needle Rinse 1		
Needle Rinse 2		
Number of Conditioning Injections		NA

NA: Not Applicable

Initial: \_\_\_\_\_ Date: \_\_\_\_\_

Additional Comments / Incidents during analysis:

SRI Form 106.202C  
08/21/19

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Obtained via FOIA by White Coat Waste Project



## INSTRUMENT REAGENT PREPARATION

**Study Number:** \_\_\_\_\_

### Mobile Phase A:

Assigned ID: \_\_\_\_\_

2% Acetic Acid in Water

Add \_\_\_\_\_ ml (nominal 20.0 ml) of acetic acid to \_\_\_\_\_ ml (nominal 1000 ml) of Milli-Q Water. This solution should be stored at room temperature for up to one month after preparation.

Expiration Date: \_\_\_\_\_

### Mobile Phase B:

Assigned ID: \_\_\_\_\_

0.1% Acetic Acid in Acetonitrile

Add \_\_\_\_\_ ml (nominal 1.00 ml) of acetic acid to \_\_\_\_\_ ml (nominal 1000 ml) of acetonitrile. This solution should be stored at room temperature for up to one month after preparation.

Expiration Date: \_\_\_\_\_

### Needle Rinse 1:

Assigned ID: \_\_\_\_\_

Acetonitrile:Isopropanol (80:20, v:v) with 1% Ammonium Hydroxide

Add \_\_\_\_\_ ml (nominal 400 ml) of acetonitrile to \_\_\_\_\_ ml (nominal 100 ml) isopropanol and add \_\_\_\_\_ ml (nominal 5.00 ml) ammonium hydroxide in a glass bottle. This solution should be stored at room temperature for up to one month after preparation.

Expiration Date: \_\_\_\_\_

### Needle Rinse 2:

Assigned ID: \_\_\_\_\_

Water:Methanol (90:10, v:v) with 1% Formic Acid

Add \_\_\_\_\_ ml (nominal 450 ml) of Milli-Q water to \_\_\_\_\_ ml (nominal 50.0 ml) methanol and add \_\_\_\_\_ ml (nominal 5.00 ml) formic acid in a glass bottle. This solution should be stored at room temperature for up to one month after preparation.

Expiration Date: \_\_\_\_\_

**Milli-Q water:** Decanted from Milli-Q unit on the day of use. Unit ID: \_\_\_\_\_ Exp: \_\_\_\_\_

Is resistivity  $\geq 18.0 \text{ M}\Omega\text{-cm}$ ? Y / N (circle) Is TOC  $< 50.0 \text{ ppb}$ ? Y / N (circle)

### Ammonium Hydroxide:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

### Acetic Acid:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

### Formic Acid:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

### Methanol:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

### Acetonitrile:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

### Isopropanol:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

Prepared by: \_\_\_\_\_ Date: \_\_\_\_\_

SRI Form 106.202D

08/21/19

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## EXTRACTION / STOCK REAGENT PREPARATION

Study Number: \_\_\_\_\_

### 0.2% Acetic Acid in Methanol (Diluent):

Assigned ID: \_\_\_\_\_

Add \_\_\_\_\_ ml (nominal 0.400 ml) of acetic acid to \_\_\_\_\_ ml (nominal 200 ml) of methanol. This solution should be stored at room temperature for up to one month after preparation.

Expiration Date: \_\_\_\_\_

### Water:Methanol (90:10, v:v) with 0.1% acetic acid (Reconstitution Solution):

Assigned ID: \_\_\_\_\_

Add \_\_\_\_\_ ml (nominal 0.200 ml) of acetic acid to \_\_\_\_\_ ml (nominal 180 ml) Milli-Q water and \_\_\_\_\_ ml (nominal 20.0 ml) of methanol. This solution should be stored at room temperature for up to one month after preparation.

Expiration Date: \_\_\_\_\_

### Test Mix (System Suitability):

Assigned ID: \_\_\_\_\_

To prepare at the LLOQ level, spike 0.0100 ml of the 1.00 mg/ml Proprietary Info stock solutions and 0.0500 ml of the 1.00 mg/ml Proprietary stock solution into 9.930 ml of Diluent Solution. Vortex, remove 0.100 ml, and add to a vial containing 0.900 ml of Diluent Solution. Vortex, remove 0.0100 ml, and add to a vial containing 0.990 ml of Diluent Solution. This solution may be stored refrigerated for up to 3 months from the date of preparation. On the day of use, remove 0.0203 ml of this solution and place into a separate vial containing 0.0203 ml of internal standard spiking solution and 0.959 ml of Reconstitution Solution.

Expiration Date: \_\_\_\_\_ Storage Unit / Temperature: \_\_\_\_\_

**Milli-Q water:** Decanted from Milli-Q unit on the day of use. Unit ID: \_\_\_\_\_ Exp: \_\_\_\_\_

Is resistivity  $\geq 18.0 \text{ M}\Omega\text{-cm}$ ? Y / N (circle) Is TOC  $< 50.0 \text{ ppb}$ ? Y / N (circle)

### Acetic Acid:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

### Methanol:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

Proprietary  
Info

**Stock Solution (1.00 mg/ml)** ID: \_\_\_\_\_ Expire: \_\_\_\_\_

**Stock Solution (1.00 mg/ml)** ID: \_\_\_\_\_ Expire: \_\_\_\_\_

**Stock Solution (1.00 mg/ml)** ID: \_\_\_\_\_ Expire: \_\_\_\_\_

**Internal Standard Spiking Solution** ID: \_\_\_\_\_ Expire: \_\_\_\_\_

**Diluent** ID: \_\_\_\_\_ Expire: \_\_\_\_\_

**Reconstitution Solution** ID: \_\_\_\_\_ Expire: \_\_\_\_\_

**Pipettes:** \_\_\_\_\_

Prepared by: \_\_\_\_\_ Date: \_\_\_\_\_

SRI Form 106.202E  
08/21/19

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**Method Validation Report for the Quantitative Analysis of [Proprietary Info] Ritonavir,  
and [Proprietary] in K<sub>2</sub> EDTA Rat Plasma  
SRI Study No. B181-18**

**Appendix B**

**PARTIAL VALIDATION PLAN OF THE BIOANALYTICAL METHOD FOR  
ANALYSIS OF [Proprietary Info] [Proprietary Info] AND [Proprietary Info] IN K<sub>2</sub> EDTA RAT  
PLASMA**

**Partial Validation Plan for the Bioanalytical Method for Analysis of**  
**Proprietary Info Proprietary Info and Proprietary Info in**  
**K<sub>2</sub> EDTA Rat Plasma**

**SRI International Study Number: B181-18**

Redacted by agreement

06/12/19

Date

06/12/19

Date



## SCOPE

This validation will be limited to the analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA rat plasma using 0.0200 ml sample volumes, using a protein precipitation extraction procedure followed by LC-MS/MS detection. The internal standards used in this assay are [Proprietary Info] [Proprietary Info] and [Proprietary Info]

This plan is based on SRI SOP 006.060, *Bioanalytical Method Validation*. Further details on the conduct of a typical validation study are described in this SOP.

## METHOD DEVELOPMENT

Based on the outcome of method development, it is determined that the validation of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA rat plasma may proceed. A summary of the method development results is provided in Appendix A. Full details of the method development experiments performed and the corresponding results can be found in the study method development raw data.

## OBJECTIVE

The objective of this validation plan is to describe the experimental procedures, the validation parameters, the statistical analyses to be performed, and the corresponding acceptance criteria, including the basis for acceptance or rejection of the validity of the method for its intended use. This method was fully validated in dog in the validation study B185-18. Therefore, it will be done as a partial validation in rat with at least one accuracy and precision batch. This partial validation investigates the following parameters: method linearity and range, maximum batch size, selectivity, carryover, accuracy (intra-batch), precision (intra-batch), recovery, matrix effects on ionization, effect of dilution, matrix effects using 6 unique lots of matrix, freeze thaw stability, room temperature matrix stability, re-injection stability, post-preparative extract stability, long term matrix storage stability, whole blood processing stability, effect of hemolysis, and effect of concomitant medications. Analyte stock solution stability for [Proprietary] [Proprietary] and [Proprietary] was established in the validation study B185-18, and therefore will not be performed in this study. Following completion of the validation, a final validation report will be written that will include a brief description of the method development outcome, a summary of the method used to validate this assay, any changes made over the course of the validation, including revalidation if applicable, the results obtained, and the validation conclusions.

## SUMMARY OF METHOD

Full details of the methodology used during the conduct of this validation study will be detailed in SRI Test Method *Analysis of* [Proprietary] [Proprietary] *and* [Proprietary] *in K<sub>2</sub> EDTA Rat Plasma*. This Test Method will be issued as a draft Test Method during the conduct of the validation, and will be formally issued and approved by department management and Quality Assurance (QA) prior to the start of sample analysis. Until an SRI Test Method number can be formally assigned, following completion of the validation, the initial draft Test Method number used during validation will be B181-18-TM-Draft 1, with subsequent revisions numbered -Draft 2, -Draft 3, etc.

During validation, this draft Test Method may be modified, if necessary, by the validation scientist providing that the integrity of the validation is not compromised, and the modifications are fully documented. Any modifications to the method occurring after the first accepted validation run may require revalidation of the method.



All details pertaining to equipment used, materials, reagents etc will be included in the raw data and detailed in the resulting final bioanalytical Test Method.

All temperatures quoted in this validation outline are nominal.

## COMPOUND INFORMATION

The actual details of [Proprietary] [Proprietary] and [Proprietary] and the stable label internal standards used will be noted in the raw data. The final report will include the supplier of the test articles and internal standards, the purities and the expiration dates.

### Stock Solution Preparation

Duplicate weighings of [Proprietary] [Proprietary] and [Proprietary] will be performed and 1.00 mg/ml stock solutions of each analyte (adjusting for water or solvent present and purity, as appropriate) will be prepared. Dimethyl sulfoxide (DMSO) will be used as the diluent for the [Proprietary] and [Proprietary] stocks, while Milli-Q water will be the diluent for the [Proprietary] stock solutions. These stock solutions will be stored in a refrigerator ( $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ ) until use. Per SRI SOP 006.063 *Reference Material Receipt and Stock, Spiking Solution and Calibration and Quality Control Sample Preparation / Expiration*, a comparison of these solutions will be performed, prior to use in the validation, to ensure equivalency and accuracy of preparation.

The internal standards [Proprietary Info] [Proprietary Info] and [Proprietary Info] will be weighed and a 1.00 mg/ml stock solution of each prepared. The diluent for the [Proprietary Info] and [Proprietary Info] stocks will be DMSO, and the diluent for the [Proprietary Info] will be Milli-Q water. It is not necessary to apply a correction factor to the internal standards following weighing.

The preparation of working solutions from the analyte and internal standard stocks, if applicable, will be detailed in the raw data.

## METHOD VALIDATION PARAMETERS

### Method Linearity and Range

On each day of method validation, a freshly prepared calibration standard curve (5.00 to 1000 ng/ml for [Proprietary] and [Proprietary] and 25.0 to 5000 ng/ml for [Proprietary] in K<sub>2</sub> EDTA rat plasma will be extracted and the peak area ratios (PAR) of analyte to internal standard in the calibration standards will be fitted to a weighted regression analysis. The eventual weighting used will be documented in the raw data and will be consistently applied across all batches.

The calibration standards (n=2 per analytical batch) will contain the following concentrations of [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA rat plasma: 5.00, 10.0, 20.0, 50.0, 100, 200, 500 and 1000 ng/ml. These standards will also contain [Proprietary] at 25.0, 50.0, 100, 250, 500, 1000, 2500, and 5000 ng/ml. Also included with each batch will be at least n=2 blank samples containing no analyte or internal standard (BI/BI) and at least n=2 blank samples containing no analyte but containing internal standard (BI/IS).

The linearity of the assay will be assessed by the correlation coefficient ( $r$ ) obtained from the regression analysis of the peak area ratios, with perfect fit of the data yielding an  $r$  value of 1.000. The minimum value for  $r$  for an assay to be acceptable is 0.990.

On each day of accuracy and precision assessment, the peak response vs. standard concentration in the calibration standards will be fitted to a regression analysis. The simplest weighting should be used wherever possible, however, other weightings may be used if the fit is more appropriate. The best fit will be determined using the sum of residual squares, where the lowest value will be determined to be the most suitable. This will be performed using linear  $1/x$  or  $1/x^2$  at a minimum, with the inclusion of quadratic regression if appropriate. Quality Control (QC) sample results from all accuracy and precision batches, across all concentrations, will be used in the calculations to determine the sum of residual squares. The specific standard curve model fit used, including weighting, will be documented in the raw data and will be consistently applied across all batches.

In order for the calibration curve to be considered acceptable there will not be more than a 15% difference between the nominal and observed concentrations, except at the lower limit of quantitation (LLOQ) where a 20% deviation is permitted. At least 75% of the calibration standards, including at least one replicate at the lowest and highest concentrations, will fulfill this criterion. Individual calibration standards which do not fulfill the criterion will be excluded from the regression.

As this validation has not yet initiated, the analytical range stated above, and the resulting QC concentrations selected, may change from stated here depending on experimental findings. This will be recorded in the raw data.

### **Maximum Batch Size**

One accuracy and precision batch of sufficient size should be analyzed to represent a typical batch when running study samples. In order to get the desired number of samples, additional blanks (BI/BI or BI/IS) may be analyzed with this batch, and no acceptance criteria will be attached to these additional samples. The maximum batch size will be counted from the first calibration standard to the last calibration standard.

### **Selectivity**

To confirm the selectivity of the assay for the analyte and the internal standard, plasma samples containing no analyte or internal standard (BI/BI) from at least 6 unique lots of K<sub>2</sub> EDTA rat plasma will be extracted in at least one analytical batch in replicates of  $n=1$ . Additionally, a BI/BI sample at 0.5% and 2% hemolysis will be extracted and analyzed in replicates of  $n=1$  to determine if there is a significant impact on either the analyte or internal standard response due to the presence of whole blood. Selectivity for the analyte will be indicated by the absence of an apparent chromatographic peak at the retention time for the analyte that shows a peak area greater than 20% of the mean peak area observed for the extracted calibration standards at the LLOQ. Selectivity for the internal standard in these samples will be indicated by the absence of an apparent chromatographic peak at the retention time for the internal standard that shows a peak area greater than 5% of the mean peak area observed for the extracted samples containing the internal standard.



In all analytical batches, at least  $n=2$  samples containing no analyte or internal standard (BI/BI) will be extracted. Any peak detected at the retention time of the analyte should be less than 20% of the analyte mean peak area observed for the extracted calibration standards at the LLOQ. Any peak detected at the retention time of the internal standard should be less than 5% of the mean peak area observed for the extracted samples containing the internal standard. The same plasma lot used to prepare the fresh calibration curve should be used for this evaluation.

In all analytical batches, at least  $n=2$  samples containing no analyte but with internal standard included (BI/IS) will be extracted to confirm the suitability of the internal standard for use in the assay at that concentration. Any peak detected at the retention time of the analyte as a result of the internal standard addition should be less than 20% of the analyte mean peak area observed for the extracted calibration standards at the LLOQ. The same plasma lot used to prepare the fresh calibration curve should be used for this evaluation.

In at least one analytical batch, at least  $n=1$  sample containing only [Proprietary] at the upper limit of quantitation but containing no internal standard (ULOQ/BI), will be extracted. Any peak detected at the retention time of the internal standard as a result of the analyte addition at the ULOQ should be less than 5% of the internal standard mean peak area observed for the extracted samples containing internal standard. This will also be performed with at least  $n=1$  sample containing only [Proprietary] and at least  $n=1$  sample containing only [Proprietary] at the ULOQ concentration.

### Carryover

Carryover of the analytes will be determined during all validation batches by injecting at least one BI/BI sample immediately following injection of each highest calibration standard. Any peak detected at the retention time of the analyte in the first injected BI/BI sample should be less than 20% of the analyte mean peak area observed for the extracted samples at the LLOQ. Any peak detected at the retention time of the internal standard should be less than 5% of the internal standard mean peak area observed for the extracted samples containing internal standard.

### Method Accuracy and Precision

Quality Control (QC) samples at four concentrations will be freshly prepared in K<sub>2</sub> EDTA rat plasma on each day of analysis for use in accuracy and precision batches. These samples will contain both [Proprietary] and [Proprietary] at 5.00 ng/ml (LLOQ), 15.0 ng/ml (low), 400 ng/ml (mid), and 800 ng/ml (high) concentrations, and also [Proprietary] at 25.0 ng/ml, 75.0 ng/ml, 2000 ng/ml, and 4000 ng/ml, respectively.

To assess the accuracy and precision of the assay for the analytes, these QCs will be processed in  $n=6$  replicates on at least one day of analysis. For intra-batch (single run) accuracy (% accuracy), the results will be acceptable if the mean of the replicates at each concentration is within  $\pm 15\%$  of the nominal concentration ( $\pm 20\%$  at the LLOQ), and precision (as determined by %CV) at each concentration is  $\pm 15\%$  ( $\pm 20\%$  at the LLOQ). Additionally, for a single batch to be considered acceptable, at least 50% of the individual replicates at each concentration must be within  $\pm 15\%$  of the nominal concentration ( $\pm 20\%$  at the LLOQ), and at least 67% of the QCs in a batch must meet this acceptance criterion. A QC sample may be excluded from the overall statistics only for analytical reasons (poor chromatography, etc), or if determined that it is a statistical outlier by means of a Grubbs test. Results will be shown both with and without the inclusion of a statistical outlier.

The LLOQ QC samples will be independently prepared from the LLOQ calibration standards used to construct the calibration curve on the day of analysis.

The validation is considered to start on acceptance of the first intra-batch accuracy and precision run. If the calibration curve for a batch satisfied acceptance criteria, the accuracy and precision run will be reported and included in the inter-batch statistics, including those where the QC samples failed to meet acceptance criteria for which an assignable cause for failure (eg. documented preparation error, instrument breakdown, poor chromatography, etc) could not be determined.

### Recovery

The recovery of the method for the analytes and internal standards will be assessed by comparing the mean peak areas of the analytes and the internal standards in the QC samples (n=6) at low, mid and high concentrations after extraction to the mean peak areas obtained from extracted BI/BI (n=6) samples which were spiked post extraction with solutions containing both analyte and internal standard, to give a final concentration equivalent to the expected concentration in the final extracts, assuming the sample extraction efficiency is 100%. Determination of recovery is to characterize the assay, and there is no acceptance criterion for recovery.

Recovery will be determined by the following:

$$\frac{\text{Mean peak area of extracted samples}}{\text{Mean peak area of post-extracted spiked standards}} \times 100$$

### Matrix Effects on Ionization

The extent of the matrix effects on ionization of the analytes and internal standards will be assessed. A comparison of the mean peak areas of analyte and internal standard in extracted BI/BI samples spiked post-extraction with solutions containing both analytes and internal standards (at concentrations equivalent to low, mid and high QCs in the final extracts, assuming 100% recovery) to injections of these neat solutions (n=6) will be performed. Determination of matrix effects is to characterize the assay, and there is no acceptance criterion for this.

Matrix effects on ionization will be determined by the following:

$$\frac{\text{Mean peak area of post extracted spiked samples}}{\text{Mean peak area of solution standards}} \times 100$$

Values over 100% indicate signal enhancement caused by the matrix while values less than 100% indicate matrix induced signal suppression.

### Effect of Dilution

The ability to effectively dilute a high concentration study sample will be assessed by preparing a dilution QC at a concentration of 5000 ng/ml [Proprietary] and [Proprietary] and 25000 ng/ml [Proprietary Info]. This QC will be diluted 1:10 and 1:50 on the day of extraction using blank K<sub>2</sub> EDTA rat plasma, and will be extracted using n= 6 replicates. Effect of dilution will be considered successful if at least



50% of the individual replicates are within  $\pm 15\%$  of the nominal concentration and that overall accuracy and precision is  $\pm 15\%$ .

### **Matrix Effects Using 6 Unique Lots of Matrix**

Matrix effects on ionization will be assessed at the low QC concentration by spiking 6 unique lots of K<sub>2</sub> EDTA rat plasma (minimum of n=4 replicates per lot) with a solution of analytes to give a final plasma concentration of 15.0 ng/ml [Proprietary] and [Proprietary] and 75.0 ng/ml [Proprietary info]. These samples will then be extracted as per the Test Method. Matrix effects on ionization are not considered significant if at least 50% of the individual replicates are within  $\pm 15\%$  of the nominal concentration and that overall accuracy and precision of each lot is  $\pm 15\%$ . In the event that two or more lots fail acceptance criteria, another (different) 6 lots should be evaluated in a similar manner, and at least 9/12 lots must meet acceptance criteria.

### **Matrix Stability**

Plasma QC samples will be prepared at the low and high concentrations, subdivided into suitable volume aliquots, and stored in polypropylene vials in an ultra-low temperature freezer ( $\leq -60^{\circ}\text{C}$ ) for at least 24 hours prior to analysis. Matrix stability assessments will be conducted against a fresh calibration curve and fresh QC samples. Stability (freeze thaw stability, room temperature matrix stability, post-preparative extract stability, and long term storage stability in matrix) will be considered successful if at least 50% of the individual replicates at each concentration are within  $\pm 15\%$  of the nominal concentration and that overall accuracy and precision at each concentration is  $\pm 15\%$ . The acceptance criteria for re-injection stability and whole blood processing stability are detailed below.

#### **Freeze Thaw Stability**

Plasma QC samples at low and high QC concentrations in at least n=4 replicates will be removed from the  $\leq -60^{\circ}\text{C}$  freezer after storage for a minimum of 24 hours after initial preparation and allowed to thaw unassisted at room temperature for a minimum period of 1 hour. The samples will then be placed back in the  $\leq -60^{\circ}\text{C}$  freezer for a minimum of 12 hours. This process will then be repeated at least twice more prior to analysis. In the event that stability cannot be established or is unlikely to be successful, a smaller number of freeze thaw cycles may be examined, or the samples may be thawed on ice instead of at room temperature. This will be noted in the raw data.

#### **Room Temperature Matrix Stability**

Plasma QC samples at low and high QC concentrations in at least n=4 replicates will be removed from the  $\leq -60^{\circ}\text{C}$  freezer after storage and allowed to thaw unassisted at room temperature for a defined period. In the event that stability cannot be established over the original room temperature storage period, room temperature stability may be conducted over a shorter time period, or the samples may be stored on ice or in a refrigerator for a defined period of time instead of at room temperature. The actual storage times will be noted in the raw data.



### **Re-injection Stability**

To determine if it is appropriate to re-inject an entire batch, all calibration standards, QCs ( $n=6$  at LLOQ, low, mid and high concentrations) and all assay BI/BI and BI/IS samples (excluding individual specificity blanks) will be re-injected on the instrument following a defined period of storage. It is not necessary to re-inject other samples from additional experiments (matrix effects, effect of dilution, etc) which may have been originally extracted and analyzed with the original batch. The sample extracts after initial injection (prior to re-injection) will be stored in the autosampler (temperature set point  $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ ) or in a refrigerator, assuming equivalency between these two locations. Re-injection stability will be considered successful if the mean (intra-batch) accuracy and precision of the QCs is  $\pm 15\%$  ( $\pm 20\%$  at the LLOQ). At least 50% of the individual replicates at each concentration must be within  $\pm 15\%$  of the nominal concentration ( $\pm 20\%$  at the LLOQ), and at least 67% of the QCs in a batch must meet this acceptance criterion. The period of re-injection stability will be defined as the time from the end of extraction to the time of injection of the first calibration standard from the re-injected batch.

### **Post Preparative Extract Stability**

To determine the stability of the analytes after extraction from the plasma and while in the autosampler (temperature set point  $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ ) or refrigerator prior to injection, plasma QC samples at low and high concentrations in replicates of  $n=4$  at each concentration, will be extracted and stored in the autosampler for a defined period of time. These QCs will then be injected alongside a freshly extracted calibration curve and QCs. The period of stability will be determined by the time between placement in storage to the time of injection of the first stored QC.

### **Long Term Matrix Storage Stability**

Plasma QC samples at low and high QC concentrations in at least  $n=4$  replicates will be removed from the  $\leq -60^{\circ}\text{C}$  freezer after storage for a defined period of time and extracted and analyzed against a fresh calibration curve alongside fresh batch acceptance QC samples. Stability evaluations will be conducted at a minimum of one time-point and will at least cover the expected duration of sample storage from future sample analysis studies.

### **Whole Blood Processing Stability**

To determine the stability of the analyte in whole blood, prior to processing the blood sample to plasma, QC samples at two concentrations (representing low and high analyte levels, assuming 100% of analyte partitions into plasma) will be prepared in a pool of whole blood, which will then be subdivided after mixing. Plasma will be collected immediately after spiking (time zero) and at defined intervals (eg. 1 hour and 4 hours, stored refrigerated) after spiking. The plasma samples will be extracted in replicates of  $n=4$  at each concentration. The mean of the values from each stability timepoint will be compared to the mean of the value obtained from the time zero assessment. Stability will be achieved if the % difference is  $\pm 15\%$ . Full details on the conduct of this experiment are provided in SRI SOP 006.060 *Bioanalytical Method Validation*.

### Effect of Hemolysis

Plasma QC samples at low and high QC concentrations will be prepared in plasma matrix containing red blood cells to represent 0%, 0.5%, and 2% hemolysis, and extracted in at least n=4 replicates. Matrix effects of hemolysis will not be considered significant if at least 50% of the individual replicates at each concentration are within  $\pm 15\%$  of the nominal concentration and that overall accuracy and precision at each concentration is  $\pm 15\%$ .

### Concomitant Medications

A QC sample will be prepared containing 15.0 ng/ml [Proprietary], 2000 ng/ml [Proprietary] and 20000 ng/ml [Proprietary] and the impact of [Proprietary] and [Proprietary] on [Proprietary] quantitation will be assessed. Similarly, the effect of [Proprietary] and [Proprietary] on the quantitation of [Proprietary] will be assessed by preparing a QC sample at 2000 ng/ml [Proprietary], 15.0 ng/ml [Proprietary] and 20000 ng/ml [Proprietary]. To determine the effect of [Proprietary] and [Proprietary] on [Proprietary] quantitation, a QC sample containing 2000 ng/ml [Proprietary] and [Proprietary] and 75.0 ng/ml [Proprietary] will be prepared. The values selected for the concomitant medication concentrations were higher than the CMax values from SRI Study Number M332-17, where the animals were dosed at similar concentrations. These samples will be extracted in at least n=4 replicates. The effect of concomitant medications will not be considered significant if at least 50% of the individual replicates at each concentration are within  $\pm 15\%$  of the nominal concentration and that overall accuracy and precision at each concentration is  $\pm 15\%$ .

### DATA GENERATION AND RESULTS

The results from this study will be generated using AB Sciex Analyst software, version 1.6.2. This software will generate the peak area ratios for all samples, which will then be used to generate the calibration curve data and resulting QC concentrations, using unrounded values. The Analyst software will then use these values to calculate the overall precision and accuracy statistics. Microsoft Excel may be used in the calculation of some statistics.

### CHANGES TO THE VALIDATION PLAN

In the event that it becomes necessary to make significant changes to the procedures outlined in this validation plan, these changes will be fully documented in the study binder and communicated to the Sponsor, with a new validation plan generated if necessary.

Any deviations to any SRI SOP will be maintained with the raw data. If a deviation is judged to have an impact on the integrity of the study, a copy of the deviation will be sent to the Sponsor and will be discussed in the final report. Deviations with no impact on the integrity of the study will be included in the final report at the discretion of the bioanalytical scientist responsible for the conduct of the validation.

## REFERENCES

SRI- Test Method B181-18: *Analysis of [Proprietary] [Proprietary] and Tenofovir in K<sub>2</sub> EDTA Rat Plasma – (DRAFT)*

B185-18 “*Method Validation Report for the Quantitative Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma*”

SRI SOP 006.060: *Bioanalytical Method Validation*

SRI SOP 006.063: *Reference Material Receipt and Stock, Spiking Solution and Calibration and Quality Control Sample Preparation / Expiration*

**APPENDIX A**  
**SUMMARY OF METHOD DEVELOPMENT RESULTS**



**Method Development Completion Checklist (Partial Validation)****Study Number: B181-18****Batches:** MD1, MD1-RI, MD2, MD3, MD4 and MD5.

Method development has been satisfactorily completed for [Proprietary] [Proprietary] and [Proprietary] [Proprietary Info] [Proprietary] in K<sub>2</sub> EDTA rat plasma. The extraction procedure utilizes a protein precipitation extraction procedure followed by LC-MS/MS detection.

**Summary Table**

Analytes	[Proprietary] [Proprietary] [Proprietary]
Internal standards	[Proprietary Info] [Proprietary Info] [Proprietary Info]
Quantitation range	5.00-1000 ng/ml [Proprietary] and [Proprietary] 25.0-5000 ng/ml [Proprietary Info]
Sample volume	0.0200 ml
Regression type, weighting	Linear, 1/x <sup>2</sup> [Proprietary] and [Proprietary] Linear, 1/x [Proprietary Info]
Maximum batch size	115 samples
QC levels:	
LLOQ	5.00 ng/ml [Proprietary] and [Proprietary] 25.0 ng/ml [Proprietary Info]
Low	15.0 ng/ml [Proprietary] and [Proprietary] 75.0 ng/ml [Proprietary Info]
Mid	400 ng/ml [Proprietary] and [Proprietary] 2000 ng/ml [Proprietary Info]
High	800 ng/ml [Proprietary] and [Proprietary] 4000 ng/ml [Proprietary Info]
Dilution QC level	5000 ng/ml [Proprietary] and [Proprietary] 25000 ng/ml [Proprietary Info] diluted 10-fold, 50-fold
Intra-batch precision	1.3% to 6.1% [Proprietary Info] 1.2% to 5.2% [Proprietary] 1.0% to 4.4% [Proprietary Info]
Intra-batch accuracy	93.9% to 102.2% [Proprietary Info] 93.9% to [Proprietary] [Proprietary] 95.9% to 100.1% [Proprietary Info]
Inter-batch precision	3.6% to 4.1% [Proprietary Info] 2.8% to 5.6% [Proprietary] 1.6% to 3.7% [Proprietary Info]
Inter-batch accuracy	95.4% to 100.8% [Proprietary Info] 95.9% to 97.8% [Proprietary] 98.2% to 99.0% [Proprietary Info]
Analyte and internal standard recovery	90.7% [Proprietary Info] 89.3% [Proprietary Info] 94.3% [Proprietary Info] 99.3% [Proprietary Info] 95.6% [Proprietary Info] 92.4% [Proprietary Info]



Matrix effect on ionization of analyte and internal standard	117.3%	Proprietary Info
	144.4%	Proprietary
	100.6%	Proprietary Info
	116.8%	Proprietary Info
	146.9%	Proprietary Info
	100.5%	Proprietary Info
Selectivity	6 out of 6 lots satisfied acceptance criteria	
Matrix effects	6 out of 6 lots satisfied acceptance criteria	
Analyte carryover	<20% of mean LLOQ peak area <span>Proprietary</span> and <span>Proprietary Info</span> Some carryover (21-29%) was observed for <span>Proprietary</span>	
Internal Standard carryover	<5% of mean internal standard peak area	
Room temperature stability	26 hours established (all three analytes)	
Freeze thaw stability	5 cycles established (all three analytes)	
Reinjection (autosampler) stability	148 hours (refrigerated) established (all three analytes)	
Post-preparative extract stability	117 hours (refrigerated) established (all three analytes)	
Whole blood processing stability	4 hours (all three analytes)	
Effect of hemolysis	0.5% and 2% hemolysis; no impact (all three analytes)	

Redacted by agreement

06/12/19

/ Date

**Method Validation Report for the Quantitative Analysis of** Proprietary Info  
**and** Proprietary **in K<sub>2</sub> EDTA Rat Plasma**  
**SRI Study No. B181-18**

**Appendix C**

**CERTIFICATES OF ANALYSIS**  
**(REFERENCE STANDARDS AND INTERNAL STANDARDS)**

# Certificate

Proprietary Info

**LABEL TEXT**

For use with specified USP compendial tests.  
Not for use as a drug. See SDS prior to use  
at [www.usp.org/sds](http://www.usp.org/sds).

**USP REFERENCE STANDARD**

Proprietary Info

**350 mg**

For quantitative applications, determine the water content  
titrimetrically at the time of use. Use as is material and correct  
weight for water content. Use a value of 0.997 mg of Proprietary per  
mg of material on the anhydrous basis. Keep container tightly  
closed. Protect from light. Store in a refrigerator.

USP, 12601 Twinbrook Pkwy, Rockville, MD, +1-301-881-0666  
Cat. No. 1370101 Material mfd. in India

LOT: R077R0



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*Quality Assurance*

**Calculation Value**

If a value is not provided on the label or accompanying documentation and the Reference Standard has a quantitative USP compendial application, a value of 100.0% is used. The purity value is not applicable for qualitative uses. Please refer to the specific Reference Standard label for further information.

**Expiration**

Current lots are identified in the current USP Catalog. In some cases, the previous lot may still be considered valid for use. If so, it is identified in the column marked "Previous Lot/Valid Use Date."

It is the responsibility of each user to determine that this lot is current or valid when used. For the most up-to-date information, please refer to the USP Store at [www.usp.org](http://www.usp.org).

**Instructions for Use**

Follow the instructions on the label of the USP Reference Standard and in the appropriate USP documentary standard(s).

**Non-Monograph Use**

The suitability of this Reference Standard for use in non-compendial applications is solely the responsibility of the user.

**LEGAL NOTICE**

USP WARRANTS GOOD TITLE TO USP REFERENCE STANDARDS ON DISPATCH FROM USP. THE FOREGOING WARRANTY IS IN LIEU OF ANY OTHER WARRANTIES, EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION ANY WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, OR ANY WARRANTY THAT THE PRODUCTS, INCLUDING THIS CERTIFICATE, ARE OF MERCHANTABILITY QUALITY. USP'S LIABILITY ARISING OUT OF OR RELATING TO THE SUPPLY OF USP REFERENCE STANDARDS AND THIS CERTIFICATE SHALL IN NO EVENT INCLUDE LOSS OF PROFITS, COST OF PROCURING SUBSTITUTE GOODS OR SERVICES, OR ANY INCIDENTAL, INDIRECT, OR CONSEQUENTIAL DAMAGES OF ANY KIND, EVEN IF USP IS AWARE OF THE POSSIBILITY OF SUCH DAMAGES. WITHOUT LIMITING THE GENERALITY OF THE FOREGOING, USP DOES NOT WARRANT THAT THE USE OR RESALE OF USP REFERENCE STANDARDS, INCLUDING THEIR USE TO PERFORM TESTS AND ASSAYS PUBLISHED BY USP, WILL NOT INFRINGE UNITED STATES OR ANY OTHER PATENTS.

USP Reference Standards are not intended for use as drugs, dietary supplements, or as medical devices.

This certificate may not be reproduced without the express written permission of USP.

# Certificate

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**LABEL TEXT**



**USP REFERENCE STANDARD**  
**Proprietary 200 mg**  
Warning! Harmful if swallowed. Causes skin irritation.  
Causes serious eye irritation.  
Do not dry. For quantitative applications, use a value of 0.993 mg of **Proprietary** per mg of material on the as is basis. Keep container tightly closed. Protect from light.  
USP, 12801 Twinbrook Pkwy, Rockville, MD, +1-301-881-0666  
CAT No. 1604803 Material mfd. in Italy

Wash thoroughly after handling. Wear protective gloves. Wear eye/face protection. If swallowed: Call a poison center/doctor if you feel unwell. Rinse mouth. If on skin: Wash with plenty of water. If skin irritation occurs: Get medical advice/attention. Take off contaminated clothing and wash before reuse. If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical advice/attention. Dispose of contents/container in accordance with local/regional/national/international regulations.

Redacted by agreement

*Quality Assurance*

**Calculation Value**

If a value is not provided on the label or accompanying documentation and the Reference Standard has a quantitative USP compendial application, a value of 100.0% is used. The purity value is not applicable for qualitative uses. Please refer to the specific Reference Standard label for further information.

**Expiration**

Current lots are identified in the current USP Catalog. In some cases, the previous lot may still be considered valid for use. If so, it is identified in the column marked "Previous Lot/Valid Use Date."

It is the responsibility of each user to determine that this lot is current or valid when used. For the most up-to-date information, please refer to the USP Store at [www.usp.org](http://www.usp.org).

**Instructions for Use**

Follow the instructions on the label of the USP Reference Standard and in the appropriate USP documentary standard(s).

**Non-Monograph Use**

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# Certificate

Proprietary Info

**LABEL TEXT**

For use with specified USP compendial tests.  
Not for use as a drug. See SDS prior to use at  
[www.usp.org/sds](http://www.usp.org/sds).



**REFERENCE STANDARD**

Proprietary Info

**15 mg**

This is the monohydrate form of Proprietary Info Do not dry.  
Keep container tightly closed. Store in the refrigerator.

USP, 12601 Twinbrook Pkwy, Rockville, MD, +1-301-881-0666  
Cat. No. 1643601 Material mfd. in China

LOT: R044C0



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*Quality Assurance*

**Calculation Value**

If a value is not provided on the label or accompanying documentation and the Reference Standard has a quantitative USP compendial application, a value of 100.0% is used. The purity value is not applicable for qualitative uses. Please refer to the specific Reference Standard label for further information.

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**MEDICAL ISOTOPES, INC.**  
100 Bridge Street  
Pelham, NH 03076 USA  
Tel: 603 635-2255, Toll Free: 800 374-9513  
Fax: 603 635-2448  
E-Mail: [info@medicalisotopes.com](mailto:info@medicalisotopes.com)  
URL: [www.medicalisotopes.com](http://www.medicalisotopes.com)

Proprietary Info

## CERTIFICATE OF ANALYSIS

**Product Name:**

Proprietary Info

**Catalog No:**

Proprietary Info

**Lot No:**

**Date:**

June 2017

**Re Test Date:**

June 2024

**Method of Analysis:**

<sup>1</sup>H-NMR and Mass Spec

**Purity:**

Chemical purity: 98%

Isotopic purity: 98%

**Molecular Formula:**

**Molecular Weight:**

Proprietary Info

**Appearance of Product:**

Pale Beige Solid

**Stability:**

N/A

**Melting Point:**

N/A

**Boiling Point:**

N/A

**Solubility:**

N/A

**Storage:**

-20°C in freezer. Under inert atmosphere

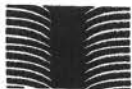
**Additional Information:**

NMR and MS conforms to structure

Personal Info

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**MEDICAL ISOTOPES, INC.**  
100 Bridge Street  
Pelham, NH 03076 USA  
Tel: 603 635-2255, Toll Free: 800 374-9513  
Fax: 603 635-2448  
E-Mail: [info@medicalisotopes.com](mailto:info@medicalisotopes.com)  
URL: [www.medicalisotopes.com](http://www.medicalisotopes.com)

Proprietary Info

## CERTIFICATE OF ANALYSIS

**Product Name:** Proprietary Info

**Catalog No:** Proprietary Info

**Lot No:**

**Date:** April 2016

**Retest Date:** April 2020

**Method of Analysis:**

$^1\text{H}$  NMR and Mass Spec

**Purity:**

Chemical purity: 97%

Isotopic purity: 99%

**Molecular Formula:**

Proprietary Info

**Molecular Weight:**

**Appearance of Product:**

Pale Yellow Solid

**Stability:**

N/A

**Melting Point:**

N/A

**Boiling Point:**

N/A

**Solubility:**

N/A

**Storage:**

-20°C in freezer, Under Inert Atmosphere

**Additional Information:**

$^1\text{H}$  NMR and mass spectra conform to structure.

TLC: Single Spot

Personal Info

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100 Bridge Street  
Pelham, NH 03076 USA  
Tel: 603 635-2255, Toll Free: 800 374-9513  
Fax: 603 635-2448  
E-Mail: [info@medicalisotopes.com](mailto:info@medicalisotopes.com) URL: [www.medicalisotopes.com](http://www.medicalisotopes.com)

Proprietary Info

## CERTIFICATE OF ANALYSIS

**Product Name:**

Proprietary Info

**Catalog No:**

**Lot No:**

**Date:**

October 2016

**Re Test Date:**

October 2020

**Method of Analysis:**

<sup>1</sup>H-NMR, HPLC, and Mass Spec

**Purity:**

Chemical purity: 97%

Isotopic Purity: 99%

**Molecular Formula:**

Proprietary Info

**Molecular Weight:**

**Appearance of Product:**

White Solid

**Stability:**

N/A

**Melting Point:**

N/A

**Boiling Point:**

N/A

**Solubility:**

N/A

**Storage:**

-20°C in freezer

**Additional Information:**

NMR and Mass Spec conforms to structure

Personal Info

Redacted by agreement

**I. SAFETY EVALUATION OF** Proprietary Info **AFTER 28 DAYS OF REPEAT DOSE ADMINISTRATION IN BEAGLE DOGS****II. SRI STUDY NUMBER: M393-18****III. SPONSOR**

National Institute of Allergy and Infectious Disease  
Division of AIDS  
5601 Fishers Lane, Room 9F39  
Bethesda, MD 20892

Contract Number: HHSN272201400006I

Sponsor's Representative:

Redacted by agreement**IV. TESTING FACILITY**

SRI International  
Biosciences Division  
333 Ravenswood Avenue  
Menlo Park, CA 94025

Study Director:

Redacted by agreement**V. PROPOSED IN-LIFE SCHEDULE**

Start of In-life (first dose): September 19, 2018

Termination (final necropsy): November 11, 2018

**VI. APPROVALS**Redacted by agreement\_\_\_\_\_  
Sponsor's Representative\_\_\_\_\_  
DateRedacted by agreement**REVIEWED BY:**\_\_\_\_\_  
Date\_\_\_\_\_  
SRI Quality Assurance\_\_\_\_\_  
Date

Safety Evaluation of [Proprietary Info] after 28 Days of  
Repeat Dose Administration in Beagle Dogs  
SRI Study No. M393-18

## **VII. PURPOSE OF STUDY**

The purpose of this study is to provide data of suitable quality and integrity to support application to the U.S. Food and Drug Administration (FDA) and other regulatory agencies. Therefore, this study will be performed in accordance with the U.S. FDA “Good Laboratory Practice for Nonclinical Laboratory Studies” (GLP) as described in 21 CFR Part 58.

## **VIII. STUDY OBJECTIVE**

The objectives of this study are: (1) to determine potential toxic effects and target organs of toxicity and to identify a no observable adverse effect level (NOAEL) of [Proprietary Info] in dogs after twice daily oral dose administration for 28 days; (2) to assess the reversibility of effects (if any) after 14 days of recovery; and (3) to determine the toxicokinetic parameters after single and multiple dose administrations.

## **IX. SPONSOR RESPONSIBILITIES**

The Sponsor is responsible for the following:

1. Documentation on the strength, purity composition, physical properties, stability and other pertinent information on the bulk test article in the form of a Certificate or Record of Analysis and a Certificate of Stability or other documentation for the bulk test article for inclusion in the final report.
2. Providing sufficient quantity of test article.

**Safety Evaluation of Amikacin-Cochleate after 28 Days of  
Repeat Dose Administration in Beagle Dogs  
SRI Study No. M393-18**

**X. EXPERIMENTAL DESIGN**

Group	Treatment	AMK Dose Level (mg/kg)	Phospholipid <sup>1</sup> Dose Level (mg/kg/day)	AMK Dose Conc. (mg/ml)	Phospholipid  Conc. (mg/ml)	Total No. of Animals	No. of Animals Sacrificed	
							Main (Day 29) <sup>8</sup>	Recovery (Day 42) <sup>9</sup>
1	Proprietary Info	0	TBD	0	TBD	5M/5F	3M/3F	2M/2F
2		0	0	0	0	3M/3F	3M/3F	-
3		2	TBD	0.2	TBD	3M/3F	3M/3F	-
4		10	TBD	1	TBD	3M/3F	3M/3F	-
5		50	TBD	5	TBD	5M/5F	3M/3F	2M/2F
6		200	TBD	187		3M/3F	3M/3F	-
Total No. of Animals						22M/22F	18M/18F	4M/4F

Proprietary Info

Proprietary Info

will be administered at 400 mg/kg BID, 8 hr interval.

Proprietary  
Info

will be administered at BID, 8 hr interval.

Proprietary Info

will be administered at 1 mg/kg BID, 8 hr interval.

will be administered as 5 mg/kg BID, 8 hr interval.

will be administered as 25 mg/kg BID, 8 hr interval.

Group 6 will receive Proprietary Info via intramuscular (IM) dose; all other groups will receive test article or vehicle by oral gavage (PO)

<sup>8</sup> Main Study animals will be euthanized on Day 29.

<sup>9</sup> Recovery Group animals will be euthanized on Day 42.

BID = twice per day QD = once daily

Any change will be approved by the Study Director and documented in the study records. Variances in volumes administered should not be more than  $\pm 30\%$  and must be within the IACUC-approved dose volume administration guideline limit.

**Species and Strain**

Beagle dog

**Route of Administration**

Proprietary Info

**Frequency**

Two daily administrations for 28 days. The interval for dosing will be  $8 \pm 1$  hr. Single daily administration for 28 days for Group 6 (Proprietary Info IM).



Safety Evaluation of [Proprietary Info] after 28 Days of  
Repeat Dose Administration in Beagle Dogs  
SRI Study No. M393-18

**Dosing Volume**

5 ml/kg for oral gavage (po) with [Proprietary Info] and vehicle (Group 1-5). 1.07 ml/kg for intramuscular (IM) with [Proprietary] (Group 6). Dose volumes will be calculated based on each animal's most recent body weight. The dose volume may be adjusted to achieve the target dose levels based on actual measured concentration of the dose solution. Any change will be approved by the Study Director and documented in the study records.

**Duration of In-Life Phase**

42 days

**XI. MATERIALS AND METHODS**

**A. Test and Control Articles**

**1. Test Article**

[Proprietary Info] [Proprietary] [Proprietary] [Proprietary] Drug Product)

**Supplier**

To be documented in the final report

**Manufacturer**

To be documented in the final report

**Lot Number**

To be documented in the final report

**Physical Description**

To be documented in the final report

**Storage Conditions**

Refrigerator, 2–8°C (36–46°F)

**Characterization of Test Article**

The Sponsor is responsible for characterization and stability of the test article and will provide a Certificate of Analysis (CofA), or equivalent documentation, to SRI for inclusion in the final report. The raw data generated by the Sponsor in support of this CofA or its equivalent will not be verified or maintained by SRI.

**2. Reference Control**

[Proprietary Info]



**Safety Evaluation of [Proprietary Info] after 28 Days of  
Repeat Dose Administration in Beagle Dogs  
SRI Study No. M393-18**

**Supplier**

[Proprietary Info]

**Manufacturer**

To be documented in the final report

**Lot Number**

To be documented in the final report

**Physical Description**

To be documented in the final report

**Storage Conditions**

Room temperature, 15–30°C (59–86°F)

**Characterization of Reference Control**

Information on the identity, purity, and stability of the reference article may be obtained by recording all of the pertinent information provided on the container labels or in a CofA provided by the supplier.

**3. Vehicle Control**

[Proprietary] [Proprietary] [Proprietary] Placebo)

**Supplier**

To be documented in the final report

**Manufacturer**

To be documented in the final report

**Lot Number**

To be documented in the final report

**Physical Description**

To be documented in the final report

**Storage Conditions**

Refrigerator, 2–8°C (36–46°F)

**Safety Evaluation of [Proprietary Info] after 28 Days of  
Repeat Dose Administration in Beagle Dogs  
SRI Study No. M393-18**

**Characterization of Vehicle Control**

Information on the identity, purity and stability of the control article may be obtained by recording all of the pertinent information provided on the container labels or in a CofA provided by the supplier.

**4. [Proprietary Info] Control**

[Proprietary Info]

**Supplier**

To be documented in the final report

**Manufacturer**

To be documented in the final report

**Lot Number**

To be documented in the final report

**Physical Description**

To be documented in the final report

**Storage Conditions**

Room temperature, 15–30°C (59–86°F)

**Characterization of Vehicle Control**

Information on the identity, purity and stability of the control article may be obtained by recording all of the pertinent information provided on the container labels or in a CofA provided by the supplier.

**5. Preparation of Dose Formulations**

Dose formulations will be provided by the Sponsor. Container should be mixed vigorously to obtain a homogeneous suspension. No other additions are necessary. The appropriate dose should be removed from the container and administered. One container per group per day will be supplied with sufficient material for that day's dosing plus 25% extra material. For [Proprietary] (generic), dose calculations will be based upon the free base amount. [Proprietary] will be used as commercially available [Proprietary Info]

[Proprietary Info] dose level will be calculated as free base at 75%, and therefore [Proprietary] dose formulation concentration is 187 mg/ml.

**Storage of Dose Formulations**

**PO dose formulations for Groups 1 -5** will be stored in the refrigerator, 2-8°C until the day of use. Formulations will be brought to room temperature prior to administration to the animals.

Safety Evaluation of Proprietary Info after 28 Days of  
Repeat Dose Administration in Beagle Dogs  
SRI Study No. M393-18

IM dose formulation for Group 6 Amiglyde-V® Solution will be stored at 15–30°C.

**6. Characterization of Dose Formulations**

The Sponsor will provide formulated, ready-to-dose test article; therefore, SRI will not conduct assays to verify dose formulations, concentration and homogeneity, and stability under the conditions of the study. Reference control Proprietary is a pre-formulated commercial veterinary product and will not be verified for stability, concentration or homogeneity

**7. Test Article Handling**

At a minimum, personnel handling the test, reference and control article formulations will wear eye protection, gloves and a protective smock or laboratory coat.

**8. Disposition**

At the end of the study any remaining partially used and unused containers of vehicle control, test article and reference article will be shipped to the Sponsor unless the Sponsor issues other directions.

Residual dose formulations will be discarded after analysis, when the final report is submitted, or when samples no longer afford evaluation.

Empty control, test and reference article containers may be destroyed by SRI on submission of the final report to the sponsor.

See Section XII.D, “Regulatory Compliance,” for information about retention of records and study samples.

**9. Method for Assuring Correct Dosing**

The administration of each dose formulation will be properly documented and the amount administered to each animal will be recorded.

**B. Test System**

**Species**

Dog

**Strain**

Beagle

**Supplier**

Proprietary Info

**Safety Evaluation of [Proprietary Info] after 28 Days of  
Repeat Dose Administration in Beagle Dogs  
SRI Study No. M393-18**

**Number of Animals**

44 assigned to test

**Sex**

22 Males and 22 females

**Age at First Dose**

6-8 months

**Weight Range at First Dose**

8–11.5 kg (males); 6.5–11 kg (females)

**10. Animal Care**

General procedures for animal care and housing will be in accordance with the current Association for assessment and Accreditation of Laboratory Animal Care (AAALAC) in recommendations, current requirements stated in the *Guide for the Care and Use of Laboratory Animals* (National Research Council), and current requirements as stated by the U.S. Department of Agriculture through the Animal Welfare Act and Animal Welfare regulations (November 2013).

**Quarantine/Acclimation**

At least 14 days. A complete physical examination will be performed on each dog before quarantine release.

**Housing**

1 per enclosed run ( $\geq 4$  ft x 6 ft)

**Light Cycle**

12 hr light/12 hr dark

**Temperature**

64–84°F

**Humidity**

30–70%. Brief excursions outside this range may occur; excursions of less than 4 hr/day will not be considered deviations from the protocol.

**Ventilation**

At least 10 room volumes per hour, with no recirculation of air.



Safety Evaluation of Proprietary Info after 28 Days of  
Repeat Dose Administration in Beagle Dogs  
SRI Study No. M393-18

**Food**

Envigo Teklad Certified Global 25% Protein Dog Diet, 2025C or equivalent. Dogs will be exposed to their daily ration of food, except for periods of fasting required by the study protocol. The quantity of the daily ration is sufficient to meet nutritional requirements. Feed is analyzed periodically to ensure that contaminants known to be capable of interfering with the study and reasonably expected to be present in such feed are not present at levels that would affect the study. Documentation of feed analyses is maintained at SRI for reference. A copy of the lot specific reports provided by the supplier will be maintained in the study records.

**Water**

Water (purified, reverse osmosis or untreated tap water) will be provided *ad libitum*. Based on previous reports, no contaminants that could interfere with and affect the results of the study are expected to be present in the water. Copies of annual analysis reports are maintained at SRI for reference.

**11. Assignment of Animals to Study**

**Day**

No more than 5 days before initiation of treatment.

**Randomization**

Animals will be randomly assigned to treatment groups via a computerized body weight stratification procedure. Animals may be excluded based on health, behavior or inappropriate weight.

**Identification**

Animals will be individually identified by a uniquely numbered ear tattoo.

**12. Welfare of the Animals**

Every effort will be made to minimize, if not eliminate, pain and suffering in all animals in this study. Moribund animals and animals experiencing undue pain and suffering will be euthanized at the discretion of the Study Director, attending veterinarian, or other qualified person. The Study Director will make every effort to protect the scientific validity of the study.

**C. Experimental Procedure (In-Life Evaluations)**

**1. Preparation of Animals**

Animals will be ~~be~~ not **fasted** before dose administration.



**Safety Evaluation of [Proprietary Info] after 28 Days of  
Repeat Dose Administration in Beagle Dogs  
SRI Study No. M393-18**

**2. Dose Administration**

Oral (po) via gavage with [Proprietary Info] and [Proprietary Info]. The oral route of administration is proposed for clinical use of the test article in humans; gavage administration is considered a reasonable surrogate for the proposed human route. [Proprietary] will be given via intramuscular (IM) injection once a day for 28 days (Group 6). Injection may be administered to a single site or multiple sites, as necessary.

**3. Mortality/Morbidity**

Animals will be checked at least once daily.

**4. Clinical Observations**

Recorded once daily and approximately 2–4 hr postdose on treatment days, or more often as clinical signs warrant, and on the day of necropsy. Animals will be examined for any altered clinical signs, including gross motor and behavioral activity, and observable changes in appearance.

**5. Body Weights**

Body weights will be recorded prior to dosing on Day 1 for the purpose of dose calculation and weekly thereafter as well as at each necropsy.

Body weights will be recorded for animals found dead and for any euthanized early, but these weights will not be included in the statistical evaluations.

**6. Food Consumption**

Quantitatively measured for approximately a 24-hr period once weekly for each run throughout the study. The total run consumption per interval will be divided by the number of animals in the run to determine the average daily food consumption per animal.

**7. Ophthalmologic Examination**

All animals will have a pre-test ophthalmic examination performed by a board-certified veterinary ophthalmologist, and all surviving animals will be re-examined by the ophthalmologist during the final week before their scheduled necropsy. (i.e., during week before Day 29 for main study animals and week before Day 42 for recovery animals)

**8. Plasma Drug Levels**

**Method of Collection**

Blood will be collected from cephalic, saphenous, or jugular veins into tubes containing K<sub>3</sub>EDTA, processed to plasma, and then stored frozen at ≤−60°C.

Safety Evaluation of [Proprietary Info] after 28 Days of  
Repeat Dose Administration in Beagle Dogs  
SRI Study No. M393-18

**Volume**

Maximum ~2 ml whole blood (~ 800 µl of plasma) per sample

**Frequency**

Plasma drug levels will be determined at the following time points after the first dose administration on Day 1 and Day 28:

**Group 1:** Blood samples will be collected on Day 1 at 1 hr (3 dogs per time-point)

**Group 2:** Blood samples will **not** be collected for TK analysis.

**Groups 3-5:** Blood samples will be collected on Day 1 and Day 28 after the **first dose** only, at the following time points: Pre-dose and at 0.5, 1, 2, 4, 8 and 24 hr post-dose (3 dogs/per time-point). The 8 hr samples will be collected *prior* to the second dose; the 24 hr samples will be collected *prior* to the next day's first dose. Pre-dose samples on Day 28 will be collected *prior* to dose administration on Day 28.

**Group 6** [Proprietary Info] **IM):** Blood samples will be collected for TK analysis **on Day 1 Pre-dose only** and on Day 28 at the following time points: 0.5, 1, 2, 4, 8 and 24 hr post-dose (3 dogs per time-point).

**Method of Analysis**

Drug levels of [Proprietary] will be determined in collected plasma samples using a bioanalytical method developed and validated at SRI. Details of the bioanalytical method and validation results are included in a separate validation report [Proprietary Info]

**9. Toxicokinetics Analysis**

The plasma drug level data will be analyzed using Phoenix<sup>®</sup> WinNonlin<sup>®</sup> (version 6.4 or higher) software to perform noncompartmental modeling. The dose administered will be input to the program as mg/kg, and as a result no additional corrections for individual body weights of the animals will be necessary.

The following parameters and constants will be determined if the data allow: maximal plasma concentration ( $C_{max}$ ), time to maximum plasma concentration ( $T_{max}$ ), area under the plasma concentration time curve (AUC), and terminal elimination half-life ( $t_{1/2}$ ).

Other methods of analysis may be used or other parameters calculated, as appropriate, based on the plasma drug level data.

**Disposition**

Residual bioanalytical samples will be discarded on submission of the final report.

Safety Evaluation of Proprietary Info after 28 Days of  
Repeat Dose Administration in Beagle Dogs  
SRI Study No. M393-18

**10. Clinical Pathology Evaluations**

**Preparation of Animals**

Animals will be **fasted** before blood collection.

**Method of Collection**

Hematology samples will be collected using K<sub>3</sub> EDTA as the anticoagulant.  
Coagulation samples will be collected using citrate as the anticoagulant.

Blood will be collected, when possible, from moribund animals before euthanasia for clinical pathology analysis. The data from moribund animals will not be included in the statistical analysis.

**Frequency**

Day 8 (only clinical chemistry). At scheduled sacrifice on Day 29 and Day 42 for all clinical chemistry, hematology and coagulation parameters.

Clinical pathology parameters that will be evaluated are listed below. In some cases automated analyzers report additional parameters not specified in the protocol. Results for the additional parameter(s) will be included in the data package, but will not be summarized, analyzed or reported, and their collection will not be considered deviations from the protocol.

If manual WBC differential counts have to be conducted, some parameters that are not specified in the protocol may be evaluated and reported. This will not be considered a deviation from the protocol.

**Hematology Parameters**

- Hematocrit (HCT)
- Hemoglobin (HGB)
- Red blood cell count (RBC)
- Red blood cell distribution width (RDW)
- White blood cell count (WBC)
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular volume (MCV)
- Mean corpuscular hemoglobin concentration (MCHC)
- Platelet count (PLT)
- Mean platelet volume (MPV)
- Absolute Reticulocyte (ARET)
- Percent Reticulocyte (PRET)
- WBC differential and absolute counts
  - Absolute neutrophil [ANE]
  - Percent neutrophil [PNE]
  - Absolute lymphocyte [ALY]



**Safety Evaluation of [Proprietary Info] after 28 Days of  
Repeat Dose Administration in Beagle Dogs  
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- Percent lymphocyte [PLY]
- Absolute monocyte [AMO]
- Percent monocyte [PMO]
- Absolute eosinophil [AEO]
- Percent eosinophil [PEO]
- Absolute basophil [ABA]
- Percent basophil [PBA]

**Clinical Chemistry Parameters**

- Total Bilirubin (TBI)
- Creatinine (CRE)
- Sodium (SOD)
- Potassium (POT)
- Chloride (CHL)
- Cholesterol (CHO)
- Triglyceride (TRI)
- Glucose (GLU)
- Blood urea nitrogen (BUN)
- Aspartate aminotransferase (AST)
- Alanine aminotransferase (ALT)
- Alkaline phosphatase (ALP)
- Calcium (CAL)
- Phosphorus (PHO)
- Total protein (TPR)
- Albumin (ALB)
- Albumin/globulin ratio (AGR)
- Globulin (GLO)

**Coagulation**

- Prothrombin time (PT)
- Activated partial thromboplastin time (PTT)
- Fibrinogen (FIB)

**11. Urinalysis**

**Method of Collection**

Urine, if available, will be collected by cystocentesis. ≤15 ml will be submitted to the clinical laboratory.

**Frequency**

Day 29 (Main necropsy) and Day 42 (Recovery necropsy)

Safety Evaluation of Proprietary Info after 28 Days of  
Repeat Dose Administration in Beagle Dogs  
SRI Study No. M393-18

**Urinalysis Parameters**

- Color (noted at time of collection)
- Clarity (noted at time of collection)
- Specific gravity
- Microscopic examination of urine sediment
- Bilirubin
- Glucose
- Ketones
- Leukocytes
- Nitrite
- Occult blood
- pH
- Protein
- Urobilinogen

**D. Necropsy**

**Interval**

Day 29 for Main Group and Day 42 for Recovery group. Necropsies will also be performed for any animals found dead or euthanized in moribund condition.

**Euthanasia**

Following sedation via subcutaneous administration of a sedative cocktail (as recommended by a LAMD veterinarian). Dogs will be euthanized via an overdose of a commercially available sodium pentobarbital-based euthanasia solution (e.g., Euthasol) administered by iv injection.

**Observations**

External examination of all body orifices and an examination of all cranial, thoracic and abdominal organs will be performed, and all gross findings will be recorded.

**Tissues Retained**

The following tissues will be collected from all animals in the Main Group and the Recovery Group, including those found dead and moribund animals. Tissues will be retained in 10% neutral buffered formalin, except where noted.

- All gross lesions (including tissue masses and abnormal regional lymph nodes)
- Adrenal glands
- Aorta
- Bone (femur, distal with joint surface)
- Bone, sternum (marrow histology)



Safety Evaluation of Proprietary Info after 28 Days of  
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SRI Study No. M393-18

- Bone marrow smear, sternum (for cytology except for found dead animals)
- Brain (fore-, mid-, and hindbrain)
- Cecum
- Cervix
- Colon
- Duodenum
- Epididymides
- Esophagus
- Eyes, with optic nerve (fixed with modified Davidson's solution)
- Gall bladder
- Heart
- Identification; (retained in formalin; not processed for histology)
- Ileum
- Injection site(s) tissue. Only representative sections of the injection site(s) will be collected (**Groups 2 and 6**)
- Jejunum
- Kidneys
- Liver
- Lungs with bronchi
- Lymph nodes, mandibular and mesenteric
- Mammary gland (females, males when present)
- Ovaries
- Pancreas
- Pituitary gland
- Prostate
- Rectum
- Salivary gland, mandibular
- Sciatic nerve
- Skeletal muscle
- Skin, ventral abdomen, taken with mammary gland
- Spinal cord ~~retained within spinal column~~ section (thoracic only)
- Spleen
- Stomach
- Tongue (**Groups 1, 3, 4-5**)
- Testes (fixed with modified Davidson's solution)
- Thymus
- Thyroid/parathyroid glands
- Trachea
- Urinary bladder
- Uterus
- Vagina

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**Final Body/Organ Weights**

Body weight will be recorded on the day of necropsy for body to organ weight ratios. The following organs will be weighted. Paired organs will be weighted together.

- Adrenal glands
- Brain
- Heart
- Kidneys
- Liver
- Ovaries
- Testes, without epididymides
- Thymus

Organ weights will be recorded for animals found dead or sacrificed in moribund condition, but these data will not be included in statistical evaluations.

**E. Histopathologic Examination**

**Tissues**

Tissues listed above will be processed and evaluated for the following:

- All Main animals in the control and high-dose groups (**Groups 1, 2, 5 and 6; oral and IM groups**)
- Animals with an unscheduled death or euthanized in moribund condition
- Any tissue identified as a target organ of toxicity by the pathologist (examined in all other dose groups)
- Any other tissue deemed necessary by the pathologist
- All gross lesions will be processed for all animals
- Bone marrow smear for cytology will be processed but only analyzed at the discretion of the Study Director and/or pathologist based on hematology and/or bone marrow histopathology findings.
- If neurological clinical signs are not present, only a representative sample of the thoracolumbar (or thoracic) section of the spinal cord will be collected, processed and evaluated. If neurological clinical signs are observed, representative sections of all available spinal cord tissues (cervical, thoracic and lumbar sections) will be collected, processed and evaluated.

**Tissue Sections**

Sections of the tissues will be embedded in paraffin, cut approximately 5 µm thick, and stained with hematoxylin and eosin by a histology laboratory qualified by SRI.

Safety Evaluation of Proprietary Info after 28 Days of  
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**Evaluated By**

A board-certified veterinary pathologist.

**Method**

Each lesion will be listed and coded by the most specific topographic and morphologic diagnoses, severity, and distribution, using International Harmonization of Nomenclature and Diagnostic Criteria for Lesions (INHAND) as a guide. A four-step grading system (minimal, mild, moderate and marked) will be used to define gradable lesions for comparison between treated and control groups. Data will be recorded and summarized using Provantis® version 9.3.1.1 or other appropriate program. Records of gross findings for a specimen from postmortem observations will be available to the pathologist when examining that specimen microscopically.

**F. Evaluation of Data Parameters**

Mean and standard deviation will be calculated for body weight, food consumption, clinical pathology, urinalysis pH, urobilinogen, and specific gravity, and organ weight data at each evaluation interval. Calculations will be performed using Provantis® version 9.3.1.1, MS Excel 2010 or later or other appropriate program.

**Proposed Statistical Tests**

Body weight, food consumption, clinical pathology, urinalysis pH, specific gravity, and urobilinogen and organ weight data will be evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's test (if the ANOVA is significant). All other numeric parameters will be evaluated by Student's *t*-test, unless specified otherwise. If appropriate, other post hoc analyses may also be performed. For clinical pathology data, values for parameters that are not within the detection threshold will not be included in the statistical evaluation.

**Criteria for Null Hypothesis Rejection**

$p \leq 0.05$

**G. Control of Bias**

While evaluating the responses of the animals and conducting the analyses, the technical staff will be aware of the treatment history of each animal and sample. Based on the relatively objective endpoints to be examined, bias is not expected to influence the results of the study.



Safety Evaluation of Proprietary Info after 28 Days of  
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## **XII. REGULATORY COMPLIANCE**

### **A. Good Laboratory Practice Compliance**

This study is intended to be submitted to and reviewed by the U.S. FDA or an equivalent regulatory agency, and this study therefore will be performed in accordance with the U.S. FDA “Good Laboratory Practice for Nonclinical Laboratory Studies,” as described in 21 CFR Part 58, with the following exceptions:

- Various pre-initiation study activities (e.g., receipt and quarantine of animals, pre-initiation body weights and randomization) may be performed prior to the approval of the protocol. These activities will be conducted according to testing facility SOPs, but because they may be conducted before the protocol is signed, they may not be considered by the FDA to have been conducted in compliance with GLP requirements.
- Animal water and food analysis will not be performed under GLP compliance by the vendors.

### **B. Standard Operating Procedures (SOPs)**

All operations pertaining to this study, unless specifically defined in this protocol, will be performed according to the SOPs of the laboratory. All deviations from any SOP and the reasons for the deviations will be documented and acknowledged by the Study Director.

### **C. Protocol Amendments and Deviations**

All changes or revisions made to the approved protocol by any involved party and the reasons for the changes and revisions will be documented, signed and dated by the Study Director and the Sponsor’s Representative. Amendments will be maintained with the protocol. Verbal or email approval for changes in the protocol may be granted by the Sponsor’s Representative, but a written amendment as described above will follow.

All deviations from the protocol and the reasons for the deviations will be documented and acknowledged by the Study Director. The Sponsor’s Representative will be informed of the occurrence of any deviations that might affect the results of the study, and any corrective actions taken.

### **D. Retention of Records and Study Samples**

The original protocol, amendments, final report, raw data, supporting documents and records as well as all pathology materials (slides, blocks and wet tissue specimens) specific to this study will be retained and stored by SRI International. All records and materials will be maintained for a period of at least 1 year. At the end of the retention period, the Sponsor will be contacted for instructions regarding disposition of these materials.

**Safety Evaluation of Proprietary Info after 28 Days of  
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**XIII. REPORTING**

The final report will accurately and completely describe the study design, procedures and findings. An analysis and summary of the data followed by the conclusions derived from the analyses will also be included. A draft report will be issued prior to submission of the final report.



Final Report • October 29, 2019

# GLP-MULTIPLE (5 WEEKLY) REPEAT SUBCUTANEOUS DOSE TOXICITY AND TOXICOKINETICS STUDY WITH Proprietary Info Proprietary IN MALE AND FEMALE BEAGLE DOGS

**Author:**Redacted by agreement**Testing Facility:**

SRI International  
Biosciences Division  
333 Ravenswood Avenue  
Menlo Park, CA 94025

**SRI Study Number:**

M397-18

**SRI Project Number:**

P25035.411

**Study Initiation:**

November 14, 2018

**Experimental Work Performed:****Start:**

November 20, 2018

**Finish:**

June 7, 2019

**Study Completion:**

October 29, 2019

**Sponsor:**

National Institute of Allergy and Infectious Diseases  
Division of AIDS  
5601 Fishers Lane, Redacted by agreement  
Bethesda, MD 20892-9830

**Sponsor's Representative:**Redacted by agreement**NIAID Contract Number:**

HHSN272201400006I/TO- HHSN27200008

GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with Proprietary Pro in Male and Female Beagle Dogs  
SRI Study No. M397-18

APPROVAL SIGNATURES

Written By:

Redacted by agreement

10/29/19  
Date

Approved By:

10-25-19  
Date

SRI International  
Biosciences Division  
333 Ravenswood Avenue  
Menlo Park, CA 94025

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with Proprietary Info Pro in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

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**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
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**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
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**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**SUMMARY**

The objective of this study was to determine potential toxicity of [Proprietary] [Prop] a new formulation of [Proprietary] [Proprietary] 12 mg/ml), [Proprietary] [Proprietary] 6.9 mg/ml) and [Proprietary] [Proprietary] 3.3 mg/ml), in adult male and female Beagle dogs following a multiple, 5-weekly repeat subcutaneous (s.c.) administration.

Male and female Beagle dogs (5/sex) were given weekly s.c. administration of [Proprietary] [Pro] at 0.4 mg/kg for 5 weeks (5 days of [Proprietary] [Pro] administration) and 3.64 and 14.55 mg/kg/day for 3 weeks (3 days of [Proprietary] [Pro] administration). A control group, (5/sex) was given weekly s.c. administration for 5 weeks of Excipient Control (5 days of Excipient Control administration) at an equivalent volume. [Proprietary Info]

[Proprietary Info]

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

Proprietary Info

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

Proprietary Info

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with**  
**Proprietary Info [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**QUALITY ASSURANCE UNIT**

**Final Report and  
Conflict of Interest Statement**

SRI's Quality Assurance Unit assures that the study *GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary [Pro] in Male and Female Beagle Dogs*, SRI Study Number M397-18 -- has been reviewed for adherence to U.S. Food and Drug Administration Good Laboratory Practice Regulations (21 CFR Part 58).

The following inspections were conducted during this study:

<u>Phase Inspected</u>	<u>Date of Inspection</u>	<u>Date Findings Reported to Management/Study Director</u>
Protocol	10-02-2018	10-02-2018
Protocol Amendment No. 1	11-14-2018	11-14-2018
Protocol Amendment No. 2	11-19-2018	11-19-2018
Protocol Amendment No. 3	12-03-2018	12-03-2018
Protocol Amendment No. 4	12-06-2018	12-06-2018
Protocol Amendment No. 5	12-14-2018	12-14-2018
Protocol Amendment No. 6	01-10-2019	01-10-2019
Protocol Amendment No. 7	01-21-2019	01-21-2019
Dispensing, Body Weights, Dosing, Bleeds, Clinical Observations	11-20-2018	11-20-2018
Necropsy	12-20-2018 01-30-2019	12-20-2018 01-31-2019
COAs	03-28-2019	03-28-2019
Plasma Analysis	06-06-2019	06-06-2019
Raw Data	04-22-2019 07-26-2019 09-26-2019	04-22-2019 07-26-2019 09-26-2019
Draft Final Report	04-22-2019 07-26-2019 09-26-2019 10-03-2019	04-22-2019 07-26-2019 09-26-2019 10-03-2019
Final Report Verification	10-29-2019	10-29-2019

This statement certifies that the personnel listed below participated in the inspections and audit of this study. These personnel have not been involved in the generation or evaluation of the data. Participation by the individuals listed below poses no conflict of interest.

Redacted by agreement

Redacted by agreement

29 Oct 19  
Date

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

Proprietary  
Info

**QUALITY ASSURANCE STATEMENT**

Proprietary Info

**QUALITY ASSURANCE FINAL CERTIFICATION**

Study Title: GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics  
Study with [Proprietary Info] [Pro] in Male and Female Beagle Dogs

Client Study: M397-18

Redacted by agreement

EPL Project Number: 748-118

The following aspects of this study were inspected by the Quality Assurance Unit of Experimental Pathology Laboratories, Inc. Dates inspections were performed and findings reported to the EPL Principal Investigator and Management are indicated below.

Area Inspected	Dates	
	Inspection	Reporting
EPL Project Sheets	12/27/18; 1/28/19; 2/11,12/19	12/27/18; 1/28/19; 2/12/19
Project Setup	12/27/18; 1/7/19; 2/11,12,13/19	12/27/18; 1/7/19; 2/12,13/19
Data Review	1/28/19; 1/29/19; 2/25,26/19; 3/6,7/19	1/28/19; 1/29/19; 2/25,26/19; 3/6,7/19

Date reported to Study Director/Management 9/9/19

Date of last annual facility inspection 8/19

Redacted by agreement

9 September 2019  
Date



**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**KEY PERSONNEL**

<b>Name</b>	<b>Functional Role</b>
Redacted by agreement	

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**I. PURPOSE OF STUDY**

The purpose of this study was to provide data of suitable quality and integrity to support application to the U.S. Food and Drug Administration (FDA) and other regulatory agencies. Therefore, this study was performed in accordance with the U.S. FDA “Good Laboratory Practice for Nonclinical Laboratory Studies” (GLP) as described in 21 CFR Part 58.

**II. OBJECTIVE OF STUDY**

The objective of this study was to determine potential toxicity of [Proprietary] [Prop] a new formulation of [Proprietary] [Proprietary] 12 mg/ml), [Proprietary] [Proprietary] 6.9 mg/ml) and [Proprietary] [Proprietary] 3.3 mg/ml), in adult male and female Beagle dogs following a multiple, 5-weekly repeat subcutaneous (s.c.) administration.

The protocol and protocol amendments are presented in Appendix A.

**III. EXPERIMENTAL DESIGN**

Group	Treatment	Dose Level (mg/kg) <sup>a</sup>	Dose Conc. (mg/ml/site)	Volume (ml/kg/site) <sup>a</sup>	Total No. of Animals	No. of Animals at Necropsy		
						Day 17 (Main)	Day 30 (Main)	Day 72 (Recovery)
1	Excipient	Eq to 16 mg/kg	0	0.5	5M/5F	--	3M/3F	2M/2F
2	[Proprietary] [Pr]	0.4 (0.13 x 3 sites)	0.27	0.5	5M/5F	--	3M/3F	2M/2F
3	[Proprietary] [Pr]	3.64 (1.21 x 3 sites)	2.43	0.5	5M/5F	3M/3F	--	2M/2F
4	[Proprietary] [Pr]	14.55 (4.85 x 3 sites)	9.72	0.5	5M/5F	3M/3F	--	2M/2F
Total No. of Animals					20M/20F	6M/6F	6M/6F	8M/8F

<sup>a</sup> Excipient and [Proprietary] [Pro] formulations were administered in 3 sites at 0.5 ml/kg/site.

[Proprietary Info]

**Species and Strain**

Beagle dog

**Route of Administration**

Subcutaneous (s.c.)

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
**SRI Study No. M397-18**

**Frequency**

Groups 1-2: once weekly for 5 weeks (divided over 3 sites per every weekly dosing)

Groups 3-4: once weekly for 3 weeks (divided over 3 sites per every weekly dosing)

**Dosing Volume**

0.5 ml/kg/site; 3 sites. Dose volumes were calculated based on the animal's most recent body weight to achieve the target dose levels based on 12 mg/ml [Proprietary Info] anchored TLC-ART101) concentration of dose solution. Maximum injection volume was in accordance with IACUC guidelines.

**Duration of In-Life Phase**

72 days

**IV. MATERIALS AND METHODS**

**A. Test and Control Articles**

**1. Test Article**

[Proprietary Info] [Proprietary] [Prop] a new formulation of [Proprietary Info] (LPV: 12 mg/ml), [Proprietary Info] 6.9 mg/ml) and [Proprietary Info]: 3.3 mg/ml)

**Supplier**

[Proprietary Info]

**Manufacturer**

[Proprietary Info]

**Lot Number**

[Proprietary Info]

**Physical Description**

Milky white suspension (white, turbid suspension)

**Storage Conditions**

2-8°C

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Characterization of Test Article**

The Sponsor was responsible for characterization and stability of the test article and provided Certificates of Analysis (CofA) to SRI for inclusion in the final report (Appendix B). The raw data generated by the Sponsor in support of these CofA were not verified or maintained by SRI.

**2. Vehicle Control:**

Excipient Control

**Supplier**

[Proprietary Info]

**Manufacturer**

[Proprietary Info]

**Lot Numbers**

[Proprietary Info]

**Physical Description**

White, turbid suspension

**Storage Conditions**

2-8°C

**Characterization of Reference Control**

The Sponsor was responsible for characterization and stability of the excipient control under the specified storage conditions and provided CofA to SRI for inclusion in the final report (Appendix B). Information on the identity, purity, and stability of the excipient control article was obtained by recording all of the pertinent information provided on the CofA provided by the supplier.

**3. Preparation of Dose Formulations**

Dose formulations were provided by the Sponsor as ready-to-dose formulations at the concentrations specified in the table in Section III.

**Storage of Dose Formulations**

Dose formulations were stored refrigerated, at 2-8°C, until the day of use. Formulations were brought to room temperature prior to administration to the animals on Weeks 1 and 2. Formulations were brought to 37°C prior to administration to the animals on Weeks 3, 4 and 5 (per Protocol Amendment #3).

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**4. Characterization of Dose Formulations**

The Sponsor was responsible for stability, homogeneity, and concentration analyses of the test articles in the vehicle. Thus, the raw analytical data generated in support of this study were not verified or maintained by SRI. SRI relied on the formal CofA provided with the formulation for confirmation of concentration, quality and stability.

**5. Test Article Handling**

At a minimum, personnel handling the test, and control article formulations wore eye protection, gloves, and a protective smock or laboratory coat.

**6. Disposition**

At the end of the study, any remaining partially used and unused containers of vehicle control, test article (kept refrigerated) will be shipped to the Sponsor unless the Sponsor issues other directions.

Residual dose formulations will be discarded after analysis, when the final report is submitted.

Empty control and test article containers may be destroyed by SRI on submission of the final report to the Sponsor.

See Section V.B, "Regulatory Compliance," for information about retention of records and study samples.

**7. Method for Assuring Correct Dosing**

The administration of each dose formulation was properly documented, and the amount administered to each animal was recorded.

**B. Test System**

**1. Species**

Dog

**Strain**

Beagle

**Supplier**

[Proprietary Info]

**Number of Animals**

40 assigned to test



**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Sex**

20 males and 20 females

**Age at First Dose**

8-9 months, which deviated from the age of 6-8 months in the protocol. This deviation did not impact the study as all animals were healthy at study initiation and were dosed based on their actual body weight.

**Weight Range at First Dose**

7.8-9.8 kg (males), 6.0-8.1 kg (females)

**2. Animal Care**

General procedures for animal care and housing were in accordance with the current Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) recommendations, current requirements stated in the Guide for the Care and Use of Laboratory Animals (National Research Council), and current requirements as stated by the U.S. Department of Agriculture through the Animal Welfare Act and Animal Welfare Regulations (November 2013).

**Quarantine/Acclimation**

14 days; A complete physical examination was performed on each dog before quarantine release.

**Housing**

1 per enclosed run ( $\geq$  4 ft x 6 ft)

**Light Cycle**

12 hr light/12 hr dark

**Temperature**

64°–74°F

**Humidity**

27-99%; brief excursions from the protocol-specified range of 30-70% occurred for less than 2 hr and, therefore, are not considered deviations from the protocol.

**Ventilation**

At least 10 room volumes per hour, with no recirculation of air.

**Food**

Envigo Teklad Certified Global 25% Protein Dog Diet, 2025C. Dogs were exposed to their daily ration of food, except for periods of fasting required by the study

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with Proprietary Info Pro in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

protocol. The quantity of the daily ration was sufficient to meet nutritional requirements. Eukanuba Puppy Food or Hills i/d Digestive Care canned food (wet food), was added to daily ration for individual dogs based on Attending Veterinarian recommendation. Feed was analyzed periodically to ensure that contaminants known to be capable of interfering with the study and reasonably expected to be present in such feed were not present at levels that would affect the study. Documentation of feed analyses is maintained at SRI for reference. A copy of the lot specific reports provided by the supplier will be maintained in the study records. Specific administration of supplemental food is summarized in the table below.

<b>Animal ID</b>	<b>¼ can Eukanuba puppy food (Royal Canin, Lot #832D2NSC02, Exp. 8/2020)</b>	<b>¼ can Eukanuba puppy food (Royal Canin, Lot #751F1NSC02, Exp. 12/2019)</b>	<b>¼ can Eukanuba puppy food (Royal Canin, Lot #819D2NSC02, Exp. 5/7/2020)</b>	<b>¼ can Hills i/d Digestive Care canned food (Hills Pet Nutrition, Inc., Lot #T1121401, Exp. 8/2020)</b>
009	-	Days 68-71	-	Days 62-64, 66, 67
013	Days 1-3, 5-8, 10-15, 17-22	Days 24-29	-	-
014	Days 1, 2, 5-8, 10-15, 17-22	Days 24-29	-	-
015	-	Days 24-29, 31	-	-
023	Day 13	-	-	-
026	Day 15 <sup>a</sup>	-	-	-
028	Days 1, 4-8, 10-15	-	-	-
034	Days 20, 21	Days 26, 27, 29, 31	-	-
036	Day 15	-	-	-
037	Days 14, 15	-	-	-
038	Day 15	Days 22-29, 31	Days 32-36, 39-50, 52-55	Day 21
039	Day 15	-	-	-
040	Day 15	Days 24-29 <sup>b</sup> , 31 <sup>c</sup>	-	-

<sup>a</sup>-Provided 1 tbs on Day 15

<sup>b</sup>-Provided 1/2 can on Day 24

<sup>c</sup>-Provided 1/8 can on Day 31

### **Water**

Water (untreated tap water) was provided, *ad libitum*. Based on previous reports, no contaminants that could interfere with and affect the results of the study were expected to be present in the water. Copies of annual analysis reports are maintained at SRI for reference.

### **3. Assignment of Animals to Study Day**

5-6 days before initiation of treatment

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**Randomization**

Animals were randomly assigned to treatment groups via a computerized body weight stratification procedure (Provantis 10.1.0.1).

**Identification**

Animals were individually identified by a uniquely numbered ear tattoo.

**4. Welfare of the Animals**

Every effort was made to minimize, if not eliminate, pain and suffering in all animals in this study. No animals were required to be euthanized prior to their scheduled sacrifice during this study due to their health status. [Proprietary Info]

[Proprietary Info]

[Proprietary Info] Per the Attending Veterinarian's recommendation, some animals received supplemental treatments as described in the following table:

Animal ID	Day	Observation	Treatment
004	63	[Proprietary Info]	
	69		
023	8		
	15		
024	11-14		
	15		
027	15		
028	1		
	3		
029	15		
030	8		
	15		
031	8		
	15		

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Animal ID	Day	Observation	Treatment
032	2	Proprietary Info	
	15		
	17		
033	8		
	15		
034	8		
	15		
	20		
035	8		
	15		
036	15		
037	1		
	15		
038	15		
	43		
	45		
039	15		
040	15		
Proprietary Info			



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L-Epinephrine (VetOne, Lot #171039, Exp. 5/2019)  
M-Sterile water (Nova-tech, Lot #B1710092, Exp. 10/2019)  
N-Chlorhexidine solution (Biomedica, Lot #B22J, Exp. 1/2019)  
O-3% hydrogen peroxide (VetUS, Lot #J4J, Exp. 9/2019)

**C. Experimental Procedure (In-Life Evaluations)**

**1. Preparation of Animals**

Animals were not fasted before dose administration.

**2. Dose Administration**

Weekly s.c. injection to 3 sites on the back and/or sides of the animal (for 3 weeks for Groups 3 and 4, and for 5 weeks for Groups 1 and 2). This route of administration is proposed for clinical use of the test article in humans.

The area was shaved prior to injection. The injection sites on the back were on either side of the spine and progressed from the base of the neck towards the shoulder blades moving ventral approximately one inch every week to avoid injecting into the same area. The third injection site on the side was similarly shaved and the injection site progressed similarly, approximately 1 inch each week. Each injection site was marked (adjacent to the injection location) and this marking was refreshed as needed to maintain a visual identification of the injection site.

On Day 1, Animals #002-004, 014 (Group 1, Excipient Control), 011-013 (Group 2, [Proprietary Info] [Pro] 0.4 mg/kg), and 021 (Group 3, [Proprietary Info] [Pro] 3.64 mg/kg) were initially dosed with a set volume of 1.5 ml rather than a dose volume of 0.5 ml/kg/site. This deviation had minimal impact to the study as the error was discovered shortly after injection, the correct administration amount was recalculated, and the missing dose volume immediately administered to complete the appropriate dose amount.

On Day 15, Animal #030 was not administered its third dose of the three site injections [Proprietary Info]

[Proprietary Info] Per Attending Veterinarian and Study Director recommendation, this animal received a total of 6.8 ml dose rather than the full 10.2 ml administration. This deviation had minimal impact to the study as there were 4 other female dogs in this group that received the proper dose volume and from which a valid data interpretation was made.

**3. Mortality/Morbidity**

Animals were checked at least once daily.

**4. Clinical Observations**

Recorded once daily and approximately 2-4 hr postdose on dosing days (Groups 1-2: Days 1, 8, 15, 22 and 29; Groups 3-4: Days 1, 8 and 15), once weekly during the recovery phase, or more often as clinical signs warranted, and at necropsy.



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Animals were examined for any altered clinical signs, including gross motor and behavioral activity, and observable changes in appearance.

**5. Body Weights**

Body weights were recorded predose on dosing days (Groups 1-2: Days 1, 8, 15, 22 and 29; Groups 3-4: Days 1, 8 and 15) for the purpose of dose calculation, weekly thereafter, and at each necropsy.

**6. Food Consumption**

Food consumption was quantitatively measured for approximately a 24 hr period once weekly for each run (1 dog/run) throughout the study.

Due to the [Proprietary Info]  
[Proprietary Info] food consumption activity was completed 2 hr earlier than scheduled for these animals. This had minimal impact to the study as this deviation was taken into consideration when analyzing food consumption results.

**7. Ophthalmologic Examination**

All animals (including extras) had a pretest ophthalmic examination performed by Ann Gratzek, DVM, DACVO, a board-certified veterinary ophthalmologist, and all surviving animals were re-examined by the ophthalmologist within the week before their scheduled necropsy (Main necropsy Day 30 and Recovery necropsy Day 72). Main animals in Groups 3 and 4 did not have an ophthalmic examination performed within the week before their scheduled necropsy on Day 17 due to the decision to terminate this group early. No adverse ophthalmologic effects were observed in the Main subset animals of Groups 1 and 2 at termination time point, therefore, ophthalmologic examinations were not performed on the Recovery subset animals.

**8. Plasma Drug Levels**

**Method of Collection**

Whole blood from animals in Groups 2-4 were collected from cephalic or jugular veins into tubes containing K<sub>2</sub>EDTA, processed to plasma, and then stored frozen at ≤-60°C.

**Volume**

Maximum ~1.5 ml whole blood (~ 700 µl of plasma) per sample.

**Frequency**

Plasma drug levels were sampled at the following time points in Group 2 animals:

Main animals (3M/3F): 1 hr predose and 1 hr postdose on dosing days (Days 1, 8, 15, 22 and 29)

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Recovery animals (2M/2F): 1 hr predose and 1 hr postdose on dosing Day 29 and on a single timepoint on Days 36, 43, 50, 57, 64 and 71

Plasma drug levels were sampled at the following time points in Groups 3 and 4 animals:

Main animals (3M/3F): 1 hr predose and 1 hr postdose on dosing days (Days 1, 8 and 15)

Recovery animals (2M/2F): on a single timepoint on Days 22, 29, 36, 43, 50, 57, 64 and 71

### **Method of Analysis**

Drug levels of [Proprietary Info] were measured in plasma samples using a bioanalytical method provided by the Sponsor and validated by SRI. Details of the bioanalytical method and validation results will be included in a separate validation report (SRI Study No. B185-18).

### **9. Toxicokinetics Analysis**

TK data analysis was performed on the measurable plasma concentrations of each test compound from Appendix G (Bioanalytical Chemistry). The plasma concentration data of each test compound were analyzed using Phoenix<sup>®</sup> WinNonlin<sup>®</sup> (version 6.3) software to perform non-compartmental analysis. Individual animal plasma concentrations at each actual time of blood collection were used in TK data analysis. The dose administered in each dose group was entered into the program as mg/kg, and as a result, no additional corrections for individual body weights of the animals were necessary.

Plasma concentrations that were less than the lower limit of quantitation (LLOQ) of 5.00 ng/ml for [Proprietary Info] and 25.00 ng/ml for [Proprie] of the bioanalytical assay were not included in TK data analysis. TK parameters were determined only for the Main animals and up to Day 15 (i.e. up to three doses), as all three dose groups had blood collections in the main animals up to Day 15. The following TK parameters were determined for each test compound in [Proprietary] [Pro] using the administration of the first dose on Day 1 as time zero: overall apparent maximal plasma concentration ( $C_{max}$ ) and area under the plasma concentration time curve up to the last blood collection time ( $AUC_{last}$ ). The time-course of mean plasma concentrations of each test compound was plotted for Main and Recovery animals at all timepoints. Mean plasma concentrations that were less than the LLOQ of each compound were assigned a value of 2.00 ng/ml only to illustrate apparent troughs in the concentration vs. time profiles.

### **Disposition**

Residual bioanalytical samples will be discarded on submission of the final report.



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**10. Clinical Pathology Evaluations**

**Preparation of Animals**

Animals were fasted before blood collection.

Due to [Proprietary Info], Animals #021-023 and 031-33 were not fasted prior to emergency clinical pathology blood collections on Day 17. The lack of fasting these animals prior to blood collections was not considered to have compromised the ability to interpret the clinical pathology data. Therefore, this deviation had minimal impact on the integrity of the study.

**Method of Collection**

Whole blood from animals in all treatment groups were collected from cephalic or jugular veins. Hematology samples were collected using K<sub>3</sub>EDTA as the anticoagulant. Coagulation samples were collected using sodium citrate as the anticoagulant. No anticoagulant was used for clinical chemistry samples.

**Frequency**

Prestudy and prior to necropsy as follows:

- On Day 17, two days after the last treatment, blood was collected for clinical pathology from Main animals in Groups 3 and 4 (3 animals/sex/group) that were then sacrificed for necropsy evaluations (per Amendment #4).
- On Day 30, one day after the last treatment, blood was collected for clinical pathology from Main animals in Groups 1 and 2 (3 animals/sex/group) that were then sacrificed for necropsy evaluations; however, Day 30 hematology analysis for Animals #002-003 and 014 (Group 1) and #011-013 (Group 2) was not performed due to apparent hemolysis of the samples. This deviation had minimal impact to the study as the other clinical pathology parameters (clinical chemistry and coagulation) were analyzed per protocol without incident.
- On Day 72, blood was collected for clinical pathology from the Recovery animals (remaining 2 animals/sex/group) that were then sacrificed for necropsy evaluations.

Clinical pathology parameters that were evaluated are listed below. In some cases, automated analyzers report additional parameters not specified in the protocol. Results for the additional parameter(s) are included in the data package, but are not summarized, analyzed, or reported, and their collection is not considered a deviation from the protocol.

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Manual WBC differential counts were conducted. Some parameters that were not specified in the protocol were evaluated and reported. This is not considered a deviation from the protocol.

**Hematology Parameters**

- Hematocrit (HCT)
- Hemoglobin (HGB)
- Red blood cell count (RBC)
- Red blood cell distribution width (RDW)
- White blood cell count (WBC)
- WBC differential and absolute counts
  - Absolute neutrophil [ANE]
  - Percent neutrophil [PNE]
  - Absolute lymphocyte [ALY]
  - Percent lymphocyte [PLY]
  - Absolute monocyte [AMO]
  - Percent monocyte [PMO]
  - Absolute eosinophil [AEO]
  - Percent eosinophil [PEO]
  - Absolute basophil [ABA]
  - Percent basophil [PBA]
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular volume (MCV)
- Mean corpuscular hemoglobin concentration (MCHC)
- Platelet count (PLT)
- Mean platelet volume (MPV)
- Absolute Reticulocyte (ARET)
- Percent Reticulocyte (PRET)

**Clinical Chemistry Parameters**

- Total Bilirubin (TBI)
- Creatinine (CRE)
- Sodium (SOD)
- Potassium (POT)
- Chloride (CHL)
- Cholesterol (CHO)
- Triglyceride (TRI)
- Glucose (GLU)
- Blood urea nitrogen (BUN)
- Aspartate aminotransferase (AST)
- Alanine aminotransferase (ALT)
- Alkaline phosphatase (ALP)
- Calcium (CAL)
- Phosphorus (PHO)

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- Total protein (TPR)
- Albumin (ALB)
- Albumin/globulin ratio (AGR)
- Globulin (GLO)

**Coagulation Parameters**

- Prothrombin time (PT)
- Activated partial thromboplastin time (PTT)
- Fibrinogen (FIB)

**11. Urinalysis**

**Method of Collection**

Urine was collected by cystocentesis; ≤15 ml was submitted to the clinical laboratory.

**Frequency**

Urine was collected on Day 17 (Groups 3 and 4 Main necropsy per Amendment #4), Day 30 (Groups 1 and 2 Main necropsy) and Day 72 (Recovery necropsy).

**Urinalysis Parameters**

- Color (noted at time of collection)
- Clarity (noted at time of collection)
- Specific gravity
- Microscopic examination of urine sediment
- Bilirubin
- Glucose
- Ketones
- Leukocytes
- Nitrite
- Occult blood
- pH
- Protein
- Urobilinogen

**D. Necropsy**

**Interval**

Necropsies were performed on Day 17 for Main Groups 3 and 4 (per Amendment #4), Day 30 for Main Groups 1 and 2; and Day 72 for the Recovery Groups 1-4.



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**Euthanasia**

Following s.c. administration of a sedative cocktail, an overdose of sodium pentobarbital was administered by intravenous injection.

**Observations**

External examination of all body orifices and an examination of all cranial, thoracic and abdominal organs was performed, and all gross findings were recorded.

**Tissues Retained**

The following tissues were collected from all animals in the Main and Recovery Groups. Tissues were retained in 10% neutral buffered formalin, except where noted.

- All gross lesions (including tissue masses and abnormal regional lymph nodes)
- Adrenal glands
- Aorta
- Bone (femur, distal with joint surface)
- Bone, sternum (marrow histology)
- Bone marrow smear, sternum
- Brain (fore-, mid-, and hindbrain)
- Cecum
- Cervix
- Colon
- Duodenum
- Epididymides
- Esophagus
- Eyes, with optic nerve (fixed with modified Davidson's solution)
- Gall bladder
- Heart
- Identification; (retained in formalin; not processed for histology)
- Ileum
- Injection site(s) tissue. Only representative sections of the injection site(s) were collected
- Jejunum
- Kidneys
- Liver
- Lungs with bronchi
- Lymph nodes, mandibular and mesenteric
- Mammary gland (females, males when present)
- Ovaries
- Pancreas
- Pituitary gland

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- Prostate
- Rectum
- Salivary gland, mandibular
- Sciatic nerve
- Skeletal muscle
- Skin, ventral abdomen, taken with mammary gland
- Skin at each of the three injection sites
- Spinal cord sections (thoracic only)
- Spleen
- Stomach
- Testes (fixed with modified Davidson's solution)
- Thymus
- Thyroid/parathyroid glands
- Trachea
- Urinary bladder
- Uterus
- Vagina

**Final Body/Organ Weights**

Body weight was recorded on the day of necropsy for body to organ weight ratios. The following organs were weighed. Paired organs were weighted together.

- Adrenal glands
- Brain
- Heart
- Kidneys
- Liver
- Ovaries
- Testes, without epididymides
- Thymus

**E. Histopathologic Examination**

**Tissues**

Tissues listed under "Retained Tissues" were processed and evaluated for the following:

- All Main animals in the control and high-dose groups (Groups 1 and 4)
- Thymus, kidney, bone marrow and injection site, identified as target tissues of toxicity by the pathologist, were examined in all dose groups
- All gross lesions were processed for all animals

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- Bone marrow smear for cytology was processed and analyzed at the discretion of the Study Director and pathologist based on bone marrow histopathology findings.

Of tissues scheduled for microscopic evaluation, the following were not available in tissue section: mammary gland from Animals #006, 008, 036, 037, and 039, sciatic nerve and skeletal muscle from Animal #007. In animals with immature/non-cycling ovaries (#006, 008, 036, and 037), mammary gland would not be developed and would, therefore, not be present in tissue sections. This deviation had no impact on the integrity of the study as the absence of these tissues was not considered to have compromised the ability to interpret the microscopic data.

### **Tissue Sections**

Sections of the tissues were embedded in paraffin, cut approximately 5 µm thick, and stained with hematoxylin and eosin by [Proprietary Info]  
[Proprietary Info] a histology laboratory qualified by SRI.

### **Evaluated By**

Histopathology specimens were evaluated by [Redacted by agreement]  
[Redacted by agreement] a board-certified veterinary pathologist.

### **Method**

Each lesion was listed and coded by the most specific topographic and morphologic diagnoses, severity, and distribution, using International Harmonization of Nomenclature and Diagnostic Criteria for Lesions (INHAND) as a guide. A four-step grading system (minimal, mild, moderate and marked) was used to define gradable lesions for comparison between treated and control groups. Data were recorded and summarized using Provantis® version 10.1.0.1. Records of gross findings for a specimen from postmortem observations were available to the pathologist when examining that specimen microscopically.

## **F. Evaluation of Data Parameters**

Mean and standard deviation were calculated for body weight, food consumption, clinical pathology; urinalysis pH, urobilinogen, and specific gravity; and organ weight data at each evaluation interval. Calculations were performed using Provantis® version 10.1.0.1.

### **Statistical Tests**

Body weight, food consumption, clinical pathology, urinalysis pH and specific gravity, and organ weight data were evaluated by one-way analysis of variance (ANOVA), followed by Dunnett's test (if the ANOVA is significant). For clinical pathology data, values for parameters that were not within the detection threshold were not included in the statistical evaluation.



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**Criteria for Null Hypothesis Rejection**

$p \leq 0.05$

**G. Control of Bias**

While evaluating the responses of the animals and conducting the analyses, the technical staff were aware of the treatment history of each animal and sample. Based on the relatively objective endpoints to be examined, bias did not influence the results of the study.

**V. REGULATORY COMPLIANCE**

**A. Good Laboratory Practice Compliance**

This study was intended to be submitted to and reviewed by the U.S. FDA or an equivalent regulatory agency, and this study therefore was performed in accordance with the U.S. FDA “Good Laboratory Practice for Nonclinical Laboratory Studies,” as described in 21 CFR Part 58, with the following exceptions:

- Receipt and quarantine of animals were performed prior to the approval of the protocol. These activities were conducted according to testing facility SOPs, but because they were conducted before the protocol was signed, they may not be considered by the FDA to have been conducted in compliance with GLP requirements. None of these activities had an impact on the study.
- Animal water and food analysis was not performed under GLP compliance by the vendors. Based on previous reports, no contaminants that could interfere with and affect the results of the study were expected to be present in the water. Feed was analyzed periodically to ensure that contaminants known to be capable of interfering with the study and reasonably expected to be present in such feed were not present at levels that would affect the study; therefore, water and food analyses not performed under GLP compliance by the vendors has no impact on the study.

**B. Retention of Records and Study Samples**

The original protocol, amendments, final report, raw data, supporting documents and records as well as all pathology materials (slides, blocks and wet tissue specimens) specific to this study will be retained and stored by SRI International. All records and materials will be maintained for a period of at least 1 year. An archival sample of the test and control articles will be maintained by SRI for at least 5 years or as long as samples afford evaluation (21 CFR 58.105[d]). At the end of the retention period, the Sponsor will be contacted for instructions regarding disposition of these materials.

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**VI. RESULTS**

**A. Mortality/Morbidity and Clinical Observations**

Proprietary Info



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(females) for [Proprietary Info] 3,071, 26,949 and 75,047 day\*ng/ml (males) and 3,914, 31,017 and  
[Proprietary Info]

[Proprietary Info]

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Proprietary Info



**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
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Proprietary Info

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
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**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
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Proprietary Info

## **VIII. REFERENCES**

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McInnes E. (2012) Dog. *In: Background Lesions in Laboratory Animals. A Color Atlas*. Saunders Elsevier. 3:37-44.

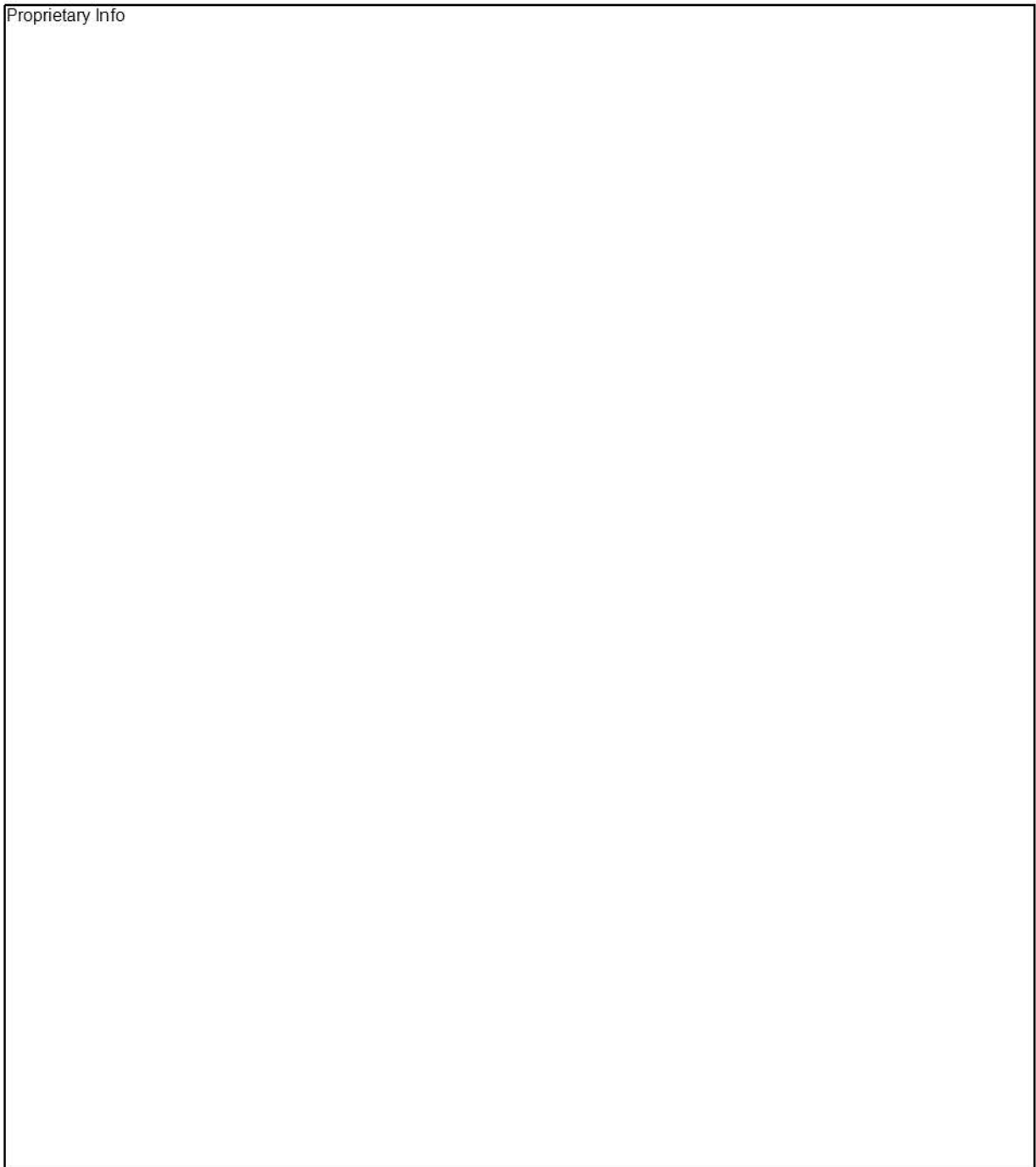


Proprietary Info

Proprietary Info

**Figure 1.** Time-course of plasma concentrations of [Proprietary] in male (top) and female (bottom) dogs in main and recovery groups after sc administration of [Proprietary] [Pro] a formulation of [Proprietary] [Proprietary] and [Proprietary] [Proprietary] [Pro] was administered weekly for 5 weeks in Group 2 and for 3 weeks in Groups 3 and 4. [Proprietary] doses were 0.400 mg/kg (Group 2), 3.64 mg/kg (Group 3), and 14.55 mg/kg (Group 4). Nominal times are plotted using dose administration on Day 1 as time zero. Plasma [Proprietary] concentrations that were less than LLOQ (5.00 ng/ml) were assigned a value of 2 ng/ml for graphical illustration purposes only.

**Figure 2.** Time-course of plasma concentrations of [Proprietary] in male (top) and female (bottom) dogs in main and recovery groups after sc administration of [Proprietary] [Proprietary] a formulation of [Proprietary] [Proprietary] and [Proprietary] [Proprietary] [Pro] was administered weekly for 5 weeks in Group 2 and for 3 weeks in Groups 3 and 4. [Proprietary] doses were 0.230 mg/kg (Group 2), 2.09 mg/kg (Group 3), and 8.37 mg/kg (Group 4). Nominal times are plotted using dose administration on Day 1 as time zero. Plasma [Proprietary] concentrations that were less than LLOQ (25.00 ng/ml) were assigned a value of 2 ng/ml for graphical illustration purposes only.



**Figure 3.** Time-course of plasma concentrations of [Proprietary] in male (top) and female (bottom) dogs in main and recovery groups after sc administration of [Proprietary]. [Prop] a formulation of [Proprietary] [Proprietary] and [Proprietary] [Proprietary] [Pro] was administered weekly for 5 weeks in Group 2 and for 3 weeks in Groups 3 and 4. [Proprietary] doses were 0.110 mg/kg (Group 2), 1.0 mg/kg (Group 3), and 4.0 mg/kg (Group 4). Nominal times are plotted using dose administration on Day 1 as time zero. Plasma [Proprietary] concentrations that were less than LLOQ (5.00 ng/ml) were assigned a value of 2 ng/ml for graphical illustration purposes only.

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Withheld pursuant to exemption

Proprietary Info

of the Freedom of Information and Privacy Act

Table 1  
Clinical Observations Summary

3/11/2019 10:40:41AM

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with [Propriet] [Pr] in Male and Female Beagle Dogs

Key Page

**General Footnotes**

Provantis version 10.1.0.1  
"-" indicates Not Applicable

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings</u>		
1	Excipient	Control	Group 1	0	mg/kg SC
2	[Propriet] [Pr] (0.4)	Dose	Group 2	0.4	mg/kg SC
3	[Propriet] [Pr] (3.64)	Dose	Group 3	3.64	mg/kg SC
4	[Propriet] [Pr] (14.55)	Dose	Group 4	14.55	mg/kg SC



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Table 2  
Body Weights Summary

2/6/2019 10:01:35AM

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Key Page

**General Footnotes**

Provantis version 10.1.0.1  
"-" indicates Not Applicable

**Measurement Descriptions**

<u>Headings Used</u>	<u>Description</u>
Body Weight	Body Weight

**Measurement/Statistics**

<u>Measurement</u>	<u>Descriptive</u>	<u>Comparative</u>	<u>Arithmetic</u> <u>/Adjusted</u>	<u>Transformation</u>
Body Weight	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic

**Automatic Transformations**

<u>Measurement</u>	<u>Transformation Order</u>
Body Weight	Identity (No Transformation), Log, Rank

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings 1-4</u>		
1	Excipient	Control	Group 1	0	mg/kg SC
2	<u>Propriet</u> <u>Pr</u> (0.4)	Dose	Group 2	0.4	mg/kg SC
3	<u>Propriet</u> <u>Pr</u> (3.64)	Dose	Group 3	3.64	mg/kg SC
4	<u>Propriet</u> <u>Pr</u> (14.55)	Dose	Group 4	14.55	mg/kg SC

**Pairwise Comparisons**

<u>Group</u>	<u>Vs</u>	<u>Group</u>
1		2
1		3
1		4

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Table 3  
Body Weight Changes Summary

2/6/2019 10:01:53AM

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with Propriet IPr in Male and Female Beagle Dogs

Key Page

**General Footnotes**

Provantis version 10.1.0.1  
"-" indicates Not Applicable

**Measurement Descriptions**

<u>Headings Used</u>	<u>Description</u>
Absolute Weight Gains	Absolute Weight Gain (kg)

**Measurement/Statistics**

<u>Measurement</u>	<u>Descriptive</u>	<u>Comparative</u>	<u>Arithmetic</u> <u>/Adjusted</u>	<u>Transformation</u>
Absolute Weight Gains	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic

**Automatic Transformations**

<u>Measurement</u>	<u>Transformation Order</u>
Absolute Weight Gains	Identity (No Transformation), Log, Rank

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings 1-4</u>		
1	Excipient	Control	Group 1	0	mg/kg SC
2	<u>Propriet</u> <u>IPr</u> (0.4)	Dose	Group 2	0.4	mg/kg SC
3	<u>Propriet</u> <u>IPr</u> (3.64)	Dose	Group 3	3.64	mg/kg SC
4	<u>Propriet</u> <u>IPr</u> (14.55)	Dose	Group 4	14.55	mg/kg SC

**Pairwise Comparisons**

<u>Group</u>	<u>Vs</u>	<u>Group</u>
1		2
1		3
1		4

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Table 4  
Food Consumption Summary

2/7/2019 8:30:37AM

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

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**General Footnotes**

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"-" indicates Not Applicable

**Measurement Descriptions**

<u>Headings Used</u>	<u>Description</u>
Food Mean Daily Consumption	Food Mean Consumption

**Measurement/Statistics**

<u>Measurement</u>	<u>Descriptive</u>	<u>Comparative</u>	<u>Arithmetic</u> <u>/Adjusted</u>	<u>Transformation</u>
Food Mean Daily Consumption	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic

**Automatic Transformations**

<u>Measurement</u>	<u>Transformation Order</u>
Food Mean Daily Consumption	Identity (No Transformation), Log, Rank

**Time-Points/Ranges**

<u>Measurement</u>	<u>From</u>	<u>To</u>	<u>Report As</u>
Food Mean Daily Consumption	2	3	2 - 3
	9	10	9 - 10
	16	17	16 - 17
	23	24	23 - 24
	30	31	30 - 31
	37	38	37 - 38
	44	45	44 - 45
	51	52	51 - 52
	58	59	58 - 59
	65	66	65 - 66

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings 1-4</u>		
1	Excipient	Control	Group 1	0	mg/kg SC
2	<u>Propriet</u> <u>Pr</u> (0.4)	Dose	Group 2	0.4	mg/kg SC

Table 4  
Food Consumption Summary

2/7/2019 8:30:37AM

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

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Key Page

**Group Information (Continued)**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings 1-4</u>		
3	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (3.64)	Dose	Group 3	3.64	mg/kg SC
4	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (14.55)	Dose	Group 4	14.55	mg/kg SC

**Pairwise Comparisons**

<u>Group</u>	<u>Vs</u>	<u>Group</u>
1		2
1		3
1		4

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Key Page

**General Footnotes**

Provantis version 10.1.0.1

"." indicates Not Applicable

Statistical significance indicated on a group with an N < 3 is not valid

**Measurement Descriptions**

<u>Headings Used</u>	<u>Description</u>
White Blood Cells	White Blood Cells
Red Blood Cells	Red Blood Cells
Hemoglobin	Hemoglobin
Hematocrit	Hematocrit
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
RDW	Red Blood Cell Distribution Width
Platelet Count	Platelet Count
MeanPlatelet Volume	Mean Platelet Volume
Percent Neutrophils	Percent Neutrophils
Percent Lymphocytes	Percent Lymphocytes
Percent Monocytes	Percent Monocytes
Percent Eosinophils	Percent Eosinophils
Percent Basophils	Percent Basophils
Neutrophils (Absolute)	Absolute Neutrophils
Lymphocytes (Absolute)	Absolute Lymphocytes
Monocytes (Absolute)	Absolute Monocytes
Eosinophils (Absolute)	Absolute Eosinophils
Basophils (Absolute)	Absolute Basophils
Percent Reticulocyte	Percent Reticulocytes
Reticulocyte (Absolute)	Absolute Reticulocytes

**Unit Descriptions**

<u>Headings Used</u>	<u>Description</u>
%	%
10^9/L	10^9/L
fL	fL
g/dL	g/dL
pg	pg
x10^3/uL	x10^3/uL
x10^6/uL	x10^6/uL

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Key Page

**Measurement/Statistics**

<u>Measurement</u>	<u>Descriptive</u>	<u>Comparative</u>	<u>Arithmetic /Adjusted</u>	<u>Transformation</u>
White Blood Cells	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Red Blood Cells	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Hemoglobin	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Hematocrit	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Mean Corpuscular Volume	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Mean Corpuscular Hemoglobin	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Mean Corpuscular HGB Conc.	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
RBC Distribution Width	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Platelet Count	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Mean Platelet Volume	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Percent Neutrophils	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Percent Lymphocytes	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Percent Monocytes	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Percent Eosinophils	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Key Page

**Measurement/Statistics (Continued)**

<u>Measurement</u>	<u>Descriptive</u>	<u>Comparative</u>	<u>Arithmetic /Adjusted</u>	<u>Transformation</u>
Percent Basophils	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Neutrophils (Absolute)	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Lymphocytes (Absolute)	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Monocytes (Absolute)	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Eosinophils (Absolute)	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Basophils (Absolute)	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Percent Reticulocytes	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Reticulocyte (Absolute)	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic

**Automatic Transformations**

<u>Measurement</u>	<u>Transformation Order</u>
White Blood Cells	Identity (No Transformation), Log, Rank
Red Blood Cells	Identity (No Transformation), Log, Rank
Hemoglobin	Identity (No Transformation), Log, Rank
Hematocrit	Identity (No Transformation), Log, Rank
Mean Corpuscular Volume	Identity (No Transformation), Log, Rank
Mean Corpuscular Hemoglobin	Identity (No Transformation), Log, Rank
Mean Corpuscular HGB Conc.	Identity (No Transformation), Log, Rank
RBC Distribution Width	Identity (No Transformation), Log, Rank
Platelet Count	Identity (No Transformation), Log, Rank
Mean Platelet Volume	Identity (No Transformation), Log, Rank
Percent Neutrophils	Identity (No Transformation), Log, Rank
Percent Lymphocytes	Identity (No Transformation), Log, Rank
Percent Monocytes	Identity (No Transformation), Log, Rank
Percent Eosinophils	Identity (No Transformation), Log, Rank

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Key Page

**Automatic Transformations (Continued)**

Measurement	Transformation Order
Percent Basophils	Identity (No Transformation), Log, Rank
Neutrophils (Absolute)	Identity (No Transformation), Log, Rank
Lymphocytes (Absolute)	Identity (No Transformation), Log, Rank
Monocytes (Absolute)	Identity (No Transformation), Log, Rank
Eosinophils (Absolute)	Identity (No Transformation), Log, Rank
Basophils (Absolute)	Identity (No Transformation), Log, Rank
Percent Reticulocytes	Identity (No Transformation), Log, Rank
Reticulocyte (Absolute)	Identity (No Transformation), Log, Rank

**Group Information**

Short Name	Long Name	Type	Report Headings 1-4		
1	Excipient	Control	Group 1	0	mg/kg SC
2	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (0.4)	Dose	Group 2	0.4	mg/kg SC
3	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (3.64)	Dose	Group 3	3.64	mg/kg SC
4	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (14.55)	Dose	Group 4	14.55	mg/kg SC

**Pairwise Comparisons**

Group	Vs	Group
1		2
1		3
1		4

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Key Page

**General Footnotes**

Provantis version 10.1.0.1

"." indicates Not Applicable

Statistical significance indicated on a group with an N < 3 is not valid

**Measurement Descriptions**

<u>Headings Used</u>	<u>Description</u>
Blood Urea Nitrogen	Blood Urea Nitrogen
Creatinine	Creatinine
Glucose	Glucose
AST	Aspartate Aminotransferase
ALT	Alanine Aminotransferase
Alkaline Phosphatase	Alkaline Phosphatase
Total Bilirubin	Total Bilirubin
Sodium	Sodium
Potassium	Potassium
Chloride	Chloride
Calcium	Calcium
Phosphorus	Phosphorus
Total Protein	Total Protein
Albumin	Albumin
Globulin	Globulin
Alb/Glo Ratio	Albumin/Globulin Ratio
Cholesterol	Cholesterol
Triglyceride	Triglyceride

**Unit Descriptions**

<u>Headings Used</u>	<u>Description</u>
g/dL	g/dL
mg/dL	mg/dL
mmol/L	mmol/L
U/L	U/L

**Measurement/Statistics**

<u>Measurement</u>	<u>Descriptive</u>	<u>Comparative</u>	<u>Arithmetic</u> <u>/Adjusted</u>	<u>Transformation</u>
Blood Urea Nitrogen	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Key Page

**Measurement/Statistics (Continued)**

<u>Measurement</u>	<u>Descriptive</u>	<u>Comparative</u>	<u>Arithmetic /Adjusted</u>	<u>Transformation</u>
Creatinine	Mean	Anova & Dunnett's	Arithmetic	Automatic
	Standard Deviation Count (N)	2 Sided		
Glucose	Mean	Anova & Dunnett's	Arithmetic	Automatic
	Standard Deviation Count (N)	2 Sided		
Aspartate Aminotransferase	Mean	Anova & Dunnett's	Arithmetic	Automatic
	Standard Deviation Count (N)	2 Sided		
Alanine Aminotransferase	Mean	Anova & Dunnett's	Arithmetic	Automatic
	Standard Deviation Count (N)	2 Sided		
Alkaline Phosphatase	Mean	Anova & Dunnett's	Arithmetic	Automatic
	Standard Deviation Count (N)	2 Sided		
Total Bilirubin	Mean	Anova & Dunnett's	Arithmetic	Automatic
	Standard Deviation Count (N)	2 Sided		
Sodium	Mean	Anova & Dunnett's	Arithmetic	Automatic
	Standard Deviation Count (N)	2 Sided		
Potassium	Mean	Anova & Dunnett's	Arithmetic	Automatic
	Standard Deviation Count (N)	2 Sided		
Chloride	Mean	Anova & Dunnett's	Arithmetic	Automatic
	Standard Deviation Count (N)	2 Sided		
Calcium	Mean	Anova & Dunnett's	Arithmetic	Automatic
	Standard Deviation Count (N)	2 Sided		
Phosphorus	Mean	Anova & Dunnett's	Arithmetic	Automatic
	Standard Deviation Count (N)	2 Sided		
Total Protein	Mean	Anova & Dunnett's	Arithmetic	Automatic
	Standard Deviation Count (N)	2 Sided		
Albumin	Mean	Anova & Dunnett's	Arithmetic	Automatic
	Standard Deviation Count (N)	2 Sided		
Globulin	Mean	Anova & Dunnett's	Arithmetic	Automatic
	Standard Deviation Count (N)	2 Sided		

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Key Page

**Measurement/Statistics (Continued)**

<u>Measurement</u>	<u>Descriptive</u>	<u>Comparative</u>	<u>Arithmetic /Adjusted</u>	<u>Transformation</u>
Albumin/Globulin Ratio	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Cholesterol	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Triglyceride	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic

**Automatic Transformations**

<u>Measurement</u>	<u>Transformation Order</u>
Blood Urea Nitrogen	Identity (No Transformation), Log, Rank
Creatinine	Identity (No Transformation), Log, Rank
Glucose	Identity (No Transformation), Log, Rank
Aspartate Aminotransferase	Identity (No Transformation), Log, Rank
Alanine Aminotransferase	Identity (No Transformation), Log, Rank
Alkaline Phosphatase	Identity (No Transformation), Log, Rank
Total Bilirubin	Identity (No Transformation), Log, Rank
Sodium	Identity (No Transformation), Log, Rank
Potassium	Identity (No Transformation), Log, Rank
Chloride	Identity (No Transformation), Log, Rank
Calcium	Identity (No Transformation), Log, Rank
Phosphorus	Identity (No Transformation), Log, Rank
Total Protein	Identity (No Transformation), Log, Rank
Albumin	Identity (No Transformation), Log, Rank
Globulin	Identity (No Transformation), Log, Rank
Albumin/Globulin Ratio	Identity (No Transformation), Log, Rank
Cholesterol	Identity (No Transformation), Log, Rank
Triglyceride	Identity (No Transformation), Log, Rank

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings 1-4</u>		
1	Excipient	Control	Group 1	0	mg/kg SC
2	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (0.4)	Dose	Group 2	0.4	mg/kg SC
3	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (3.64)	Dose	Group 3	3.64	mg/kg SC
4	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (14.55)	Dose	Group 4	14.55	mg/kg SC

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

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Key Page

**Pairwise Comparisons**

<u>Group</u>	<u>Vs</u>	<u>Group</u>
1		2
1		3
1		4

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and  
Female Beagle Dogs

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Key Page

**General Footnotes**

Provantis version 10.1.0.1

"." indicates Not Applicable

Statistical significance indicated on a group with an N < 3 is not valid

**Measurement Descriptions**

<u>Headings Used</u>	<u>Description</u>
Prothrombin Time	PT (Prothrombin Time)
Activated PTT	Activated partial thromboplastin time
Fibrinogen	Fibrinogen

**Unit Descriptions**

<u>Headings Used</u>	<u>Description</u>
mg/dL	mg/dL
Seconds	Seconds

**Measurement/Statistics**

<u>Measurement</u>	<u>Descriptive</u>	<u>Comparative</u>	<u>Arithmetic /Adjusted</u>	<u>Transformation</u>
Prothrombin Time	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Activated PTT	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Fibrinogen	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic

**Automatic Transformations**

<u>Measurement</u>	<u>Transformation Order</u>
Prothrombin Time	Identity (No Transformation), Log, Rank
Activated PTT	Identity (No Transformation), Log, Rank
Fibrinogen	Identity (No Transformation), Log, Rank

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings 1-4</u>	
1	Excipient	Control	Group 1	0 mg/kg SC

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

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Key Page

**Group Information (Continued)**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings 1-4</u>		
2	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (0.4)	Dose	Group 2	0.4	mg/kg SC
3	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (3.64)	Dose	Group 3	3.64	mg/kg SC
4	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (14.55)	Dose	Group 4	14.55	mg/kg SC

**Pairwise Comparisons**

<u>Group</u>	<u>Vs</u>	<u>Group</u>
1		2
1		3
1		4

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Key Page

**General Footnotes**

Provantis version 10.1.0.1

"." indicates Not Applicable

Statistical significance indicated on a group with an N < 3 is not valid

**Measurement Descriptions**

<u>Headings Used</u>	<u>Description</u>
Specific Gravity	Specific Gravity
UA pH	UA pH
UA Urobilinogen	UA Urobilinogen

**Unit Descriptions**

<u>Headings Used</u>	<u>Description</u>
EU/dL	EU/dL

**Measurement/Statistics**

<u>Measurement</u>	<u>Descriptive</u>	<u>Comparative</u>	<u>Arithmetic /Adjusted</u>	<u>Transformation</u>
Specific Gravity	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Identity (No Transformation)
UA pH	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
UA Urobilinogen	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic

**Automatic Transformations**

<u>Measurement</u>	<u>Transformation Order</u>
UA pH	Identity (No Transformation), Log, Rank
UA Urobilinogen	Identity (No Transformation), Log, Rank

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings 1-4</u>		
1	Excipient	Control	Group 1	0	mg/kg SC
2	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (0.4)	Dose	Group 2	0.4	mg/kg SC
3	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (3.64)	Dose	Group 3	3.64	mg/kg SC



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

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Key Page

**Group Information (Continued)**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings 1-4</u>
4	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (14.55)	Dose	Group 4      14.55      mg/kg SC

**Pairwise Comparisons**

<u>Group</u>	<u>Vs</u>	<u>Group</u>
1		2
1		3
1		4

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

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End of Print

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Withheld pursuant to exemption

Proprietary Info

of the Freedom of Information and Privacy Act

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Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Key Page

**General Footnotes**

Provantis version 10.1.0.1  
"-" indicates Not Applicable

**Measurement Descriptions**

<u>Headings Used</u>	<u>Description</u>
Adrenal Glands Wt	Adrenal Glands Weight
Brain Weight	Brain Weight
Heart Weight	Heart Weight
Kidneys Weight	Kidneys Weight
Liver Weight	Liver Weight
Ovaries Weight	Ovaries Weight
Testes Weight	Testes Weight
Thymus Weight	Thymus Weight
Adrenal/BW Ratio	Adrenals/Terminal BW Ratio (kg)
Brain/BW Ratio	Brain/Terminal BW Ratio (kg)
Heart/BW Ratio	Heart/Terminal BW Ratio (kg)
Kidney/BW Ratio	Kidney/Terminal BW Ratio (kg)
Liver/BW Ratio	Liver/Terminal BW Ratio (kg)
Ovaries/BW Ratio	Ovaries/Terminal BW Ratio (kg)
Testes/BW Ratio	Testes/Terminal BW Ratio (kg)
Thymus/BW Ratio	Thymus/Terminal BW Ratio (kg)
Adrenal/ Brain	Adrenals/Brain Ratio
Heart/ Brain	Heart/Brain Ratio
Kidneys/ Brain	Kidneys/Brain Ratio
Liver/ Brain	Liver/Brain Ratio
Ovaries/ Brain	Ovaries/Brain Ratio
Testes/ Brain	Testes/Brain Ratio
Thymus/ Brain	Thymus/Brain Ratio

**Unit Descriptions**

<u>Headings Used</u>	<u>Description</u>
%	%
g	g

**Measurement/Statistics**

<u>Measurement</u>	<u>Descriptive</u>	<u>Comparative</u>	<u>Arithmetic</u> <u>/Adjusted</u>	<u>Transformation</u>
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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
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Key Page

**Measurement/Statistics (Continued)**

<u>Measurement</u>	<u>Descriptive</u>	<u>Comparative</u>	<u>Arithmetic /Adjusted</u>	<u>Transformation</u>
Adrenal Glands Weight	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Brain Weight	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Heart Weight	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Kidneys Weight	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Liver Weight	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Ovaries Weight	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Testes Weight	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Thymus Weight	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Adrenals/Terminal BW Ratio (kg)	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Brain/Terminal BW Ratio (kg)	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Heart/Terminal BW Ratio (kg)	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Kidney/Terminal BW Ratio (kg)	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Liver/Terminal BW Ratio (kg)	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Ovaries/Terminal BW Ratio (kg)	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Key Page

**Measurement/Statistics (Continued)**

<u>Measurement</u>	<u>Descriptive</u>	<u>Comparative</u>	<u>Arithmetic /Adjusted</u>	<u>Transformation</u>
Testes/Terminal BW Ratio (kg)	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Thymus/Terminal BW Ratio (kg)	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Adrenals/Brain Ratio	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Heart/Brain Ratio	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Kidneys/Brain Ratio	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Liver/Brain Ratio	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Ovaries/Brain Ratio	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Testes/Brain Ratio	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic
Thymus/Brain Ratio	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic

**Automatic Transformations**

<u>Measurement</u>	<u>Transformation Order</u>
Adrenal Glands Weight	Identity (No Transformation), Log, Rank
Brain Weight	Identity (No Transformation), Log, Rank
Heart Weight	Identity (No Transformation), Log, Rank
Kidneys Weight	Identity (No Transformation), Log, Rank
Liver Weight	Identity (No Transformation), Log, Rank
Ovaries Weight	Identity (No Transformation), Log, Rank
Testes Weight	Identity (No Transformation), Log, Rank
Thymus Weight	Identity (No Transformation), Log, Rank
Adrenals/Terminal BW Ratio (kg)	Identity (No Transformation), Log, Rank
Brain/Terminal BW Ratio (kg)	Identity (No Transformation), Log, Rank
Heart/Terminal BW Ratio (kg)	Identity (No Transformation), Log, Rank

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
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Key Page

**Automatic Transformations (Continued)**

Measurement	Transformation Order
Kidney/Terminal BW Ratio (kg)	Identity (No Transformation), Log, Rank
Liver/Terminal BW Ratio (kg)	Identity (No Transformation), Log, Rank
Ovaries/Terminal BW Ratio (kg)	Identity (No Transformation), Log, Rank
Testes/Terminal BW Ratio (kg)	Identity (No Transformation), Log, Rank
Thymus/Terminal BW Ratio (kg)	Identity (No Transformation), Log, Rank
Adrenals/Brain Ratio	Identity (No Transformation), Log, Rank
Heart/Brain Ratio	Identity (No Transformation), Log, Rank
Kidneys/Brain Ratio	Identity (No Transformation), Log, Rank
Liver/Brain Ratio	Identity (No Transformation), Log, Rank
Ovaries/Brain Ratio	Identity (No Transformation), Log, Rank
Testes/Brain Ratio	Identity (No Transformation), Log, Rank
Thymus/Brain Ratio	Identity (No Transformation), Log, Rank

**Group Information**

Short Name	Long Name	Type	Report Headings 1-4		
1	Excipient	Control	Group 1	0	mg/kg SC
2	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (0.4)	Dose	Group 2	0.4	mg/kg SC
3	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (3.64)	Dose	Group 3	3.64	mg/kg SC
4	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (14.55)	Dose	Group 4	14.55	mg/kg SC

**Pairwise Comparisons**

Group	Vs	Group
1		2
1		3
1		4

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix A**

**PROTOCOL AND AMENDMENTS**

**I. GLP-MULTIPLE (5 WEEKLY) REPEAT SUBCUTANEOUS TOXICITY AND TOXICOKINETICS STUDY WITH [Proprietary] [Pro] IN MALE AND FEMALE BEAGLE DOGS**

**II. SRI STUDY NUMBER: M397-18**

**III. SPONSOR**

National Institute of Allergy and Infectious Diseases  
Division of AIDS  
5601 Fishers Lane, [Redacted by agreement]  
Bethesda, MD 20892-9830

Contract and TO Number: HHSN272201400006I/TO- HHSN27200007  
Sponsor's Representative: [Redacted by agreement]

**IV. TESTING FACILITY**

SRI International  
Biosciences Division  
333 Ravenswood Avenue  
Menlo Park, CA 94025

Study Director: [Redacted by agreement]

**V. PROPOSED IN-LIFE SCHEDULE**

Start of In-life (first dose): November 20, 2018  
Termination (final necropsy): January 31, 2019

**VI. APPROVALS**

[Redacted by agreement]

Date

11/14/2018

Date

11/14/2018

Date

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**VII. PURPOSE OF STUDY**

The purpose of this study is to provide data of suitable quality and integrity to support application to the U.S. Food and Drug Administration (FDA) and other regulatory agencies. Therefore, this study will be performed in accordance with the U.S. FDA “Good Laboratory Practice for Nonclinical Laboratory Studies” (GLP) as described in 21 CFR Part 58.

**VIII. STUDY OBJECTIVE**

The objective of this study is to determine potential toxicity of [Proprietary] [Prop] a new formulation of [Proprietary Info] 12 mg/ml), [Proprietary Info] 6.9 mg/ml) and [Proprietary Info] 3.3 mg/ml), in adult male and female Beagle dogs following a multiple, 5-weekly repeat subcutaneous (s.c.) administration.

**IX. SPONSOR RESPONSIBILITIES**

The Sponsor is responsible for the following:

1. Documentation on the strength, purity, composition, physical properties, stability, and other pertinent information on the bulk test article in the form of a Certificate or Record of Analysis and a Certificate of Stability or other documentation for the bulk test article for inclusion in the final report.
2. Stability, homogeneity and concentration of the formulated test article under conditions of use.
3. Providing sufficient quantity of test article.

**X. EXPERIMENTAL DESIGN**

The design of the study is summarized in the table on the next page.



**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

Group	Treatment	Dose Level (mg/kg) <sup>a</sup>	Dose Conc. (mg/ml/site)	Volume (ml/kg/site) <sup>a</sup>	Total No. of Animals	No. of Animals at Necropsy	
						Day 30 (Main)	Day 72 (Recovery)
1	Excipient	Eq to 16 mg/kg	0	0.5	5M/5F	3M/3F	2M/2F
2	[Proprietary] [Pro]	0.4 (0.13 x 3 sites)	0.26	0.5	5M/5F	3M/3F	2M/2F
3	[Proprietary] [Pro]	4 (1.33 x 3 sites)	2.66	0.5	5M/5F	3M/3F	2M/2F
4	[Proprietary] [Pro]	16 (5.33 x 3 sites)	10.66	0.5	5M/5F	3M/3F	2M/2F
<b>Total No. of Animals</b>					<b>20M/20F</b>	<b>12M/12F</b>	<b>8M/8F</b>

<sup>a</sup> Excipient and [Proprietary] [Pro] formulations will be administered in 3 sites at 0.5 ml/kg/site. The dose volume may be adjusted to achieve the target dose levels based on actual measured concentration of dose solution. Any change will be approved by the Study Director and documented in the study records.

<sup>b</sup> [Proprietary] [Pro]

### Species and Strain

Beagle dog

### Route of Administration

Subcutaneous (s.c.)

### Frequency

Once weekly for 5 weeks (divided over 3 sites per every weekly dosing)

### Dosing Volume

0.5 ml/kg/site; 3 sites. Dose volumes will be calculated based on the animal's most recent body weight to achieve the target dose levels based on 12 mg/ml [Proprietary Info]

[Proprietary Info] concentration of dose solution. Maximum injection volume will be in accordance with IACUC guidelines.

### Duration of In-Life Phase

72 days

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**XI. MATERIALS AND METHODS**

**A. Test and Control Articles**

**1. Test Article**

Proprietary Info

**Supplier**

To be included in the final report

**Manufacturer**

To be included in the final report

**Lot Number**

To be included in the final report

**Physical Description**

Milky white suspension

**Storage Conditions**

2-8°C

**Characterization of Test Article**

The Sponsor is responsible for characterization and stability of the test article and will provide a Certificate of Analysis (CofA), or equivalent documentation, to SRI for inclusion in the final report. The raw data generated by the Sponsor in support of this CofA or its equivalent will not be verified or maintained by SRI.

**2. Vehicle Control:**

Excipient Control

**Supplier**

To be included in the final report

**Manufacturer**

To be included in the final report

**Lot Number**

To be included in the final report

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Physical Description**

To be included in the final report

**Storage Conditions**

2-8°C

**Characterization of Reference Control**

The Sponsor is responsible for characterization and stability of the excipient control under the specified storage conditions and will provide the CofA to SRI for inclusion in the final report. Information on the identity, purity, and stability of the excipient control article may be obtained by recording all of the pertinent information provided on the container labels or in a CofA provided by the supplier.

**3. Preparation of Dose Formulations**

Dose formulations will be provided by the Sponsor as ready-to-dose formulations at the concentrations specified in the table in Section X.

**Storage of Dose Formulations**

Dose formulations will be stored refrigerated at 2°-8°C, protected from light until the day of use. Formulation(s) will be brought to room temperature prior to administration to the animals.

**4. Characterization of Dose Formulations**

The Sponsor is responsible for stability, homogeneity, and concentration analyses of the test articles in the vehicle. Thus, the raw analytical data generated in support of this study will not be verified or maintained by SRI. SRI will rely on the formal Certificate of Analysis provided with the formulation for confirmation of concentration, quality and stability.

**5. Test Article Handling**

At a minimum, personnel handling the test, and control article formulations will wear eye protection, gloves, and a protective smock or laboratory coat.

**6. Disposition**

At the end of the study, any remaining partially used and unused containers of vehicle control, test article (kept refrigerated) will be shipped to the Sponsor unless the Sponsor issues other directions.

Residual dose formulations will be discarded after analysis, when the final report is submitted, or when samples no longer afford evaluation.

Empty control, test article containers may be destroyed by SRI on submission of the final report to the Sponsor.

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

See Section XII.D, "Regulatory Compliance," for information about retention of records and study samples.

**7. Method for Assuring Correct Dosing**

The administration of each dose formulation will be properly documented, and the amount administered to each animal will be recorded.

**B. Test System**

**Species**

Dog

**Strain**

Beagle

**Supplier**

[Proprietary Info]

**Number of Animals**

40 assigned to test

**Sex**

20 males and 20 females

**Age at First Dose**

6-8 months

**Weight Range at First Dose**

7-10 kg (males), 5-9 kg (females)

**Animal Care**

General procedures for animal care and housing will be in accordance with the current Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) recommendations, current requirements stated in the Guide for the Care and Use of Laboratory Animals (National Research Council), and current requirements as stated by the U.S. Department of Agriculture through the Animal Welfare Act and Animal Welfare Regulations (November 2013).

**Quarantine/Acclimation**

At least 14 days. A complete physical examination will be performed on each dog before quarantine release.



**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
with Proprietary Info Pro in Male and Female Beagle Dogs  
SRI Study No. M397-18

**Housing**

1 per enclosed run ( $\geq 4$  ft x 6 ft)

**Light Cycle**

12 hr light/12 hr dark

**Temperature**

64–84°F

**Humidity**

30–70% Brief excursions outside this range may occur; excursions of less than 4 hr/day will not be considered deviations from the protocol.

**Ventilation**

At least 10 room volumes per hour, with no recirculation of air.

**Food**

Envigo Teklad Certified Global 25% Protein Dog Diet, 2025C or equivalent. Dogs will be exposed to their daily ration of food, except for periods of fasting required by the study protocol. The quantity of the daily ration is sufficient to meet nutritional requirements. Eukanuba Puppy Food (wet food), or equivalent, may be added to daily ration by attending veterinarian recommendation. Feed is analyzed periodically to ensure that contaminants known to be capable of interfering with the study and reasonably expected to be present in such feed are not present at levels that would affect the study. Documentation of feed analyses is maintained at SRI for reference. A copy of the lot specific reports provided by the supplier will be maintained in the study records.

**Water**

Water (purified, reverse osmosis or untreated tap water) will be provided ad libitum. Based on previous reports, no contaminants that could interfere with and affect the results of the study are expected to be present in the water. Copies of annual analysis reports are maintained at SRI for reference.

**8. Assignment of Animals to Study Day**

No more than 3 days before initiation of treatment

**Randomization**

Animals will be randomly assigned to treatment groups via a computerized or manual body weight stratification procedure. Animals may be excluded based on health, behavior, or inappropriate weight.



**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Identification**

Animals will be individually identified by a uniquely numbered ear tattoo.

**9. Welfare of the Animals**

Every effort will be made to minimize, if not eliminate, pain and suffering in all animals in this study. Moribund animals and animals experiencing undue pain and suffering will be euthanized at the discretion of the Study Director, attending veterinarian, or other qualified person. The Study Director will make every effort to protect the scientific validity of the study.

**C. Experimental Procedure (In-Life Evaluations)**

**1. Preparation of Animals**

Animals will **not be fasted** before dose administration.

**2. Dose Administration**

Weekly s.c. injection to 3 sites on the back and/or sides of the animal (for 5 weeks). The area will be shaved prior to injection. The injection sites on the back will be on either side of the spine and will progress from the base of the neck towards the shoulder blades moving ventral approximately one inch every week to avoid injecting on the same area. The third injection site on the side will be similarly shaved and the injection site will progress similarly, approximately 1 inch each week to avoid injecting into the same area. Each injection site will be marked (adjacent to the injection location) and this marking will be refreshed as needed to maintain a visual identification of the injection site. This route of administration is proposed for clinical use of the test article in humans.

**3. Mortality/Morbidity**

Animals will be checked at least once daily.

**4. Clinical Observations**

Recorded once daily and approximately 2–4 hr postdose on dosing days (Days 1, 8, 15, 22 and 29) and once weekly during the recovery phase, or more often as clinical signs warrant and at necropsy. Animals will be examined for any altered clinical signs, including gross motor and behavioral activity, and observable changes in appearance.

**5. Body Weights**

Body weights will be recorded predose on dosing days (Days 1, 8, 15, 22 and 29) for the purpose of dose calculation, weekly thereafter and at each necropsy.

Body weights will be recorded for animals found dead and for any dogs euthanized early, but these weights will not be included in the statistical evaluations.

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
with Proprietary Info Pro in Male and Female Beagle Dogs  
**SRI Study No. M397-18**

**6. Food Consumption**

Food consumption will be quantitatively measured for approximately a 24 hr period once weekly for each run (1 dog/run) throughout the study.

**7. Ophthalmologic Examination**

All animals (including extras) will have a pretest ophthalmic examination performed by a board-certified veterinary ophthalmologist, and all surviving animals will be re-examined by the ophthalmologist within the week before their scheduled necropsy (Main necropsy Day 30 and Recovery necropsy Day 72). If there are no adverse ophthalmologic effects seen in the Main subset animals at termination time point, then ophthalmologic examinations will not be performed on the Recovery subset animals.

**8. Plasma Drug Levels**

**Method of Collection**

Whole blood from animals in Groups 2-4 will be collected from cephalic, saphenous, or jugular veins into tubes containing K<sub>2</sub>EDTA, processed to plasma, and then stored frozen at  $\leq -60^{\circ}\text{C}$ .

**Volume**

Maximum ~1.5 ml whole blood (~ 700  $\mu\text{l}$  of plasma) per sample.

**Frequency**

Plasma drug levels will be sampled at the following time points in Groups 2-4 animals:

Main animals (3M/3F): 1 hr predose and 1 hr postdose on dosing days (Days 1, 8, 15, 22 and 29)

Recovery animals (2M/2F): 1 hr predose and 1 hr postdose on dosing Day 29 and on a single timepoint on Days 36, 43, 50, 57, 64 and 71

**Method of Analysis**

Drug levels of Proprietary Info will be measured in plasma samples using a bioanalytical method provided by the Sponsor and validated by SRI. Details of the bioanalytical method and validation results will be included in a separate validation report (SRI Study No. B185-18).

**9. Toxicokinetics Analysis**

Plasma concentrations of the test articles will be plotted versus time for each animal. The plasma drug level data will be analyzed using Phoenix® WinNonlin® (version 6.3 or higher) software to perform noncompartmental assessments. The dose administered will be input to the program as mg/kg, and as a result no additional corrections for individual body weights of the animals will be necessary.



**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

The following parameters and constants will be determined if the data allow: maximal plasma concentration ( $C_{max}$ ), time to maximum plasma concentration ( $T_{max}$ ), and area under the plasma concentration-time curve to the last time point ( $AUC_{last}$ ). Values will be calculated for each individual animal.

Other methods of analysis may be used or other parameters calculated, as appropriate, based on the plasma drug level data.

**Disposition**

Residual bioanalytical samples will be discarded on submission of the final report.

**10. Clinical Pathology Evaluations**

**Preparation of Animals**

Animals will be fasted before blood collection.

**Method of Collection**

Whole blood from animals in all treatment groups will be collected from cephalic, saphenous, or jugular veins. Hematology samples will be collected using K<sub>3</sub>EDTA as the anticoagulant. No anticoagulant will be used for clinical chemistry samples.

**Frequency**

Prestudy and prior to necropsy as follows:

- On Day 30, one day after the last treatment, blood will be collected for clinical pathology from Main animals (3 animals/sex/group) that will then be sacrificed for necropsy evaluations.
- On Day 72, blood will be collected from the Recovery animals (remaining 2 animals/sex/group) for clinical pathology, and then sacrificed for necropsy evaluations.

Clinical pathology parameters that will be evaluated are listed below. In some cases, automated analyzers report additional parameters not specified in the protocol. Results for the additional parameter(s) will be included in the data package, but will not be summarized, analyzed, or reported, and their collection will not be considered deviations from the protocol.

If manual WBC differential counts have to be conducted some parameters that are not specified in the protocol may be evaluated and reported. This will not be considered a deviation from the protocol.

**Hematology Parameters**

- Hematocrit (HCT)
- Hemoglobin (HGB)

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

- Red blood cell count (RBC)
- Red blood cell distribution width (RDW)
- White blood cell count (WBC)
- WBC differential and absolute counts
  - Absolute neutrophil [ANE]
  - Percent neutrophil [PNE]
  - Absolute lymphocyte [ALY]
  - Percent lymphocyte [PLY]
  - Absolute monocyte [AMO]
  - Percent monocyte [PMO]
  - Absolute eosinophil [AEO]
  - Percent eosinophil [PEO]
  - Absolute basophil [ABA]
  - Percent basophil [PBA]
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular volume (MCV)
- Mean corpuscular hemoglobin concentration (MCHC)
- Platelet count (PLT)
- Mean platelet volume (MPV)
- Absolute Reticulocyte (ARET)
- Percent Reticulocyte (PRET)

**Clinical Chemistry Parameters**

- Total Bilirubin (TBI)
- Creatinine (CRE)
- Sodium (SOD)
- Potassium (POT)
- Chloride (CHL)
- Cholesterol (CHO)
- Triglyceride (TRI)
- Glucose (GLU)
- Blood urea nitrogen (BUN)
- Aspartate aminotransferase (AST)
- Alanine aminotransferase (ALT)
- Alkaline phosphatase (ALP)
- Calcium (CAL)
- Phosphorus (PHO)
- Total protein (TPR)
- Albumin (ALB)
- Albumin/globulin ratio (AGR)
- Globulin (GLO)

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
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**Coagulation Parameters**

- Prothrombin time (PT)
- Activated partial thromboplastin time (PTT)
- Fibrinogen (FIB)

**11. Urinalysis**

**Method of Collection**

Urine, if available, will be collected by cystocentesis; ≤15 ml will be submitted to the clinical laboratory.

**Frequency**

Urine will be collected on Day 30 (Main necropsy) and Day 72 (Recovery necropsy)

**Urinalysis Parameters**

- Color (noted at time of collection)
- Clarity (noted at time of collection)
- Specific gravity
- Microscopic examination of urine sediment
- Bilirubin
- Glucose
- Ketones
- Leukocytes
- Nitrite
- Occult blood
- pH
- Protein
- Urobilinogen

**D. Necropsy**

**Interval**

Necropsies will be performed on Day 30 for Main Group and Day 72 for Recovery Group. Necropsies will also be performed for any animal found dead or euthanized in moribund condition.

**Euthanasia**

Following s.c. administration of a sedative cocktail, an overdose of sodium pentobarbital will be administered by intravenous injection.



**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Observations**

External examination of all body orifices and an examination of all cranial, thoracic and abdominal organs will be performed, and all gross findings will be recorded.

**Tissues Retained**

The following tissues will be collected from all animals in the Main Group and the Recovery Group, including those found dead and moribund animals. Tissues will be retained in 10% neutral buffered formalin, except where noted.

- All gross lesions (including tissue masses and abnormal regional lymph nodes)
- Adrenal glands
- Aorta
- Bone (femur, distal with joint surface)
- Bone, sternum (marrow histology)
- Bone marrow smear, sternum (for cytology except for found dead animals)
- Brain (fore-, mid-, and hindbrain)
- Cecum
- Cervix
- Colon
- Duodenum
- Epididymides
- Esophagus
- Eyes, with optic nerve (fixed with modified Davidson's solution)
- Gall bladder
- Heart
- Identification; (retained in formalin; not processed for histology)
- Ileum
- Injection site(s) tissue. Only representative sections of the injection site(s) will be collected
- Jejunum
- Kidneys
- Liver
- Lungs with bronchi
- Lymph nodes, mandibular and mesenteric
- Mammary gland (females, males when present)
- Ovaries
- Pancreas
- Pituitary gland
- Prostate
- Rectum
- Salivary gland, mandibular

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

- Sciatic nerve
- Skeletal muscle
- Skin, ventral abdomen, taken with mammary gland
- Skin at each of the three injection sites
- Spinal cord sections (thoracic only)
- Spleen
- Stomach
- Testes (fixed with modified Davidson's solution)
- Thymus
- Thyroid/parathyroid glands
- Trachea
- Urinary bladder
- Uterus
- Vagina

**Final Body/Organ Weights**

Body weight will be recorded on the day of necropsy for body to organ weight ratios. The following organs will be weighed. Paired organs will be weighted together.

- Adrenal glands
- Brain
- Heart
- Kidneys
- Liver
- Ovaries
- Testes, without epididymides
- Thymus

Organ weights will be recorded for animals found dead or sacrificed in moribund condition, but these data will not be included in statistical evaluations.

**E. Histopathologic Examination**

**Tissues**

Tissues listed under "Retained Tissues" will be processed and evaluated for the following:

- All Main animals in the control and high-dose groups (Groups 1 and 4)
- Animals with an unscheduled death or euthanized in moribund condition
- Any tissue identified as a target organ of toxicity by the pathologist (examined in all other dose groups)
- Any other tissue deemed necessary by the pathologist

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

- All gross lesions will be processed for all animals
- Bone marrow smear for cytology will be processed but only analyzed at the discretion of the Study Director and/or pathologist based on hematology and/or bone marrow histopathology findings.

**Tissue Sections**

Sections of the tissues will be embedded in paraffin, cut approximately 5 µm thick, and stained with hematoxylin and eosin by a histology laboratory qualified by SRI.

**Evaluated By**

Histopathology specimens will be evaluated by a board-certified veterinary pathologist.

**Method**

Each lesion will be listed and coded by the most specific topographic and morphologic diagnoses, severity, and distribution, using International Harmonization of Nomenclature and Diagnostic Criteria for Lesions (INHAND) as a guide. A four-step grading system (minimal, mild, moderate and marked) will be used to define gradable lesions for comparison between treated and control groups. Data will be recorded and summarized using Provantis® version 9.3.1.1 or other appropriate program. Records of gross findings for a specimen from postmortem observations will be available to the pathologist when examining that specimen microscopically.

**F. Evaluation of Data Parameters**

Mean and standard deviation will be calculated for body weight, food consumption, clinical pathology; urinalysis pH, urobilinogen, and specific gravity; and organ weight data at each evaluation interval. Calculations will be performed using Provantis® version 9.3.1.1, MS Excel 2010 or later, or other appropriate program.

**Proposed Statistical Tests**

Body weight, food consumption, clinical pathology, urinalysis pH and specific gravity, and organ weight data will be evaluated by one-way analysis of variance (ANOVA), followed by Dunnett's test (if the ANOVA is significant). All other numeric parameters will be evaluated by Student's t-test, unless specified otherwise. If appropriate, other post hoc analyses may also be performed. For clinical pathology data, values for parameters that are not within the detection threshold will not be included in the statistical evaluation.

**Criteria for Null Hypothesis Rejection**

$p \leq 0.05$



**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**G. Control of Bias**

While evaluating the responses of the animals and conducting the analyses, the technical staff will be aware of the treatment history of each animal and sample. Based on the relatively objective endpoints to be examined, bias is not expected to influence the results of the study.

**XII. REGULATORY COMPLIANCE**

**A. Good Laboratory Practice Compliance**

This study is intended to be submitted to and reviewed by the U.S. FDA or an equivalent regulatory agency, and this study therefore will be performed in accordance with the U.S. FDA “Good Laboratory Practice for Nonclinical Laboratory Studies,” as described in 21 CFR Part 58, with the following exceptions:

- Various pre-initiation study activities (e.g., receipt and quarantine of animals, pre-initiation body weights and randomization) may be performed prior to the approval of the protocol. These activities will be conducted according to testing facility SOPs, but because they may be conducted before the protocol is signed, they may not be considered by the FDA to have been conducted in compliance with GLP requirements.
- Animal water and food analysis will not be performed under GLP compliance by the vendors.

**B. Standard Operating Procedures (SOPs)**

All operations pertaining to this study, unless specifically defined in this protocol, will be performed according to the SOPs of the laboratory. All deviations from any SOP and the reasons for the deviations will be documented and acknowledged by the Study Director.

**C. Protocol Amendments and Deviations**

All changes or revisions made to the approved protocol by any involved party and the reasons for the changes and revisions will be documented, signed, and dated by the Study Director and the Sponsor’s Representative. Amendments will be maintained with the protocol. Verbal or email approval for changes in the protocol may be granted by the Sponsor’s Representative, but a written amendment as described above will follow.

All deviations from the protocol and the reasons for the deviations will be documented and acknowledged by the Study Director. The Sponsor’s Representative will be informed of the occurrence of any deviations that might affect the results of the study, and any corrective actions taken.

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**D. Retention of Records and Study Samples**

The original protocol, amendments, final report, raw data, supporting documents and records as well as all pathology materials (slides, blocks and wet tissue specimens) specific to this study will be retained and stored by SRI International. All records and materials will be maintained for a period of at least 1 year. For studies with an in-life phase longer than 28 days, an archival sample of the test and control articles will be maintained by SRI for at least 5 years or as long as samples afford evaluation (21 CFR 58.105[d]). At the end of the retention period, the Sponsor will be contacted for instructions regarding disposition of these materials.

**XIII. REPORTING**

The final report will accurately and completely describe the study design, procedures, and findings. An analysis and summary of the data followed by the conclusions derived from the analyses will also be included. A draft report will be issued prior to submission of the final report.



## PROTOCOL AMENDMENT NO. 1

**PROTOCOL TITLE** GLP-Multiple (5 Weekly) Repeat Subcutaneous Toxicity and Toxicokinetics Study with Proprietary Pro in Male and Female Beagle Dogs

**SRI Study Number:** M397-18

**Sponsor:** National Institute of Allergy and Infectious Diseases  
Division of AIDS  
5601 Fishers Lane, Redacted by agreement  
Bethesda, MD 20892-9830

**Sponsor's Representative:** Redacted by agreement

**SRI Study Director**

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This amendment modifies the following lines/sections of the study protocol. Additions are in bold and italics: *addition*. Deleted text has been struck through: ~~deleted~~.

**Section XI. MATERIALS AND METHODS, page 7:**

**B. Test System**

**8. Assignment of Animals to Study Day**

No more than ~~3~~ **6** days before initiation of treatment

**Reason for Change:** Text clarification in the Test System section. We are clarifying that dogs will be assigned to study not more than 6 days prior to initiation of treatment.

**Effect on the Study:** No impact to study because study has not started yet.

**Effective Date:** 11/14/2018

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**APPROVALS**

Sponsor's Representative

Date

11/15/2018

Date

**PROTOCOL AMENDMENT NO. 1**

**PROTOCOL TITLE** GLP-Multiple (5 Weekly) Repeat Subcutaneous Toxicity and Toxicokinetics Study with [Proprietary] [Pro] in Male and Female Beagle Dogs

**SRI Study Number:** M397-18

**Sponsor:** National Institute of Allergy and Infectious Diseases  
Division of AIDS  
5601 Fishers Lane [Redacted by agreement]  
Bethesda, MD 20892-9830

**Sponsor's Representative:** [Redacted by agreement]

**SRI Study Director**

This amendment modifies the following lines/sections of the study protocol. Additions are in bold and italics: *addition*. Deleted text has been struck through: ~~deleted~~.

**Section XI. MATERIALS AND METHODS, page 7:**

**B. Test System**

**8. Assignment of Animals to Study Day**

No more than 3 6 days before initiation of treatment

**Reason for Change:** Text clarification in the Test System section. We are clarifying that dogs will be assigned to study not more than 6 days prior to initiation of treatment.

**Effect on the Study:** No impact to study because study has not started yet.

**Effective Date:** 11/14/2018

**APPROVALS**

[Redacted by agreement]

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Date

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Date

# **PROTOCOL AMENDMENT NO. 2**

**PROTOCOL TITLE** GLP-Multiple (5 Weekly) Repeat Subcutaneous *Dose* Toxicity and Toxicokinetics Study with Proprietary Pro in Male and Female Beagle Dogs

**SRI Study Number:** M397-18

**Sponsor:** National Institute of Allergy and Infectious Diseases  
Division of AIDS  
5601 Fishers Lane Redacted by  
Bethesda, MD 20892-9830

**Sponsor's Representative:** Redacted by agreement

**SRI Study Director**

This amendment modifies the following lines/sections of the study protocol. Additions are in bold and italics: *addition*. Deleted text has been struck through: ~~deleted~~.

## **Section X. EXPERIMENTAL DESIGN, page 3:**

Group	Treatment	Dose Level (mg/kg) <sup>a</sup> <sub>b</sub>	Dose Conc. (mg/ml/ site)	Volume (ml/kg/site) <sup>a</sup>	Total No. of Animals	No. of Animals at Necropsy	
						Day 30 (Main)	Day 72 (Recovery)
1	Excipient	Eq to 16 mg/kg	0	0.5	5M/5F	3M/3F	2M/2F
2	<span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> <span style="border: 1px solid black; padding: 0 5px;">Pro</span>	0.4 (0.13 x 3 sites)	<del>0.26</del> <b>0.27</b>	0.5	5M/5F	3M/3F	2M/2F
3	<span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> <span style="border: 1px solid black; padding: 0 5px;">Pro</span>	<del>4-3.64</del> ( <del>1.33</del> <b>1.21</b> x 3 sites)	<del>2.66</del> <b>2.43</b>	0.5	5M/5F	3M/3F	2M/2F
4	<span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> <span style="border: 1px solid black; padding: 0 5px;">Pro</span>	<del>16-14.55</del> ( <del>5.33</del> <b>4.85</b> x 3 sites)	<del>10.66</del> <b>9.72</b>	0.5	5M/5F	3M/3F	2M/2F
<b>Total No. of Animals</b>					<b>20M/20F</b>	<b>12M/12F</b>	<b>8M/8F</b>

<sup>a</sup> Excipient and Proprietary Pr formulations will be administered in 3 sites at 0.5 ml/kg/site. The dose volume may be adjusted to achieve the target dose levels based on actual measured concentration of dose solution. Any change will be approved by the Study Director and documented in the study records.

Proprietary Info

**PROTOCOL AMENDMENT NO. 2****Section XI. MATERIALS AND METHODS, pages 5, 6-8, 10 and 15:****A. Test and Control Articles****3. Preparation of Dose Formulations**

Dose formulations will be provided by the Sponsor as ready-to-dose formulations at the concentrations specified in the table in Section X.

**Storage of Dose Formulations**

Dose formulations will be stored refrigerated at 2°-8°C, ~~protected from light~~ until the day of use. Formulation(s) will be brought to room temperature prior to administration to the animals.

**B. Test System****1. Species**

Dog

**Strain**

Beagle

**Supplier**

Marshall (or another approved vendor)

**Number of Animals**

40 assigned to test

**Sex**

20 males and 20 females

**Age at First Dose**

6-8 months

**Weight Range at First Dose**

7-10 kg (males), 5-9 kg (females)

**2. Animal Care**

General procedures for animal care and housing will be in accordance with the current Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) recommendations, current requirements stated in the Guide for the Care and Use of Laboratory Animals (National Research Council), and current requirements as stated by the U.S. Department of Agriculture through the Animal Welfare Act and Animal Welfare Regulations (November 2013).



**PROTOCOL AMENDMENT NO. 2****Quarantine/Acclimation**

At least 14 days. A complete physical examination will be performed on each dog before quarantine release.

**Housing**

1 per enclosed run ( $\geq 4$  ft x 6 ft)

**Light Cycle**

12 hr light/12 hr dark

**Temperature**

64–84°F

**Humidity**

30–70% Brief excursions outside this range may occur; excursions of less than 4 hr/day will not be considered deviations from the protocol.

**Ventilation**

At least 10 room volumes per hour, with no recirculation of air.

**Food**

Envigo Teklad Certified Global 25% Protein Dog Diet, 2025C or equivalent. Dogs will be exposed to their daily ration of food, except for periods of fasting required by the study protocol. The quantity of the daily ration is sufficient to meet nutritional requirements. Eukanuba Puppy Food (wet food), or equivalent, may be added to daily ration by attending veterinarian recommendation. Feed is analyzed periodically to ensure that contaminants known to be capable of interfering with the study and reasonably expected to be present in such feed are not present at levels that would affect the study. Documentation of feed analyses is maintained at SRI for reference. A copy of the lot specific reports provided by the supplier will be maintained in the study records.

**Water**

Water (purified, reverse osmosis or untreated tap water) will be provided ad libitum. Based on previous reports, no contaminants that could interfere with and affect the results of the study are expected to be present in the water. Copies of annual analysis reports are maintained at SRI for reference.

**83. Assignment of Animals to Study Day**

No more than 6 days before initiation of treatment

**Randomization**

Animals will be randomly assigned to treatment groups via a computerized or manual body weight stratification procedure. Animals may be excluded based on health, behavior, or inappropriate weight.



## PROTOCOL AMENDMENT NO. 2

**Identification**

Animals will be individually identified by a uniquely numbered ear tattoo.

**94. Welfare of the Animals**

Every effort will be made to minimize, if not eliminate, pain and suffering in all animals in this study. Moribund animals and animals experiencing undue pain and suffering will be euthanized at the discretion of the Study Director, attending veterinarian, or other qualified person. The Study Director will make every effort to protect the scientific validity of the study.

**C. Experimental Procedure (In-Life Evaluations)****10. Clinical Pathology Evaluations****Preparation of Animals**

Animals will be fasted before blood collection.

**Method of Collection**

Whole blood from animals in all treatment groups will be collected from cephalic, saphenous, or jugular veins. Hematology samples will be collected using K3EDTA as the anticoagulant. *Coagulation samples will be collected using citrate as the anticoagulant.* No anticoagulant will be used for clinical chemistry samples.

**E. Histopathologic Examination****Method**

Each lesion will be listed and coded by the most specific topographic and morphologic diagnoses, severity, and distribution, using International Harmonization of Nomenclature and Diagnostic Criteria for Lesions (INHAND) as a guide. A four-step grading system (minimal, mild, moderate and marked) will be used to define gradable lesions for comparison between treated and control groups. Data will be recorded and summarized using Provantis® version ~~9.3.1.1~~ **10.1.0.1** or *later*. ~~other~~ **Other** appropriate programs *may be used*. Records of gross findings for a specimen from postmortem observations will be available to the pathologist when examining that specimen microscopically.

**F. Evaluation of Data Parameters**

Mean and standard deviation will be calculated for body weight, food consumption, clinical pathology; urinalysis pH, urobilinogen, and specific gravity; and organ weight data at each evaluation interval. Calculations will be performed using Provantis® version ~~9.3.1.1~~ **10.1.0.1**, and MS Excel 2010 or later, ~~or other~~. **Other** appropriate programs *may be used*.

**Reason for Change:** Clarification of concentration and dose formulations levels in Experimental Design Table. Text clarification in the following

**PROTOCOL AMENDMENT NO. 2**

sections: Title, Test and Control Articles, Test System,  
Experimental Procedure (In-Life Evaluations), Histopathologic  
Examination and Evaluation of Data Parameters.

**Effect on the Study:** No impact to study because study has not started yet.

**Effective Date:** 11/19/2018

**APPROVALS**

Redacted by agreement

Date

11/19/18

Date

**PROTOCOL AMENDMENT NO. 3**

**PROTOCOL TITLE** GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with [Proprietary] [Pro] in Male and Female Beagle Dogs

**SRI Study Number:** M397-18

**Sponsor:** National Institute of Allergy and Infectious Diseases  
Division of AIDS  
5601 Fishers Lane, [Redacted by agreement]  
Bethesda, MD 20892-9830

**Sponsor's Representative:**

[Redacted by agreement]

**SRI Study Director**

[Redacted]

This amendment modifies the following lines/sections of the study protocol. Additions are in bold and italics: ***addition***. Deleted text has been struck through: ~~deleted~~.

**Section XI. MATERIALS AND METHODS, page 5:**

**A. Test and Control Articles**

**3. Preparation of Dose Formulations**

Dose formulations will be provided by the Sponsor as ready-to-dose formulations at the concentrations specified in the table in Section X.

**Storage of Dose Formulations**

Dose formulations will be stored refrigerated at 2°-8°C, until the day of use. Formulation(s) will be brought to ~~37°C room~~ temperature prior to administration to the animals.

**Reason for Change:** Clarification of temperature at which dose formulation should be administered to animals.

**Effect on the Study:** Upon observation of some animal's painful response during dosing formulations at room temperature (25°C), the Sponsor suggested that warming to body temperature may aid in the movement of the formulation from the injection site because the lipids may flow somewhat more easily relative to room temperature. Given that the volume per injection is significant, avoiding a 12°C temperature differential may be beneficial and animals will show a decreased pain response to dose administration.

**Effective Date:** 12/4/2018

PROTOCOL AMENDMENT NO. 3

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**APPROVALS**

Redacted by agreement

Date

2/4/18

Date



## PROTOCOL AMENDMENT NO. 4

**PROTOCOL TITLE** GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with [Proprietary] [Pro] in Male and Female Beagle Dogs

**SRI Study Number:** M397-18

**Sponsor:** National Institute of Allergy and Infectious Diseases  
Division of AIDS  
5601 Fishers Lane, [Redacted by]  
Bethesda, MD 20892-9830

**Sponsor's Representative:**

[Redacted by agreement]

**SRI Study Director**

This amendment modifies the following lines/sections of the study protocol. Additions are in bold and italics: ***addition***. Deleted text has been struck through: ~~deleted~~.

**Section X. EXPERIMENTAL DESIGN, page 2-3:**

Group	Treatment	Dose Level (mg/kg) <sup>a</sup>	Dose Conc. (mg/ml/site)	Volume (ml/kg/site) <sup>a</sup>	Total No. of Animals	No. of Animals at Necropsy		
						Day 17 (Main)	Day 30 (Main)	Day 72 (Recovery)
1	Excipient	Eq to 16 mg/kg	0	0.5	5M/5F	--	3M/3F	2M/2F
2	[Proprietary] [Pro]	0.4 (0.13 x 3 sites)	0.26	0.5	5M/5F	--	3M/3F	2M/2F
3	[Proprietary] [Pro]	4 (1.33 x 3 sites)	2.66	0.5	5M/5F	<b>3M/3F</b>	3M/3F --	2M/2F
4	[Proprietary] [Pro]	16 (5.33 x 3 sites)	10.66	0.5	5M/5F	<b>3M/3F</b>	3M/3F --	2M/2F
<b>Total No. of Animals</b>					<b>20M/20F</b>	<b>6M/6F</b>	<b>12M/12F 6M/6F</b>	<b>8M/8F</b>

<sup>a</sup> Excipient and [Proprietary] [Pro] formulations will be administered in 3 sites at 0.5 ml/kg/site. The dose volume may be adjusted to achieve the target dose levels based on actual measured concentration of dose solution. Any change will be approved by the Study Director and documented in the study records.

Proprietary Info

**Frequency**

***Groups 1-2:*** Once weekly for 5 weeks (divided over 3 sites per every weekly dosing)

***Groups 3-4:*** Once weekly for 3 weeks (divided over 3 sites per every weekly dosing)



## PROTOCOL AMENDMENT NO. 4

## Section XI. MATERIAL AND METHODS, page 8-10, 12:

## C. Experimental Procedure (In-Life Evaluations)

## 2. Dose Administration

Weekly s.c. injection to 3 sites on the back and/or sides of the animal (*for 3 weeks for Groups 3 and 4, and for 5 weeks for Groups 1 and 2*). The area will be shaved prior to injection. The injection sites on the back will be on either side of the spine and will progress from the base of the neck towards the shoulder blades moving ventral approximately one inch every week to avoid injecting on the same area. The third injection site on the side will be similarly shaved and the injection site will progress similarly, approximately 1 inch each week to avoid injecting into the same area. Each injection site will be marked (adjacent to the injection location) and this marking will be refreshed as needed to maintain a visual identification of the injection site. This route of administration is proposed for clinical use of the test article in humans.

## 4. Clinical Observations

Recorded once daily and approximately 2–4 hr postdose on dosing days (*Groups 1-2: Days 1, 8, 15, 22 and 29; Groups 3-4: Days 1, 8 and 15*) and once weekly during the recovery phase, or more often as clinical signs warrant and at necropsy. Animals will be examined for any altered clinical signs, including gross motor and behavioral activity, and observable changes in appearance.

## 5. Body Weights

Body weights will be recorded predose on dosing days (*Groups 1-2: Days 1, 8, 15, 22 and 29; Groups 3-4: Days 1, 8 and 15*) for the purpose of dose calculation, weekly thereafter and at each necropsy.

Body weights will be recorded for animals found dead and for any dogs euthanized early, but these weights will not be included in the statistical evaluations.

## 7. Ophthalmologic Examination

All animals (including extras) will have a pretest ophthalmic examination performed by a board-certified veterinary ophthalmologist, and all surviving animals will be re-examined by the ophthalmologist within the week before their scheduled necropsy (Main necropsy Day 30 and Recovery necropsy Day 72). *Main animals in Groups 3 and 4 will not have ophthalmic examination performed within the week before their scheduled necropsy on Day 17.* If there are no adverse ophthalmologic effects seen in the Main subset animals *of Groups 1 and 2* at termination time point, then ophthalmologic examinations will not be performed on the Recovery subset animals. *Recovery animals in Groups 3 and 4 will have ophthalmic examination performed within the week before their scheduled necropsy on Day 72.*

## PROTOCOL AMENDMENT NO. 4

**8. Plasma Drug Levels****Frequency**

Plasma drug levels will be sampled at the following time points in **Group 2**  
Groups 2-4 animals:

Main animals (3M/3F): 1 hr predose and 1 hr postdose on dosing days (Days 1, 8, 15, 22 and 29)

Recovery animals (2M/2F): 1 hr predose and 1 hr postdose on dosing Day 29 and on a single timepoint on Days 36, 43, 50, 57, 64 and 71

*Plasma drug levels will be sampled at the following time points in Groups 3-4 animals:*

Main animals (3M/3F): 1 hr predose and 1 hr postdose on dosing days (Days 1, 8 and 15)

Recovery animals (2M/2F): on a single timepoint on Days 22, 29, 36, 43, 50, 57, 64 and 71

**10. Clinical Pathology Evaluations****Method of Collection**

Whole blood from animals in all treatment groups will be collected from cephalic, saphenous, or jugular veins. Hematology samples will be collected using K<sub>3</sub>EDTA as the anticoagulant. Coagulation samples will be collected using *sodium* citrate as the anticoagulant. No anticoagulant will be used for clinical chemistry samples.

**Frequency**

Prestudy and prior to necropsy as follows:

- *On Day 17, one day after the last treatment, blood will be collected for clinical pathology from Main animals in Groups 3 and 4 (3 animals/sex/group) that will then be sacrificed for necropsy evaluations.*
- On Day 30, one day after the last treatment, blood will be collected for clinical pathology from Main animals **in Groups 1 and 2** (3 animals/sex/group) that will then be sacrificed for necropsy evaluations.
- On Day 72, blood will be collected from the Recovery animals (remaining 2 animals/sex/group) for clinical pathology, and then sacrificed for necropsy evaluations.

## PROTOCOL AMENDMENT NO. 4

**11. Urinalysis****Frequency**

Urine will be collected on **Day 17 (Groups 3 and 4 Main necropsy)** and Day 30 (**Groups 1 and 2 Main necropsy**) and Day 72 (Recovery necropsy).

**D. Necropsy****Interval**

Necropsies will be performed on **Day 17 for Main Groups 3 and 4** and Day 30 for Main Groups **1 and 2**; and Day 72 for Recovery Group. Necropsies will also be performed for any animal found dead or euthanized in moribund condition.

**Reason for Change:**

Proprietary Info

**Effect on the Study:****Effective Date:**

12/6/2018

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**APPROVALS**

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**Sponsor's Representative**

Redacted by agreement

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**Date**

12/11/18

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**Date**

## PROTOCOL AMENDMENT NO. 4

**11. Urinalysis****Frequency**

Urine will be collected on *Day 17 (Groups 3 and 4 Main necropsy)* and Day 30 (*Groups 1 and 2 Main necropsy*) and Day 72 (Recovery necropsy).

**D. Necropsy****Interval**

Necropsies will be performed on *Day 17 for Main Groups 3 and 4 and* Day 30 for Main Groups *1 and 2;* and Day 72 for Recovery Group. Necropsies will also be performed for any animal found dead or euthanized in moribund condition.

**Reason for Change:**

Proprietary Info

**Effect on the Study:****Effective Date:**

12/6/2018

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**APPROVALS**

Redacted by agreement

\_\_\_\_\_  
Date\_\_\_\_\_  
Date



**PROTOCOL AMENDMENT NO. 5**

**PROTOCOL TITLE** GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pro in Male and Female Beagle Dogs

**SRI Study Number:** M397-18

**Sponsor:** National Institute of Allergy and Infectious Diseases  
Division of AIDS  
5601 Fishers Lane, Redacted by agreement  
Bethesda, MD 20892-9830

**Sponsor's Representative:**

**SRI Study Director**

Redacted by agreement

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This amendment modifies the following lines/sections of the study protocol. Additions are in bold and italics: *addition*. Deleted text has been struck through: ~~deleted~~.

**Section IV. TESTING FACILITY, page 1:**

**Study Director:**

Redacted by agreement

**Reason for Change:**

Personal Info

**Effect on the Study:** This change has no impact on the validity of the study because the replacement Study Director is qualified to take on the study.

**Effective Date:** 12/20/2018

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**APPROVALS**

Redacted by agreement



## PROTOCOL AMENDMENT NO. 6

**PROTOCOL TITLE** GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pro in Male and Female Beagle Dogs

**SRI Study Number:** M397-18

**Sponsor:** National Institute of Allergy and Infectious Diseases  
Division of AIDS  
5601 Fishers Lane, Redacted by agreement  
Bethesda, MD 20892-9830

**Sponsor's Representative:**

Redacted by agreement

**SRI Study Director**

This amendment modifies the following lines/sections of the study protocol. Additions are in bold and italics: ***addition***. Deleted text has been struck through: ~~deleted~~.

**Section IV. TESTING FACILITY, page 1:**

**Study Director:**

Redacted by agreement

**Reason for Change:**

Personal Info

**Effect on the Study:**

**Effective Date:**

**APPROVALS**

Redacted by agreement

Date

1/11/19

Date

## PROTOCOL AMENDMENT NO. 7

**PROTOCOL TITLE** GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pro in Male and Female Beagle Dogs

**SRI Study Number:** M397-18

**Sponsor:** National Institute of Allergy and Infectious Diseases  
Division of AIDS  
5601 Fishers Lane, Redacted by agreement  
Bethesda, MD 20892-9830

**Sponsor's Representative:** Redacted by agreement

**SRI Study Director**

This amendment modifies the following lines/sections of the study protocol. Additions are in bold and italics: *addition*. Deleted text has been struck through: ~~deleted~~.

**Section X. EXPERIMENTAL DESIGN, page 2-3:**

Group	Treatment	Dose Level (mg/kg) <sup>a</sup> <sub>b</sub>	Dose Conc. (mg/ml/site)	Volume (ml/kg/site) <sup>a</sup>	Total No. of Animals	No. of Animals at Necropsy		
						Day 17 (Main)	Day 30 (Main)	Day 72 (Recovery)
1	Excipient	Eq to 16 mg/kg	0	0.5	5M/5F	--	3M/3F	2M/2F
2	<span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> <span style="border: 1px solid black; padding: 0 5px;">Pro</span>	0.4 (0.13 x 3 sites)	<del>0.26</del> <b>0.27</b>	0.5	5M/5F	--	3M/3F	2M/2F
3	<span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> <span style="border: 1px solid black; padding: 0 5px;">Pr</span>	<del>4-3.64</del> (4.33 <b>1.21</b> x 3 sites)	<del>2.66</del> <b>2.43</b>	0.5	5M/5F	3M/3F	--	2M/2F
4	<span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> <span style="border: 1px solid black; padding: 0 5px;">Pro</span>	<del>16-14.55</del> (5.33 <b>4.85</b> x 3 sites)	<del>10.66</del> <b>9.72</b>	0.5	5M/5F	3M/3F	--	2M/2F
<b>Total No. of Animals</b>					<b>20M/20F</b>	<b>6M/6F</b>	<b>6M/6F</b>	<b>8M/8F</b>

<sup>a</sup> Excipient and Proprietary Pro formulations will be administered in 3 sites at 0.5 ml/kg/site. The dose volume may be adjusted to achieve the target dose levels based on actual measured concentration of dose solution. Any change will be approved by the Study Director and documented in the study records.

Proprietary Info

## PROTOCOL AMENDMENT NO. 7

**Reason for Change:** Clarification of concentration and dose formulations levels in Experimental Design Table. These had been changed in Amendment #2 but they were not reflected in Amendment #4. The present amendment corrects this discrepancy.

**Effect on the Study:** No impact to study as this is only a text clarification that had been mistakenly carried over from a previous amendment.

**Effective Date:** Upon signature of Study Director

---

**APPROVALS**

Redacted by agreement

19

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix B**

**CERTIFICATES OF ANALYSIS**

Proprietary Info

## CERTIFICATE OF ANALYSIS

Proprietary Pro Drug Combination Suspension

Lot Number

Proprietary Info

Date of Manufacturing  
11/06/2018

Page 1 of 1

Product Name:

Manufacturer:  
Lot Number:

Proprietary Info

Proprietary Info

## Storage and Expiration

Store at 2-8°C

Expiration / Retest Date 4/29/19

Test	Specification	Result
Appearance	White, turbid suspension	White turbid suspension
Identity	Proprietary Info	conforms
Proprietary Info	0 mg/mL $\pm$ 0 mg/mL	0 mg/ml
	0 mg/mL $\pm$ 0 mg/mL	0 mg/m
	0 mg/mL $\pm$ 0 mg/mL	0 mg/ml
	45.8 mg/mL $\pm$ 10 mg/mL	47.21 mg/ml
	116 mg/mL $\pm$ 23 mg/mL	101.37 mg/ml
Osmolality	250 – 450 mmol/Kg	350 mmol/Kg
pH	6.5 – 8.5	8.5
Endotoxin USP <85>	< 50 EU/mL	< 5 EU/ml
Sterility USP <71>	No growth on FTM or TSB	Pass

## Approval Signatures

Author	Name Title
Approver	Redacted by agreement

COPY

LN 5/2/19

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COPY

04/13/19

Proprietary Info

## CONFIDENTIAL DOCUMENT

<b>Specification No.</b> <b>SPC2016001-00</b>	<div><div>Proprietary</div><div>Pro</div></div> <b>Drug Combination Suspension</b>	
<b>Rev. No</b> <b>01</b>	<b>Effective Date</b> <b>11/14/2018</b>	<b>Page 1 of 2</b>

**Product Name:** **Proprietary** **Pro** **Excipient (Group 1) for subcutaneous injection (Group 1—0 mg/kg)**

**Drug Content:** **Proprietary** (0), **Proprietary** (0), **Proprietary** (0)

**Manufacturer:** **Proprietary Info**

**Specification Type:** Specification for Product Release

**Lot:** 20181106 blank lipid (0.0 mg/mL for **Excipient control; Group 1** dose)

## 1. Specifications

<b>Drug Suspension</b>	
<b>Test</b>	<b>Specification</b>
Appearance	White, turbid suspension
Identity	<b>Proprietary Info</b>
<b>Proprietary</b> assay	0 mg/mL
<b>Proprietary</b> assay	0 mg/mL
<b>Proprietary</b> assay	0 mg/mL
Osmolality, USP <785>	350 mOsmol/kg
pH, USP <791>	7.8
Residual solvents, USP <467>	Ethanol (Class III): < 5000ppm
Particulate matter, USP <788>	≤3000 particles/dosage unit of size ≥ 10µm diameter, and ≤300 particles/dosage unit of size ≥25µm diameter
Heavy Metals, USP <232> and <233>	<0.25 µg/g cadmium; <0.5 µg/g mercury <0.15 µg/g arsenic; <0.15 µg/g mercury

<b>Dosage Form</b>	
<b>Test</b>	<b>Specification</b>
<b>Proprietary Info</b>	Acceptance Value < 15 for up to 30 units
	Acceptance Value < 15 for up to 30 units
	Acceptance Value < for up to 30 units
Bacterial Endotoxin, USP <85>	Pending analysis
Sterility, USP <71>	Pending analysis

COPY

M397-18

JW 1/3/19

Proprietary Info

## CONFIDENTIAL DOCUMENT

<b>Specification No.</b> <b>SPC2016001-00</b>	<div><div>Proprietary</div><div>Pro</div></div> <b>Drug Combination Suspension</b>	
<b>Rev. No</b> <b>01</b>	<b>Effective Date</b> <b>11/14/2018</b>	<b>Page 2 of 2</b>

<u>Excipients</u>	
Inactive ingredients	Concentration (mg/mL)
Proprietary Info	

2. **Authorized Uses**
  - 2.1. Experimental use
  - 2.2. GLP exploratory use
3. **References**
  - 3.1. USP <1> Injections and implanted drug products (parenterals)-product quality tests
  - 3.2. Code of Federal Regulations, Title 21, Part 210.
  - 3.3. FDA Guidance for Industry "CGMP for Phase 1 Investigational Drugs" (published July 2008)
4. **Revision History**

Revision Number	Revisions	Revising Author
00	Original	Redacted by agreement
01	Updated drug content, nominal targets for drug assays, particle size, residual solvents, and bacterial endotoxin	

<b>Approval Signatures</b>		
	Name	Signature and Date
<b>Author</b>	Redacted by agreement	
<b>Approved by</b>		

Proprietary Info

## CERTIFICATE OF ANALYSIS

Proprietary Pro Drug Combination Suspension

Lot Number  
Proprietary InfoDate of Manufacturing  
12/10/2018

Page 1 of 1

Product Name: Proprietary Pro Drug Combination Suspension for subcutaneous  
injection (Group 1 — 0.0 mg/kg)

Manufacturer: Proprietary Info

Lot Number: 20181210

Proprietary Info

## Storage and Expiration

Store at 2-8°C

Expiration / Retest Date 4/29/19

Test	Specification	Result
Appearance	White, turbid suspension	White turbid suspension
Identity	Proprietary Info	conforms
Proprietary Info	0 mg/mL $\pm$ 0 mg/mL	0 mg/ml
	0 mg/mL $\pm$ 0 mg/mL	0 mg/ml
	0 mg/mL $\pm$ 0 mg/mL	0 mg/ml
	45.8 mg/mL $\pm$ 5 mg/mL	43.9 mg/ml
	116 mg/mL $\pm$ 12 mg/mL	114.19 mg/ml
Osmolality	250 – 450 mmol/Kg	410 mmol/Kg
pH	6.5 – 8.5	8.00
Endotoxin USP <85>	< 50 EU/mL	< 5 EU/ml
Sterility USP <71>	No growth on FTM or TSB	Pass

## Approval Signatures

	Name, Title	Signature and Date
Author	Redacted by agreement	
Approver		

COPY

LN 5/2/19

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Proprietary Info

## CERTIFICATE OF ANALYSIS

Proprietary Pro Drug Combination Suspension

Proprietary Info

Date of Manufacturing  
10/31/2018

Page 1 of 1

Product Name:

Proprietary Pro Drug Combination Suspension for subcutaneous  
injection (Group 2 — 0.4 mg/kg)

Manufacturer:

Proprietary Info

Lot Number:

Proprietary Info

## Storage and Expiration

Store at 2-8°C

Expiration / Retest Date 4/29/19

Test	Specification	Result
Appearance	White, turbid suspension	White turbid suspension
Identity	Proprietary Info	conforms
Proprietary Info	0.27 mg/mL $\pm$ 0.027 mg/mL	0.24 mg/ml
	0.08 mg/mL $\pm$ 0.008 mg/mL	0.07 mg/ml
	0.15 mg/mL $\pm$ 0.015 mg/mL	0.14 mg/ml
Osmolality	250 – 350 mmol/Kg	254 mmol/Kg
pH	6.5 – 8.5	7.80
Endotoxin USP <85>	< 50 EU/mL	< 5 EU/ml
Sterility USP <71>	No growth on FTM or TSB	Pass

## Approval Signatures

	Name Title	Signature and Date
Author	Redacted by agreement	
Approver		

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JW 1/3/19

Proprietary Info

## CONFIDENTIAL DOCUMENT

<b>Specification No.</b> SPC2016001-00	<div>Proprietary</div> <div>Prop</div> <b>Drug Combination Suspension</b>	
<b>Rev. No</b> 01	<b>Effective Date</b> 11/14/2018	<b>Page 1 of 2</b>

**Product Name:**

Proprietary

Pro

**Drug Combination Suspension for  
subcutaneous injection (Group 2—0.4 mg/kg)**
**Drug Content:**

Proprietary Info

**Manufacturer:****Specification Type:****Specification for Product Release****Lot:**

Proprietary Info

0.27 mg/mL for **0.4 mg/kg—Group 2** dose)**1. Specifications**

<b>Drug Suspension</b>	
<b>Test</b>	<b>Specification</b>
Appearance	White, turbid suspension
Identity	Proprietary Info
Proprietary Info	0.27 mg/mL
	0.08 mg/mL
	0.18 mg/mL
Osmolality, USP <785>	250 mOsmol/kg
pH, USP <791>	7.8
Residual solvents, USP <467>	Ethanol (Class III): < 5000ppm
Particulate matter, USP <788>	≤3000 particles/dosage unit of size ≥ 10µm diameter, and ≤300 particles/dosage unit of size ≥25µm diameter
Heavy Metals, USP <232> and <233>	<0.25 µg/g cadmium; <0.5 µg/g mercury <0.15 µg/g arsenic; <0.15 µg/g mercury

<b>Dosage Form</b>	
<b>Test</b>	<b>Specification</b>
Proprietary Info	Acceptance Value < 15 for up to 30 units
	Acceptance Value < 15 for up to 30 units
	Acceptance Value < for up to 30 units
Bacterial Endotoxin, USP <85>	Pending analysis
Sterility, USP <71>	Pending analysis



UW 113/19

Proprietary Info

CONFIDENTIAL DOCUMENT

<b>Specification No.</b> <b>SPC2016001-00</b>	<div> <div>Proprietary</div> <div>Prop</div> </div> <b>Drug Combination Suspension</b>	
<b>Rev. No</b> <b>01</b>	<b>Effective Date</b> <b>11/14/2018</b>	<b>Page 2 of 2</b>

<u>Excipients</u>	
Inactive ingredients	Concentration (mg/mL)
Proprietary Info	

**2. Authorized Uses**

2.1. Experimental use

2.2. GLP exploratory use

**3. References**

3.1. USP <1> Injections and implanted drug products (parenterals)-product quality tests

3.2. Code of Federal Regulations, Title 21, Part 210.

3.3. FDA Guidance for Industry "CGMP for Phase 1 Investigational Drugs" (published July 2008)

**4. Revision History**

Revision Number	Revisions	Revising Author
00	Original	Redacted by agreement
01	Updated drug content, nominal targets for drug assays, particle size, residual solvents, and bacterial endotoxin	

Approval Signatures		
	Name	Signature and Date
<b>Author</b>	Redacted by agreement	
<b>Approved by</b>		

Proprietary Info

## CERTIFICATE OF ANALYSIS

Proprietary Pro Drug Combination Suspension

Date of Manufacturing  
10/31/2018

Page 1 of 1

Product Name:

Proprietary Pro Drug Combination Suspension for subcutaneous  
injection (Group 3 — 3.64 mg/kg)

Manufacturer:

Lot Number:

Proprietary Info

Proprietary Info

## Storage and Expiration

Store at 2-8°C

Expiration / Retest Date 4/29/19

Test	Specification	Result
Appearance	White, turbid suspension	White turbid suspension
Identity	Proprietary Info	conforms
Proprietary Info		Proprietary Info
Osmolality	250 – 350 mmol/Kg	272 mmol/Kg
pH	6.5 – 8.5	7.85
Endotoxin USP <85>	< 50 EU/mL	< 5 EU/ml
Sterility USP <71>	No growth on FTM or TSB	Pass

## Approval Signatures

	Name, Title	Signature and Date
Author	Redacted by agreement	
Approver		

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COPY

JW 1/3/19

Proprietary Info

## CONFIDENTIAL DOCUMENT

<b>Specification No.</b> <b>SPC2016001-00</b>	<div><div>Proprietary</div><div>Prop</div></div> <b>Drug Combination Suspension</b>	
<b>Rev. No</b> <b>01</b>	<b>Effective Date</b> <b>11/14/2018</b>	<b>Page 1 of 2</b>

**Product Name:** **Proprietary** **Pro** **Drug Combination Suspension for subcutaneous injection (Group 3—3.64 mg/kg)**

**Drug Content:**

**Manufacturer:**

**Specification Type:**

**Lot:**

Proprietary Info

Specification for Product Release

Proprietary Info

(for 2.43 mg/mL for 3.64 mg/kg; **Group 3 dose**)1. **Specifications**

<b>Drug Suspension</b>	
<b>Test</b>	<b>Specification</b>
Appearance	White, turbid suspension
Identity	Proprietary Info
Proprietary Info	
Osmolality, USP <785>	250 mOsmol/kg
pH, USP <791>	7.8
Residual solvents, USP <467>	Ethanol (Class III): < 5000ppm
Particulate matter, USP <788>	≤3000 particles/dosage unit of size ≥ 10µm diameter, and ≤300 particles/dosage unit of size ≥25µm diameter
Heavy Metals, USP <232> and <233>	<0.25 µg/g cadmium; <0.5 µg/g mercury <0.15 µg/g arsenic; <0.15 µg/g mercury

<b>Dosage Form</b>	
<b>Test</b>	<b>Specification</b>
Proprietary Info	Acceptance Value < 15 for up to 30 units
	Acceptance Value < 15 for up to 30 units
	Acceptance Value < for up to 30 units
Bacterial Endotoxin, USP <85>	Pending analysis
Sterility, USP <71>	Pending analysis

COPY

JW 1/3/19

Proprietary Info

## CONFIDENTIAL DOCUMENT

<b>Specification No.</b> SPC2016001-00	<input type="checkbox"/> Proprietary <input type="checkbox"/> Prop <b>Drug Combination Suspension</b>
<b>Rev. No</b> 01	<b>Effective Date</b> 11/14/2018
<b>Page 2 of 2</b>	

<u>Excipients</u>	
Inactive ingredients	Concentration (mg/mL)
Proprietary Info	

2. **Authorized Uses**

- 2.1. Experimental use
- 2.2. GLP exploratory use

3. **References**

- 3.1. USP <1> Injections and implanted drug products (parenterals)-product quality tests
- 3.2. Code of Federal Regulations, Title 21, Part 210.
- 3.3. FDA Guidance for Industry "CGMP for Phase 1 Investigational Drugs" (published July 2008)

4. **Revision History**

Revision Number	Revisions	Revising Author
00	Original	Redacted by agreement
01	Updated drug content, nominal targets for drug assays, particle size, residual solvents, and bacterial endotoxin	

<b>Approval Signatures</b>		
	Name	Signature and Date
<b>Author</b>	Redacted by agreement	
<b>Approved by</b>		

Proprietary Info

## CERTIFICATE OF ANALYSIS

Proprietary Pro Drug Combination Suspension

Date of Manufacturing  
10/31/2018

Page 1 of 1

Product Name:

Proprietary Pro Drug Combination Suspension for subcutaneous  
injection (Group 4 — 14.55 mg/kg)

Manufacturer:

Lot Number:

Proprietary Info

Proprietary Info

## Storage and Expiration

Store at 2-8°C

Expiration / Retest Date 4/29/19

Test	Specification	Result
Appearance	White, turbid suspension	White turbid suspension
Identity	Proprietary Info	conforms
Proprietary Info		Proprietary Info
Osmolality	250 – 350 mmol/Kg	320 mmol/kg
pH	6.5 – 8.5	7.55
Endotoxin USP <85>	< 50 EU/mL	< 5 EU/ml
Sterility USP <71>	No growth on FTM or TSB	Pass

## Approval Signatures

	Name, Title	Signature and Date
Author	Redacted by agreement	
Approver		

COPY

LN-5/2/19



COPY

JW 1/3/19

Proprietary Info

## CONFIDENTIAL DOCUMENT

<b>Specification No.</b> <b>SPC2016001-00</b>	<div><div>Proprietary</div><div>Prop</div><b>Drug Combination Suspension</b></div>	
<b>Rev. No</b> <b>01</b>	<b>Effective Date</b> <b>11/14/2018</b>	<b>Page 1 of 2</b>

**Product Name:** **Proprietary** **Pro** **Drug Combination Suspension for subcutaneous injection (Group 4—14.55mg/kg)**

**Drug Content:**

**Manufacturer:**

**Specification Type:** **Specification for Product Release**

**Lot:** **Proprietary Info** (9.72 mg/mL for 14.55 mg/kg dose; **Group 4**)

1. **Specifications**

<b>Drug Suspension</b>	
<b>Test</b>	<b>Specification</b>
Appearance	White, turbid suspension
Identity	Proprietary Info
Proprietary Info	
Osmolality, USP <785>	250 mOsmol/kg
pH, USP <791>	7.8
Residual solvents, USP <467>	Ethanol (Class III): < 5000ppm
Particulate matter, USP <788>	≤3000 particles/dosage unit of size ≥ 10µm diameter, and ≤300 particles/dosage unit of size ≥25µm diameter
Heavy Metals, USP <232> and <233>	<0.25 µg/g cadmium; <0.5 µg/g mercury <0.15 µg/g arsenic; <0.15 µg/g mercury

<b>Dosage Form</b>	
<b>Test</b>	<b>Specification</b>
Proprietary Info	Acceptance Value < 15 for up to 30 units
	Acceptance Value < 15 for up to 30 units
	Acceptance Value < for up to 30 units
Bacterial Endotoxin, USP <85>	Pending analysis
Sterility, USP <71>	Pending analysis

COPY

JW 1/3/19

Proprietary Info

## CONFIDENTIAL DOCUMENT

<b>Specification No.</b> <b>SPC2016001-00</b>	<div><div>Proprietary</div><div>Prop</div></div> <b>Drug Combination Suspension</b>	
<b>Rev. No</b> <b>01</b>	<b>Effective Date</b> <b>11/14/2018</b>	<b>Page 2 of 2</b>

<u>Excipients</u>	
Inactive ingredients	Concentration (mg/mL)
Proprietary Info	

2. **Authorized Uses**

- 2.1. Experimental use
- 2.2. GLP exploratory use

3. **References**

- 3.1. USP <1> Injections and implanted drug products (parenterals)-product quality tests
- 3.2. Code of Federal Regulations, Title 21, Part 210.
- 3.3. FDA Guidance for Industry "CGMP for Phase 1 Investigational Drugs" (published July 2008)

4. **Revision History**

Revision Number	Revisions	Revising Author
00	Original	Redacted by agreement
01	Updated drug content, nominal targets for drug assays, particle size, residual solvents, and bacterial endotoxin	

**Approval Signatures**

	Name	Signature and Date
<b>Author</b>	Redacted by agreement	
<b>Approved by</b>		

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix C**

**INDIVIDUAL CLINICAL OBSERVATIONS**

Page 0684 of 3822 to Page 0897 of 3822

Withheld pursuant to exemption

Proprietary Info

of the Freedom of Information and Privacy Act

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with Proprietary Pro in Male and Female Beagle Dogs

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Key Page

**General Footnotes**

Provantis version 10.1.0.1  
"." indicates Not Applicable

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings</u>		
1	Excipient	Control	Group 1	0	mg/kg SC
2	<span>Proprietary</span> <span>Pro</span> (0.4)	Dose	Group 2	0.4	mg/kg SC
3	<span>Proprietary</span> <span>Pro</span> (3.64)	Dose	Group 3	3.64	mg/kg SC
4	<span>Proprietary</span> <span>Pro</span> (14.55)	Dose	Group 4	14.55	mg/kg SC



**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix D**

**INDIVIDUAL BODY WEIGHTS**

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix D-1**

**INDIVIDUAL BODY WEIGHTS**

Page 0901 of 3822 to Page 0917 of 3822

Withheld pursuant to exemption

Proprietary Info

of the Freedom of Information and Privacy Act

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with [Propriet] [Pr] in Male and Female Beagle Dogs

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Key Page

**General Footnotes**

Provantis version 10.1.0.1

"-" indicates Not Applicable

**Measurement Descriptions**

Headings Used

Body Weight

Description

Body Weight

**Measurement/Statistics**

Measurement

Body Weight

Descriptive

Mean

Standard Deviation

Count (N)

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings 1-4</u>		
1	Excipient	Control	Group 1	0	mg/kg SC
2	[Propriet] [Pr] (0.4)	Dose	Group 2	0.4	mg/kg SC
3	[Propriet] [Pr] (3.64)	Dose	Group 3	3.64	mg/kg SC
4	[Propriet] [Pr] (14.55)	Dose	Group 4	14.55	mg/kg SC

**Comment Abbreviations**

RC = Result Comment

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix D-2**

**INDIVIDUAL BODY WEIGHT CHANGES**



Page 0920 of 3822 to Page 0935 of 3822

Withheld pursuant to exemption

Proprietary Info

of the Freedom of Information and Privacy Act

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with [Propriet] [Pr] in Male and Female Beagle Dogs

---

Key Page

**General Footnotes**

Provantis version 10.1.0.1

"-" indicates Not Applicable

**Measurement Descriptions**

Headings Used

Absolute Weight Gains

Description

Absolute Weight Gain (kg)

**Measurement/Statistics**

Measurement

Absolute Weight Gains

Descriptive

Mean

Standard Deviation

Count (N)

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings 1-4</u>		
1	Excipient	Control	Group 1	0	mg/kg SC
2	[Propriet] [Pr] (0.4)	Dose	Group 2	0.4	mg/kg SC
3	[Propriet] [Pr] (3.64)	Dose	Group 3	3.64	mg/kg SC
4	[Propriet] [Pr] (14.55)	Dose	Group 4	14.55	mg/kg SC

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix E**

**INDIVIDUAL FOOD CONSUMPTION**

Page 0938 of 3822 to Page 0953 of 3822

Withheld pursuant to exemption

Proprietary Info

of the Freedom of Information and Privacy Act

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with [Propriet] [Pr] in Male and Female Beagle Dogs

---

Key Page

**General Footnotes**

Provantis version 10.1.0.1

"-" indicates Not Applicable

**Measurement Descriptions**

Headings Used

Food Mean Daily Consumption

Description

Food Mean Consumption

**Measurement/Statistics**

Measurement

Food Mean Daily Consumption

Descriptive

Mean

Standard Deviation

Count (N)

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings 1-4</u>		
1	Excipient	Control	Group 1	0	mg/kg SC
2	[Propriet] [Pr] (0.4)	Dose	Group 2	0.4	mg/kg SC
3	[Propriet] [Pr] (3.64)	Dose	Group 3	3.64	mg/kg SC
4	[Propriet] [Pr] (14.55)	Dose	Group 4	14.55	mg/kg SC



**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix F**

**OPHTHALMOLOGY REPORTS**

Redacted by agreement

Redacted by agreement

SRI International  
333 Ravenswood Avenue  
Menlo Park, CA 94025

Dear Redacted by agreement

I examined 44 prestudy dogs (22 males, 22 females) associated with **Study m397-18** on October 22, 2018. Eyes were examined with a Kowa SL-16 hand held slit lamp biomicroscope and indirect ophthalmoscopy using a Volk 40 diopter double aspheric lens. The pupils were pharmacologically dilated with 1% tropicamide prior to examination. The eyelids, conjunctiva, cornea and the anterior compartment including the aqueous humor iris, lens and anterior vitreous humor were examined with a biomicroscope. The retinal vessels, optic nerve and posterior vitreous were examined with indirect ophthalmoscopy.

All animals examined were normal.

Sincerely,

Redacted by agreement

Redacted by agreement

Redacted by agreement

SRI International  
333 Ravenswood Avenue  
Menlo Park, CA 94025

Dear Redacted by agreement

I examined 12 dogs (6 males, 6 females) associated with **Study m397-18** on December 17, 2018. Eyes were examined with a Kowa SL-16 hand held slit lamp biomicroscope and indirect ophthalmoscopy using a Volk 40 diopter double aspheric lens. The pupils were pharmacologically dilated with 1% tropicamide prior to examination. The eyelids, conjunctiva, cornea and the anterior compartment including the aqueous humor iris, lens and anterior vitreous humor were examined with a biomicroscope. The retinal vessels, optic nerve and posterior vitreous were examined with indirect ophthalmoscopy.

All animals examined were normal.

Sincerely,

Redacted by agreement

GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with Proprietary Pro in Male and Female Beagle Dogs  
SRI Study No. M397-18

Appendix G

BIOANALYTICAL CHEMISTRY

Bioanalytical Scientist:

Redacted by agreement

10/22/19  
Date

Approved by:

10/25/2019  
Date

Biosciences Division  
SRI International

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix G-1**

**BIOANALYTICAL METHOD**



**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**I. INTRODUCTION**

Dog plasma samples from SRI Study No. M397-18 were analyzed for [Proprietary Info] and [Proprietary Info] according to the Bioanalytical Sample Analysis Plan (Appendix G-3) and the analytical method detailed in SRI Test Method 106.201 (Appendix G-4); K<sub>2</sub> EDTA was used as the anticoagulant. This method was validated using a methanol protein precipitation extraction followed by tandem mass spectrometric (LC-MS/MS) detection. Refer to SRI Report No. B185-18 for details and results from this validation. The analytical range was 5.00-1000 ng/ml for [Proprietary Info] and [Proprietary Info] and 25.0-5000 ng/ml for [Proprietary Info] with 0.0200 ml sample volumes. The internal standards used for this assay were [Proprietary Info] [Proprietary Info] and [Proprietary Info]

Incurred sample reanalysis (ISR) was performed during this study to ensure assay performance and to verify the results.

**II. REFERENCE STANDARDS**

The certificates of analysis for the analytes and internal standards are provided in Appendix G-5.

**Reference Standard Description**

Reference Standard	Supplier	Lot Number	Correction Factor	Storage Conditions	Expiration
[Proprietary Info]	U.S. Pharmacopeia	[Proprietary Info]	0.997	Refrigerated, Protected from light	Current lot
[Proprietary Info]	U.S. Pharmacopeia		0.993	Refrigerated, Protected from light	Current lot
[Proprietary Info]	U.S. Pharmacopeia		0.940	Refrigerated	Current lot
[Proprietary Info]	Medical Isotopes, Inc.		0.980 <sup>b</sup>	-20°C	Retest 06-30-24
[Proprietary Info]	Medical Isotopes, Inc.		0.970 <sup>b</sup>	-20°C	Retest 04-30-20
[Proprietary Info]	Medical Isotopes, Inc.		0.970 <sup>b</sup>	-20°C	Retest 10-31-20

<sup>a</sup> [Proprietary Info] was supplied as [Proprietary Info] monohydrate. The final correction factor used during stock solution preparation is based on the amount of [Proprietary Info] present when weighing.

<sup>b</sup> The purity was assumed as 100% during weighing.

Pooled control K<sub>2</sub> EDTA dog plasma (lot number BGL110446, expiration 04-30-21) which was used in the preparation of calibration curves, QC samples, assay blanks, and sample

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dilution, was obtained from BioIVT (Westbury, NY) on 04-02-19 and stored in a -20°C freezer until use.

### **III. SAMPLE RECEIPT AND STORAGE**

The Bioanalytical Chemistry group at SRI received dog plasma samples from the Toxicology group on 02-04-19. A total of 228 frozen, intact samples were received and transferred to an ultra-low temperature freezer ( $\leq -60^{\circ}\text{C}$ ) prior to analysis. A total of 228 samples were analyzed during this study.

The freezer storage temperatures stated in this report are nominal. The temperature of the sample storage freezers did not go above  $-60^{\circ}\text{C}$  from the dates of sample receipt until the final analysis of the samples. Any recorded departure from the manufacturer's specifications for a particular freezer unit would result in a facility deviation. No deviations were generated as a result of this over the course of this study.

### **IV. RESULTS**

#### **A. Summary of Runs Performed**

A description of all analytical runs performed, including the date of extraction, date of analysis, and run outcome, is presented in Table G-1. Representative chromatographs from Run MF1, including blank plasma extracts, calibration standards, quality control (QC) samples, and study samples, are included in Appendix G-6.

#### **B. Calibration Standard Acceptance Criteria and Results**

The calibration curve ranged from 5.00 ng/ml (lower limit of quantitation, LLOQ) to 1000 ng/ml (upper limit of quantitation, ULOQ) for [Proprietary] and [Proprietary] and from 25.0 ng/ml to 5000 ng/ml for [Proprietary]. As freeze thaw stability and sufficient long-term storage stability in matrix were generated during the SRI validation study no. B185-18, calibration standards were prepared in bulk on 05-28-19 and stored in a  $\leq -60^{\circ}\text{C}$  freezer until use. A total of eight calibration standards, extracted in duplicate, were used in the construction of each calibration curve. The correlation coefficient (r) in each run was at least 0.9995 for [Proprietary] and 0.9997 for [Proprietary] using a least-squares linear regression with a  $1/x$  weighting (Tables G-2 and G-3). The correlation coefficient (r) in each run was at least 0.9992 for [Proprietary] using a least-squares linear regression with a  $1/x^2$  weighting (Table G-4). A representative calibration curve for each analyte from Run MF1 are shown in Figures G-1 to G-3.

For a calibration curve to be considered acceptable for each analytical run, at least 75% of the calibration standards must be accurate to within 15% of the nominal concentration ( $\pm 20\%$  at the LLOQ).



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**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
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Table G-5 shows the inter-batch back-calculated concentrations of [Proprietary] in the calibration standards from all of the accepted analytical runs. The inter-batch precision (defined as %CV) ranged from 1.8% to 4.2%, and the inter-batch accuracy ranged from 98.5% to 103.2%.

Table G-6 shows the inter-batch back-calculated concentrations of [Proprietary] in the calibration standards from all of the accepted analytical runs. The inter-batch precision ranged from 1.4% to 4.4%, and the inter-batch accuracy ranged from 98.2% to [Proprietary]

Table G-7 shows the inter-batch back-calculated concentrations of [Proprietary] in the calibration standards from all of the accepted analytical runs. The inter-batch precision ranged from 1.3% to 4.0%, and the inter-batch accuracy ranged from 98.3% to 102.2%.

### **C. Quality Control Sample Acceptance Criteria and Results**

QC samples in K<sub>2</sub> EDTA dog plasma were spiked with [Proprietary] and [Proprietary] at low (15.0 ng/ml), mid (400 ng/ml), and high (800 ng/ml) concentrations. These QC samples were also spiked with [Proprietary] at 75.0 ng/ml (low), 2000 ng/ml (mid) and 4000 ng/ml (high) concentrations. These QC samples were prepared in bulk on 05-28-19 before being aliquoted into smaller volumes and stored in an ultra-low temperature freezer ( $\leq -60^{\circ}\text{C}$ ) prior to analysis. These QC samples were extracted in replicates of two at each concentration in each analytical batch. For an analytical batch to be acceptable, at least 67% of the low, mid and high QC samples must be within 15% of the nominal concentration, with at least one QC sample at each concentration satisfying this criterion.

Table G-8 shows the inter-batch back-calculated concentrations of [Proprietary] in QC samples from all reported analytical runs. The inter-batch precision ranged from 3.8% to 4.8%, and the inter-batch accuracy ranged from 98.7% to 103.4%.

Table G-9 shows the inter-batch back-calculated concentrations of [Proprietary] in QC samples from all reported analytical runs. The inter-batch precision ranged from 2.5% to 2.7%, and the inter-batch accuracy ranged from 101.2% to 103.2%.

Table G-10 shows the inter-batch back-calculated concentrations of [Proprietary] in QC samples from all reported analytical runs. The inter-batch precision ranged from 1.4% to 1.7%, and the inter-batch accuracy ranged from 100.5% to 102.2%.

In addition to calibration standards and QC samples, pooled plasma samples containing no analyte or internal standard (BI/BI) and pooled plasma samples containing internal standard only (BI/IS) were analyzed in each run. These samples were positioned throughout the run in order to identify either analyte contamination or auto-injector carryover. For [Proprietary] there was no evidence of significant chromatographic interferences in these samples at the analyte or internal standard retention time, defined as a peak at the analyte retention time with a peak area  $>20\%$  of the mean analyte peak area at the LLOQ, or a peak at the internal standard retention time with a peak area  $>5\%$  of the mean peak area of the extracted internal standard across samples. One out of two

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**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
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[Proprietary] carryover blanks from runs MF2 and MF4 showed significant analyte carryover, but as this was just outside the 20% criteria defined above, and as the other blank was acceptable, no further action was taken. For [Proprietary] significant carryover was observed in both carryover blanks in runs MF1-MF4. In MF1 and MF3, the second BI/BI sample also showed evidence of significant carryover, as the injection preceding this was a high QC sample. The first BI/BI sample and both BI/IS samples did not show a significant response at the analyte retention time. As both carryover blanks were affected in runs MF1-MF4, a carryover impact assessment was performed in these batches. Results from the carryover impact assessment, which are stored with the raw data and are not reported here, indicated that no study sample was significantly affected by carryover by more than 5%, which would warrant sample reanalysis per SRI SOP 006.061. Carryover for [Proprietary] was observed during the SRI validation study no. B185-18. The carryover originates from the column and not the autosampler, and as chromatographic limitations already existed because of the differences in analyte polarity, it was not practical to fully eliminate the carryover. Tables G-11 to G-13 compares the analyte peak areas from the BI/BI, BI/IS, and carryover samples with the mean LLOQ calibration standard analyte peak areas from all reported runs.

**D. Incurred Sample Reanalysis**

Incurred sample reanalysis (ISR) was performed on 24 study samples (10.5%) in order to confirm the integrity of the bioanalytical method. Samples that had been previously analyzed and had the results accepted were reanalyzed in replicates of n=1 and the resulting concentration compared with the original reported concentration. The results generated from ISR are not intended to replace the original result and are generated for comparative purposes only. According to SRI SOP 006.062, in order for an ISR result to be considered comparable to the original result, the difference between the two results must be  $\leq 20\%$ . At least 67% of the ISR samples must meet the acceptance criteria in order for the overall ISR evaluation to be considered successful.

ISR of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA dog plasma samples satisfied the acceptance criterion, with 100% of all reassayed samples meeting the acceptance criterion as defined above. The complete results and outcome of ISR analysis are reported in Tables G-14 to G-16.

**E. Sample Analysis Results**

Tables G-18 to G-35 show the final concentrations of each analyte from individual K<sub>2</sub> EDTA dog plasma samples. Also included for toxicokinetic (TK) purposes are the mean and standard deviation (SD) per time point per sex. Samples with values < LLOQ were excluded from the calculation of the mean  $\pm$  SD. Note that the complete TK analysis is separate from the bioanalytical report and will appear as an appendix to the report for SRI Study No. M397-18.

Samples with values less than the LLOQ are reported as lower than the level of quantitation (<LLOQ).



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**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
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Samples 017 and 018 (Female, Group 2 Day 1, 1 hr postdose) in run MF2 showed a [Proprietary] peak which appeared to have a small shoulder when compared to the [Proprietary] peaks from other study samples, calibration standards, and QC samples. These samples were reassayed again in run MF4 to see if this could be resolved. However, the same shoulder was observed in the chromatographs on reassay, particularly with sample 017. As this was observed only in these samples and not in others with detectable analyte, this appears to be an issue with the samples themselves and not with the extraction or instrumentation. As it cannot be confirmed what this shoulder is, the peak has been integrated without inclusion of this shoulder. The original and reassayed values are shown in Table G-17, and as the values are within 15% of each other, the mean value was reported as the final result. Representative chromatographs for [Proprietary] for these samples from run MF2 are shown in Appendix G-7.

## **V. DEVIATIONS**

There were no deviations to SRI SOPs that had any impact on the integrity of the study.

## **VI. DATA MANAGEMENT**

LC-MS/MS data were acquired, peak areas were integrated, the calibration line regressions were calculated, and the final concentrations were generated using AB Sciex Analyst software, version 1.6.2. All concentration values calculated by the Analyst software have been rounded to three significant figures. The inter-batch calibration curve and QC statistics described in this report were generated, using unrounded values, with this software. The mean and standard deviation values for each sample analysis timepoint were calculated using Microsoft Excel 2016, and are reported to three significant figures.

## **VII. CONCLUSION**

Dog plasma samples from SRI Study No. M397-18 were analyzed for [Proprietary] [Proprietary] and [Proprietary] using the validated analytical method detailed in SRI Test Method 106.201 (Appendix G-4). All sample results generated from this study were acceptable.

Interim long-term matrix storage stability for 21 days at  $\leq -60^{\circ}\text{C}$  was established during the SRI validation study no. B185-18. Additional stability is still ongoing, and an amended validation report will be created on completion.

## **VIII. DEFINITIONS AND ABBREVIATIONS**

Accuracy:	$\frac{\text{Calculated concentration}}{\text{Nominal concentration}} * 100$
Bl/Bl:	Double blank, containing no analyte or internal standard
Bl/IS:	Blank, containing only internal standard



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CV: Coefficient of variation; SD/mean \*100  
DMSO: Dimethyl sulfoxide  
K<sub>2</sub> EDTA: Di-potassium ethylenediaminetetraacetic acid  
IS: Internal standard [Proprietary Info] [Proprietary Info] [Proprietary Info]  
ISR: Incurred sample reanalysis  
LC-MS/MS: Liquid chromatography-mass spectrometer; tandem or triple-quadrupole mass spectrometer  
LLOQ: Lower limit of quantitation  
QC: Quality Control  
SD: Standard Deviation  
TK: Toxicokinetic  
ULOQ: Upper limit of quantitation

**IX. REFERENCES**

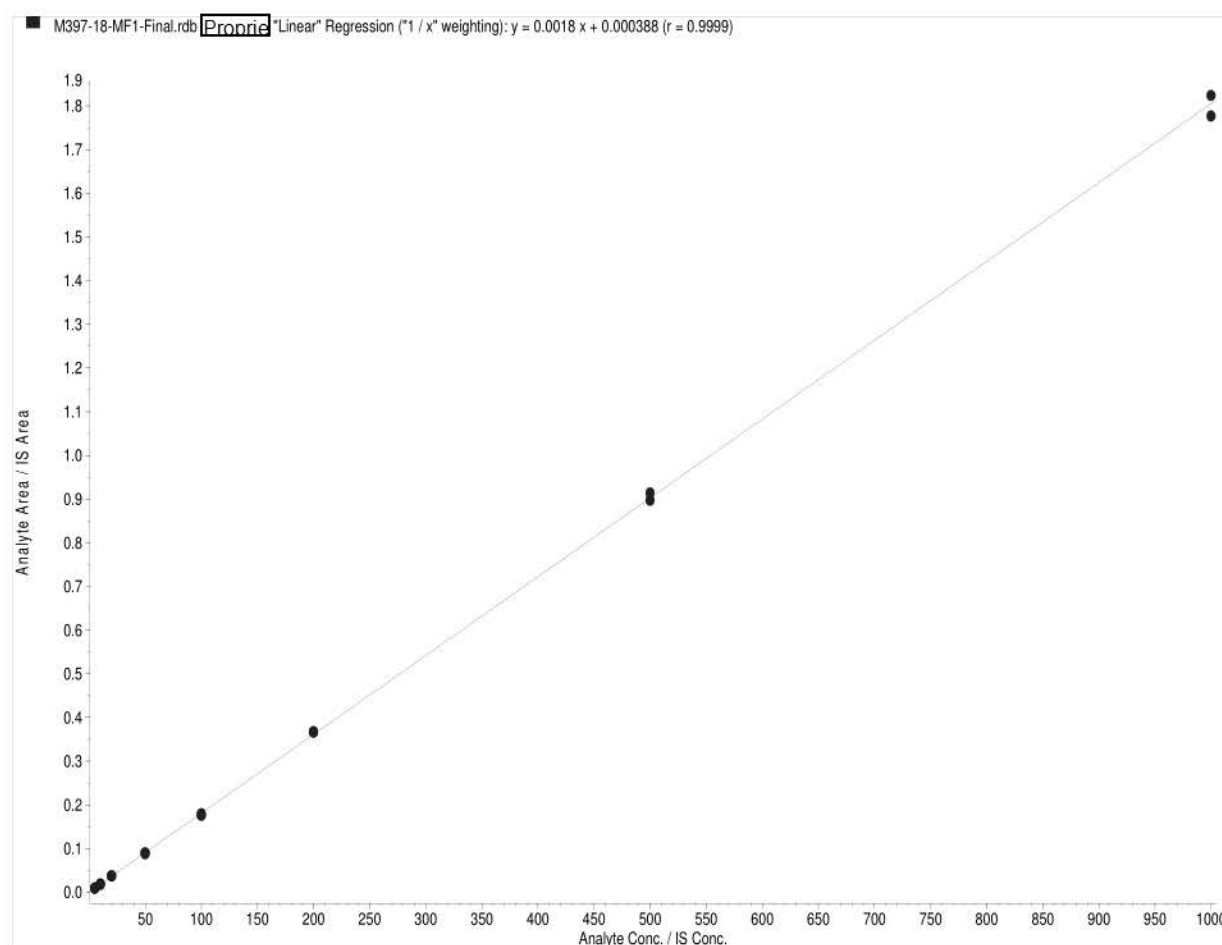
1. SRI Test Method 106.201 *Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma*
2. SRI Report B185-18 "Method Validation Report for the Quantitative Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma"
3. SRI SOP 006.061 *Bioanalytical Sample Analysis*
4. SRI SOP 006.062 *Bioanalytical Sample Reanalysis*

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix G-2**

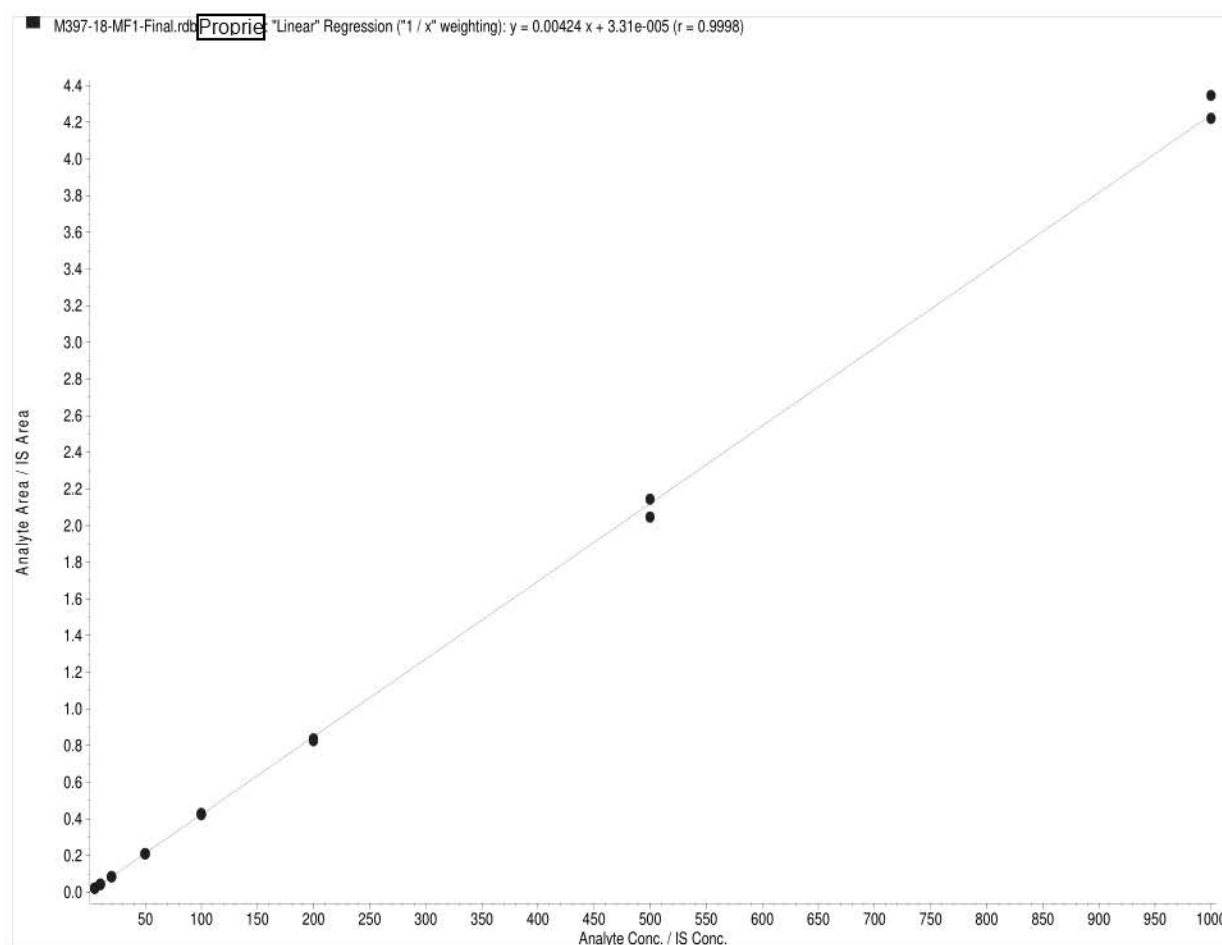
**BIOANALYTICAL FIGURES AND TABLES**

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**



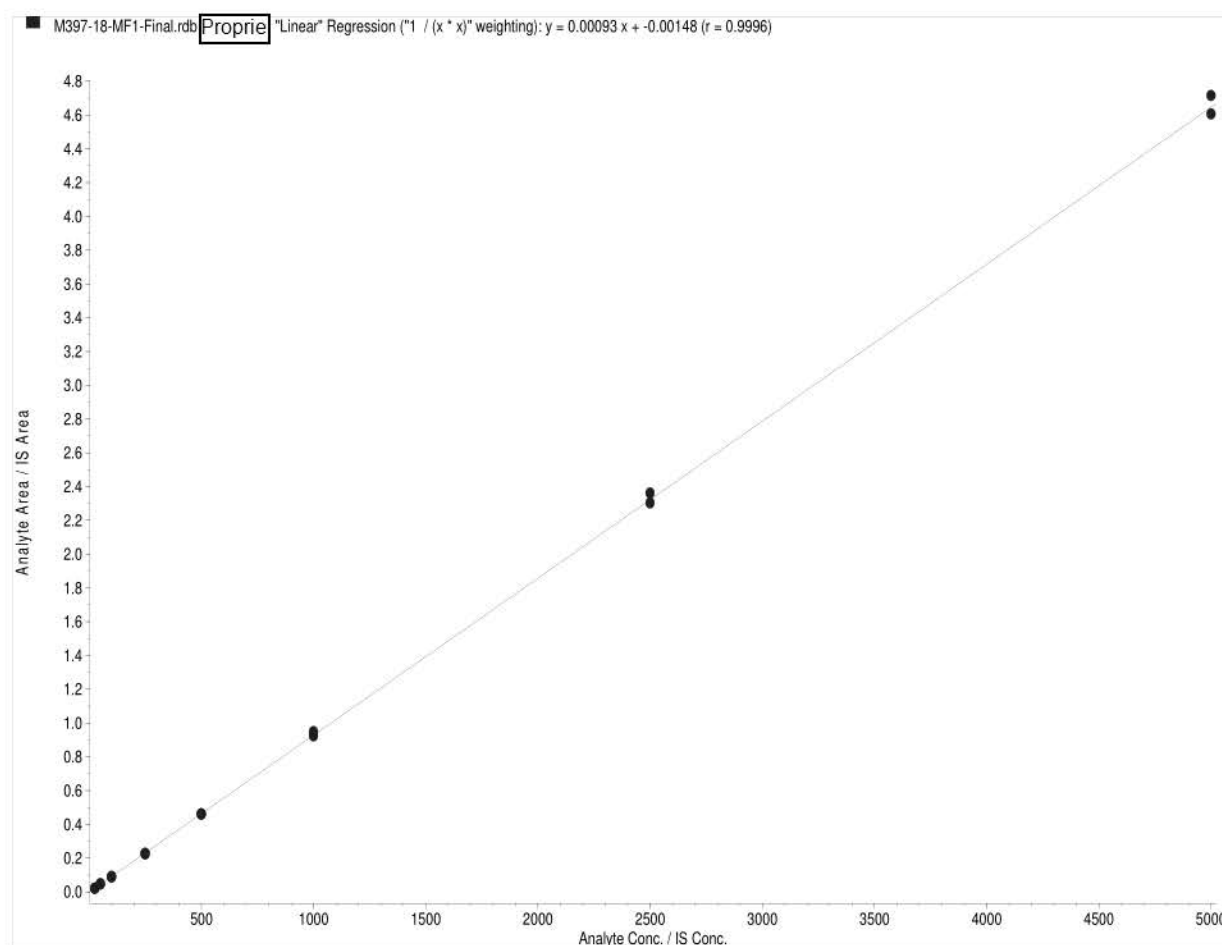
**Figure G-1.** Representative [Proprietary] calibration curve from run MF1.

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
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**Figure G-2.** Representative [Proprietary] calibration curve from run MF1.

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**Figure G-3.** Representative [Proprietary] calibration curve from run MF1.



**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Prop] in Male and Female Beagle Dogs**  
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**Table G-1**

**Summary of Analytical Runs**

Run Number	Date Extracted	Date Analyzed	Outcome (Pass/Fail)	Run Description
MF1	30-May-19	30-May-19	Pass	Group 2 samples
MF2	3-Jun-19	3-Jun-19	Pass	Group 2 and Group 3 samples
MF3	4-Jun-19	4-Jun-19	Pass	Group 3 and Group 4 samples
MF4	5-Jun-19	5-Jun-19	Pass	Group 4 samples and reassays
MF5	6-Jun-19	6-Jun-19	Pass	Incurred Sample Reanalysis (ISR)

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**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-2**

**Summary of Calibration Curve Parameters for [Proprietary]**

Run Number	Slope	Intercept	Correlation Coefficient (r)
MF1	0.00180	0.000388	0.9999
MF2	0.00182	-0.0000899	0.9995
MF3	0.00179	-0.000186	0.9997
MF4	0.00174	0.000682	0.9999
MF5	0.00174	-0.0000564	0.9998

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
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**Table G-3**

**Summary of Calibration Curve Parameters for [Proprietary]**

Run Number	Slope	Intercept	Correlation Coefficient (r)
MF1	0.00424	0.0000331	0.9998
MF2	0.00415	0.000173	0.9997
MF3	0.00412	-0.000522	0.9999
MF4	0.00403	0.0000755	0.9997
MF5	0.00407	0.000836	0.9998

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
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**Table G-4**

**Summary of Calibration Curve Parameters for [Proprietary]**

Run Number	Slope	Intercept	Correlation Coefficient (r)
MF1	0.000930	-0.00148	0.9996
MF2	0.000928	0.000196	0.9995
MF3	0.000922	-0.000700	0.9992
MF4	0.000917	-0.000893	0.9995
MF5	0.000897	-0.0000560	0.9996

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Prop] in Male and Female Beagle Dogs**  
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**Table G-5**

**Back-Calculated Concentrations of [Proprietary] Calibration Standards (ng/ml)**

Run Number	Standard Description															
	5.00	%Acc	10.0	%Acc	20.0	%Acc	50.0	%Acc	100	%Acc	200	%Acc	500	%Acc	1000	%Acc
MF1	4.75	95.1	10.2	102.3	20.6	103.2	49.9	99.7	97.4	97.4	203	101.6	497	99.4	984	98.4
	5.17	103.4	9.71	97.1	20.4	102.2	48.6	97.1	99.3	99.3	203	101.5	506	101.3	1010	101.0
MF2	5.08	101.5	10.0	100.2	20.3	101.4	51.2	102.5	96.8	96.8	198	99.0	497	99.3	973	97.3
	5.20	104.0	9.96	99.6	19.6	98.1	46.0	91.9	106	105.8	193	96.5	523	104.6	1020	101.6
MF3	5.30	106.0	10.3	103.3	19.7	98.4	50.5	101.0	98.6	98.6	197	98.3	493	98.6	1040	103.6
	5.47	109.5	10.1	101.4	18.8	93.8	48.5	96.9	94.3	94.3	197	98.5	494	98.7	992	99.2
MF4	5.23	104.5	9.70	97.0	19.5	97.3	52.4	104.9	105	105.5	206	103.2	499	99.8	1000	100.2
	4.99	99.9	9.35	93.5	20.1	100.3	50.0	100.0	98.0	98.0	195	97.4	492	98.4	1000	100.1
MF5	5.22	104.4	9.69	96.9	19.1	95.6	51.5	103.1	95.3	95.3	205	102.3	495	98.9	1020	102.0
	5.17	103.4	10.3	103.4	20.2	101.0	50.2	100.4	94.3	94.3	200	100.0	500	100.1	989	98.9
Mean	5.16		9.95		19.8		49.9		98.5		200		500		1000	
SD	0.191		0.327		0.606		1.85		4.11		4.48		9.20		19.0	
%CV	3.7		3.3		3.1		3.7		4.2		2.2		1.8		1.9	
%Accuracy	103.2		99.5		99.1		99.7		98.5		99.8		99.9		100.2	
n	10		10		10		10		10		10		10		10	



**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-6**

**Back-Calculated Concentrations of [Proprietary] Calibration Standards (ng/ml)**

Run Number	Standard Description															
	5.00	%Acc	10.0	%Acc	20.0	%Acc	50.0	%Acc	100	%Acc	200	%Acc	500	%Acc	1000	%Acc
MF1	5.00	100.1	10.3	103.1	19.8	99.0	49.2	98.3	99.6	99.6	197	98.6	505	101.1	1020	102.5
	5.26	105.3	9.80	98.0	20.1	100.4	49.7	99.3	101	101.3	195	97.4	482	96.5	995	99.5
MF2	4.91	98.3	9.61	96.1	19.5	97.5	49.0	98.0	102	102.4	204	101.8	522	104.4	975	97.5
	4.97	99.4	10.2	101.5	19.9	99.5	50.8	101.5	102	101.9	200	99.8	507	101.4	990	99.0
MF3	5.12	102.3	10.1	100.8	19.1	95.6	48.7	97.4	99.4	99.4	196	97.8	505	101.0	994	99.4
	5.43	108.6	10.2	102.2	19.6	97.9	48.8	97.6	99.6	99.6	197	98.4	508	101.5	1010	100.5
MF4	4.82	96.4	9.38	93.8	19.8	98.8	48.5	96.9	102	102.4	207	103.3	506	101.2	988	98.8
	5.11	102.2	10.4	104.4	19.4	97.0	50.1	100.2	101	100.8	206	102.9	516	103.2	977	97.7
MF5	4.65	93.0	9.96	99.6	19.8	98.9	51.2	102.4	100	100.4	195	97.7	509	101.8	987	98.7
	5.08	101.7	10.5	104.6	19.5	97.7	52.3	104.7	98.0	98.0	196	98.0	518	103.6	993	99.3
Mean	5.04		10.0		19.6		49.8		101		199		508		993	
SD	0.220		0.356		0.274		1.27		1.46		4.55		10.7		14.3	
%CV	4.4		3.5		1.4		2.6		1.4		2.3		2.1		1.4	
%Accuracy	100.7		100.4		98.2		99.6		100.6		99.6		101.6		99.3	
n	10		10		10		10		10		10		10		10	

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Prop] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-7**

**Back-Calculated Concentrations of [Proprietary] Calibration Standards (ng/ml)**

Run Number	Standard Description															
	25.0	%Acc	50.0	%Acc	100	%Acc	250	%Acc	500	%Acc	1000	%Acc	2500	%Acc	5000	%Acc
MF1	25.1	100.6	52.5	105.0	95.6	95.6	243	97.2	499	99.8	995	99.5	2480	99.2	5070	101.5
	24.3	97.0	51.1	102.3	101	101.0	247	99.0	496	99.2	1020	102.2	2540	101.7	4960	99.1
MF2	25.3	101.1	51.1	102.3	99.8	99.8	246	98.5	489	97.9	996	99.6	2600	103.8	5220	104.4
	24.7	98.9	50.9	101.7	94.9	94.9	241	96.5	484	96.7	998	99.8	2550	101.9	5110	102.3
MF3	26.2	104.9	52.3	104.6	99.9	99.9	250	99.8	482	96.4	997	99.7	2590	103.5	4950	98.9
	23.1	92.2	50.8	101.7	100	100.5	250	99.8	492	98.4	958	95.8	2550	102.1	5090	101.8
MF4	26.2	104.8	51.1	102.2	97.3	97.3	244	97.7	501	100.3	988	98.8	2590	103.7	5110	102.1
	23.9	95.6	49.4	98.7	101	100.7	248	99.3	487	97.3	997	99.7	2570	102.9	4940	98.8
MF5	25.3	101.4	48.7	97.4	99.2	99.2	249	99.8	516	103.2	1010	101.4	2560	102.6	4950	99.0
	25.2	100.7	50.6	101.3	94.1	94.1	251	100.5	505	100.9	1000	100.2	2520	100.7	4880	97.6
Mean	24.9		50.9		98.3		247		495		997		2560		5030	
SD	0.986		1.16		2.60		3.27		10.6		16.9		36.2		107	
%CV	4.0		2.3		2.6		1.3		2.1		1.7		1.4		2.1	
%Accuracy	99.7		101.7		98.3		98.8		99.0		99.7		102.2		100.6	
n	10		10		10		10		10		10		10		10	

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-8**

**Back-Calculated Concentrations of [Proprietary] Quality Control Samples (ng/ml)**

Run Number	QC Description					
	15.0	%Acc	400	%Acc	800	%Acc
MF1	15.4	102.8	408	102.1	801	100.2
	16.5	109.9	386	96.6	769	96.2
MF2	15.5	103.5	376	93.9	763	95.3
	16.3	108.8	408	102.0	769	96.1
MF3	15.0	100.3	399	99.8	793	99.1
	15.4	102.7	370	92.5	754	94.2
MF4	14.0	93.0	413	103.3	813	101.6
	16.3	108.7	413	103.3	845	105.6
MF5	15.4	103.0	409	102.2	770	96.2
	15.3	101.7	403	100.9	822	102.8
Mean	15.5		399		790	
SD	0.744		15.7		29.9	
%CV	4.8		3.9		3.8	
%Accuracy	103.4		99.7		98.7	
n	10		10		10	

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-9**

**Back-Calculated Concentrations of [Proprietary] Quality Control Samples (ng/ml)**

Run Number	QC Description					
	15.0	%Acc	400	%Acc	800	%Acc
MF1	15.5	103.0	393	98.3	785	98.1
	15.3	102.1	402	100.4	785	98.1
MF2	15.3	101.7	390	97.5	812	101.4
	16.1	107.1	404	101.0	828	103.5
MF3	14.8	98.7	398	99.6	794	99.3
	15.6	104.3	406	101.5	799	99.8
MF4	15.0	100.2	419	104.8	812	101.5
	15.8	105.6	420	105.0	854	106.8
MF5	15.7	104.4	415	103.7	806	100.7
	15.7	104.9	399	99.9	825	103.2
Mean	15.5		405		810	
SD	0.385		10.4		21.5	
%CV	2.5		2.6		2.7	
%Accuracy	103.2		101.2		101.2	
n	10		10		10	

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-10**

**Back-Calculated Concentrations of [Proprietary] Quality Control Samples (ng/ml)**

Run Number	QC Description					
	75.0	%Acc	2000	%Acc	4000	%Acc
MF1	74.4	99.3	2010	100.6	4060	101.5
	74.4	99.1	2020	101.1	3970	99.2
MF2	76.0	101.3	2090	104.3	4170	104.3
	74.8	99.7	2070	103.5	4090	102.2
MF3	77.0	102.7	2030	101.4	4040	101.0
	75.8	101.1	2050	102.3	4040	101.0
MF4	76.3	101.7	2010	100.3	4160	103.9
	74.5	99.4	2070	103.3	4000	100.1
MF5	73.4	97.9	2080	103.9	4070	101.8
	77.3	103.1	2030	101.6	4090	102.1
Mean	75.4		2040		4070	
SD	1.28		28.7		62.7	
%CV	1.7		1.4		1.5	
%Accuracy	100.5		102.2		101.7	
n	10		10		10	



**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Prop] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-11**

**Selectivity Determination and Carryover in [Proprietary] Assay BI/BI and BI/IS samples**

Run Number	Peak Area							
	LLOQ	Mean LLOQ	BI/BI	% Difference	BI/IS	% Difference	Carryover	% Difference
MF1	9249	9474	100	1.1	496	5.2	2377	25.1
	9698		2111	22.3	712	7.5	2028	21.4
MF2	11925	12449	1500	12.0	1586	12.7	4080	32.8
	12973		2224	17.9	896	7.2	3518	28.3
MF3	12864	13029	786	6.0	468	3.6	3358	25.8
	13193		2838	21.8	1155	8.9	3258	25.0
MF4	14280	14767	107	0.7	95	0.6	3067	20.8
	15253		2514	17.0	578	3.9	3609	24.4
MF5	16090	15792	0	0.0	0	0.0	2862	18.1
	15493		2387	15.1	926	5.9	2281	14.4

% Difference is calculated using the mean of the LLOQ peak areas.

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Prop] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-12**

**Selectivity Determination and Carryover in [Proprietary] Assay BI/BI and BI/IS samples**

Run Number	Peak Area							
	LLOQ	Mean LLOQ	BI/BI	% Difference	BI/IS	% Difference	Carryover	% Difference
MF1	12654	12719	377	3.0	0	0.0	1919	15.1
	12784		2237	17.6	501	3.9	2417	19.0
MF2	14327	14869	337	2.3	250	1.7	2854	19.2
	15411		2643	17.8	814	5.5	3192	21.5
MF3	15795	16327	0	0.0	0	0.0	3019	18.5
	16859		2370	14.5	657	4.0	2694	16.5
MF4	16493	18123	0	0.0	0	0.0	3602	19.9
	19752		3067	16.9	771	4.3	3673	20.3
MF5	19132	19341	0	0.0	243	1.3	3320	17.2
	19550		2750	14.2	946	4.9	3592	18.6

% Difference is calculated using the mean of the LLOQ peak areas.

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Prop] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-13**

**Selectivity Determination and Carryover in [Proprietary] Assay BI/BI and BI/IS samples**

Run Number	Peak Area							
	LLOQ	Mean LLOQ	BI/BI	% Difference	BI/IS	% Difference	Carryover	% Difference
MF1	29124	29176	0	0.0	1128	3.9	1566	5.4
	29227		1529	5.2	0	0.0	3207	11.0
MF2	30364	29967	0	0.0	0	0.0	0	0.0
	29569		0	0.0	0	0.0	0	0.0
MF3	30778	28219	0	0.0	0	0.0	0	0.0
	25659		0	0.0	867	3.1	0	0.0
MF4	37147	37419	295	0.8	1218	3.3	3185	8.5
	37690		2078	5.6	974	2.6	2356	6.3
MF5	42012	39112	0	0.0	0	0.0	1880	4.8
	36211		0	0.0	0	0.0	2180	5.6

% Difference is calculated using the mean of the LLOQ peak areas.

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Prop] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-14**

**Incurred Sample Re-analysis (ISR) of [Proprietary] in K<sub>2</sub> EDTA Dog Plasma Samples (ng/ml)**

Animal Number	Sex	Group	Day	Timepoint (H)	Original Concentration	ISR Concentration	% Difference	Analytical Run (Original; ISR)
12	M	2	1	1	12.6	13.3	5.4	MF1; MF5
13	M	2	15	1	20.0	20.2	1.0	MF1; MF5
13	M	2	22	1	17.9	18.0	0.6	MF1; MF5
1	M	2	29	1	12.2	12.1	-0.8	MF1; MF5
15	M	2	29	1	11.8	12.1	2.5	MF1; MF5
16	F	2	1	1	15.1	15.8	4.5	MF1; MF5
16	F	2	8	1	20.2	19.9	-1.5	MF1; MF5
18	F	2	8	1	20.9	23.1	10.0	MF2; MF5
17	F	2	15	1	13.9	13.6	-2.2	MF2; MF5
16	F	2	29	1	22.7	23.1	1.7	MF1; MF5
17	F	2	29	1	13.4	13.8	2.9	MF2; MF5
23	M	3	1	1	91.9	84.7	-8.2	MF2; MF5
21	M	3	8	1	127	127	0.0	MF2; MF5
22	M	3	15	1	256	252	-1.6	MF2; MF5
27	F	3	1	1	182	186	2.2	MF3; MF5
28	F	3	8	1	133	139	4.4	MF3; MF5
26	F	3	15	1	126	136	7.6	MF3; MF5
33	M	4	1	1	121	130	7.2	MF4; MF5
31	M	4	8	1	685	686	0.1	MF4; MF5
32	M	4	8	1	246	238	-3.3	MF4; MF5
37	F	4	1	1	462	419	-9.8	MF4; MF5
38	F	4	1	1	779	778	-0.1	MF4; MF5
37	F	4	8	1	1830	1820	-0.5	MF4; MF5
37	F	4	15	1	1480	1490	0.7	MF4; MF5
						Passed # ISR /Total # ISR	24/24	
						% Acceptable Results	100.0	

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Prop] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-15**

**Incurred Sample Re-analysis (ISR) of Ritonavir in K<sub>2</sub> EDTA Dog Plasma Samples (ng/ml)**

Animal Number	Sex	Group	Day	Timepoint (H)	Original Concentration	ISR Concentration	% Difference	Analytical Run (Original; ISR)
12	M	2	1	1	20.9	21.2	1.4	MF1; MF5
13	M	2	15	1	18.5	17.7	-4.4	MF1; MF5
13	M	2	22	1	18.3	19.2	4.8	MF1; MF5
1	M	2	29	1	19.3	19.1	-1.0	MF1; MF5
15	M	2	29	1	13.0	12.6	-3.1	MF1; MF5
16	F	2	1	1	12.3	12.4	0.8	MF1; MF5
16	F	2	8	1	16.8	16.4	-2.4	MF1; MF5
18	F	2	8	1	32.1	30.7	-4.5	MF2; MF5
17	F	2	15	1	16.9	15.8	-6.7	MF2; MF5
16	F	2	29	1	19.2	19.2	0.0	MF1; MF5
17	F	2	29	1	17.7	16.9	-4.6	MF2; MF5
23	M	3	1	1	146	146	0.0	MF2; MF5
21	M	3	8	1	159	165	3.7	MF2; MF5
22	M	3	15	1	192	204	6.1	MF2; MF5
27	F	3	1	1	230	241	4.7	MF3; MF5
28	F	3	8	1	231	240	3.8	MF3; MF5
26	F	3	15	1	146	150	2.7	MF3; MF5
33	M	4	1	1	203	198	-2.5	MF4; MF5
31	M	4	8	1	479	467	-2.5	MF4; MF5
32	M	4	8	1	217	207	-4.7	MF4; MF5
37	F	4	1	1	614	595	-3.1	MF4; MF5
38	F	4	1	1	1060	1080	1.9	MF4; MF5
37	F	4	8	1	1280	1290	0.8	MF4; MF5
37	F	4	15	1	656	663	1.1	MF4; MF5
Passed # ISR /Total # ISR							24/24	
% Acceptable Results							100.0	



**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Prop] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-16**

**Incurred Sample Re-analysis (ISR) of [Proprietary] in K<sub>2</sub> EDTA Dog Plasma Samples (ng/ml)**

Animal Number	Sex	Group	Day	Timepoint (H)	Original Concentration	ISR Concentration	% Difference	Analytical Run (Original; ISR)
12	M	2	1	1	218	222	1.8	MF1; MF5
13	M	2	15	1	186	185	-0.5	MF1; MF5
13	M	2	22	1	167	167	0.0	MF1; MF5
1	M	2	29	1	232	237	2.1	MF1; MF5
15	M	2	29	1	227	223	-1.8	MF1; MF5
16	F	2	1	1	233	233	0.0	MF1; MF5
16	F	2	8	1	279	270	-3.3	MF1; MF5
18	F	2	8	1	300	294	-2.0	MF2; MF5
17	F	2	15	1	261	264	1.1	MF2; MF5
16	F	2	29	1	269	274	1.8	MF1; MF5
17	F	2	29	1	236	241	2.1	MF2; MF5
23	M	3	1	1	2130	2100	-1.4	MF2; MF5
21	M	3	8	1	1970	1960	-0.5	MF2; MF5
22	M	3	15	1	2130	2160	1.4	MF2; MF5
27	F	3	1	1	2410	2420	0.4	MF3; MF5
28	F	3	8	1	1990	1960	-1.5	MF3; MF5
26	F	3	15	1	1930	1930	0.0	MF3; MF5
33	M	4	1	1	9640	9410	-2.4	MF4; MF5
31	M	4	8	1	7280	7600	4.3	MF4; MF5
32	M	4	8	1	8250	7910	-4.2	MF4; MF5
37	F	4	1	1	11400	11500	0.9	MF4; MF5
38	F	4	1	1	10200	10400	1.9	MF4; MF5
37	F	4	8	1	10200	10200	0.0	MF4; MF5
37	F	4	15	1	10200	10000	-2.0	MF4; MF5
Passed # ISR /Total # ISR							24/24	
% Acceptable Results							100.0	

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Prop] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Table G-17**

**Sample Reassay of [Proprietary] in K<sub>2</sub> EDTA Dog Plasma Samples (ng/ml)**

Animal Number-Sex-Group-Day-Timepoint	Original Concentration	Reassay Concentration	% Difference (Between Reassay and Original)	Mean Value	Reported Value	Reason for Reported Value	Analytical Run (Original; Reassay)
Animal 17-F-Gp2-D1-Post 1H	19.2	19.9	3.6	19.6	19.6	a	MF2; MF4
Animal 18-F-Gp2-D1-Post 1H	23.1	24.0	3.8	23.6	23.6	a	MF2; MF4

<sup>a</sup> This sample was reassayed because of a chromatographic interference on original analysis. Refer to the report text for additional information.

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-18**

**Back-Calculated Concentrations of [Proprietary] in Group 2 (0.400 mg/kg [Proprietary Info] Male**  
**K<sub>2</sub> EDTA Dog Plasma Samples**

Animal Number	Day	Timepoint (Hours)	Dilution Factor	Concentration (ng/ml)	Mean Concentration (ng/ml)	SD	Analytical Run
11	1	Pre	1	<LLOQ	< LLOQ	NA	MF1
12	1	Pre	1	<LLOQ			MF1
13	1	Pre	1	<LLOQ			MF1
11	1	1	1	9.70	14.1	5.37	MF1
12	1	1	1	12.6			MF1
13	1	1	1	20.1			MF1
11	8	Pre	1	<LLOQ	< LLOQ	NA	MF1
12	8	Pre	1	<LLOQ			MF1
13	8	Pre	1	<LLOQ			MF1
11	8	1	1	8.91	18.4	9.04	MF1
12	8	1	1	19.5			MF1
13	8	1	1	26.9			MF1
11	15	Pre	1	<LLOQ	< LLOQ	NA	MF1
12	15	Pre	1	<LLOQ			MF1
13	15	Pre	1	<LLOQ			MF1
11	15	1	1	13.4	16.6	3.31	MF1
12	15	1	1	16.3			MF1
13	15	1	1	20.0			MF1
11	22	Pre	1	<LLOQ	< LLOQ	NA	MF1
12	22	Pre	1	<LLOQ			MF1
13	22	Pre	1	<LLOQ			MF1
11	22	1	1	12.5	14.9	2.76	MF1
12	22	1	1	14.2			MF1
13	22	1	1	17.9			MF1
11	29	Pre	1	<LLOQ	< LLOQ	NA	MF1
12	29	Pre	1	<LLOQ			MF1
13	29	Pre	1	<LLOQ			MF1
1	29	Pre	1	<LLOQ			MF1
15	29	Pre	1	<LLOQ			MF1
11	29	1	1	11.6	14.2	5.26	MF1
12	29	1	1	11.8			MF1
13	29	1	1	23.6			MF1
1	29	1	1	12.2			MF1
15	29	1	1	11.8			MF1
1	36	Pre	1	<LLOQ	< LLOQ	NA	MF1
15	36	Pre	1	<LLOQ			MF1
1	43	Pre	1	<LLOQ	< LLOQ	NA	MF1
15	43	Pre	1	<LLOQ			MF1
1	50	Pre	1	<LLOQ	< LLOQ	NA	MF1
15	50	Pre	1	<LLOQ			MF1
1	57	Pre	1	<LLOQ	< LLOQ	NA	MF1
15	57	Pre	1	<LLOQ			MF1
1	64	Pre	1	<LLOQ	< LLOQ	NA	MF1
15	64	Pre	1	<LLOQ			MF1
1	71	Pre	1	<LLOQ	< LLOQ	NA	MF1
15	71	Pre	1	<LLOQ			MF1

LLOQ: Lower Limit of Quantitation (5.00 ng/ml).  
NA: Not Applicable.

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-19**

**Back-Calculated Concentrations of [Proprietary] in Group 2 (0.400 mg/kg [Proprietary Info] Female**  
**K<sub>2</sub> EDTA Dog Plasma Samples**

Animal Number	Day	Timepoint (Hours)	Dilution Factor	Concentration (ng/ml)	Mean Concentration (ng/ml)	SD	Analytical Run
16	1	Pre	1	<LLOQ	< LLOQ	NA	MF1
17	1	Pre	1	<LLOQ			MF2
18	1	Pre	1	<LLOQ			MF2
16	1	1	1	15.1	14.6	0.987	MF1
17	1	1	1	15.3			MF2
18	1	1	1	13.5			MF2
16	8	Pre	1	<LLOQ	< LLOQ	NA	MF1
17	8	Pre	1	<LLOQ			MF2
18	8	Pre	1	<LLOQ			MF2
16	8	1	1	20.2	20.0	1.01	MF1
17	8	1	1	18.9			MF2
18	8	1	1	20.9			MF2
16	15	Pre	1	<LLOQ	< LLOQ	NA	MF1
17	15	Pre	1	<LLOQ			MF2
18	15	Pre	1	<LLOQ			MF2
16	15	1	1	16.7	14.2	2.41	MF1
17	15	1	1	13.9			MF2
18	15	1	1	11.9			MF2
16	22	Pre	1	<LLOQ	< LLOQ	NA	MF1
17	22	Pre	1	<LLOQ			MF2
18	22	Pre	1	<LLOQ			MF2
16	22	1	1	13.2	11.4	1.95	MF1
17	22	1	1	11.6			MF2
18	22	1	1	9.32			MF2
16	29	Pre	1	<LLOQ	< LLOQ	NA	MF1
17	29	Pre	1	<LLOQ			MF2
18	29	Pre	1	<LLOQ			MF2
19	29	Pre	1	<LLOQ			MF2
20	29	Pre	1	<LLOQ			MF2
16	29	1	1	22.7	13.9	5.09	MF1
17	29	1	1	13.4			MF2
18	29	1	1	10.2			MF2
19	29	1	1	12.8			MF2
20	29	1	1	10.6			MF2
19	36	Pre	1	<LLOQ	< LLOQ	NA	MF2
20	36	Pre	1	<LLOQ			MF2
19	43	Pre	1	<LLOQ	< LLOQ	NA	MF2
20	43	Pre	1	<LLOQ			MF2
19	50	Pre	1	<LLOQ	< LLOQ	NA	MF2
20	50	Pre	1	<LLOQ			MF2
19	57	Pre	1	<LLOQ	< LLOQ	NA	MF2
20	57	Pre	1	<LLOQ			MF2
19	64	Pre	1	<LLOQ	< LLOQ	NA	MF2
20	64	Pre	1	<LLOQ			MF2
19	71	Pre	1	<LLOQ	< LLOQ	NA	MF2
20	71	Pre	1	<LLOQ			MF2

LLOQ: Lower Limit of Quantitation (5.00 ng/ml).

NA: Not Applicable.

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-20**

**Back-Calculated Concentrations of [Proprietary] in Group 3 (3.64 mg/kg [Proprietary Info] Male  
K<sub>2</sub> EDTA Dog Plasma Samples**

Animal Number	Day	Timepoint (Hours)	Dilution Factor	Concentration (ng/ml)	Mean Concentration (ng/ml)	SD	Analytical Run
21	1	Pre	1	<LLOQ	< LLOQ	NA	MF2
22	1	Pre	1	<LLOQ			MF2
23	1	Pre	1	<LLOQ			MF2
21	1	1	1	63.0	81.4	16.0	MF2
22	1	1	1	89.3			MF2
23	1	1	1	91.9			MF2
21	8	Pre	1	11.8	14.8	2.65	MF2
22	8	Pre	1	15.8			MF2
23	8	Pre	1	16.8			MF2
21	8	1	1	127	154	58.4	MF2
22	8	1	1	221			MF2
23	8	1	1	114			MF2
21	15	Pre	1	14.3	25.3	18.0	MF2
22	15	Pre	1	46.0			MF2
23	15	Pre	1	15.5			MF2
21	15	1	1	197	197	59.0	MF2
22	15	1	1	256			MF2
23	15	1	1	138			MF2
24	22	Pre	1	11.2	18.3	NA	MF2
25	22	Pre	1	25.4			MF3
24	29	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	29	Pre	1	<LLOQ			MF3
24	36	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	36	Pre	1	<LLOQ			MF3
24	43	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	43	Pre	1	<LLOQ			MF3
24	50	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	50	Pre	1	<LLOQ			MF3
24	57	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	57	Pre	1	<LLOQ			MF3
24	64	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	64	Pre	1	<LLOQ			MF3
24	71	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	71	Pre	1	<LLOQ			MF3

LLOQ: Lower Limit of Quantitation (5.00 ng/ml).

NA: Not Applicable.



**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-21**

**Back-Calculated Concentrations of [Proprietary] in Group 3 (3.64 mg/kg [Proprietary Info] Female  
K<sub>2</sub> EDTA Dog Plasma Samples**

Animal Number	Day	Timepoint (Hours)	Dilution Factor	Concentration (ng/ml)	Mean Concentration (ng/ml)	SD	Analytical Run
26	1	Pre	1	<LLOQ	< LLOQ	NA	MF3
27	1	Pre	1	<LLOQ			MF3
28	1	Pre	1	<LLOQ			MF3
26	1	1	1	13.7	178	162	MF3
27	1	1	1	182			MF3
28	1	1	1	338			MF3
26	8	Pre	1	<LLOQ	11.8	NA	MF3
27	8	Pre	1	13.3			MF3
28	8	Pre	1	10.3			MF3
26	8	1	1	125	141	21.7	MF3
27	8	1	1	166			MF3
28	8	1	1	133			MF3
26	15	Pre	1	11.6	16.6	4.37	MF3
27	15	Pre	1	18.8			MF3
28	15	Pre	1	19.5			MF3
26	15	1	1	126	121	27.0	MF3
27	15	1	1	91.8			MF3
28	15	1	1	145			MF3
29	22	Pre	1	12.4	10.1	NA	MF3
30	22	Pre	1	7.77			MF3
29	29	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	29	Pre	1	<LLOQ			MF3
29	36	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	36	Pre	1	<LLOQ			MF3
29	43	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	43	Pre	1	<LLOQ			MF3
29	50	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	50	Pre	1	<LLOQ			MF3
29	57	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	57	Pre	1	<LLOQ			MF3
29	64	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	64	Pre	1	<LLOQ			MF3
29	71	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	71	Pre	1	<LLOQ			MF3

LLOQ: Lower Limit of Quantitation (5.00 ng/ml).

NA: Not Applicable.

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-22**

**Back-Calculated Concentrations of [Proprietary] in Group 4 (14.55 mg/kg [Proprietary Info] Male  
K<sub>2</sub> EDTA Dog Plasma Samples**

Animal Number	Day	Timepoint (Hours)	Dilution Factor	Concentration (ng/ml)	Mean Concentration (ng/ml)	SD	Analytical Run
31	1	Pre	1	<LLOQ	< LLOQ	NA	MF4
32	1	Pre	1	<LLOQ			MF4
33	1	Pre	1	<LLOQ			MF4
31	1	1	10	145	110	40.9	MF4
32	1	1	10	65.2			MF4
33	1	1	10	121			MF4
31	8	Pre	1	40.0	49.6	19.0	MF4
32	8	Pre	1	37.3			MF4
33	8	Pre	1	71.5			MF4
31	8	1	10	685	612	336	MF4
32	8	1	10	246			MF4
33	8	1	10	905			MF4
31	15	Pre	1	58.4	75.3	21.2	MF4
32	15	Pre	1	68.4			MF4
33	15	Pre	1	99.0			MF4
31	15	1	10	418	583	202	MF4
32	15	1	10	522			MF4
33	15	1	10	808			MF4
34	22	Pre	1	70.0	84.6	NA	MF3
35	22	Pre	1	99.1			MF3
34	29	Pre	1	11.5	12.2	NA	MF3
35	29	Pre	1	12.9			MF3
34	36	Pre	1	<LLOQ	< LLOQ	NA	MF3
35	36	Pre	1	<LLOQ			MF3
34	43	Pre	1	<LLOQ	< LLOQ	NA	MF3
35	43	Pre	1	<LLOQ			MF3
34	50	Pre	1	<LLOQ	< LLOQ	NA	MF3
35	50	Pre	1	<LLOQ			MF3
34	57	Pre	1	<LLOQ	< LLOQ	NA	MF3
35	57	Pre	1	<LLOQ			MF3
34	64	Pre	1	<LLOQ	< LLOQ	NA	MF3
35	64	Pre	1	<LLOQ			MF3
34	71	Pre	1	<LLOQ	< LLOQ	NA	MF3
35	71	Pre	1	<LLOQ			MF3

LLOQ: Lower Limit of Quantitation (5.00 ng/ml).

NA: Not Applicable.

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-23**

**Back-Calculated Concentrations of [Proprietary] in Group 4 (14.55 mg/kg [Proprietary Info] Female  
K<sub>2</sub> EDTA Dog Plasma Samples**

Animal Number	Day	Timepoint (Hours)	Dilution Factor	Concentration (ng/ml)	Mean Concentration (ng/ml)	SD	Analytical Run
36	1	Pre	1	<LLOQ	< LLOQ	NA	MF4
37	1	Pre	1	<LLOQ			MF4
38	1	Pre	1	<LLOQ			MF4
36	1	1	10	1240	827	391	MF4
37	1	1	10	462			MF4
38	1	1	10	779			MF4
36	8	Pre	1	53.0	55.2	4.28	MF4
37	8	Pre	1	60.1			MF4
38	8	Pre	1	52.4			MF4
36	8	1	10	863	1190	557	MF4
37	8	1	10	1830			MF4
38	8	1	10	867			MF4
36	15	Pre	1	66.8	84.8	15.7	MF4
37	15	Pre	1	92.0			MF4
38	15	Pre	1	95.6			MF4
36	15	1	10	1080	1160	291	MF4
37	15	1	10	1480			MF4
38	15	1	10	913			MF4
38	22	Pre	1	104	153	NA	MF4
40	22	Pre	1	201			MF4
38	29	Pre	1	7.36	12.1	NA	MF4
40	29	Pre	1	16.8			MF4
38	36	Pre	1	<LLOQ	< LLOQ	NA	MF4
40	36	Pre	1	<LLOQ			MF4
38	43	Pre	1	<LLOQ	< LLOQ	NA	MF4
40	43	Pre	1	<LLOQ			MF4
38	50	Pre	1	<LLOQ	< LLOQ	NA	MF4
40	50	Pre	1	<LLOQ			MF4
38	57	Pre	1	<LLOQ	< LLOQ	NA	MF4
40	57	Pre	1	<LLOQ			MF4
38	64	Pre	1	<LLOQ	< LLOQ	NA	MF4
40	64	Pre	1	<LLOQ			MF4
38	71	Pre	1	<LLOQ	< LLOQ	NA	MF4
40	71	Pre	1	<LLOQ			MF4

LLOQ: Lower Limit of Quantitation (5.00 ng/ml).

NA: Not Applicable.

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-24**

**Back-Calculated Concentrations of [Proprietary] in Group 2 (0.110 mg/kg [Proprietary] Male**  
**K<sub>2</sub> EDTA Dog Plasma Samples**

Animal Number	Day	Timepoint (Hours)	Dilution Factor	Concentration (ng/ml)	Mean Concentration (ng/ml)	SD	Analytical Run
11	1	Pre	1	<LLOQ	< LLOQ	NA	MF1
12	1	Pre	1	<LLOQ			MF1
13	1	Pre	1	<LLOQ			MF1
11	1	1	1	16.0	20.7	4.65	MF1
12	1	1	1	20.9			MF1
13	1	1	1	25.3			MF1
11	8	Pre	1	<LLOQ	< LLOQ	NA	MF1
12	8	Pre	1	<LLOQ			MF1
13	8	Pre	1	<LLOQ			MF1
11	8	1	1	12.2	19.6	6.89	MF1
12	8	1	1	25.8			MF1
13	8	1	1	20.9			MF1
11	15	Pre	1	<LLOQ	< LLOQ	NA	MF1
12	15	Pre	1	<LLOQ			MF1
13	15	Pre	1	<LLOQ			MF1
11	15	1	1	18.7	20.1	2.54	MF1
12	15	1	1	23.0			MF1
13	15	1	1	18.5			MF1
11	22	Pre	1	<LLOQ	< LLOQ	NA	MF1
12	22	Pre	1	<LLOQ			MF1
13	22	Pre	1	<LLOQ			MF1
11	22	1	1	16.6	17.0	1.12	MF1
12	22	1	1	16.2			MF1
13	22	1	1	18.3			MF1
11	29	Pre	1	<LLOQ	< LLOQ	NA	MF1
12	29	Pre	1	<LLOQ			MF1
13	29	Pre	1	<LLOQ			MF1
1	29	Pre	1	<LLOQ			MF1
15	29	Pre	1	<LLOQ			MF1
11	29	1	1	15.7	17.1	2.73	MF1
12	29	1	1	18.4			MF1
13	29	1	1	19.2			MF1
1	29	1	1	19.3			MF1
15	29	1	1	13.0			MF1
1	36	Pre	1	<LLOQ	< LLOQ	NA	MF1
15	36	Pre	1	<LLOQ			MF1
1	43	Pre	1	<LLOQ	< LLOQ	NA	MF1
15	43	Pre	1	<LLOQ			MF1
1	50	Pre	1	<LLOQ	< LLOQ	NA	MF1
15	50	Pre	1	<LLOQ			MF1
1	57	Pre	1	<LLOQ	< LLOQ	NA	MF1
15	57	Pre	1	<LLOQ			MF1
1	64	Pre	1	<LLOQ	< LLOQ	NA	MF1
15	64	Pre	1	<LLOQ			MF1
1	71	Pre	1	<LLOQ	< LLOQ	NA	MF1
15	71	Pre	1	<LLOQ			MF1

LLOQ: Lower Limit of Quantitation (5.00 ng/ml).

NA: Not Applicable.

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-25**

**Back-Calculated Concentrations of [Proprietary] in Group 2 (0.110 mg/kg [Proprietary] Female**  
**K<sub>2</sub> EDTA Dog Plasma Samples**

Animal Number	Day	Timepoint (Hours)	Dilution Factor	Concentration (ng/ml)	Mean Concentration (ng/ml)	SD	Analytical Run
16	1	Pre	1	<LLOQ	< LLOQ	NA	MF1
17	1	Pre	1	<LLOQ			MF2
18	1	Pre	1	<LLOQ			MF2
16	1	1	1	12.3	18.5	5.73	MF1
17	1	1	1	19.6			MF2/MF4
18	1	1	1	23.6			MF2/MF4
16	8	Pre	1	<LLOQ	< LLOQ	NA	MF1
17	8	Pre	1	<LLOQ			MF2
18	8	Pre	1	<LLOQ			MF2
16	8	1	1	16.8	22.7	8.25	MF1
17	8	1	1	19.1			MF2
18	8	1	1	32.1			MF2
16	15	Pre	1	<LLOQ	< LLOQ	NA	MF1
17	15	Pre	1	<LLOQ			MF2
18	15	Pre	1	<LLOQ			MF2
16	15	1	1	17.3	21.7	7.97	MF1
17	15	1	1	16.9			MF2
18	15	1	1	30.9			MF2
16	22	Pre	1	<LLOQ	< LLOQ	NA	MF1
17	22	Pre	1	<LLOQ			MF2
18	22	Pre	1	<LLOQ			MF2
16	22	1	1	17.4	16.6	0.764	MF1
17	22	1	1	15.9			MF2
18	22	1	1	16.4			MF2
16	29	Pre	1	<LLOQ	< LLOQ	NA	MF1
17	29	Pre	1	<LLOQ			MF2
18	29	Pre	1	<LLOQ			MF2
19	29	Pre	1	<LLOQ			MF2
20	29	Pre	1	<LLOQ			MF2
16	29	1	1	19.2	16.9	1.98	MF1
17	29	1	1	17.7			MF2
18	29	1	1	16.7			MF2
19	29	1	1	13.8			MF2
20	29	1	1	17.2			MF2
19	36	Pre	1	<LLOQ	< LLOQ	NA	MF2
20	36	Pre	1	<LLOQ			MF2
19	43	Pre	1	<LLOQ	< LLOQ	NA	MF2
20	43	Pre	1	<LLOQ			MF2
19	50	Pre	1	<LLOQ	< LLOQ	NA	MF2
20	50	Pre	1	<LLOQ			MF2
19	57	Pre	1	<LLOQ	< LLOQ	NA	MF2
20	57	Pre	1	<LLOQ			MF2
19	64	Pre	1	<LLOQ	< LLOQ	NA	MF2
20	64	Pre	1	<LLOQ			MF2
19	71	Pre	1	<LLOQ	< LLOQ	NA	MF2
20	71	Pre	1	<LLOQ			MF2

LLOQ: Lower Limit of Quantitation (5.00 ng/ml).

NA: Not Applicable.



**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-26**

**Back-Calculated Concentrations of [Proprietary] in Group 3 (1.00 mg/kg [Proprietary] Male  
K<sub>2</sub> EDTA Dog Plasma Samples**

Animal Number	Day	Timepoint (Hours)	Dilution Factor	Concentration (ng/ml)	Mean Concentration (ng/ml)	SD	Analytical Run
21	1	Pre	1	<LLOQ	< LLOQ	NA	MF2
22	1	Pre	1	<LLOQ			MF2
23	1	Pre	1	<LLOQ			MF2
21	1	1	1	116	135	16.8	MF2
22	1	1	1	144			MF2
23	1	1	1	146			MF2
21	8	Pre	1	<LLOQ	< LLOQ	NA	MF2
22	8	Pre	1	<LLOQ			MF2
23	8	Pre	1	<LLOQ			MF2
21	8	1	1	159	190	36.2	MF2
22	8	1	1	230			MF2
23	8	1	1	182			MF2
21	15	Pre	1	<LLOQ	< LLOQ	NA	MF2
22	15	Pre	1	<LLOQ			MF2
23	15	Pre	1	<LLOQ			MF2
21	15	1	1	185	179	17.4	MF2
22	15	1	1	192			MF2
23	15	1	1	159			MF2
24	22	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	22	Pre	1	<LLOQ			MF3
24	29	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	29	Pre	1	<LLOQ			MF3
24	36	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	36	Pre	1	<LLOQ			MF3
24	43	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	43	Pre	1	<LLOQ			MF3
24	50	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	50	Pre	1	<LLOQ			MF3
24	57	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	57	Pre	1	<LLOQ			MF3
24	64	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	64	Pre	1	<LLOQ			MF3
24	71	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	71	Pre	1	<LLOQ			MF3

LLOQ: Lower Limit of Quantitation (5.00 ng/ml).

NA: Not Applicable.

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-27**

**Back-Calculated Concentrations of [Proprietary] in Group 3 (1.00 mg/kg [Proprietary] Female  
K<sub>2</sub> EDTA Dog Plasma Samples**

Animal Number	Day	Timepoint (Hours)	Dilution Factor	Concentration (ng/ml)	Mean Concentration (ng/ml)	SD	Analytical Run
26	1	Pre	1	<LLOQ	< LLOQ	NA	MF3
27	1	Pre	1	<LLOQ			MF3
28	1	Pre	1	<LLOQ			MF3
26	1	1	1	19.5	181	143	MF3
27	1	1	1	230			MF3
28	1	1	1	292			MF3
26	8	Pre	1	<LLOQ	< LLOQ	NA	MF3
27	8	Pre	1	<LLOQ			MF3
28	8	Pre	1	<LLOQ			MF3
26	8	1	1	261	245	15.0	MF3
27	8	1	1	244			MF3
28	8	1	1	231			MF3
26	15	Pre	1	<LLOQ	< LLOQ	NA	MF3
27	15	Pre	1	<LLOQ			MF3
28	15	Pre	1	<LLOQ			MF3
26	15	1	1	146	127	38.3	MF3
27	15	1	1	83.4			MF3
28	15	1	1	153			MF3
29	22	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	22	Pre	1	<LLOQ			MF3
29	29	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	29	Pre	1	<LLOQ			MF3
29	36	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	36	Pre	1	<LLOQ			MF3
29	43	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	43	Pre	1	<LLOQ			MF3
29	50	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	50	Pre	1	<LLOQ			MF3
29	57	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	57	Pre	1	<LLOQ			MF3
29	64	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	64	Pre	1	<LLOQ			MF3
29	71	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	71	Pre	1	<LLOQ			MF3

LLOQ: Lower Limit of Quantitation (5.00 ng/ml).  
NA: Not Applicable.

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-28**

**Back-Calculated Concentrations of [Proprietary] in Group 4 (4.00 mg/kg [Proprietary] Male  
K<sub>2</sub> EDTA Dog Plasma Samples**

Animal Number	Day	Timepoint (Hours)	Dilution Factor	Concentration (ng/ml)	Mean Concentration (ng/ml)	SD	Analytical Run
31	1	Pre	1	<LLOQ	< LLOQ	NA	MF4
32	1	Pre	1	<LLOQ			MF4
33	1	Pre	1	<LLOQ			MF4
31	1	1	10	380	243	122	MF4
32	1	1	10	145			MF4
33	1	1	10	203			MF4
31	8	Pre	1	<LLOQ	< LLOQ	NA	MF4
32	8	Pre	1	<LLOQ			MF4
33	8	Pre	1	<LLOQ			MF4
31	8	1	10	479	427	189	MF4
32	8	1	10	217			MF4
33	8	1	10	585			MF4
31	15	Pre	1	<LLOQ	< LLOQ	NA	MF4
32	15	Pre	1	<LLOQ			MF4
33	15	Pre	1	<LLOQ			MF4
31	15	1	10	291	341	49.1	MF4
32	15	1	10	344			MF4
33	15	1	10	389			MF4
34	22	Pre	1	<LLOQ	< LLOQ	NA	MF3
35	22	Pre	1	<LLOQ			MF3
34	29	Pre	1	<LLOQ	< LLOQ	NA	MF3
35	29	Pre	1	<LLOQ			MF3
34	36	Pre	1	<LLOQ	< LLOQ	NA	MF3
35	36	Pre	1	<LLOQ			MF3
34	43	Pre	1	<LLOQ	< LLOQ	NA	MF3
35	43	Pre	1	<LLOQ			MF3
34	50	Pre	1	<LLOQ	< LLOQ	NA	MF3
35	50	Pre	1	<LLOQ			MF3
34	57	Pre	1	<LLOQ	< LLOQ	NA	MF3
35	57	Pre	1	<LLOQ			MF3
34	64	Pre	1	<LLOQ	< LLOQ	NA	MF3
35	64	Pre	1	<LLOQ			MF3
34	71	Pre	1	<LLOQ	< LLOQ	NA	MF3
35	71	Pre	1	<LLOQ			MF3

LLOQ: Lower Limit of Quantitation (5.00 ng/ml).  
NA: Not Applicable.

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-29**

**Back-Calculated Concentrations of [Proprietary] in Group 4 (4.00 mg/kg [Proprietary] Female  
K<sub>2</sub> EDTA Dog Plasma Samples**

Animal Number	Day	Timepoint (Hours)	Dilution Factor	Concentration (ng/ml)	Mean Concentration (ng/ml)	SD	Analytical Run
36	1	Pre	1	<LLOQ	< LLOQ	NA	MF4
37	1	Pre	1	<LLOQ			MF4
38	1	Pre	1	<LLOQ			MF4
36	1	1	10	2650	1440	1070	MF4
37	1	1	10	614			MF4
38	1	1	10	1060			MF4
36	8	Pre	1	<LLOQ	< LLOQ	NA	MF4
37	8	Pre	1	<LLOQ			MF4
38	8	Pre	1	<LLOQ			MF4
36	8	1	10	644	795	430	MF4
37	8	1	10	1280			MF4
38	8	1	10	461			MF4
36	15	Pre	1	<LLOQ	< LLOQ	NA	MF4
37	15	Pre	1	<LLOQ			MF4
38	15	Pre	1	<LLOQ			MF4
36	15	1	10	618	576	107	MF4
37	15	1	10	656			MF4
38	15	1	10	455			MF4
38	22	Pre	1	<LLOQ	< LLOQ	NA	MF4
40	22	Pre	1	<LLOQ			MF4
38	29	Pre	1	<LLOQ	< LLOQ	NA	MF4
40	29	Pre	1	<LLOQ			MF4
38	36	Pre	1	<LLOQ	< LLOQ	NA	MF4
40	36	Pre	1	<LLOQ			MF4
38	43	Pre	1	<LLOQ	< LLOQ	NA	MF4
40	43	Pre	1	<LLOQ			MF4
38	50	Pre	1	<LLOQ	< LLOQ	NA	MF4
40	50	Pre	1	<LLOQ			MF4
38	57	Pre	1	<LLOQ	< LLOQ	NA	MF4
40	57	Pre	1	<LLOQ			MF4
38	64	Pre	1	<LLOQ	< LLOQ	NA	MF4
40	64	Pre	1	<LLOQ			MF4
38	71	Pre	1	<LLOQ	< LLOQ	NA	MF4
40	71	Pre	1	<LLOQ			MF4

LLOQ: Lower Limit of Quantitation (5.00 ng/ml).  
NA: Not Applicable.

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-30**

**Back-Calculated Concentrations of [Proprietary] in Group 2 (0.230 mg/kg [Proprietary Info] Male**  
**K<sub>2</sub> EDTA Dog Plasma Samples**

Animal Number	Day	Timepoint (Hours)	Dilution Factor	Concentration (ng/ml)	Mean Concentration (ng/ml)	SD	Analytical Run
11	1	Pre	1	<LLOQ	< LLOQ	NA	MF1
12	1	Pre	1	<LLOQ			MF1
13	1	Pre	1	<LLOQ			MF1
11	1	1	1	223	210	18.4	MF1
12	1	1	1	218			MF1
13	1	1	1	189			MF1
11	8	Pre	1	<LLOQ	< LLOQ	NA	MF1
12	8	Pre	1	<LLOQ			MF1
13	8	Pre	1	<LLOQ			MF1
11	8	1	1	208	224	22.5	MF1
12	8	1	1	250			MF1
13	8	1	1	215			MF1
11	15	Pre	1	<LLOQ	< LLOQ	NA	MF1
12	15	Pre	1	<LLOQ			MF1
13	15	Pre	1	<LLOQ			MF1
11	15	1	1	245	220	30.4	MF1
12	15	1	1	228			MF1
13	15	1	1	186			MF1
11	22	Pre	1	<LLOQ	< LLOQ	NA	MF1
12	22	Pre	1	<LLOQ			MF1
13	22	Pre	1	<LLOQ			MF1
11	22	1	1	242	210	38.6	MF1
12	22	1	1	220			MF1
13	22	1	1	167			MF1
11	29	Pre	1	<LLOQ	< LLOQ	NA	MF1
12	29	Pre	1	<LLOQ			MF1
13	29	Pre	1	<LLOQ			MF1
1	29	Pre	1	<LLOQ			MF1
15	29	Pre	1	<LLOQ			MF1
11	29	1	1	230	217	17.5	MF1
12	29	1	1	194			MF1
13	29	1	1	203			MF1
1	29	1	1	232			MF1
15	29	1	1	227			MF1
1	36	Pre	1	<LLOQ	< LLOQ	NA	MF1
15	36	Pre	1	<LLOQ			MF1
1	43	Pre	1	<LLOQ	< LLOQ	NA	MF1
15	43	Pre	1	<LLOQ			MF1
1	50	Pre	1	<LLOQ	< LLOQ	NA	MF1
15	50	Pre	1	<LLOQ			MF1
1	57	Pre	1	<LLOQ	< LLOQ	NA	MF1
15	57	Pre	1	<LLOQ			MF1
1	64	Pre	1	<LLOQ	< LLOQ	NA	MF1
15	64	Pre	1	<LLOQ			MF1
1	71	Pre	1	<LLOQ	< LLOQ	NA	MF1
15	71	Pre	1	<LLOQ			MF1

LLOQ: Lower Limit of Quantitation (25.0 ng/ml).

NA: Not Applicable.



**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Table G-31**

**Back-Calculated Concentrations of [Proprietary] in Group 2 (0.230 mg/kg [Proprietary Info] Female  
K<sub>2</sub> EDTA Dog Plasma Samples**

Animal Number	Day	Timepoint (Hours)	Dilution Factor	Concentration (ng/ml)	Mean Concentration (ng/ml)	SD	Analytical Run
16	1	Pre	1	<LLOQ	< LLOQ	NA	MF1
17	1	Pre	1	<LLOQ			MF2
18	1	Pre	1	<LLOQ			MF2
16	1	1	1	233	269	31.2	MF1
17	1	1	1	291			MF2
18	1	1	1	282			MF2
16	8	Pre	1	<LLOQ	< LLOQ	NA	MF1
17	8	Pre	1	<LLOQ			MF2
18	8	Pre	1	<LLOQ			MF2
16	8	1	1	279	286	12.1	MF1
17	8	1	1	279			MF2
18	8	1	1	300			MF2
16	15	Pre	1	<LLOQ	< LLOQ	NA	MF1
17	15	Pre	1	<LLOQ			MF2
18	15	Pre	1	<LLOQ			MF2
16	15	1	1	276	275	13.5	MF1
17	15	1	1	261			MF2
18	15	1	1	288			MF2
16	22	Pre	1	<LLOQ	< LLOQ	NA	MF1
17	22	Pre	1	<LLOQ			MF2
18	22	Pre	1	<LLOQ			MF2
16	22	1	1	236	236	6.51	MF1
17	22	1	1	242			MF2
18	22	1	1	229			MF2
16	29	Pre	1	<LLOQ	< LLOQ	NA	MF1
17	29	Pre	1	<LLOQ			MF2
18	29	Pre	1	<LLOQ			MF2
19	29	Pre	1	<LLOQ			MF2
20	29	Pre	1	<LLOQ			MF2
16	29	1	1	269	232	23.7	MF1
17	29	1	1	236			MF2
18	29	1	1	230			MF2
19	29	1	1	217			MF2
20	29	1	1	207			MF2
19	36	Pre	1	<LLOQ	< LLOQ	NA	MF2
20	36	Pre	1	<LLOQ			MF2
19	43	Pre	1	<LLOQ	< LLOQ	NA	MF2
20	43	Pre	1	<LLOQ			MF2
19	50	Pre	1	<LLOQ	< LLOQ	NA	MF2
20	50	Pre	1	<LLOQ			MF2
19	57	Pre	1	<LLOQ	< LLOQ	NA	MF2
20	57	Pre	1	<LLOQ			MF2
19	64	Pre	1	<LLOQ	< LLOQ	NA	MF2
20	64	Pre	1	<LLOQ			MF2
19	71	Pre	1	<LLOQ	< LLOQ	NA	MF2
20	71	Pre	1	<LLOQ			MF2

LLOQ: Lower Limit of Quantitation (25.0 ng/ml).

NA: Not Applicable.

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-32**

**Back-Calculated Concentrations of [Proprietary] in Group 3 (2.09 mg/kg [Proprietary Info] Male  
K<sub>2</sub> EDTA Dog Plasma Samples**

Animal Number	Day	Timepoint (Hours)	Dilution Factor	Concentration (ng/ml)	Mean Concentration (ng/ml)	SD	Analytical Run
21	1	Pre	1	<LLOQ	< LLOQ	NA	MF2
22	1	Pre	1	<LLOQ			MF2
23	1	Pre	1	<LLOQ			MF2
21	1	1	1	1780	2040	225	MF2
22	1	1	1	2200			MF2
23	1	1	1	2130			MF2
21	8	Pre	1	<LLOQ	< LLOQ	NA	MF2
22	8	Pre	1	<LLOQ			MF2
23	8	Pre	1	<LLOQ			MF2
21	8	1	1	1970	1920	342	MF2
22	8	1	1	2240			MF2
23	8	1	1	1560			MF2
21	15	Pre	1	<LLOQ	< LLOQ	NA	MF2
22	15	Pre	1	<LLOQ			MF2
23	15	Pre	1	<LLOQ			MF2
21	15	1	1	1920	1830	348	MF2
22	15	1	1	2130			MF2
23	15	1	1	1450			MF2
24	22	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	22	Pre	1	<LLOQ			MF3
24	29	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	29	Pre	1	<LLOQ			MF3
24	36	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	36	Pre	1	<LLOQ			MF3
24	43	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	43	Pre	1	<LLOQ			MF3
24	50	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	50	Pre	1	<LLOQ			MF3
24	57	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	57	Pre	1	<LLOQ			MF3
24	64	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	64	Pre	1	<LLOQ			MF3
24	71	Pre	1	<LLOQ	< LLOQ	NA	MF2
25	71	Pre	1	<LLOQ			MF3

LLOQ: Lower Limit of Quantitation (25.0 ng/ml).

NA: Not Applicable.

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-33**

**Back-Calculated Concentrations of [Proprietary] in Group 3 (2.09 mg/kg [Proprietary Info] Female  
K<sub>2</sub> EDTA Dog Plasma Samples**

Animal Number	Day	Timepoint (Hours)	Dilution Factor	Concentration (ng/ml)	Mean Concentration (ng/ml)	SD	Analytical Run
26	1	Pre	1	<LLOQ	< LLOQ	NA	MF3
27	1	Pre	1	<LLOQ			MF3
28	1	Pre	1	<LLOQ			MF3
26	1	1	1	240	1780	1340	MF3
27	1	1	1	2410			MF3
28	1	1	1	2690			MF3
26	8	Pre	1	<LLOQ	< LLOQ	NA	MF3
27	8	Pre	1	<LLOQ			MF3
28	8	Pre	1	<LLOQ			MF3
26	8	1	1	2770	2480	427	MF3
27	8	1	1	2680			MF3
28	8	1	1	1990			MF3
26	15	Pre	1	<LLOQ	< LLOQ	NA	MF3
27	15	Pre	1	<LLOQ			MF3
28	15	Pre	1	<LLOQ			MF3
26	15	1	1	1930	2130	178	MF3
27	15	1	1	2210			MF3
28	15	1	1	2260			MF3
29	22	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	22	Pre	1	<LLOQ			MF3
29	29	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	29	Pre	1	<LLOQ			MF3
29	36	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	36	Pre	1	<LLOQ			MF3
29	43	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	43	Pre	1	<LLOQ			MF3
29	50	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	50	Pre	1	<LLOQ			MF3
29	57	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	57	Pre	1	<LLOQ			MF3
29	64	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	64	Pre	1	<LLOQ			MF3
29	71	Pre	1	<LLOQ	< LLOQ	NA	MF3
30	71	Pre	1	<LLOQ			MF3

LLOQ: Lower Limit of Quantitation (25.0 ng/ml).

NA: Not Applicable.

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-34**

**Back-Calculated Concentrations of [Proprietary] in Group 4 (8.37 mg/kg [Proprietary Info] Male  
K<sub>2</sub> EDTA Dog Plasma Samples**

Animal Number	Day	Timepoint (Hours)	Dilution Factor	Concentration (ng/ml)	Mean Concentration (ng/ml)	SD	Analytical Run
31	1	Pre	1	<LLOQ	< LLOQ	NA	MF4
32	1	Pre	1	<LLOQ			MF4
33	1	Pre	1	<LLOQ			MF4
31	1	1	10	8700	9210	475	MF4
32	1	1	10	9290			MF4
33	1	1	10	9640			MF4
31	8	Pre	1	<LLOQ	< LLOQ	NA	MF4
32	8	Pre	1	<LLOQ			MF4
33	8	Pre	1	<LLOQ			MF4
31	8	1	10	7280	7910	544	MF4
32	8	1	10	8250			MF4
33	8	1	10	8190			MF4
31	15	Pre	1	309	238	62.6	MF4
32	15	Pre	1	191			MF4
33	15	Pre	1	214			MF4
31	15	1	10	8760	9050	365	MF4
32	15	1	10	8930			MF4
33	15	1	10	9460			MF4
34	22	Pre	1	249	300	NA	MF3
35	22	Pre	1	351			MF3
34	29	Pre	1	<LLOQ	< LLOQ	NA	MF3
35	29	Pre	1	<LLOQ			MF3
34	36	Pre	1	<LLOQ	< LLOQ	NA	MF3
35	36	Pre	1	<LLOQ			MF3
34	43	Pre	1	<LLOQ	< LLOQ	NA	MF3
35	43	Pre	1	<LLOQ			MF3
34	50	Pre	1	<LLOQ	< LLOQ	NA	MF3
35	50	Pre	1	<LLOQ			MF3
34	57	Pre	1	<LLOQ	< LLOQ	NA	MF3
35	57	Pre	1	<LLOQ			MF3
34	64	Pre	1	<LLOQ	< LLOQ	NA	MF3
35	64	Pre	1	<LLOQ			MF3
34	71	Pre	1	<LLOQ	< LLOQ	NA	MF3
35	71	Pre	1	<LLOQ			MF3

LLOQ: Lower Limit of Quantitation (25.0 ng/ml).

NA: Not Applicable.

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Table G-35**

**Back-Calculated Concentrations of [Proprietary] in Group 4 (8.37 mg/kg [Proprietary Info] Female  
K<sub>2</sub> EDTA Dog Plasma Samples**

Animal Number	Day	Timepoint (Hours)	Dilution Factor	Concentration (ng/ml)	Mean Concentration (ng/ml)	SD	Analytical Run
36	1	Pre	1	<LLOQ	< LLOQ	NA	MF4
37	1	Pre	1	<LLOQ			MF4
38	1	Pre	1	<LLOQ			MF4
36	1	1	10	11200	10900	643	MF4
37	1	1	10	11400			MF4
38	1	1	10	10200			MF4
36	8	Pre	1	<LLOQ	< LLOQ	NA	MF4
37	8	Pre	1	<LLOQ			MF4
38	8	Pre	1	<LLOQ			MF4
36	8	1	10	9030	9610	585	MF4
37	8	1	10	10200			MF4
38	8	1	10	9600			MF4
36	15	Pre	1	88.8	122	42.8	MF4
37	15	Pre	1	106			MF4
38	15	Pre	1	170			MF4
36	15	1	10	9740	10100	338	MF4
37	15	1	10	10200			MF4
38	15	1	10	10400			MF4
38	22	Pre	1	341	331	NA	MF4
40	22	Pre	1	320			MF4
38	29	Pre	1	<LLOQ	< LLOQ	NA	MF4
40	29	Pre	1	<LLOQ			MF4
38	36	Pre	1	<LLOQ	< LLOQ	NA	MF4
40	36	Pre	1	<LLOQ			MF4
38	43	Pre	1	<LLOQ	< LLOQ	NA	MF4
40	43	Pre	1	<LLOQ			MF4
38	50	Pre	1	<LLOQ	< LLOQ	NA	MF4
40	50	Pre	1	<LLOQ			MF4
38	57	Pre	1	<LLOQ	< LLOQ	NA	MF4
40	57	Pre	1	<LLOQ			MF4
38	64	Pre	1	<LLOQ	< LLOQ	NA	MF4
40	64	Pre	1	<LLOQ			MF4
38	71	Pre	1	<LLOQ	< LLOQ	NA	MF4
40	71	Pre	1	<LLOQ			MF4

LLOQ: Lower Limit of Quantitation (25.0 ng/ml).

NA: Not Applicable.



**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix G-3**

**BIOANALYTICAL SAMPLE ANALYSIS PLAN**

**Bioanalytical Sample Analysis Plan for the Analysis of  
Study Samples from SRI International Study Number  
M397-18 “GLP-Multiple (5 weekly) Repeat Subcutaneous  
Toxicity and Toxicokinetics Study with Proprietary Info Propri in  
Male and Female Beagle Dogs”**

Redacted by agreement

05/29/19

Date

5/29/2019

Date

## SCOPE

This sample analysis plan will be limited to the analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA dog plasma samples from SRI International Study M397-18 "GLP-Multiple (5 weekly) Repeat Subcutaneous Toxicity and Toxicokinetics Study with [Proprietary] [Pro] in Male and Female Beagle Dogs". [Proprietary] [Proprietary] and [Proprietary] will be extracted from dog plasma using 0.0200 ml sample volumes, using a protein precipitation extraction procedure followed by LC-MS/MS detection. The internal standards used in this assay are [Proprietary Info] [Proprietary Info] and [Proprietary Info]. This method was fully validated, with long term storage stability in matrix still ongoing at this time. Refer to SRI Report B185-18 for full details of this validation.

This sample analysis plan is based on SRI SOP 006.061, *Bioanalytical Sample Analysis*. Further details on the conduct of a typical study are described in this SOP.

## OBJECTIVE

The objective of this sample analysis plan is to describe the laboratory procedure used, the analytical curve range, the anticoagulant, matrix, and species used, the study samples, and the assay acceptance criteria. Following completion of analysis, a report will be written that will detail the assay performance and the results obtained from sample analysis.

## SUMMARY OF METHOD

Full details of the methodology used during the conduct of sample analysis will be detailed in SRI Test Method 106.201: *Analysis of* [Proprietary] [Proprietary] *and* [Proprietary] *in K<sub>2</sub> EDTA Dog Plasma*.

[Proprietary] [Proprietary] [Proprietary] and the internal standards are extracted from K<sub>2</sub> EDTA dog plasma, using 0.0200 ml sample volumes, using a protein precipitation procedure. Following centrifugation, the supernatant is then further diluted with water:methanol (90:10 v:v) with 0.1% acetic acid before analysis by high performance liquid chromatography with tandem mass spectrometric detection (LC-MS/MS).

## CALIBRATION STANDARD AND QUALITY CONTROL SAMPLES

The calibration standards (n=2 per analytical batch) contain the following concentrations of [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA dog plasma: 5.00, 10.0, 20.0, 50.0, 100, 200, 500, and 1000 ng/ml. These calibration standards also contain [Proprietary] at 25.0, 50.0, 100, 250, 500, 1000, 2500, and 5000 ng/ml. Calibration standards may be either freshly prepared on the day of analysis or prepared in advance and stored at ≤-60°C for no longer than 21 days, the period of matrix storage stability established to date in the validation study. Also included with each batch will be at least n=2 blank samples containing no analyte or

internal standard (BI/BI) and at least  $n=2$  blank samples containing no analyte but containing internal standard (BI/IS).

Quality Control (QC) samples contain the following concentrations of [Proprietary] and [Proprietary] in  $K_2$  EDTA dog plasma: 15.0 ng/ml (low), 400 ng/ml (mid), and 800 ng/ml (high). These calibration standards also contain [Proprietary] at 75.0 ng/ml (low), 2000 ng/ml (mid), and 4000 ng/ml (high) concentrations. These QC samples will be prepared in advance and stored in a  $\leq -60^\circ\text{C}$  freezer. A dilution QC, prepared at 5000 ng/ml [Proprietary] and [Proprietary] and 25000 ng/ml [Proprietary Info] has been successfully validated using a 10-fold and a 50-fold dilution. QC samples will be analyzed in at least  $n \geq 2$  replicates in each analytical batch. The dilution QC, if used, should be extracted in multiples of least  $n=3$ , although it is not necessary to extract a dilution QC in a batch where samples have been diluted providing that the dilution factor selected was previously validated. If a new dilution scheme is required, then this will be validated in replicates of 6.

## ACCEPTANCE CRITERIA

The linearity of the assay will be assessed by the correlation coefficient ( $r$ ) obtained from the linear regression analysis of the peak area ratios, with perfect fit of the data to the linear equation yielding an  $r$  value of 1.000. The minimum value for  $r$  for an assay to be acceptable is 0.990. A linear weighting of  $1/x$  will be applied to the [Proprietary] and [Proprietary] calibration curves, while a linear weighting of  $1/x^2$  will be applied to the [Proprietary] calibration curves.

In order for the calibration curve to be considered acceptable there will not be more than a 15% difference between the nominal and observed concentrations, except at the lower limit of quantitation (LLOQ) where a 20% deviation is permitted. At least 75% of the calibration standards will fulfill this criterion. Individual calibration standards which do not fulfill the criterion will be excluded from the regression.

For the QC samples to be considered acceptable, at least 50% of the individual replicates at each concentration must be within  $\pm 15\%$  of the nominal concentration, and at least 67% of the QCs in a batch must meet this acceptance criterion. For dilution QCs, at least 67% of the QCs at each validated dilution factor used must be within 15% of their nominal concentrations.

In all analytical batches, at least  $n=2$  samples containing no analyte or internal standard (BI/BI) will be extracted. Any peak detected at the retention time of the analyte should be less than 20% of the analyte mean peak area observed for the extracted samples at the LLOQ. Any peak detected at the retention time of the internal standard should be less than 5% of the mean peak area observed for the extracted samples containing the internal standard.



In all analytical batches, at least n=2 samples containing no analyte but with internal standard included (BI/IS) will be extracted to confirm the suitability of the internal standard for use in the assay at that concentration. Any peak detected at the retention time of the analyte as a result of the internal standard addition should be less than 20% of the analyte mean peak area observed for the extracted samples at the LLOQ.

### STABILITY INFORMATION

The following stability parameters were successfully established for all analytes during the validation study B185-18.

Room temperature stability in matrix	26 hours established
Freeze thaw stability in matrix	5 cycles established
Reinjection (autosampler) stability	188 hours (refrigerated) established
Post-preparative extract stability	85 hours (refrigerated) established
Whole blood processing stability	4 hours
Effect of hemolysis	0.5% and 2% hemolysis; no impact
Refrigerated stock stability	Ongoing
Long term matrix storage stability	21 days at $\leq -60^{\circ}\text{C}$ ; ongoing

### SAMPLE INFORMATION

The Bioanalytical Chemistry group at SRI received K<sub>2</sub> EDTA dog plasma samples from the Toxicology group on February 04, 2019. A total of 228 frozen samples were received and transferred to an ultra-low temperature freezer ( $\leq -60^{\circ}\text{C}$ ) upon arrival. A total of 228 samples will be analyzed per the study protocol.

### INCURRED SAMPLE REANALYSIS

Incurred sample reanalysis (ISR) will be performed during this study on at least 10% of study samples, or 21 samples, whichever number is greater. Per SRI SOP 006.062 *Bioanalytical Sample Reanalysis*, there must be a  $\leq 20\%$  difference between the two results in order for an ISR result to be considered comparable to the original result. At least 67% of the ISR samples must meet the acceptance criteria in order for the overall ISR evaluation to be considered successful.

### REFERENCES

- SRI Test Method 106.201: *Analysis of* [Proprietary] [Proprietary] *and* [Proprietary] *in K<sub>2</sub> EDTA Dog Plasma*
- SRI SOP 006.061: *Bioanalytical Sample Analysis*
- SRI SOP 006.062: *Bioanalytical Sample Reanalysis*
- SRI Report B185-18: Method Validation Report for the Quantitative Analysis of [Proprietary] [Proprietary] *and* [Proprietary] *in K<sub>2</sub> EDTA Dog Plasma*



**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix G-4**

**SRI TEST METHOD 106.201: “ANALYSIS OF [Proprietary Info] [Proprietary Info] AND  
[Proprietary Info] IN K<sub>2</sub> EDTA DOG PLASMA”**

**TEST METHOD**

**Classification:** Project  
**Supersedes:** 106.201 (06/12/19)

**TM No.:** 106.201**Page:** 1 of 26**Effective:** JUL 12 2019

**Subject:** Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma

**A. PURPOSE/SCOPE**

This Test Method describes procedures to be employed for the analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA dog plasma using a protein precipitation extraction procedure and analysis by LC-MS/MS.

During sample analysis, SRI SOPs 006.061 *Bioanalytical Sample Analysis* and 006.062 *Bioanalytical Sample Reanalysis* will also be followed.

**B. BACKGROUND/GENERAL**

This Test Method will fully detail the experimental procedures used in the analysis, detection and quantitation of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA dog plasma. To summarize, [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA dog plasma (0.0200 ml sample size) will be extracted, using [Proprietary Info] [Proprietary Info] and [Proprietary Info] as the internal standards, by a protein precipitation extraction procedure. The supernatant is then diluted prior to injection on the LC-MS/MS system. The range of the assay is 5.00 – 1000 ng/ml [Proprietary] and [Proprietary] and 25.0 – 5000 ng/ml [Proprietary Info]

**SIGNATURES**

Revised by:

Reviewed by:

Management Approval:

QAU Review:

Redacted by agreement

07/11/19

Date

07/11/19

Date


07/11/19

Date

07/12/2019

Date

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	<b>Subject:</b> Analysis of [Proprietary] [Proprietary] and [Proprietary] in K <sub>2</sub> EDTA Dog Plasma	

## C. HEALTH AND SAFETY

All personnel must observe standard laboratory safety practices. All personnel must wear protective equipment appropriate to the area in which they will work, which may include, but not be limited to: safety glasses, protective clothing and gloves.

## D. TRAINING

All personnel involved in handling chemicals, equipment, and instruments must have attended the pertinent laboratory safety classes from SRI's Environmental Health & Safety Department and must have attended GLP training courses. Training must be documented.

## E. EQUIPMENT AND MATERIALS

Chemicals, consumables or equipment may be substituted provided that equivalent assay performance is obtained.


### E.1 Chemicals

- [Proprietary] [Proprietary] and [Proprietary] USP, Current Lot
- [Proprietary Info] [Proprietary Info] and [Proprietary Info] Medical Isotopes, Inc.
- Ammonium hydroxide, reagent grade
- Acetic acid, LC-MS grade
- Milli-Q water, Millipore
- Formic acid, reagent grade
- Dimethyl Sulfoxide (DMSO), reagent grade
- Methanol, HPLC grade
- Acetonitrile, HPLC grade
- Isopropanol, reagent grade
- K<sub>2</sub> EDTA beagle dog plasma, BioIVT

### E.2 Consumables

- HPLC column: Phenomenex Synergi Polar RP 100 x 2mm, 4µm
- 0.5 µm stainless-steel pre-column frit (Upchurch Scientific)
- Assorted disposable pipette tips

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	<b>Subject:</b> Analysis of <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> and <span style="border: 1px solid black; padding: 0 5px;">Proprietary</span> in K <sub>2</sub> EDTA Dog Plasma	

- Disposable 1.5 ml and 2.0 ml polypropylene microcentrifuge tubes
- Disposable 15 ml conical polypropylene test tubes and caps
- Disposable glass vials and caps, assorted sizes
- Glass autosampler vials with inserts and caps

### E.3 Equipment

- Air displacement pipettor, Rainin
- Positive displacement pipettor, Gilson
- Repeater pipettor, Eppendorf
- Mettler Toledo AG 285 balance
- VWR Mini Vortexer
- Beckman Coulter Microfuge® 18 Centrifuge, 20 Centrifuge
- Shimadzu Corp. LC-20AD Prominence Pumps (incorporates Shimadzu Corp. CBM-20A Prominence Communications Bus Module and Shimadzu DGU-20A<sub>3R</sub> Prominence degasser
- Shimadzu Corp. CTO-20AC Prominence Column Oven
- CTC Analytics HTS-xt Autosampler
- AB Sciex 5500 Mass Spectrometer

## F. PROCEDURES

Note: SRI Forms 106.201A through 106.201E will be used to assist in raw data recording for the experimental phases of this study. The completed attachments or other documentation must be stored in the study file.

### F.1 Preparation of Reagents

Volumes of these reagents can be adjusted as long as proportionality is maintained and their preparation is documented in the raw data.

#### F.1.1 2% Acetic Acid in Water (Mobile Phase A)

Add 20.0 ml of acetic acid to 1000 ml of Milli-Q water in a glass bottle and store the solution at room temperature. The expiration date for this solution is one month after preparation.

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**TEST METHOD****Classification:** Project**Supersedes:** 106.201 (06/12/19)**Subject:** Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**TM No.:** 106.201**Page:** 4 of 26**Effective:** July 12, 2019**F.1.2 0.1% Acetic Acid in Acetonitrile (Mobile Phase B)**

Add 1.00 ml of acetic acid to 1000 ml of acetonitrile in a glass bottle and store the solution at room temperature. The expiration date for this solution is one month after preparation.

**F.1.3 Acetonitrile : Isopropanol (80:20 v:v) with 1% Ammonium Hydroxide (Needle Rinse 1)**

Add 400 ml acetonitrile to 100 ml isopropanol and 5.00 ml ammonium hydroxide in a glass bottle and store the solution at room temperature. The expiration date for this solution is one month after preparation.

**F.1.4 Water : Methanol (90:10 v:v) with 1% Formic Acid (Needle Rinse 2)**

Add 450 ml Milli-Q water to 50.0 ml methanol and 5.00 ml formic acid in a glass bottle and store the solution at room temperature. The expiration date for this solution is one month after preparation.

**F.1.5 0.2% Acetic Acid in Methanol (Diluent Solution)**

Add 0.400 ml acetic acid to 200 ml methanol in a glass bottle and store the solution at room temperature. The expiration date for this solution is one month after preparation.

**F.1.6 Water : Methanol (90:10 v:v) with 0.1% Acetic Acid (Reconstitution Solution)**


Add 180 ml Milli-Q water to 20.0 ml methanol and 0.200 ml acetic acid in a glass bottle and store the solution at room temperature. The expiration date for this solution is one month after preparation.

**F.2 Preparation of Stock and Spiking Solutions**

The following standard preparation scheme is a suggested approach. Appropriate modifications to reach the targeted nominal calibrant and quality control (QC) standard concentrations are acceptable. For example, if the targeted nominal concentration is not achieved when the analyte calibration standard primary stock solution is obtained, the volume of this stock solution used in subsequent dilutions can be modified in order to achieve the targeted nominal calibration standard matrix concentrations. The actual volumes of standards used will be documented in the raw data. Volumes of these stock solutions can be adjusted as long as proportionality is maintained and their preparation is documented in the study binder.

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Analytical reference standards are corrected for purity, water and salt content, if applicable. Internal standard stocks may not be corrected for purity, water or salt.

Example purity calculation:

$$\text{Purity} = \frac{[\text{HPLC \% purity} \times (100 - \% \text{ water} - \% \text{ residual solvent})]}{100} \times \frac{\text{Free base molecular weight}}{\text{Salt form molecular weight}}$$

#### F.2.1 Preparation of [Proprietary] [Proprietary] and [Proprietary] Stock Solutions (1.00 mg/ml)

Accurately weigh out approximately 5.00 mg of [Proprietary] into a glass vial and dilute to a concentration of 1.00 mg/ml using dimethyl sulfoxide (DMSO). The purity of the compound must be taken into account when preparing this stock (Stock A). Repeat this step to produce a second stock solution at the same concentration (Stock B). Repeat to get duplicate weighings for [Proprietary] at the same concentration.

To prepare [Proprietary] stock solutions, weigh approximately 5.00 mg of [Proprietary] into a glass vial and dilute to a concentration of 1.00 mg/ml using Milli-Q water. Repeat this step to produce a second stock solution at the same concentration.

Store all stock solutions refrigerated (set point 5°C ± 3°C) until use.

#### F.2.2 Preparation of [Proprietary Info] [Proprietary Info] and [Proprietary Info] Stock Solutions (Internal Standard), 1.00 mg/ml

These Internal Standards are supplied by Medical Isotopes, Inc. as 1.00 mg amounts in a glass vial. Add 1.00 ml of DMSO to the [Proprietary Info] and [Proprietary Info] to produce a 1.00 mg/ml stock. Repeat for [Proprietary Info] using Milli-Q water instead of DMSO.

Store all internal standard stock solutions refrigerated (set point 5°C ± 3°C) until use.

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**TEST METHOD****Classification:** Project**Supersedes:** 106.201 (06/12/19)**Subject:** Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**TM No.:** 106.201**Page:** 6 of 26**Effective:** July 12, 2019**F.2.3** Preparation of [Proprietary Info] [Proprietary Info] and [Proprietary Info] Internal Standard Secondary Stock

Accurately add 0.0100 ml of the 1.00 mg/ml [Proprietary Info] and the d<sub>6</sub>-[Proprietary] internal standard stock solutions, and 0.0500 ml of the 1.00 mg/ml [Proprietary Info] internal standard stock solution into a glass vial containing 9.930 ml of 0.2% acetic acid in methanol. The final concentration of the internal standard secondary stock solution will be 1.00 µg/ml [Proprietary Info] and [Proprietary Info] and 5.00 µg/ml [Proprietary Info]. This solution can be stored refrigerated (set point 5°C ± 3°C) until use.

**F.2.4** Preparation of [Proprietary Info] [Proprietary Info] and [Proprietary Info] Internal Standard Spiking Solution

Accurately add 2.50 ml of the internal standard secondary stock solution into a glass bottle containing 97.5 ml of 0.2% acetic acid in methanol. The final concentration of the internal standard spiking solution will be 25.0 ng/ml [Proprietary Info] and [Proprietary Info] and 125 ng/ml [Proprietary Info]. This solution can be stored refrigerated (set point 5°C ± 3°C) until use.

Per SRI SOP 006.063, *Reference Material Receipt and Stock, Spiking Solution and Calibration and Quality Control Sample Preparation / Expiration* internal standard stock and spiking solutions will be given a default expiration date of 6 months after preparation.

**F.2.5** Stock Verification

In order to determine the accuracy of preparation, the duplicate stock solutions will be verified prior to use. A suggested approach for the preparation of stock verification solutions is given here, although alternative final concentrations may be used providing that a suitable analyte and internal standard response is achieved. The duplicate stock solutions prepared in step F.2.1 should be diluted by spiking 0.0100 ml of the 1.00 mg/ml [Proprietary] and [Proprietary] stock solutions and 0.0500 ml of the 1.00 mg/ml [Proprietary] stock solutions into 9.930 ml of Diluent Solution. These duplicate vials are briefly vortexed and 0.100 ml is removed and added to a vial containing 0.900 ml Diluent Solution. Vortex, then remove 0.0325 ml of this solution and place into a separate vial containing 0.0203 ml of internal standard spiking solution and 0.947 ml of Reconstitution Solution. The final concentration of [Proprietary] and [Proprietary] is 3.25 ng/ml and the final concentration of [Proprietary] is 16.3 ng/ml. The final concentration of [Proprietary Info] and [Proprietary Info] is 0.508

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ng/ml and the final concentration of [Proprietary Info] is 2.54 ng/ml. The duplicate samples are injected in replicates of  $n \geq 3$  onto the LC-MS/MS system.

To be considered acceptable for use, the stocks must agree to within 5% of each other, calculated as follows:

% difference =

$$\frac{(\text{Mean of peak area ratio of stock A} - \text{Mean of peak area ratio of stock B})}{(\text{Mean of peak area ratio of stock A} + \text{Mean of peak area ratio of stock B})/2} \times 100$$

If these stocks agree within 5% of each other, one single stock may be used for the preparation of both calibrants and Quality Control (QC) samples. Alternatively, one stock may be used for the preparation of calibrants while the other stock can be used for the preparation of QC samples.

If these stocks do not agree, a third weighing may be performed and the three stocks compared against each other. If two stocks agree with each other these may be used to prepare calibrants and QCs, and the other stock can be discarded.

## F.2.6 Test Mix Preparation

A solution prepared at the LLOQ level (or below Low QC concentration) shall be prepared and injected at the start and the end of each bioanalytical run (system suitability). This solution will contain both analyte and internal standard. To prepare at the LLOQ level, spike 0.0100 ml of the 1.00 mg/ml [Proprietary] and [Proprietary] stock solutions and 0.0500 ml of the 1.00 mg/ml [Proprietary] stock solution into 9.930 ml of Diluent Solution. Vortex remove 0.100 ml and add to a vial containing 0.900 ml of Diluent Solution. Vortex remove 0.0100 ml and add to a vial containing 0.990 ml of Diluent Solution. This solution may be stored refrigerated for up to 3 months from the date of preparation. On the day of use, remove 0.0203 ml of this solution and place into a separate vial containing 0.0203 ml of internal standard spiking solution and 0.959 ml of Reconstitution Solution. The final concentration of [Proprietary] and [Proprietary] is 0.0203 ng/ml and the final concentration of [Proprietary] is 0.102 ng/ml. The final concentration of [Proprietary Info] and [Proprietary Info] is 0.508 ng/ml and the final concentration of [Proprietary Info] is 2.54 ng/ml. The final concentrations mimic the final theoretical concentrations of [Proprietary] [Proprietary] and [Proprietary] and internal standards seen in LLOQ samples post-extraction.

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**F.2.7** Remove approximately 10.0 ml of control K<sub>2</sub> EDTA dog plasma from storage and allow it to equilibrate to room temperature. The matrix may be centrifuged at approximately 3000 RPM for 10 minutes prior to use to remove excess particulates. Prepare QC samples in polypropylene vials as shown in the table below. Note that the Solution Spiking Volumes are combined with the Matrix Volumes. Volumes of these QC samples can be adjusted as long as proportionality is maintained and their preparation is properly documented in the study raw data.

Quality Control Sample Preparation						
QC ID	Spiking Solution ID	Spiking Solution Concentration (µg/ml)	Spiking Volume (ml)	Matrix Volume (ml)	Final Volume (ml)	Nominal Matrix Concentration (ng/ml)
QC- Low	QC-Mid	0.400 / 0.400 / 2.00	0.0375	0.9625	1.00	15.0 / 15.0 / 75.0
QC- Mid	QC-Dil	5.00 / 5.00 / 25.0	0.0800	0.920	1.00	400 / 400 / 2000
QC- High	QC-Dil	5.00 / 5.00 / 25.0	0.160	0.840	1.00	800 / 800 / 4000
QC- Dil	[Proprietary] Stock A/B;	1000	0.0100	1.930	2.00	5000
	[Proprietary] Stock A/B;	1000	0.0100			5000
	[Proprietary] Stock A/B	1000	0.0500			25000

Either Stock A or Stock B may be used, assuming equivalency is achieved.

The values in the “Spiking Solution Concentration” and the “Nominal Matrix Concentration” columns represent the concentrations of [Proprietary] [Proprietary] and [Proprietary] respectively.


These QC samples may be aliquoted into appropriate volumes into polypropylene tubes (suggested 150 µl volumes) and stored in a ≤-60°C freezer until use, providing that sufficient stability in matrix has been successfully validated under these conditions. QC samples may also be freshly prepared on the day of extraction.

**F.3 Extraction Procedure**

Each bioanalytical run will be comprised of bracketing calibration curves (8 points) each with a matrix blank sample (matrix with neither analyte nor internal standard spiked) and a control blank (matrix with only internal standard included). It is recommended that a carryover blank (matrix blank) be injected after each upper limit of quantitation (ULOQ) calibration standard to assess any carryover present. Duplicate System Suitability samples will be injected, one before the first calibration curve and one at the end of the batch. Interspersed between the calibration curves will be  $n \geq 2$  QC samples at low, mid and high concentration.

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The dilution QC, when used, should be extracted in multiples of n=3. It is not required to run the dilution QC with a batch where samples are diluted, provided that the level of sample dilution did not exceed what was previously validated. However, for troubleshooting practices, the dilution QC may be analyzed with each batch, at the discretion of the bioanalytical scientist. If study samples are diluted with multiple dilution factors, then the dilution QC may be similarly diluted (with replicates of n=3 for each dilution factor used), or alternatively, the highest dilution factor used for study samples will be used for the extraction of the dilution QC. It is also acceptable if low dilution factors are applied to study samples to use a high QC in place of the dilution QC, in order that the diluted sample falls within the calibration range. If the level of dilution required for study samples exceeds the dilution factor previously validated, then the dilution QC will need to be revalidated at the dilution factor required, in replicates of 6.

In order to facilitate the equilibration of the instrument, multiple injections of extracts (Conditioning Samples) may be injected before each batch. It is recommended to prepare conditioning samples near the LLOQ level, but providing that internal standard is present in the sample, the actual concentration used may change. Conditioning samples will be pooled, where more than one calibration standard or QC sample, at different concentrations, are combined. Individual study samples, calibration standards, and QC samples will not be used as conditioning samples without pooling. It is not acceptable to condition a batch using an old standard curve from a previous batch. It is acceptable to prepare either multiple pooled conditioning samples, or to re-inject the pooled sample from the same vial, depending on the final extract volume. The conditioning injections will be included as part of the analytical batch and will be printed with the rest of the batch. Approximately 10 conditioning injections will be analyzed prior to the start of each batch. If one batch is analyzed immediately following another, later batches may not require conditioning injections, although duplicate system suitability injections will be included.

Remove the calibration standard spiking solutions, internal standard spiking solution, QC samples and control matrix (approximately 10.0 ml) from storage and allow them to equilibrate to room temperature.

Follow the scheme listed below to prepare the calibration standards. The calibration standards are prepared in polypropylene tubes. Note that the Spiking Solution Volumes are combined with the Matrix Volumes.

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## Preparation of Calibration Standards in Matrix

Calibration Standard ID	Spiking Solution ID	Spiking Solution Concentration (µg/ml)	Spiking Volume (ml)	Matrix Volume (ml)	Final Volume (ml)	Nominal Matrix Concentration (ng/ml)
Std-1	Std-5	0.100 / 0.100 / 0.500	0.0250	0.475	0.500	5.00 / 5.00 / 25.0
Std-2	Std-5	0.100 / 0.100 / 0.500	0.0500	0.450	0.500	10.0 / 10.0 / 50.0
Std-3	Std-5	0.100 / 0.100 / 0.500	0.100	0.400	0.500	20.0 / 20.0 / 100
Std-4	Std-8	1.00 / 1.00 / 5.00	0.0250	0.475	0.500	50.0 / 50.0 / 250
Std-5	Std-8	1.00 / 1.00 / 5.00	0.0500	0.450	0.500	100 / 100 / 500
Std-6	Std-8	1.00 / 1.00 / 5.00	0.100	0.400	0.500	200 / 200 / 1000
Std-7	Std-9	10.0 / 10.0 / 50.0	0.0250	0.475	0.500	500 / 500 / 2500
Std-8	Std-9	10.0 / 10.0 / 50.0	0.0500	0.450	0.500	1000 / 1000 / 5000
Std-9	[Proprietary] Stock A/B;	1000	0.0100	0.930	1.00	10000
	[Proprietary] Stock A/B;	1000	0.0100			10000
	[Proprietary] Stock A/B	1000	0.0500			50000

Std-9 is only used to prepared the standard curve, and is not extracted.

Either Stock A or Stock B may be used, assuming equivalency is achieved.

The values in the “Spiking Solution Concentration” and the “Nominal Matrix Concentration” columns represent the concentrations of [Proprietary] [Proprietary] and [Proprietary] respectively.

These calibration standards may be aliquoted into appropriate volumes into polypropylene tubes (suggested 150 µl volumes) and stored in a ≤-60°C freezer until use, providing that sufficient stability in matrix has been successfully validated under these conditions. Calibration standards may also be freshly prepared on the day of extraction.

- F.3.1** Transfer 0.0200 ml of each calibration standard, QC sample, study sample and blank into separate 1.50 ml microcentrifuge tubes. If needed, extra samples may be extracted in order to be used as Conditioning Samples. These should be pooled before use.
- F.3.2** Add 0.100 ml of 0.2% acetic acid in methanol to the matrix blanks. Cap tubes and vortex for approximately 5 seconds.
- F.3.3** Add 0.100 ml of the Internal Standard Spiking Solution to each calibration standard, QC standard, study sample and control blank. Cap tubes and vortex for approximately 5 seconds.
- F.3.4** Centrifuge tubes at approximately 18000g for approximately 10 minutes.
- F.3.5** Transfer 0.0250 ml of the supernatant into a 2.00 ml HPLC vial containing 1.00 ml Reconstitution Solution. Cap and vortex briefly to mix.

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**F.3.6** Store on the autosampler (set point 5°C ± 3°C) or refrigerated (set point 5°C ± 3°C).

**F.4 Analytical Conditions**

Equipment can be substituted provided that equivalent assay performance is obtained.

Refer to Figures 1-3 in this Test Method for an example of a representative chromatogram for each analyte at the LLOQ level.

**F.4.1 HPLC Conditions**

Autosampler:	CTC Analytics HTS-xt
Pumps:	Shimadzu LC-20AD Prominence. Incorporates Shimadzu CBM-20A Prominence communications bus module and Shimadzu DGU-20A <sub>3R</sub> Prominence degasser
Column Oven:	Shimadzu CTO-20AC Prominence
Autosampler Temp:	Set point 5°C
Column Oven Temp:	Set point 25°C
Column:	Phenomenex Synergi Polar RP 100 x 2 mm, 4µ
Pre-column Frit:	0.5 µm stainless-steel Precolumn Frit (Upchurch Scientific)
Flow Rate:	0.350 ml/min
Run Time:	8.0 minutes
Injection Volume:	10 µl*
Mobile Phase A:	2% acetic acid in water
Mobile Phase B:	0.1% acetic acid in acetonitrile
Needle Rinse 1:	Acetonitrile:isopropanol (80:20 v:v) with 1% ammonium hydroxide
Needle Rinse 2:	Water:methanol (90:10 v:v) with 1% formic acid
Pre clean with Needle	1*, 1*
Rinses 1 and 2:	
Post clean with Needle	2*, 2*
Rinse 1 and 2:	
Valve clean with	2*, 2*
Needle Rinse 1 and 2:	
Retention Time:	4.3 minutes [Proprietary] and IS)
	4.2 minutes [Proprietary] and IS)
	1.4 minutes [Proprietary] and IS)

\* may be modified to improve performance

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**TEST METHOD****Classification:** Project**Supersedes:** 106.201 (06/12/19)**Subject:** Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**TM No.:** 106.201**Page:** 12 of 26**Effective:** July 12, 2019**F.4.2 LC Program**

Time (min)	% A	% B
0.01	98	2
2.00	98	2
2.10	50	50
4.00	2	98
5.50	2	98
5.51	98	2
8.00	98	2

## Switching Valve program

Total Time (minutes)	Position
0.0 to 0.2*	Divert to Waste
0.2 to 5.0*	Divert to MS
5.0 to 8.0*	Divert to Waste

\* may be modified to improve performance

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**TEST METHOD****Classification:** Project**Supersedes:** 106.201 (06/12/19)**Subject:** Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma**TM No.:** 106.201**Page:** 13 of 26**Effective:** July 12, 2019**F.4.3 MS/MRM Conditions**

Mass Spectrometer: AB Sciex 5500 Mass Spectrometer  
Interface: Turbo IonSpray positive-ion mode  
Scan Mode: Multiple Reaction Monitoring (MRM)  
IS: 5500V\*  
EP: 10V\*  
DP: 71V\* [Proprietary Info] 81V\* [Proprietary Info]  
121V\* [Proprietary Info] 76V\* [Proprietary Info]  
76V\* [Proprietary Info] 56V\* [Proprietary Info]  
CE: 21V\* [Proprietary Info] 19V\* [Proprietary Info]  
25V\* [Proprietary Info] 25V\* [Proprietary Info]  
33V\* [Proprietary Info] 35V\* [Proprietary Info]  
  
CXP: 38V\* [Proprietary Info] 20V\* [Proprietary Info]  
26V\* [Proprietary Info] 24V\* [Proprietary Info]  
14V\* [Proprietary Info] 14V\* [Proprietary Info]  
  
Resolution Q1, Q3: Unit, Unit  
CUR Gas: 20\*  
CAD Gas: 8\*  
GS1: 60\*  
GS2: 60\*  
Source Temp: 650°C\*  
Dwell: 80\* ms  
  
Nominal [Proprietary] m/z 629.3\* → 447.2\*  
Transitions: [Proprietary] m/z 721.3\* → 296.1\*  
[Proprietary] m/z 288.1\* → 176.1\*  
[Proprietary Info] m/z 637.4\* → 447.2\*  
[Proprietary Info] m/z 727.4\* → 302.2\*  
[Proprietary Info] m/z 294.1\* → 182.1\*

May be modified to improve performance. The eventual m/z ratios used must be within  $\pm 0.3$  amu from the masses quoted above.)

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## F.5 Calculations

**F.5.1** Chromatograms will be automatically integrated using AB Sciex Analyst software (Version 1.6.2) or equivalent and visually inspected for an acceptable integration.

**F.5.2** Compute the  $1/x$  weighted least-squares linear regression [Proprietary] and [Proprietary] and the  $1/x^2$  weighted least-squares linear regression [Proprietary Info] using Analyst software, relating the peak area ratios (relative to internal standard) of the calibration standards to their respective nominal concentrations (ng/ml in plasma) for [Proprietary] [Proprietary] and [Proprietary]

**F.5.3** Using the peak area ratios (relative to the internal standard) of the standards and the regression equation constants, concentrations for analyte in the QC samples and study samples can be interpolated.

**F.5.4** Compute the correlation coefficient for the standard data.

## F.6 Acceptance Criteria

### F.6.1 System Suitability Standard

There are no formal acceptance criteria for the System Suitability samples. The system suitability sample will be injected at the beginning and at the end of a run and inspected to ensure signal-to-noise ratio and peak shape are adequate for quantitation. Any chromatographic change between these injections which may have an impact on the ability to accurately quantitate the samples will be noted, however there is no formal acceptance criteria for this. The system suitability injections will be printed with the other chromatograms in the analytical batch.

### F.6.2 Calibration Standard Acceptance Criteria


**F.6.2.1** The lower limit of quantitation (LLOQ) standard back-calculated concentration must be within  $\pm 20\%$  of theoretical nominal concentration.

**F.6.2.2** To meet acceptance criteria, the back-calculated concentration of a calibration standards (excluding at the LLOQ level) must be within 15% of their nominal theoretical concentrations.

**F.6.2.3** A minimum of three-quarters of calibration standards must meet these criteria.

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**F.6.2.4** Any standards failing to meet the acceptance criteria will be excluded from the regression, starting with the calibration standard which is furthest away from the nominal concentration.

**F.6.3** Quality Control (QC) Sample Acceptance Criteria.

**F.6.3.1** To meet acceptance criteria, the back-calculated concentration of a QC sample must be within 15% of their nominal theoretical concentrations.

**F.6.3.2** At least two-thirds of all assay QCs (low, mid and high) must meet the acceptance criteria.

**F.6.3.3** At least 50% of the QCs at each level must meet the acceptance criteria.

**F.6.3.4** For dilution QCs, which are generally assayed using multiples of n=3 replicates, at least 67% (rounded) of the QCs must be within 15% of their nominal theoretical concentrations. Failure of a dilution QC does not mean that the batch itself has failed if the low, mid and high QCs meet acceptance criteria as defined above. However, any samples diluted in a batch with a failed dilution QC should be repeated and the value from this batch discarded. If more than one dilution scheme was followed in a batch of samples, with corresponding dilution QCs prepared using different dilution factors, only the dilution QC which failed acceptance criteria will be rejected and the associated samples repeated.


**F.6.4** Blank Acceptance Criteria

At least 50% of matrix blanks (including carryover blanks, BI/BI) and 50% of control blanks (BI/IS) must have a response (peak area) less than or equal to 20% of the mean accepted LLOQ calibration standards. Carryover blanks should be positioned in the run in a manner capable of determining assay carryover, for example, after each ULOQ calibration standard injection.

**F.7** **Data Reporting**

Concentrations found below the lowest calibration standard concentration, will be reported as below the quantitation limit (<LLOQ). Where no peak is detected (ND), the result will be flagged as (<LLOQ). Over-diluted samples falling below

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the calibration range (assuming insufficient to reassay) will be reported as <LLOQ (LLOQ value x dilution factor).

## G. STABILITY/METHOD PARAMETERS

### G.1 Solutions

<u>Parameter Evaluated</u>	<u>Validated Result</u>	<u>Study Reference</u>
Analyte Stability in Stock Solutions at 5±3°C:	40 days	B185-18

### G.2 Matrix, K<sub>2</sub> EDTA Dog Plasma

<u>Parameter Evaluated</u>	<u>Validated Result</u>	<u>Study Reference</u>
Room Temperature Stability in Matrix:	26 hours	B185-18
Freeze/Thaw Stability in Matrix:	5 cycles	B185-18
Re-injection Stability:	188 hours, refrigerated	B185-18
Post Preparative Extract Stability:	85 hours, refrigerated	B185-18
Validated Dilution Factor:	10-fold, 50-fold	B185-18
Long-Term Stability in Matrix at ≤-60°C:	21 days	B185-18
Effect of 2% Hemolysis:	No impact	B185-18
Whole Blood Stability:	4 hours, refrigerated	B185-18
Maximum Batch Size:	93 samples	B185-18
Incurred Sample Reanalysis:	Successful	M397-18

## H. REFERENCES

- H.1** B185-18: "Method Validation Report for the Quantitative Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma"
- H.2** M397-18: GLP-Multiple (5 weekly) Repeat Subcutaneous Toxicity and Toxicokinetics Study with [Proprietary] [Pro] in Male and Female Beagle Dogs
- H.3** SRI SOP 006.061, *Bioanalytical Sample Analysis*
- H.4** SRI SOP 006.062, *Bioanalytical Sample Reanalysis*
- H.5** SRI SOP 006.063, *Reference Material Receipt and Stock, Spiking Solution and Calibration and Quality Control Sample Preparation / Expiration*

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**Subject:** Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma

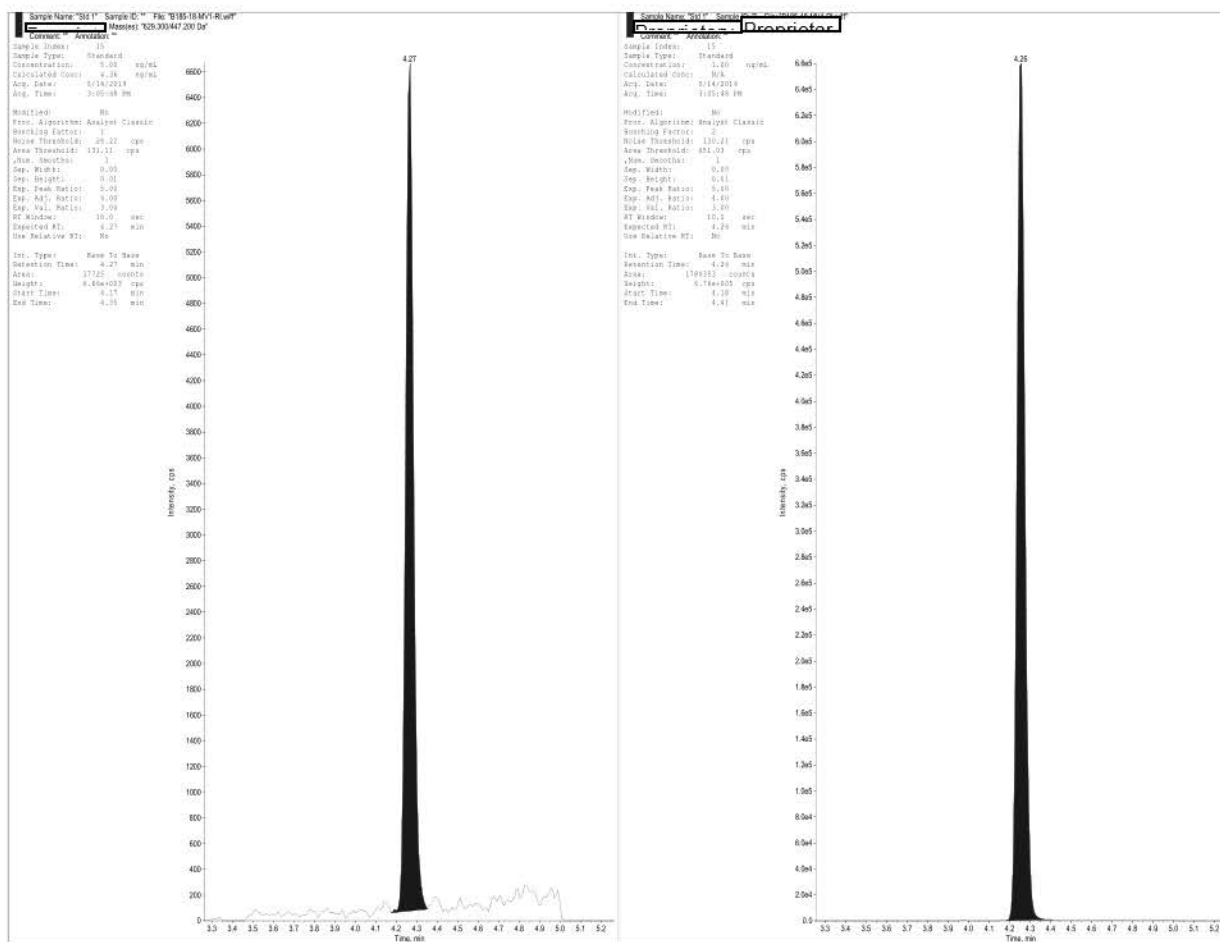
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## I. FIGURES

**I.1** Figure 1. Representative [Proprietary] Chromatogram of a K<sub>2</sub> EDTA Dog Plasma Sample Spiked at the Lower Limit of Quantitation (LLOQ)



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## TEST METHOD

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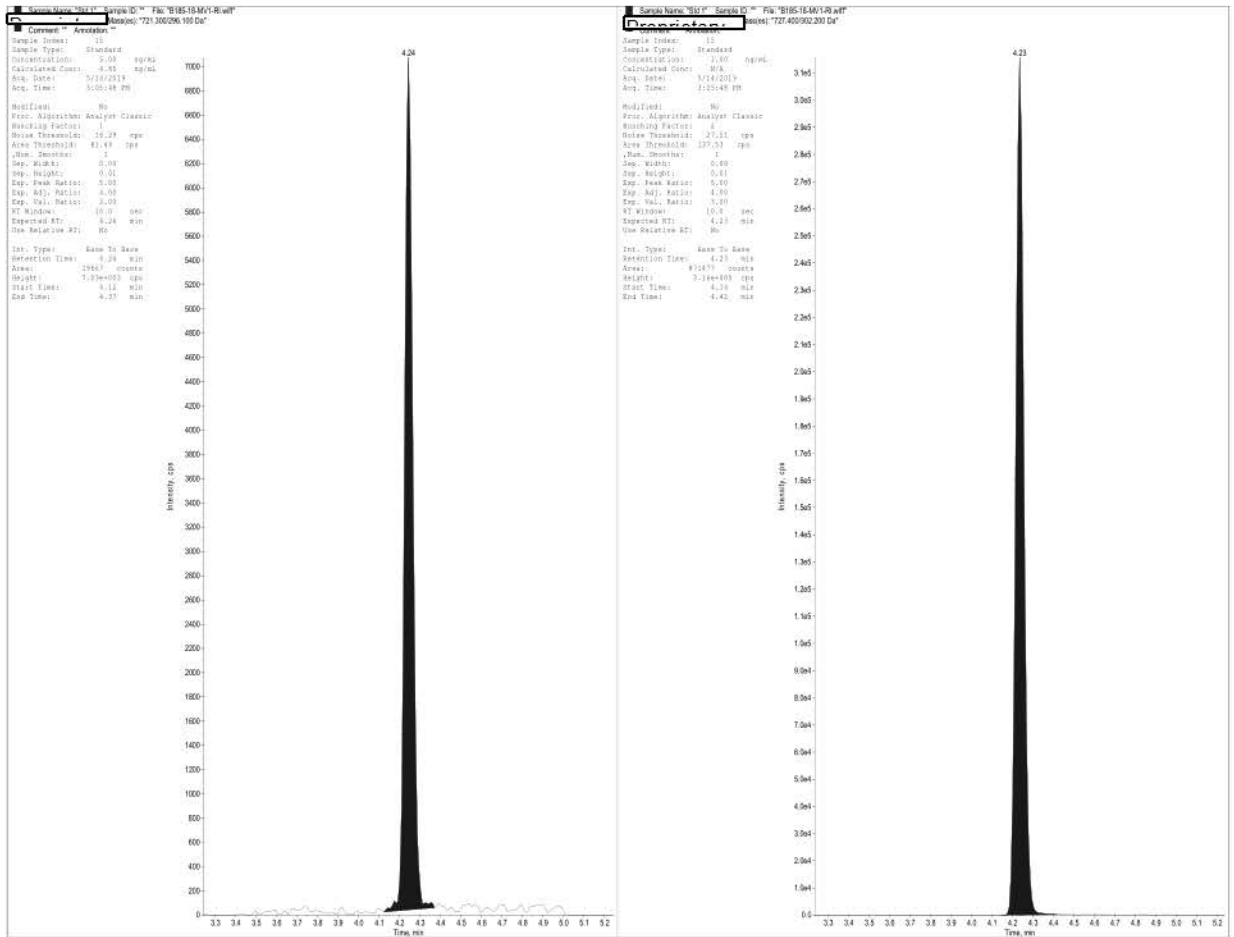
**Subject:** Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma

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**I.2** Figure 2. Representative [Proprietary] Chromatogram of a K<sub>2</sub> EDTA Dog Plasma Sample Spiked at the Lower Limit of Quantitation (LLOQ)



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## TEST METHOD

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**Supersedes:** 106.201 (06/12/19)

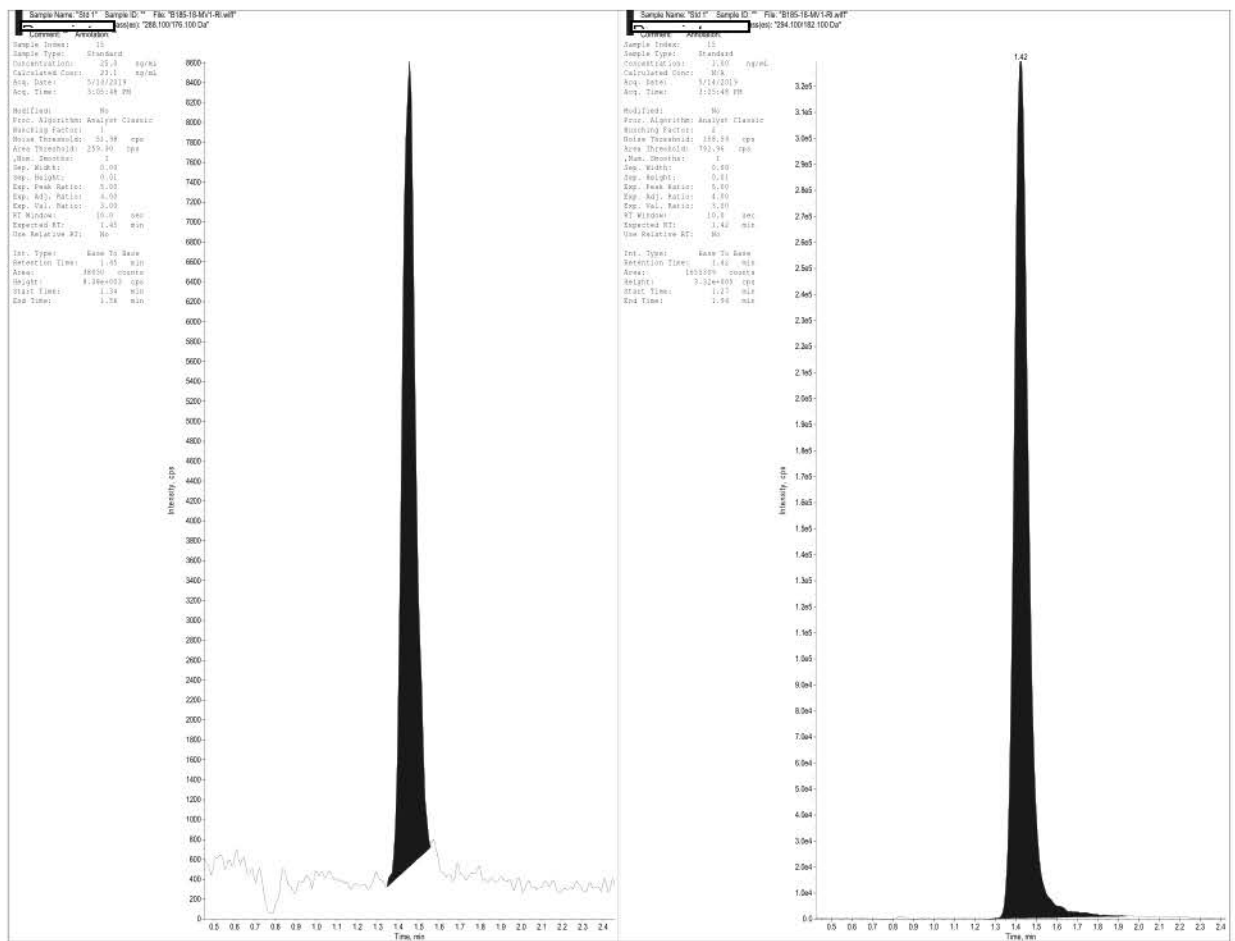
**Subject:** Analysis of [Proprietary] [Proprietary] and [Proprietary] in K<sub>2</sub> EDTA Dog Plasma

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**I.3** Figure 3. Representative [Proprietary] Chromatogram of a K<sub>2</sub> EDTA Dog Plasma Sample Spiked at the Lower Limit of Quantitation (LLOQ)



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## J. ATTACHMENTS

- J.1** Extraction Form (SRI Form 106.201A)
- J.2** Methodology and Reagent List (SRI Form 106.201B)
- J.3** Instrument Analytical Conditions and Reagents (SRI Form 106.201C)
- J.4** Instrument Reagent Preparation (SRI Form 106.201D)
- J.5** Extraction Reagent Preparation (SRI Form 106.201E)

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Study ID:

Batch ID:

**EXTRACTION FORM**

Preparation of Calibration Standards in Matrix						
Calibration Standard ID	Spiking Solution ID	Spiking Solution Concentration (µg/ml)	Spiking Volume (ml)	Matrix Volume (ml)	Final Volume (ml)	Nominal Matrix Concentration (ng/ml)
Std-1	Std-5	0.100 / 0.100 / 0.500	0.0250	0.475	0.500	5.00 / 5.00 / 25.0
Std-2	Std-5	0.100 / 0.100 / 0.500	0.0500	0.450	0.500	10.0 / 10.0 / 50.0
Std-3	Std-5	0.100 / 0.100 / 0.500	0.100	0.400	0.500	20.0 / 20.0 / 100
Std-4	Std-8	1.00 / 1.00 / 5.00	0.0250	0.475	0.500	50.0 / 50.0 / 250
Std-5	Std-8	1.00 / 1.00 / 5.00	0.0500	0.450	0.500	100 / 100 / 500
Std-6	Std-8	1.00 / 1.00 / 5.00	0.100	0.400	0.500	200 / 200 / 1000
Std-7	Std-9	10.0 / 10.0 / 50.0	0.0250	0.475	0.500	500 / 500 / 2500
Std-8	Std-9	10.0 / 10.0 / 50.0	0.0500	0.450	0.500	1000 / 1000 / 5000
Std-9	Proprietary Stock A/B;	1000	0.0100	0.930	1.00	10000
	Proprietary Stock A/B;	1000	0.0100			10000
	Proprietary Stock A/B	1000	0.0500			50000

Preparation of Quality Control Samples in Matrix						
Quality Control Sample ID	Spiking Solution ID	Spiking Solution Concentration (µg/ml)	Spiking Volume (ml)	Matrix Volume (ml)	Final Volume (ml)	Nominal Matrix Concentration (ng/ml)
QC- Low	QC-Mid	0.400 / 0.400 / 2.00	0.0375	0.9625	1.00	15.0 / 15.0 / 75.0
QC- Mid	QC-Dil	5.00 / 5.00 / 25.0	0.0800	0.920	1.00	400 / 400 / 2000
QC- High	QC-Dil	5.00 / 5.00 / 25.0	0.160	0.840	1.00	800 / 800 / 4000
QC- Dil	Proprietary Stock A/B;	1000	0.0100	1.930	2.00	5000
	Proprietary Stock A/B;	1000	0.0100			5000
	Proprietary Stock A/B	1000	0.0500			25000

Species \_\_\_\_\_ Anticoagulant/Matrix: \_\_\_\_\_ Matrix Supplier: \_\_\_\_\_

Lot: \_\_\_\_\_ Matrix Expiration Date \_\_\_\_\_

Pipette IDs: \_\_\_\_\_

Calibration Spiking Solution: \_\_\_\_\_ Expiration Date: \_\_\_\_\_

QC Sample Spiking Solution: \_\_\_\_\_ Expiration Date: \_\_\_\_\_

Prepared by: \_\_\_\_\_ Date: \_\_\_\_\_

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Study ID:	Batch ID:
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### METHODOLOGY AND REAGENT LIST

Step	Description	Equipment or Pipettes used	Step completed (check)
1	Transfer 0.0200 ml of each calibration standard, QC sample, study sample and blank into separate 1.50 ml microcentrifuge tubes		
2	Add 0.100 ml of 0.2% acetic acid in methanol to the matrix blanks. Cap and vortex for approximately 5 seconds.		
3	Add 0.100 ml of the Internal Standard Spiking Solution to each calibration standard, QC sample, study sample and control blank. Cap and vortex for approximately 5 seconds.		
4	Centrifuge tubes at approximately 18000 g for approximately 10 minutes.		
5	Transfer 0.0250 ml of the supernatant into a 2.00 ml HPLC vial containing 1.00 ml Reconstitution Solution. Cap and vortex briefly to mix.		
6	Store on the autosampler (set point 5°C ± 3°C) or refrigerated (5°C ± 3°C).	End of extraction (time):	

Ⓐ Eppendorf Repeater Plus / M4 (circle one) Equipment ID: \_\_\_\_\_ Exp: \_\_\_\_\_

Dilution Scheme (I: _____)	Add _____ ul sample to _____ ul control matrix and vortex.	Pipettes:
Dilution Scheme (I: _____)	Add _____ ul sample to _____ ul control matrix and vortex.	
Dilution Scheme (I: _____)	Add _____ ul sample to _____ ul control matrix and vortex.	

Procedure Performed by: \_\_\_\_\_ Date: \_\_\_\_\_

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Study ID:	Batch ID:
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### METHODOLOGY AND REAGENT LIST (cont.)

Additional information, if required:

Test Mix Dilution: Remove the Test Mix in 2% acetic acid in methanol from storage (ID: \_\_\_\_\_ Exp: \_\_\_\_\_) and remove 0.0203 ml of this solution and place into a separate HPLC vial containing 0.0203 ml of internal standard spiking solution and 0.959 ml of Reconstitution Solution. Store with batch.  
Pipettes: \_\_\_\_\_

### REAGENT LIST

Reagent Description	Assigned ID	Supplier	Lot #	Grade	Exp.
K <sub>2</sub> EDTA Dog Plasma	NA			NA	
Internal Standard		NA	NA	NA	
0.2% Acetic Acid in Methanol (Diluent)		NA	NA	NA	
Water:Methanol (90:10 v:v) with 0.1% Acetic Acid (Reconstitution Solution)		NA	NA	NA	
Initial/Date:					

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Study ID:	Batch ID:
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# **INSTRUMENT ANALYTICAL CONDITIONS AND REAGENTS**

<b>HPLC Column ID.</b>	<b>Vendor:</b>	<b>Calibration due date</b>
Phenomenex Synergi Polar RP 100 x 2 mm, 4µm	<b>Description:</b>	NA
	<b>Dimension:</b>	
	<b>S/N:</b>	
<b>Column Heater</b>	Equipment Tracking #-	
<b>Column Temp (Set Point 25°C)</b>	Set Point _____ °C	NA
<b>Pump ID</b>	Equipment Tracking #-	
<b>Pump Pressures at Start</b>	psi	NA
<b>Autosampler ID</b>	Equipment Tracking #-	
<b>Autosampler Temp (Set Point 5°C ± 3°C)</b>	Set Point _____ °C	NA
<b>Mass Spectrometer</b>	Equipment Tracking #-	
		<b>Exp. Date</b>
<b>Mobile Phase A</b>		
<b>Mobile Phase B</b>		
<b>Needle Rinse 1</b>		
<b>Needle Rinse 2</b>		
<b>Number of Conditioning Injections</b>		NA

NA: Not Applicable

Initial: \_\_\_\_\_ Date: \_\_\_\_\_

Additional Comments / Incidents during analysis:

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## INSTRUMENT REAGENT PREPARATION

**Study Number:** \_\_\_\_\_

### Mobile Phase A:

Assigned ID: \_\_\_\_\_

2% Acetic Acid in Water

Add \_\_\_\_\_ ml (nominal 20.0 ml) of acetic acid to \_\_\_\_\_ ml (nominal 1000 ml) of Milli-Q Water. This solution should be stored at room temperature for up to one month after preparation.

Expiration Date: \_\_\_\_\_

### Mobile Phase B:

Assigned ID: \_\_\_\_\_

0.1% Acetic Acid in Acetonitrile

Add \_\_\_\_\_ ml (nominal 1.00 ml) of acetic acid to \_\_\_\_\_ ml (nominal 1000 ml) of acetonitrile. This solution should be stored at room temperature for up to one month after preparation.

Expiration Date: \_\_\_\_\_

### Needle Rinse 1:

Assigned ID: \_\_\_\_\_

Acetonitrile:Isopropanol (80:20, v:v) with 1% Ammonium Hydroxide

Add \_\_\_\_\_ ml (nominal 400 ml) of acetonitrile to \_\_\_\_\_ ml (nominal 100 ml) isopropanol and add \_\_\_\_\_ ml (nominal 5.00 ml) ammonium hydroxide in a glass bottle. This solution should be stored at room temperature for up to one month after preparation.

Expiration Date: \_\_\_\_\_

### Needle Rinse 2:

Assigned ID: \_\_\_\_\_

Water:Methanol (90:10, v:v) with 1% Formic Acid

Add \_\_\_\_\_ ml (nominal 450 ml) of Milli-Q water to \_\_\_\_\_ ml (nominal 50.0 ml) methanol and add \_\_\_\_\_ ml (nominal 5.00 ml) formic acid in a glass bottle. This solution should be stored at room temperature for up to one month after preparation.

Expiration Date: \_\_\_\_\_

**Milli-Q water:** Decanted from Milli-Q unit on the day of use. Unit ID: \_\_\_\_\_ Exp: \_\_\_\_\_  
Is resistivity  $\geq 18.0 \text{ M}\Omega\text{-cm}$ ? Y / N (circle) Is TOC  $< 50.0 \text{ ppb}$ ? Y / N (circle)

### Ammonium Hydroxide:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

### Acetic Acid:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

### Formic Acid:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

### Methanol:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

### Acetonitrile:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

### Isopropanol:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

Prepared by: \_\_\_\_\_ Date: \_\_\_\_\_

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## EXTRACTION / STOCK REAGENT PREPARATION

Study Number: \_\_\_\_\_

### 0.2% Acetic Acid in Methanol (Diluent):

Assigned ID: \_\_\_\_\_

Add \_\_\_\_\_ ml (nominal 0.400 ml) of acetic acid to \_\_\_\_\_ ml (nominal 200 ml) of methanol. This solution should be stored at room temperature for up to one month after preparation.

Expiration Date: \_\_\_\_\_

### Water:Methanol (90:10 v:v) with 0.1% acetic acid (Reconstitution Solution):

Assigned ID: \_\_\_\_\_

Add \_\_\_\_\_ ml (nominal 0.200 ml) of acetic acid to \_\_\_\_\_ ml (nominal 180 ml) Milli-Q water and \_\_\_\_\_ ml (nominal 20.0 ml) of methanol. This solution should be stored at room temperature for up to one month after preparation.

Expiration Date: \_\_\_\_\_

### Test Mix (System Suitability):

Assigned ID: \_\_\_\_\_

To prepare at the LLOQ level, spike 0.0100 ml of the 1.00 mg/ml **Proprietary** and **Proprietary** stock solutions and 0.0500 ml of the 1.00 mg/ml **Proprietary** stock solution into 9.930 ml of Diluent Solution. Vortex, remove 0.100 ml, and add to a vial containing 0.900 ml of Diluent Solution. Vortex, remove 0.0100 ml, and add to a vial containing 0.990 ml of Diluent Solution. This solution may be stored refrigerated for up to 3 months from the date of preparation. On the day of use, remove 0.0203 ml of this solution and place into a separate vial containing 0.0203 ml of internal standard spiking solution and 0.959 ml of Reconstitution Solution. Expiration Date: \_\_\_\_\_

Storage Unit / Temperature: \_\_\_\_\_

**Milli-Q water:** Decanted from Milli-Q unit on the day of use. Unit ID: \_\_\_\_\_ Exp: \_\_\_\_\_  
Is resistivity  $\geq 18.0 \text{ M}\Omega\text{-cm}$ ? Y / N (circle) Is TOC  $< 50.0 \text{ ppb}$ ? Y / N (circle)

### Acetic Acid:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

### Methanol:

Supplier: \_\_\_\_\_ Lot: \_\_\_\_\_ Grade: \_\_\_\_\_ Expire: \_\_\_\_\_

**Proprietary** Stock Solution (1.00 mg/ml) ID: \_\_\_\_\_ Expire: \_\_\_\_\_

**Proprietary** Stock Solution (1.00 mg/ml) ID: \_\_\_\_\_ Expire: \_\_\_\_\_

**Proprietary** Stock Solution (1.00 mg/ml) ID: \_\_\_\_\_ Expire: \_\_\_\_\_

**Internal Standard Spiking Solution** ID: \_\_\_\_\_ Expire: \_\_\_\_\_

**Diluent** ID: \_\_\_\_\_ Expire: \_\_\_\_\_

**Reconstitution Solution** ID: \_\_\_\_\_ Expire: \_\_\_\_\_

**Pipettes:** \_\_\_\_\_

Prepared by: \_\_\_\_\_ Date: \_\_\_\_\_

SRI Form 106.201E

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**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix G-5**

**BIOANALYTICAL CERTIFICATES OF ANALYSIS**



**MEDICAL ISOTOPES, INC.**  
100 Bridge Street  
Pelham, NH 03076 USA  
Tel: 603 635-2255, Toll Free: 800 374-9513  
Fax: 603 635-2448  
E-Mail: [info@medicalisotopes.com](mailto:info@medicalisotopes.com)  
URL: [www.medicalisotopes.com](http://www.medicalisotopes.com)

R120718-1 p.34  
LWF 12/17/18

## CERTIFICATE OF ANALYSIS

**Product Name:**

Proprietary Info

**Catalog No:**

Proprietary  
Info

**Lot No:**

**Date:**

June 2017

**Re Test Date:**

June 2024

**Method of Analysis:**

<sup>1</sup>H-NMR and Mass Spec

**Purity:**

Chemical purity: 98%

Isotopic purity: 98%

**Molecular Formula:**

Proprietary Info

**Molecular Weight:**

**Appearance of Product:**

Pale Beige Solid

**Stability:**

N/A

**Melting Point:**

N/A

**Boiling Point:**

N/A

**Solubility:**

N/A

**Storage:**

-20°C in freezer. Under inert atmosphere

**Additional Information:**

NMR and MS conforms to structure

Redacted by agreement



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100 Bridge Street  
Pelham, NH 03076 USA  
Tel: 603 635-2255, Toll Free: 800 374-9513  
Fax: 603 635-2448  
E-Mail: [info@medicalisotopes.com](mailto:info@medicalisotopes.com)  
URL: [www.medicalisotopes.com](http://www.medicalisotopes.com)

R120718-2 p. 3

7  
CW 12/08/18

city of  
CW 12/07/18

## CERTIFICATE OF ANALYSIS

**Product Name:** Proprietary Info

**Catalog No:** Proprietary Info

**Lot No:**

**Date:** April 2016

**Retest Date:** April 2020

**Method of Analysis:**

<sup>1</sup>H NMR and Mass Spec

**Purity:**

Chemical purity: 97%

Isotopic purity: 99%

**Molecular Formula:**

Proprietary Info

**Molecular Weight:**

**Appearance of Product:**

Pale Yellow Solid

**Stability:**

N/A

**Melting Point:**

N/A

**Boiling Point:**

N/A

**Solubility:**

N/A

**Storage:**

-20°C in freezer, Under Inert Atmosphere

**Additional Information:**

<sup>1</sup>H NMR and mass spectra conform to structure.

TLC: Single Spot

Redacted by agreement





MEDICAL ISOTOPES, INC.  
100 Bridge Street  
Pelham, NH 03076 USA  
Tel: 603 635-2255, Toll Free: 800 374-9513  
Fax: 603 635-2448  
E-Mail: [info@medicalisotopes.com](mailto:info@medicalisotopes.com) URL: [www.medicalisotopes.com](http://www.medicalisotopes.com)

R120718-3 p. 3  
LW12/07/18

## CERTIFICATE OF ANALYSIS

**Product Name:**

Proprietary Info

**Catalog No:**

**Lot No:**

**Date:**

October 2016

**Re Test Date:**

October 2020

**Method of Analysis:**

<sup>1</sup>H-NMR, HPLC, and Mass Spec

**Purity:**

Chemical purity: 97%

Isotopic Purity: 99%

**Molecular Formula:**

**Molecular Weight:**

Proprietary Info

**Appearance of Product:**

White Solid

**Stability:**

N/A

**Melting Point:**

N/A

**Boiling Point:**

N/A

**Solubility:**

N/A

**Storage:**

-20°C in freezer

**Additional Information:**

NMR and Mass Spec conforms to structure

Redacted by agreement

# Certificate

Proprietary Info

**LABEL TEXT**

For use with specified USP compendial tests.  
Not for use as a drug. See SDS prior to use  
at [www.usp.org/sds](http://www.usp.org/sds).

**USP REFERENCE STANDARD**

Proprietary Info

**350 mg**

For quantitative applications, determine the water content  
titrimetrically at the time of use. Use as is material and correct  
weight for water content. Use a value of 0.997 mg of Proprietary per  
mg of material on the anhydrous basis. Keep container tightly  
closed. Protect from light. Store in a refrigerator.

USP, 12601 Twinbrook Pkwy, Rockville, MD, +1-301-881-0666  
Cat. No. 1370101 Material mfd. in India

LOT: R077R0



Redacted by agreement

*Quality Assurance*

**Calculation Value**

If a value is not provided on the label or accompanying documentation and the Reference Standard has a quantitative USP compendial application, a value of 100.0% is used. The purity value is not applicable for qualitative uses. Please refer to the specific Reference Standard label for further information.

**Expiration**

Current lots are identified in the current USP Catalog. In some cases, the previous lot may still be considered valid for use. If so, it is identified in the column marked "Previous Lot/Valid Use Date."

It is the responsibility of each user to determine that this lot is current or valid when used. For the most up-to-date information, please refer to the USP Store at [www.usp.org](http://www.usp.org).

**Instructions for Use**

Follow the instructions on the label of the USP Reference Standard and in the appropriate USP documentary standard(s).

**Non-Monograph Use**

The suitability of this Reference Standard for use in non-compendial applications is solely the responsibility of the user.

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# Certificate

Proprietary Info

**LABEL TEXT**

For use with specified USP compendial tests. Not for use as a drug. See SDS prior to use at [www.usp.org/sds](http://www.usp.org/sds).

Wash thoroughly after handling. Wear protective gloves. Wear eye/face protection. If swallowed: Call a poison center/doctor if you feel unwell. Rinse mouth. If on skin: Wash with plenty of water. If skin irritation occurs: Get medical advice/attention. Take off contaminated clothing and wash before reuse. If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical advice/attention. Dispose of contents/container in accordance with local/regional/national/international regulations.

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**Calculation Value**

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# Certificate

Proprietary Info

**LABEL TEXT**

For use with specified USP compendial tests.  
Not for use as a drug. See SDS prior to use at  
[www.usp.org/sds](http://www.usp.org/sds).



**REFERENCE STANDARD**

Proprietary Info

**15 mg**

This is the monohydrate form of Proprietary Info Do not dry.  
Keep container tightly closed. Store in the refrigerator.

USP, 12601 Twinbrook Pkwy, Rockville, MD, +1-301-881-0666  
Cat. No. 1643601 Material mfd. in China

LOT: R044C0



Redacted by agreement

**Calculation Value**

If a value is not provided on the label or accompanying documentation and the Reference Standard has a quantitative USP compendial application, a value of 100.0% is used. The purity value is not applicable for qualitative uses. Please refer to the specific Reference Standard label for further information.

**Expiration**

Current lots are identified in the current USP Catalog. In some cases, the previous lot may still be considered valid for use. If so, it is identified in the column marked "Previous Lot/Valid Use Date."

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**Instructions for Use**

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**Non-Monograph Use**

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**LEGAL NOTICE**

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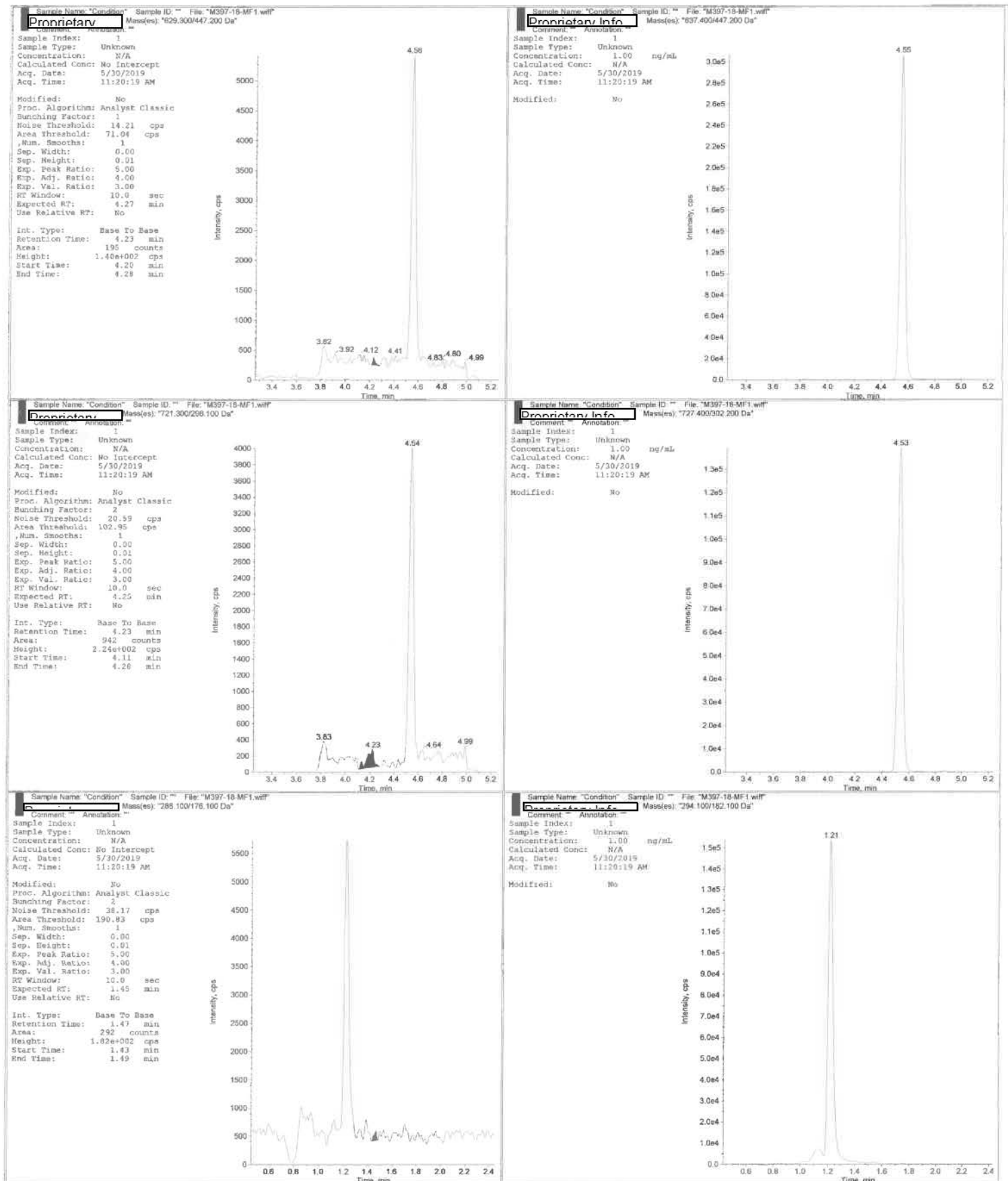
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**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

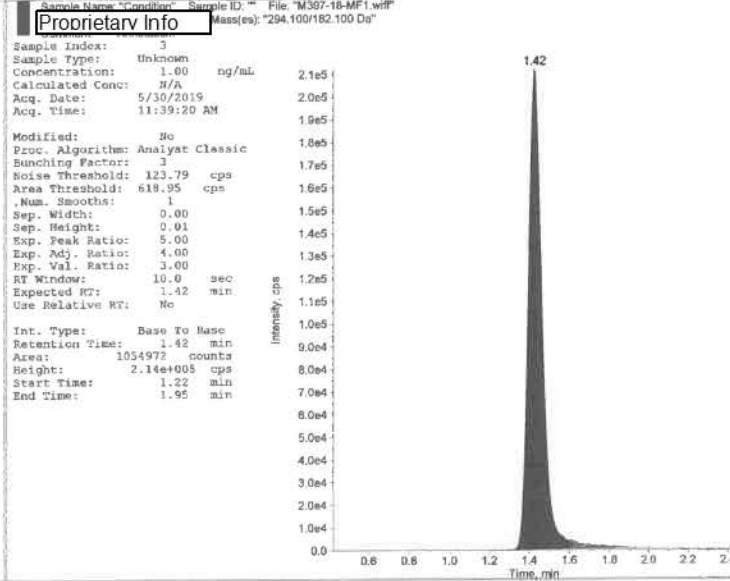
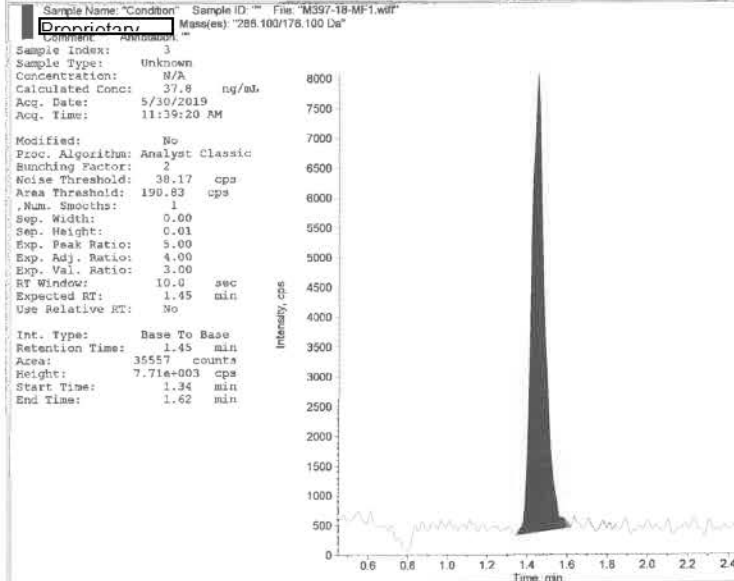
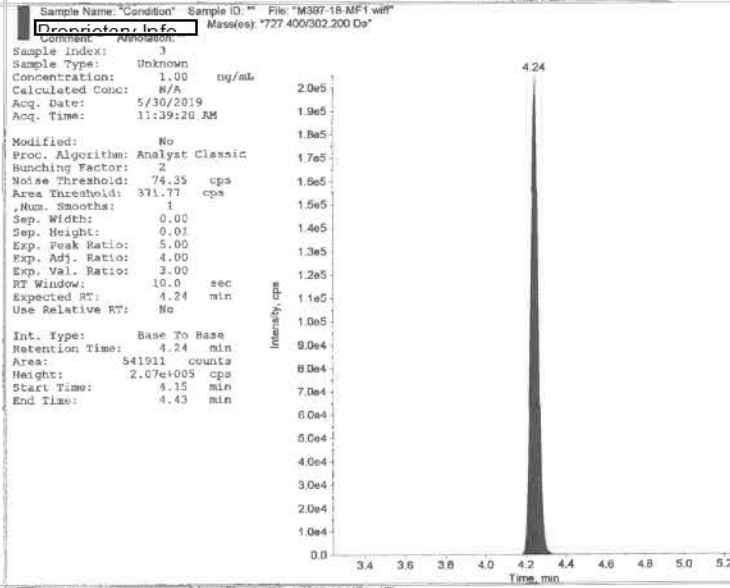
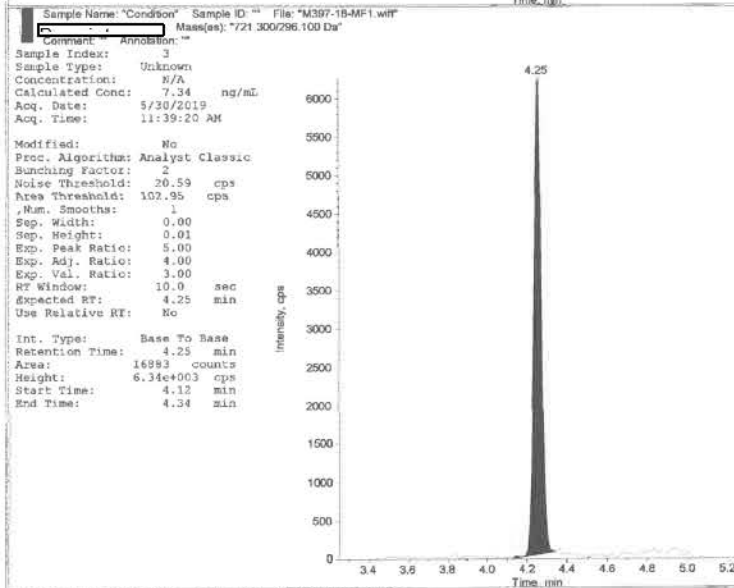
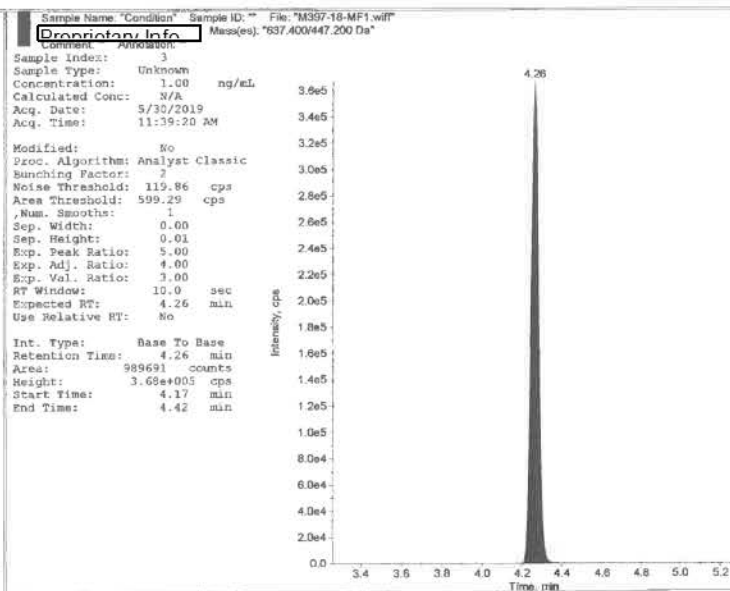
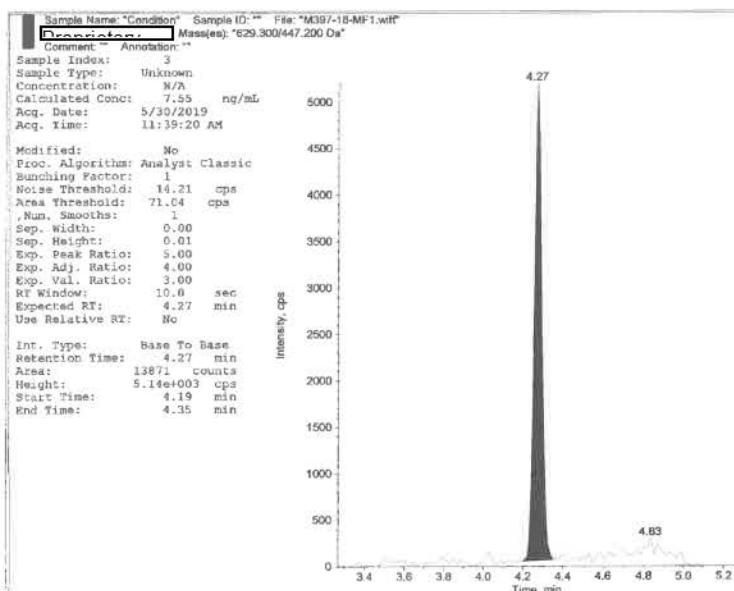
**Appendix G-6**

**REPRESENTATIVE CHROMATOGRAPHS**

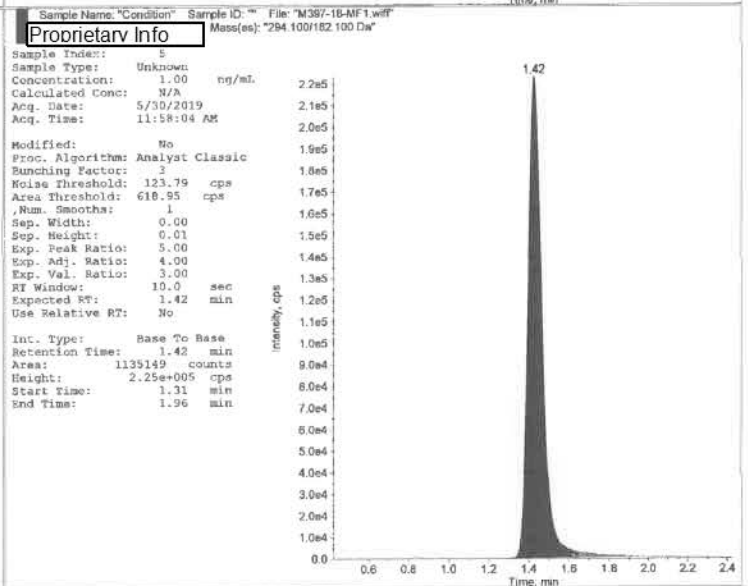
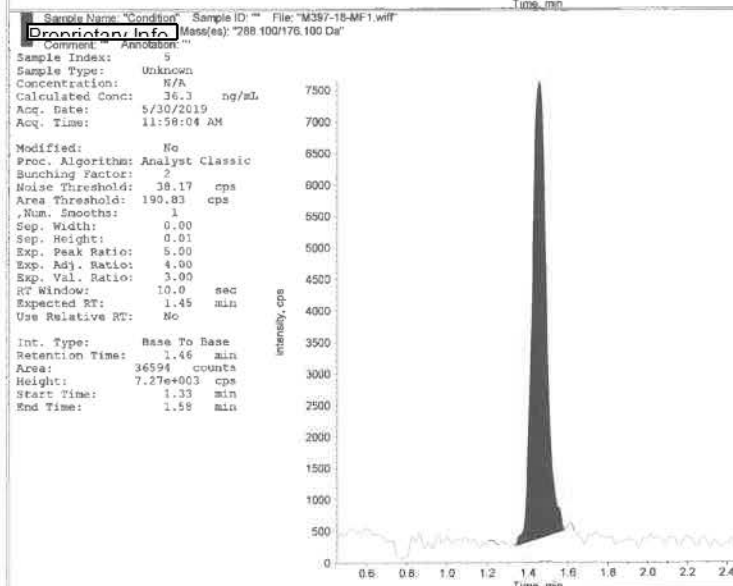
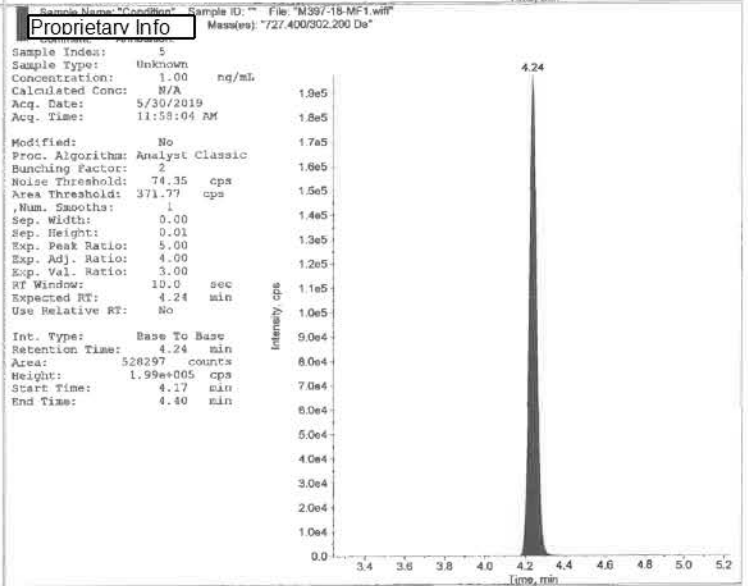
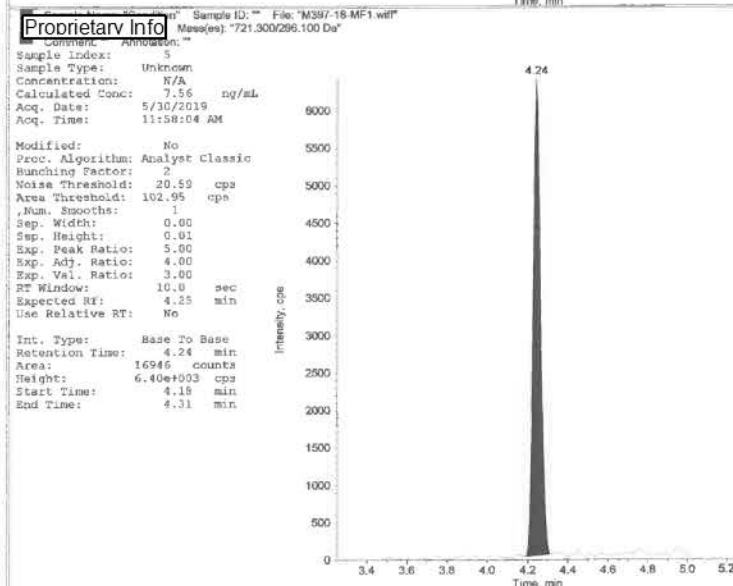
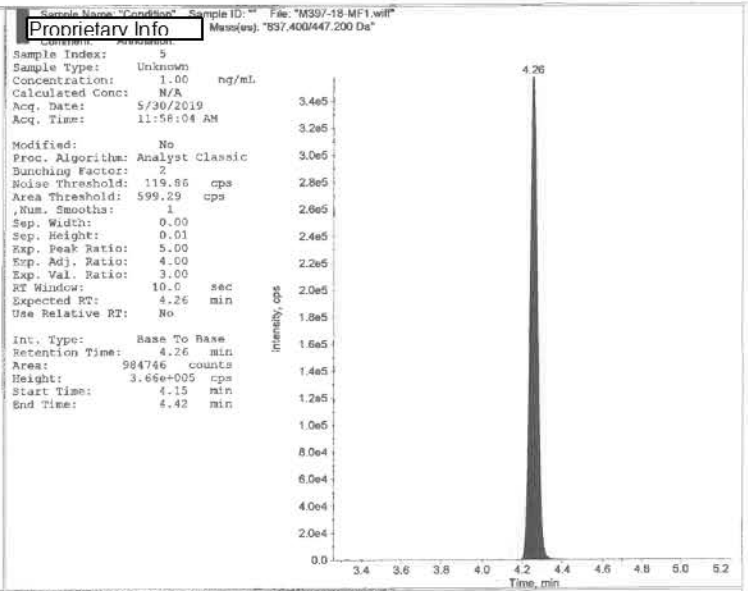
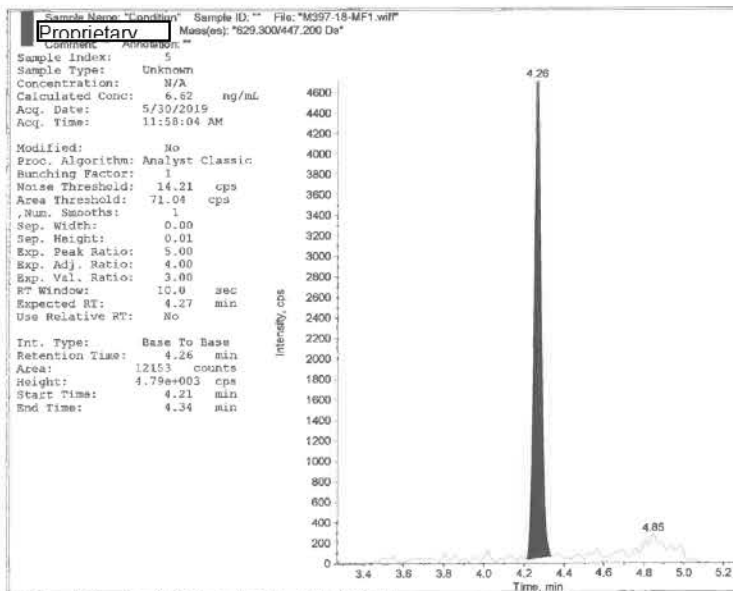




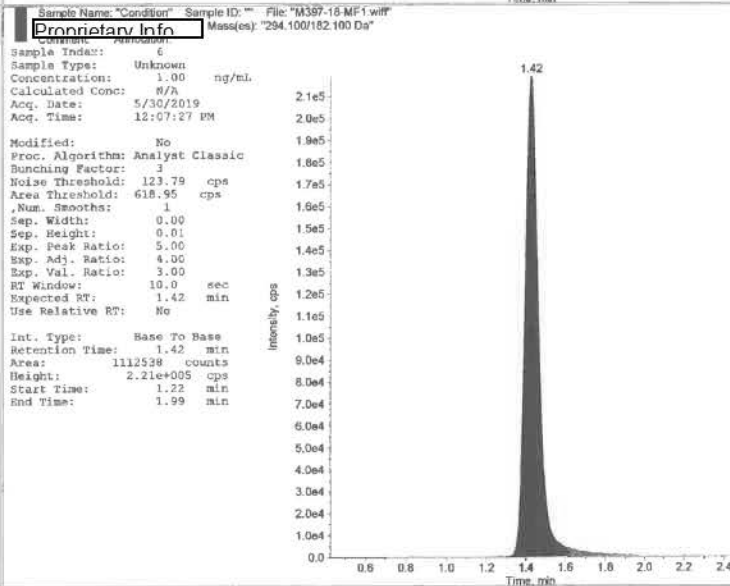
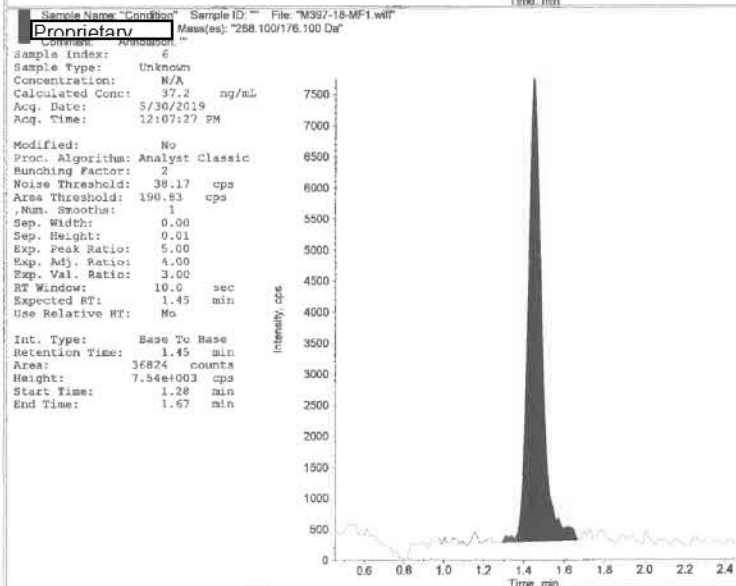
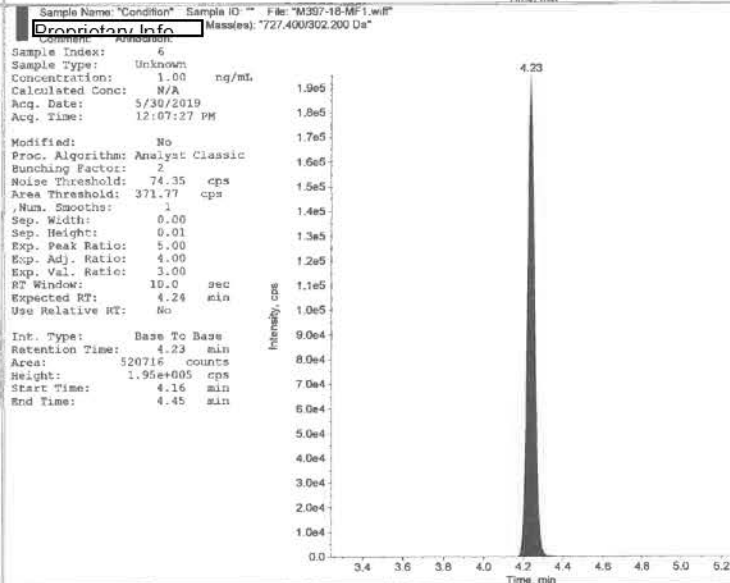
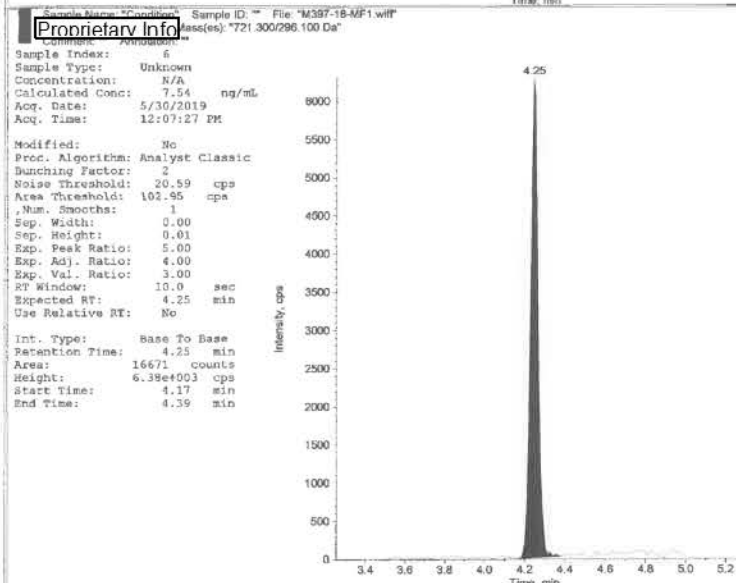
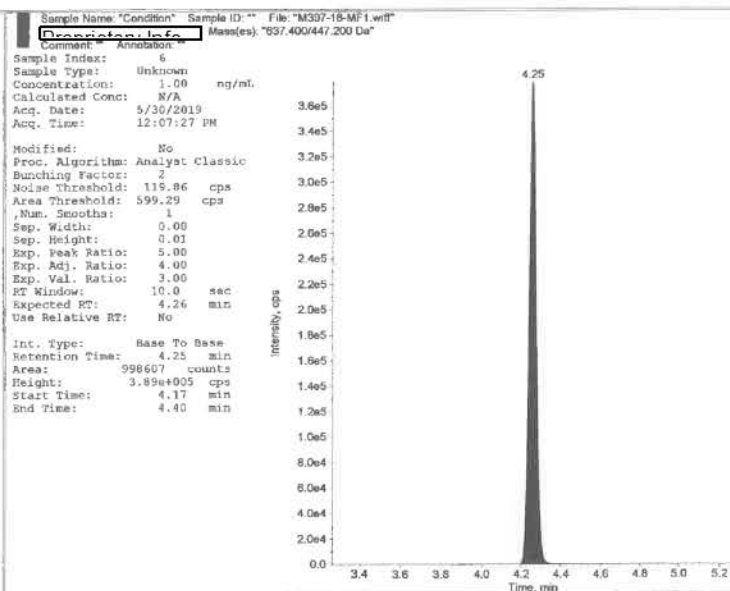
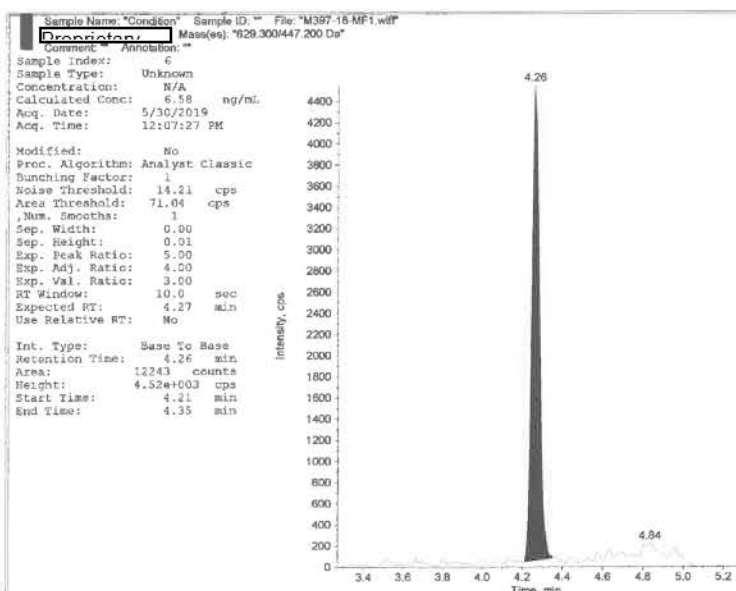


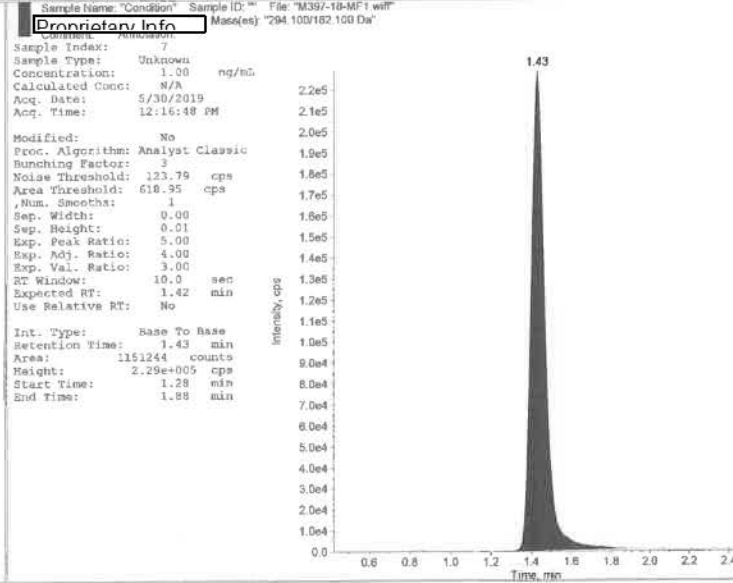
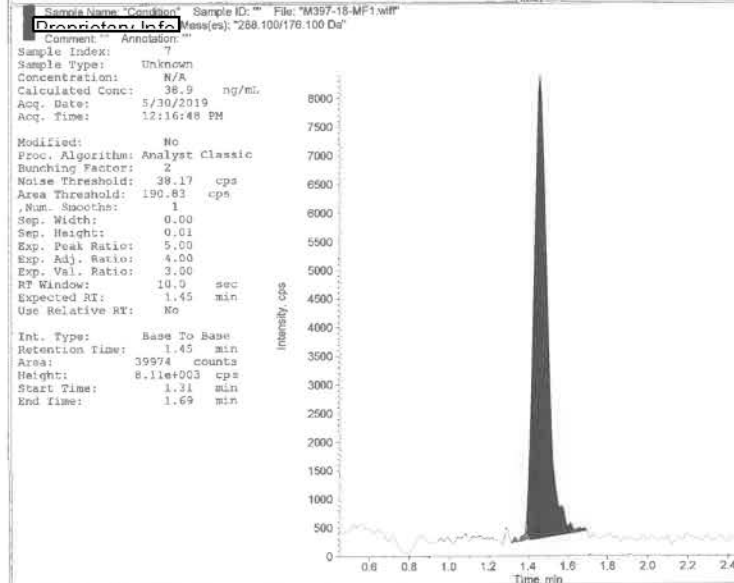
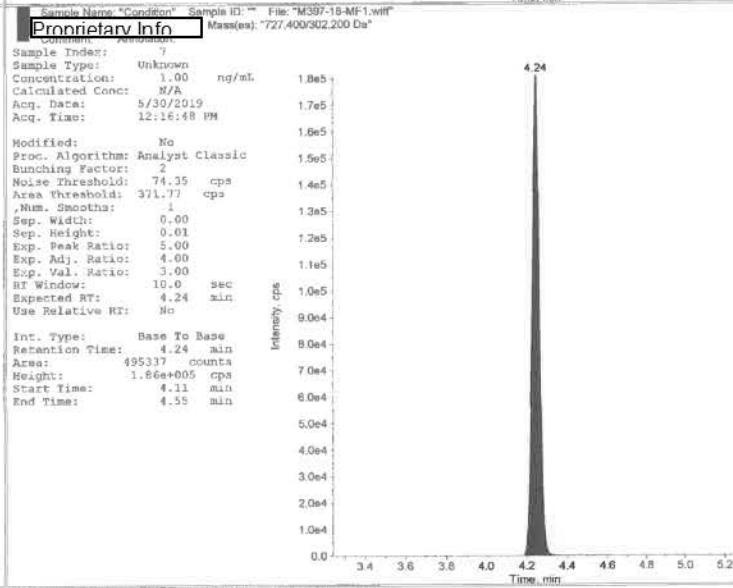
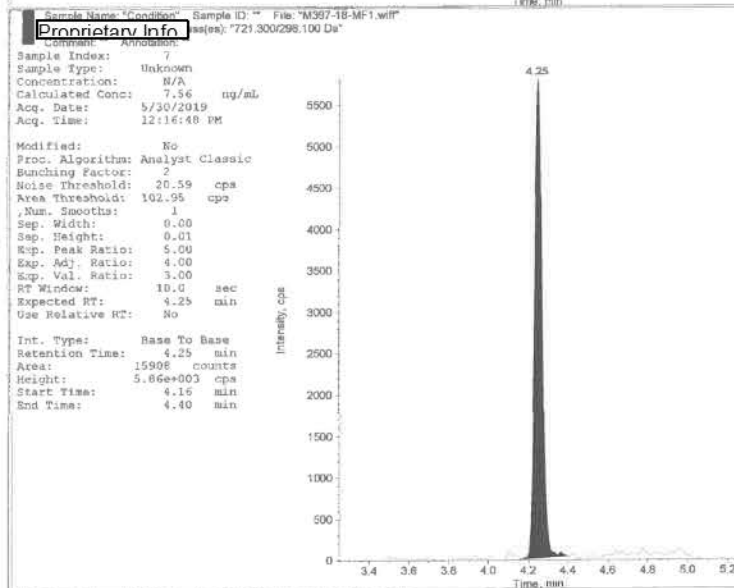
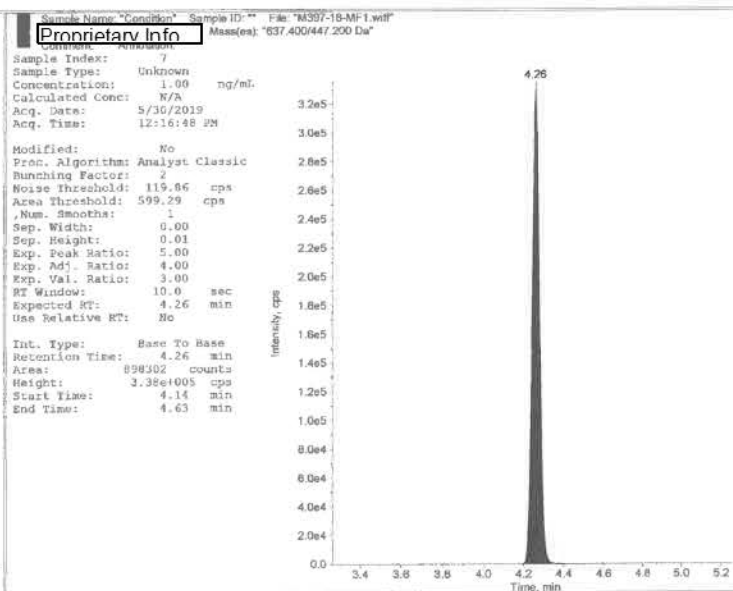
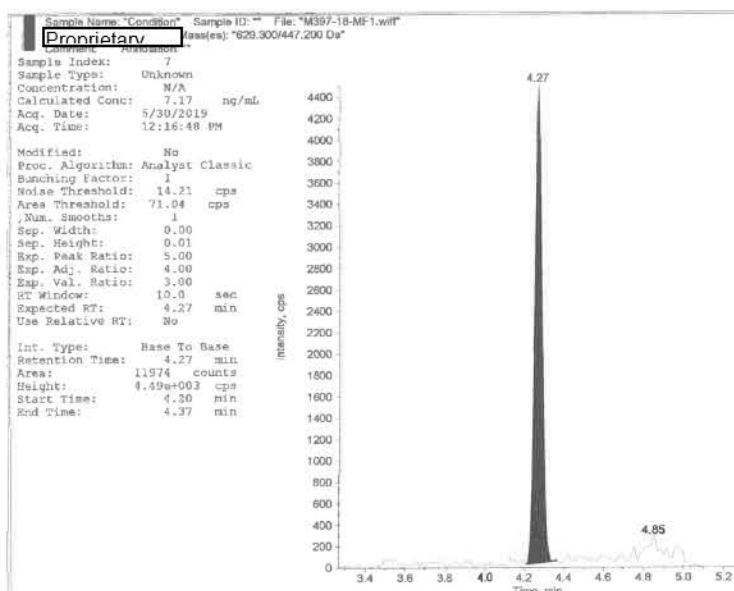






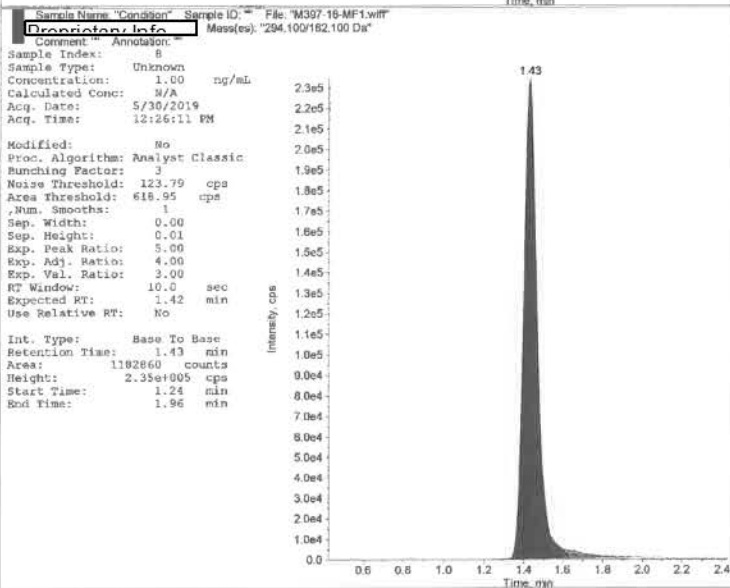
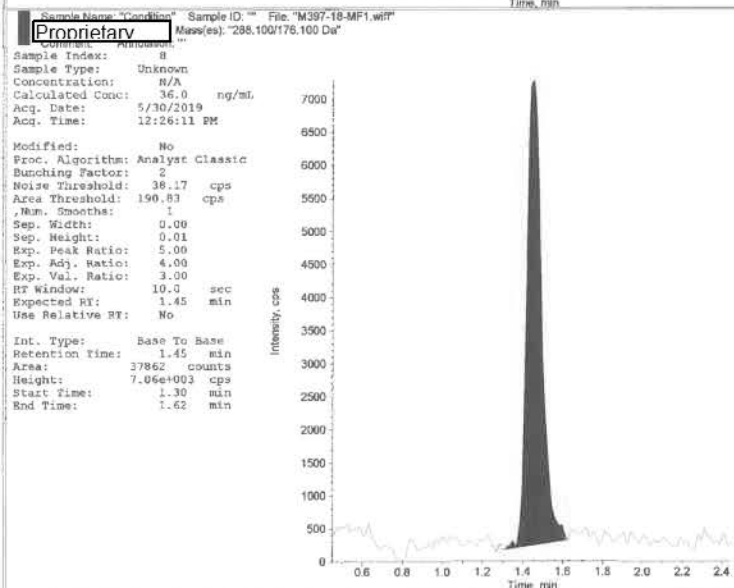
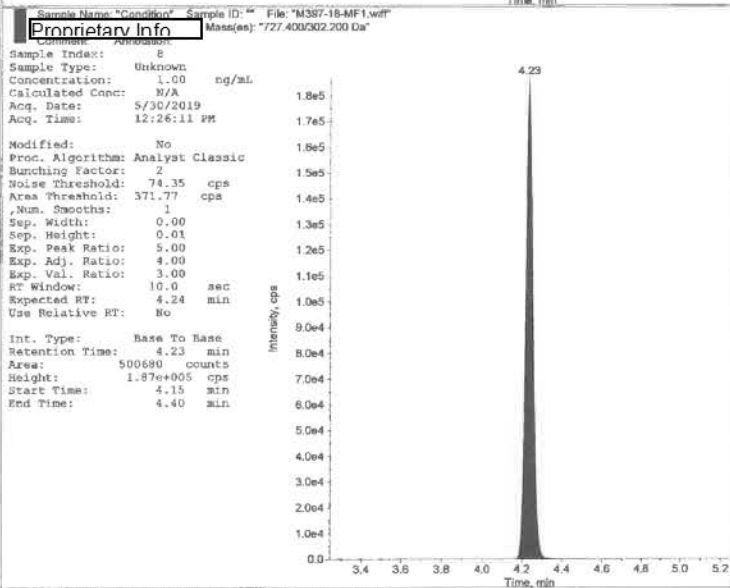
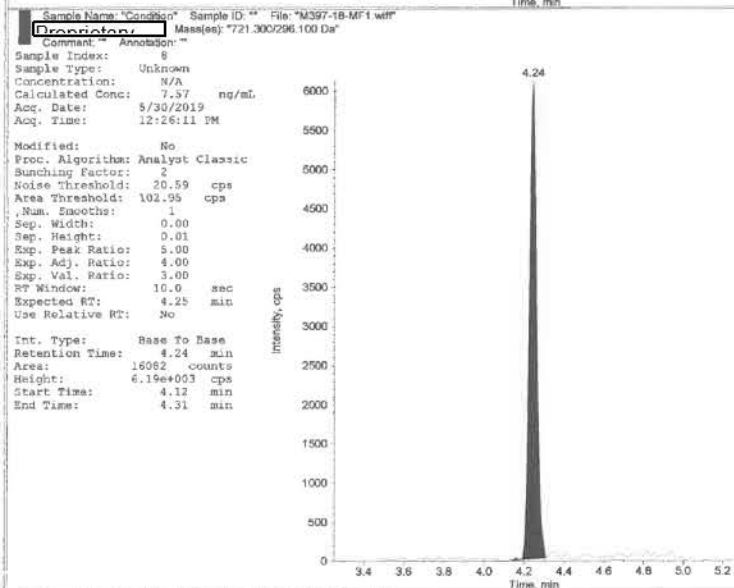
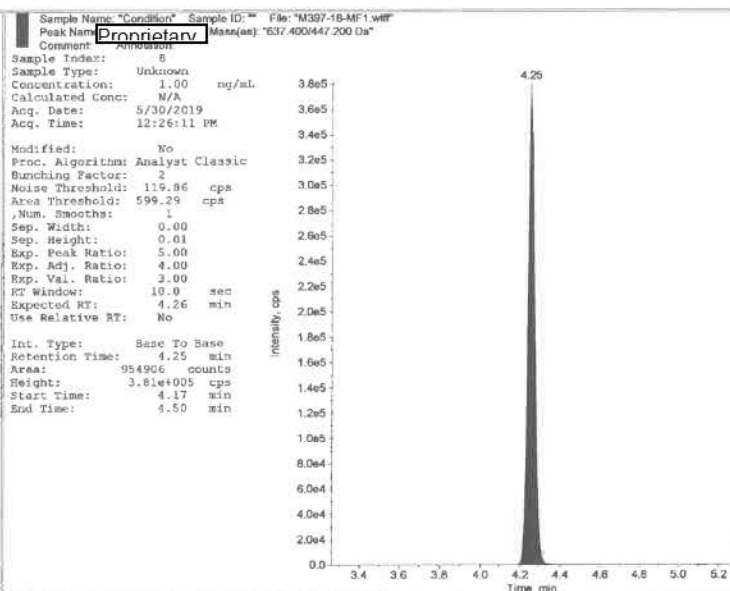
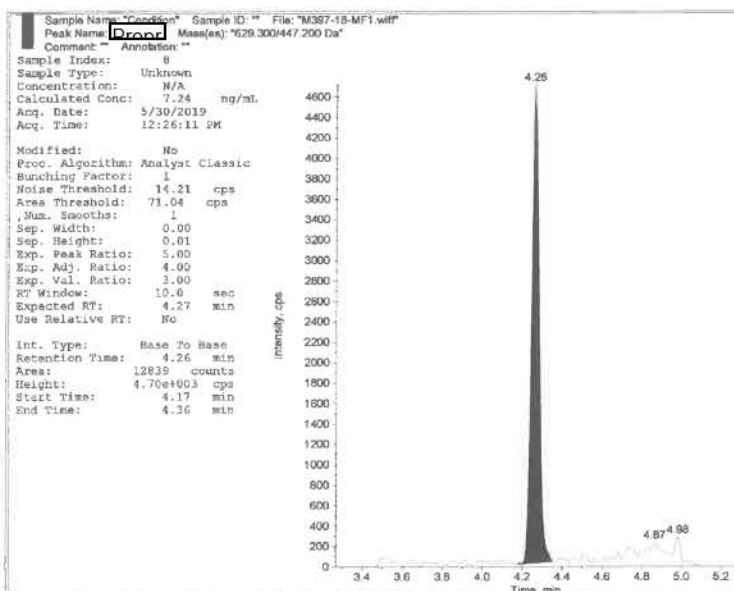


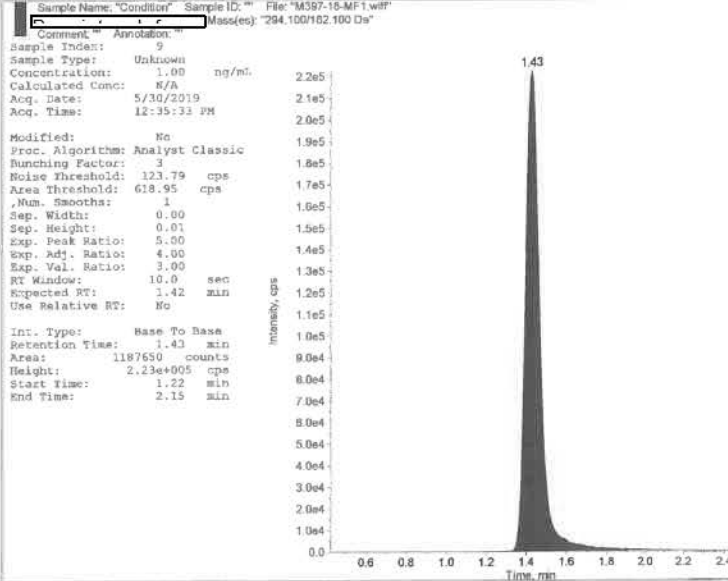
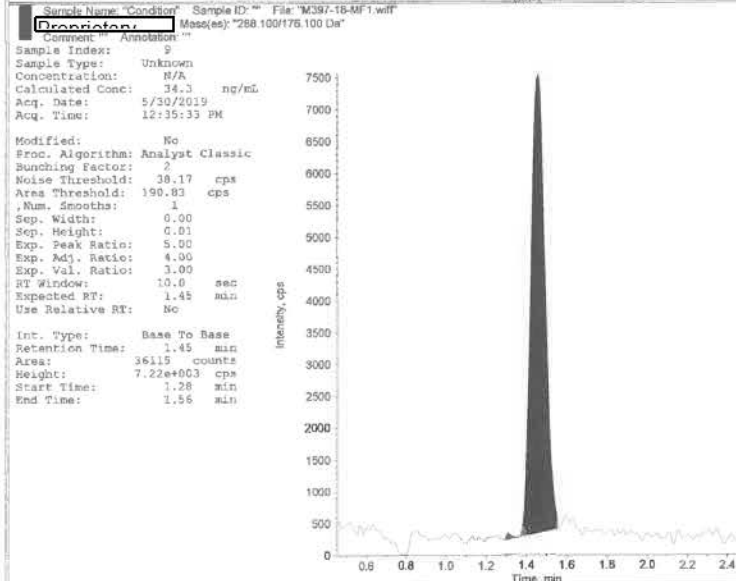
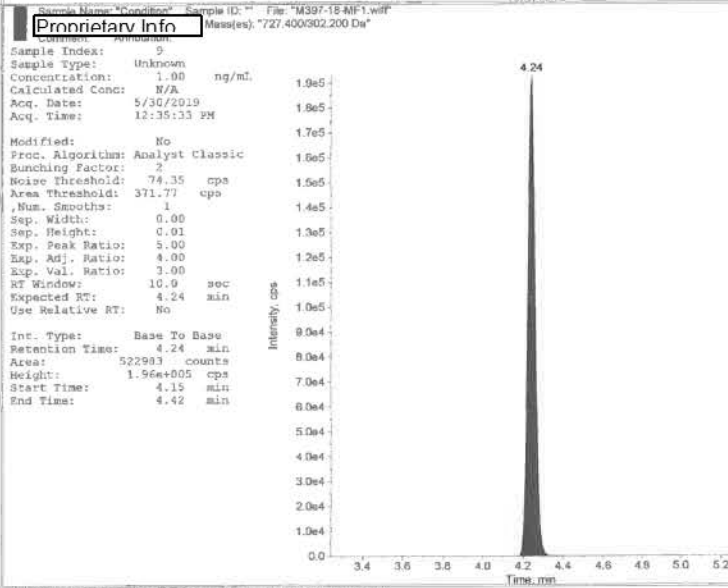
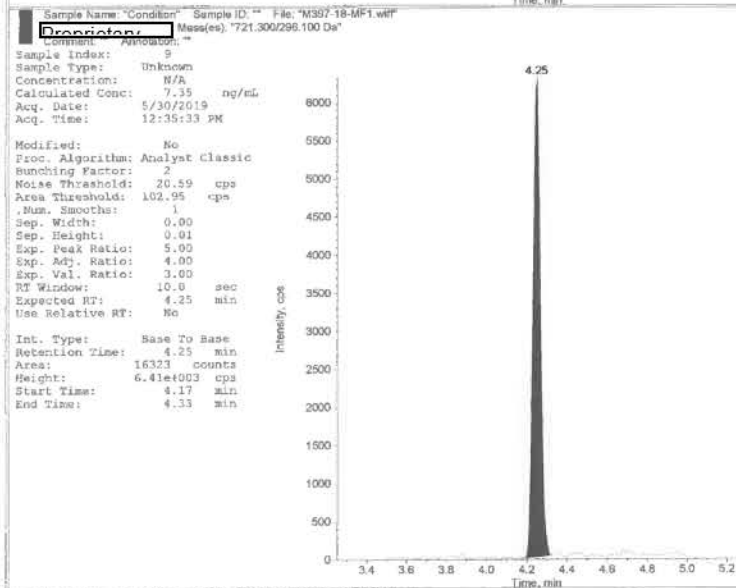
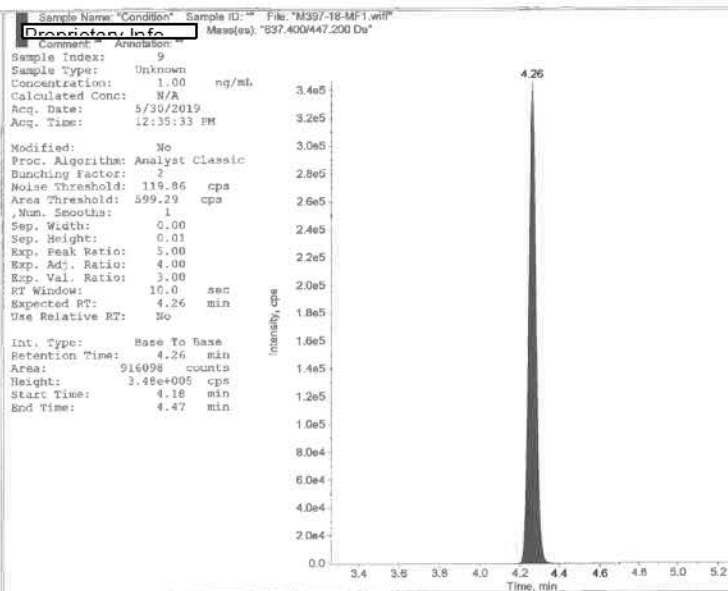
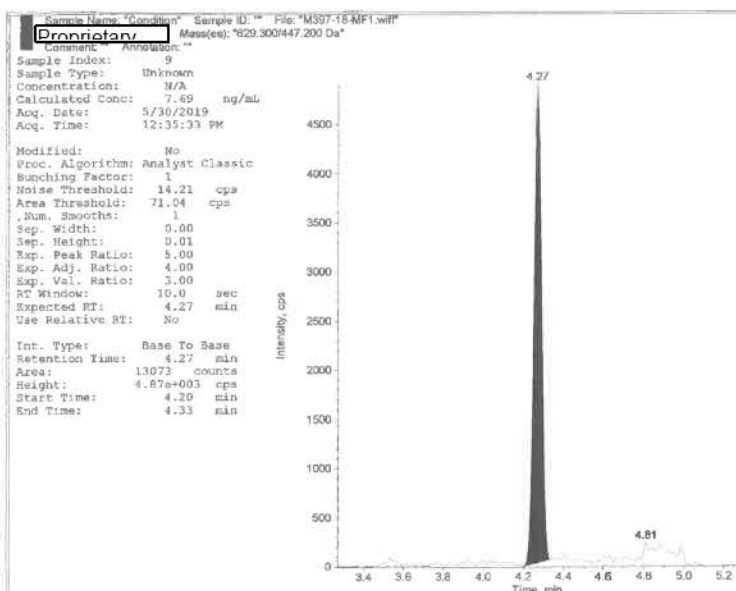


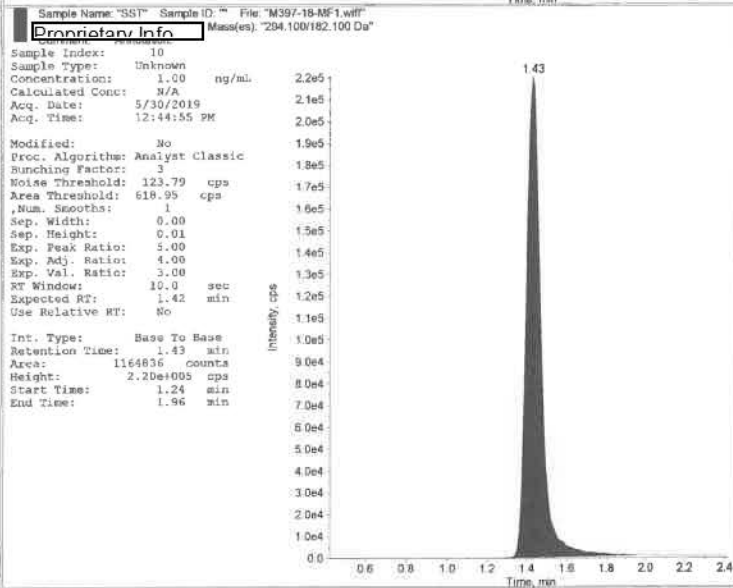
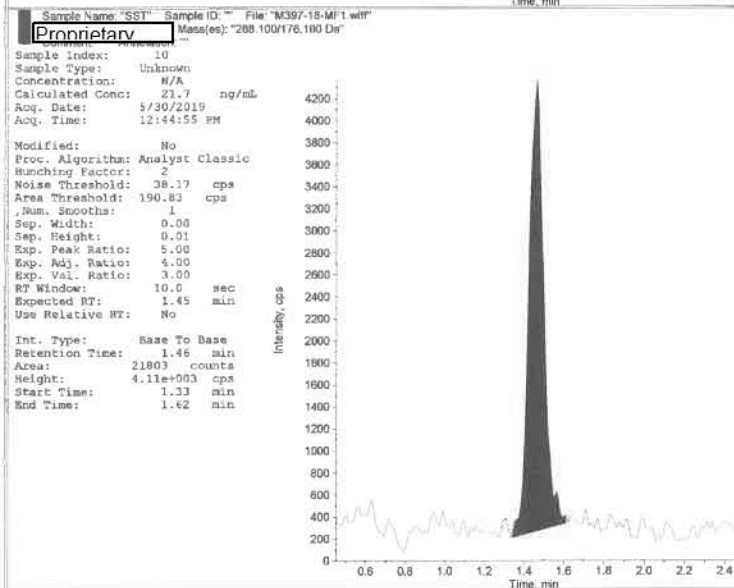
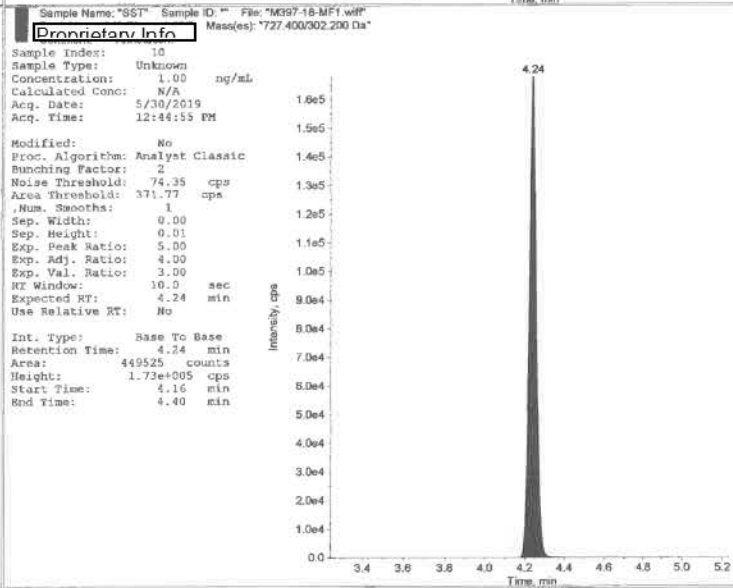
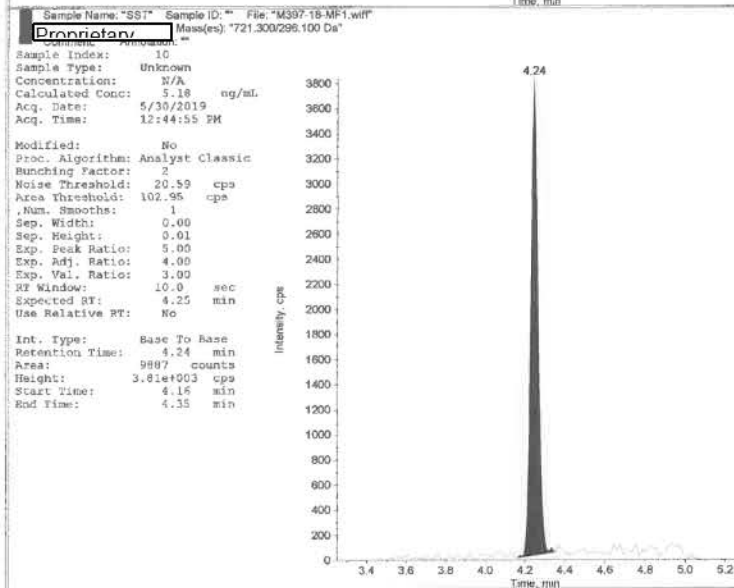
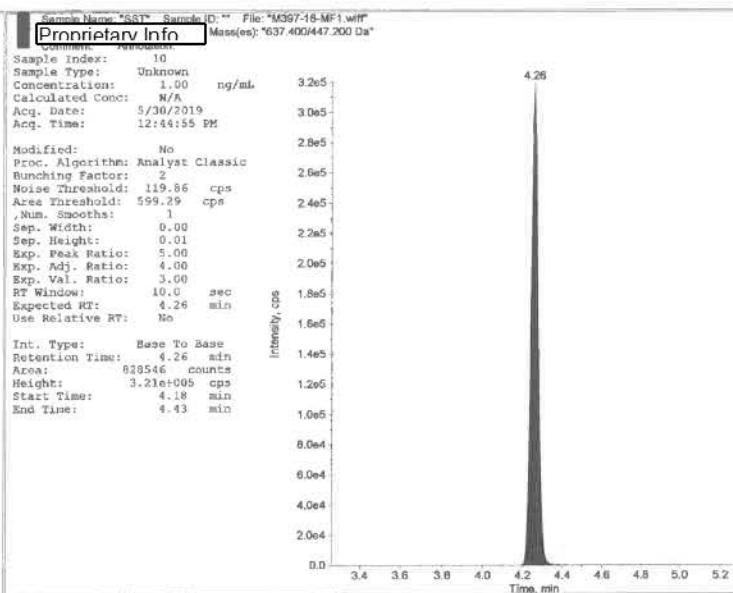
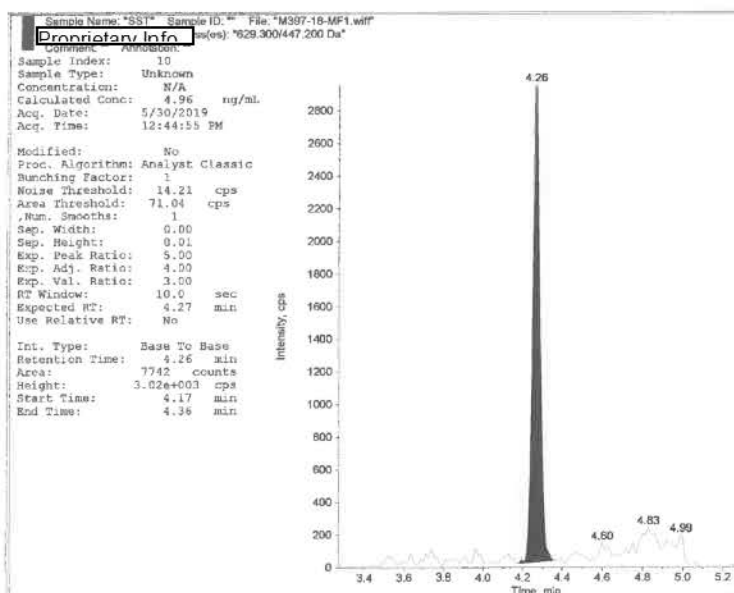




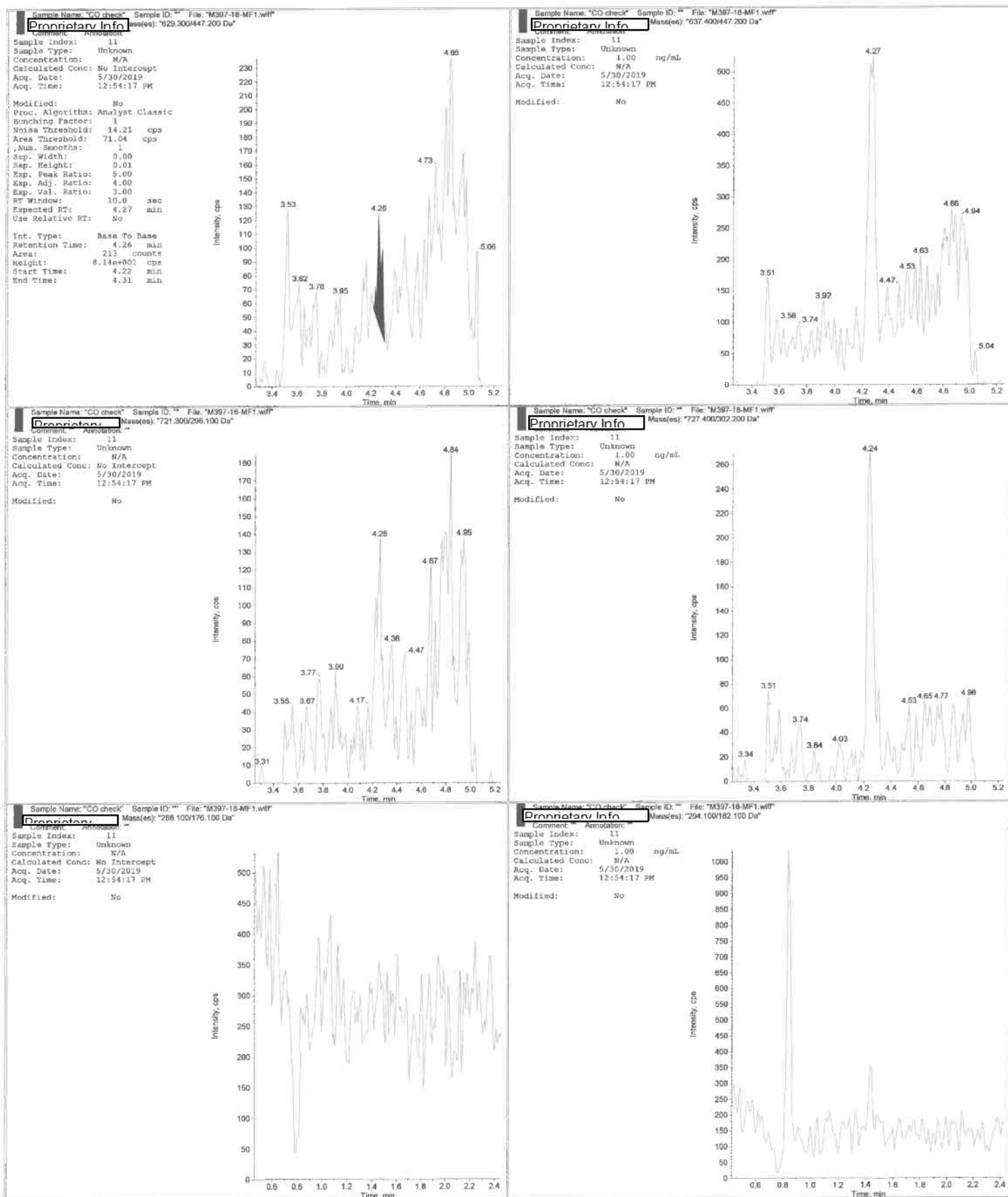
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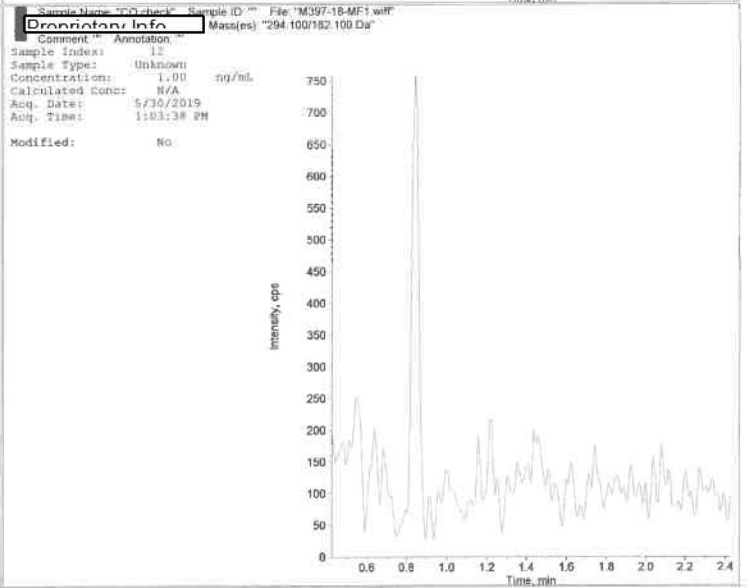
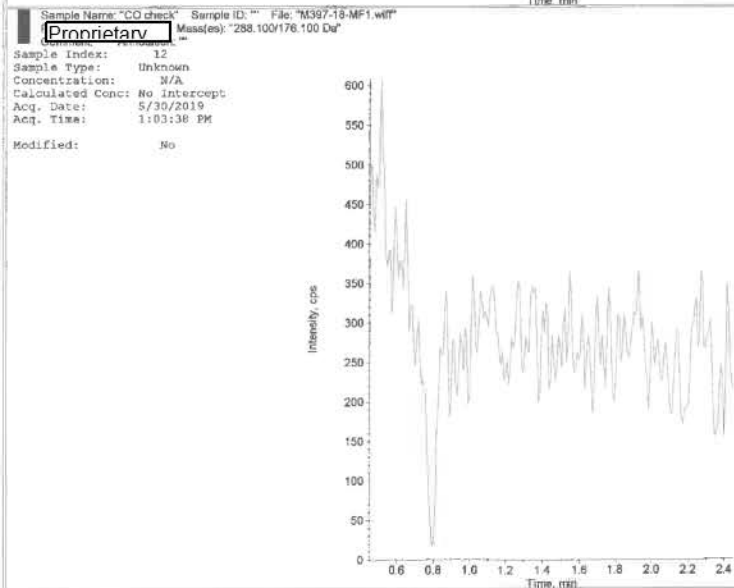
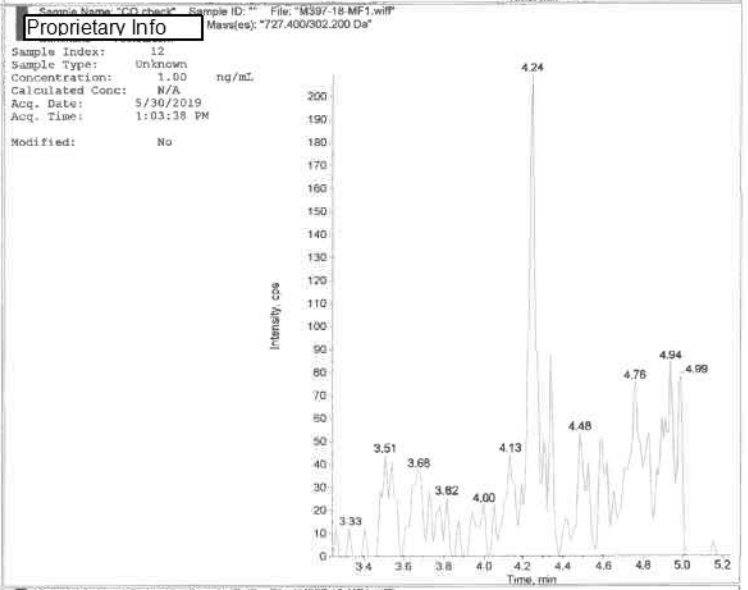
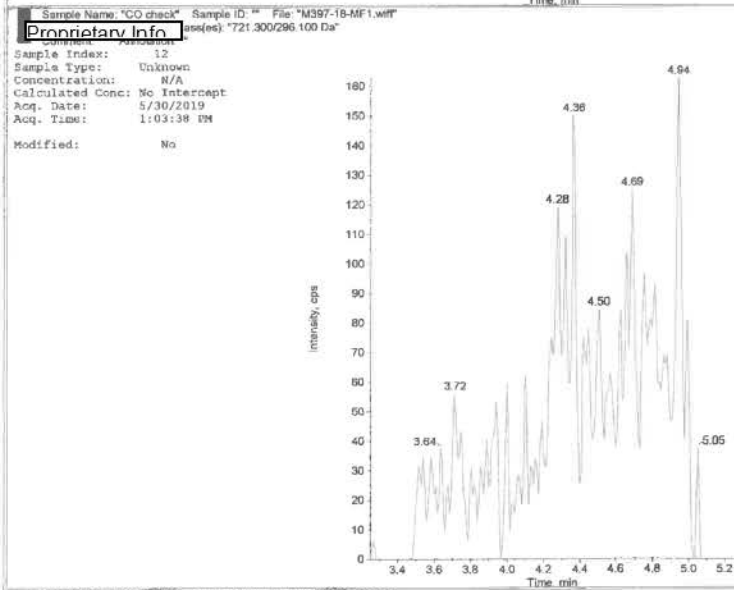
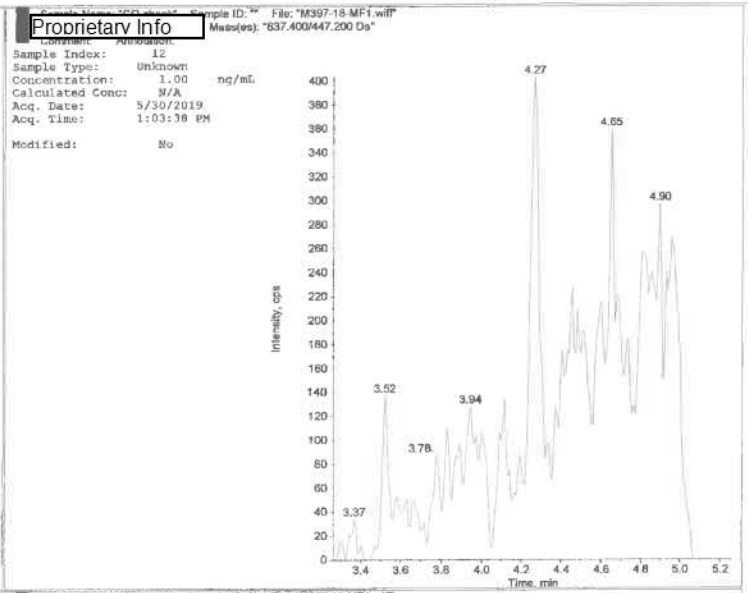
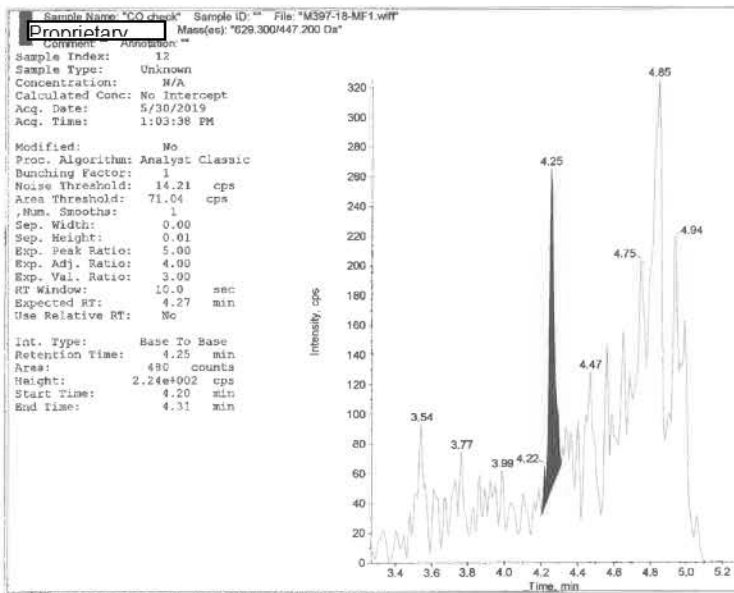




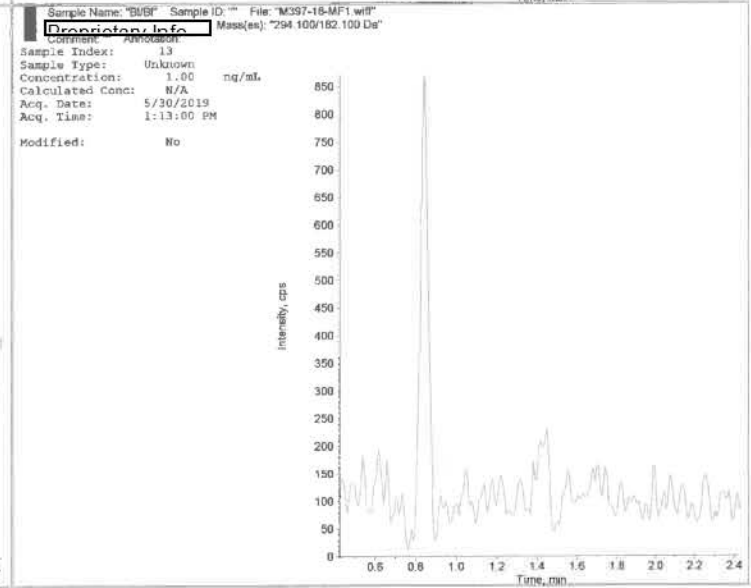
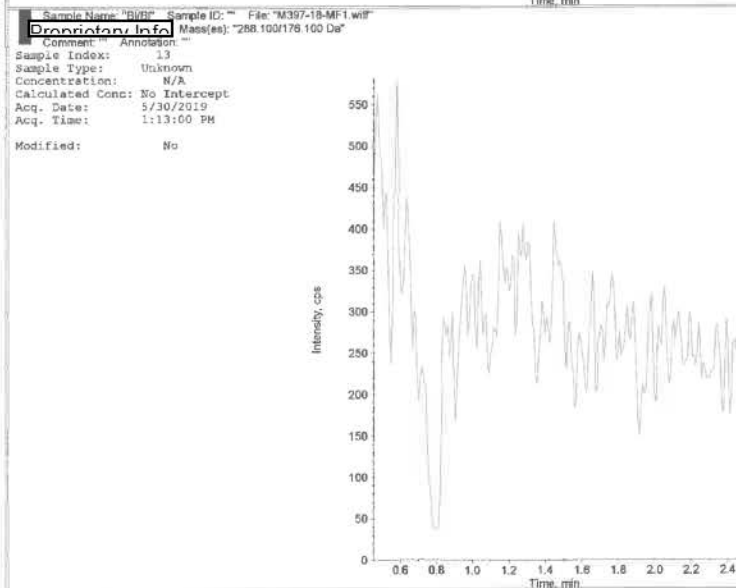
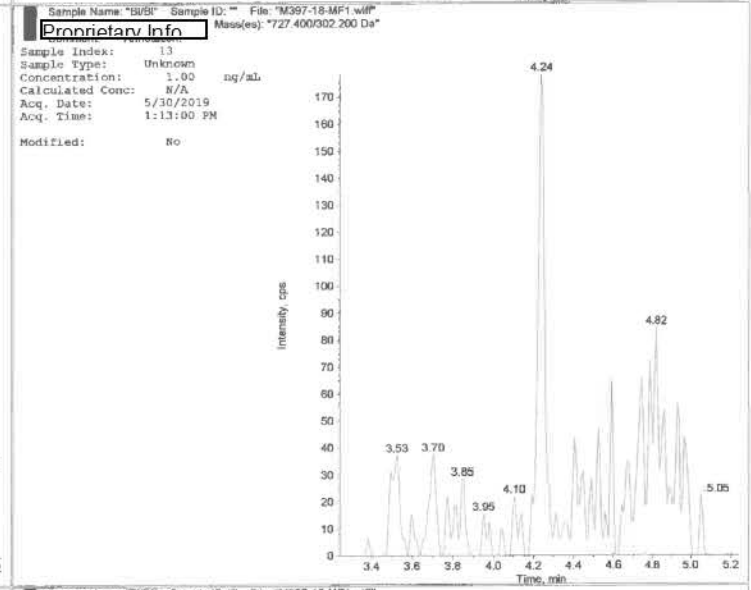
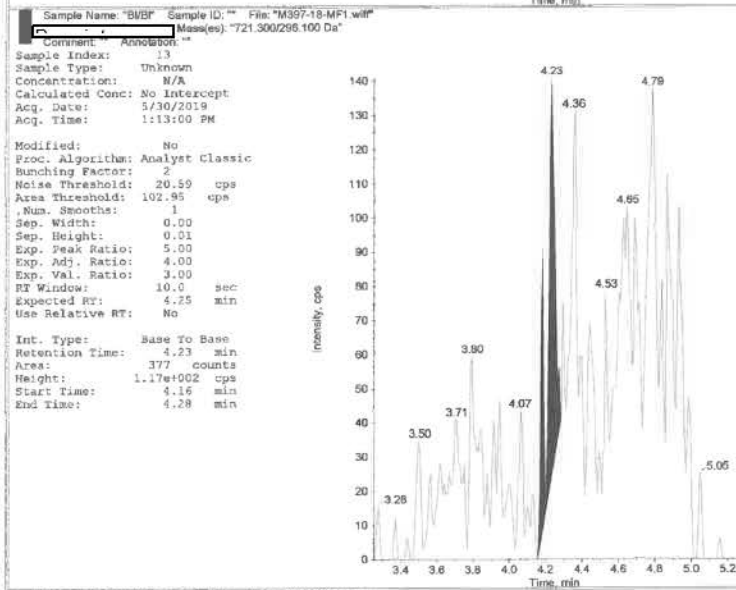
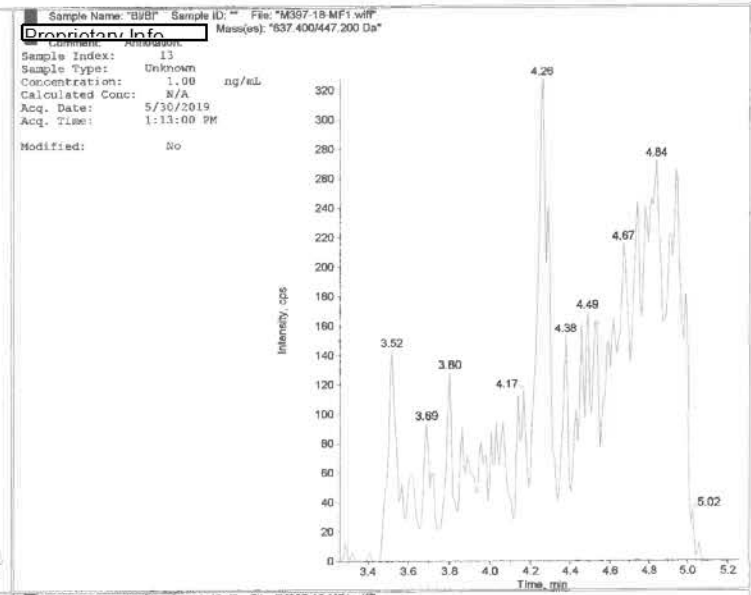
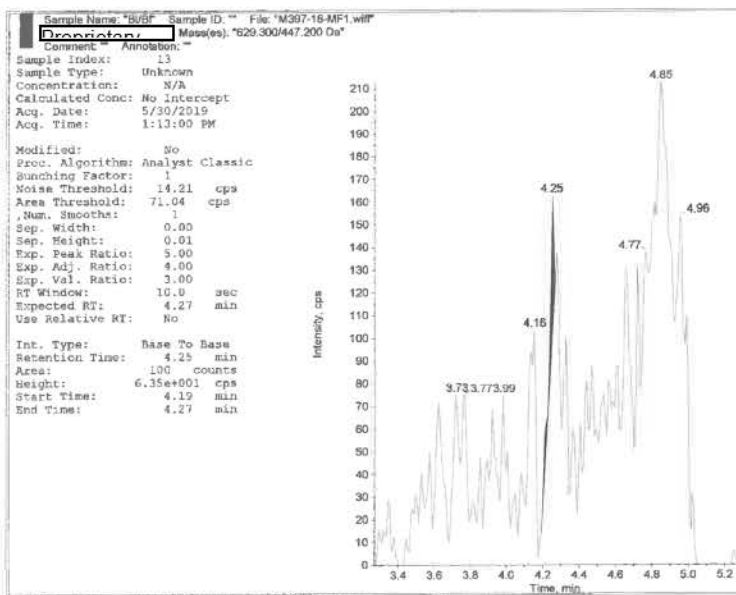


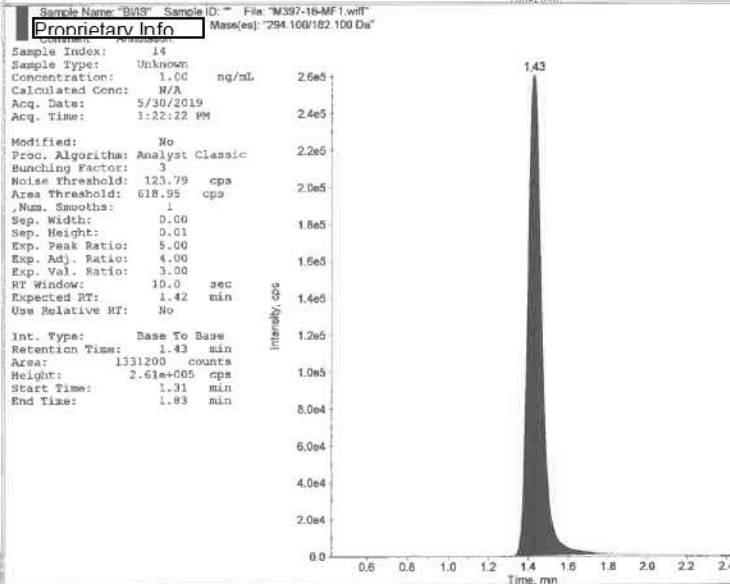
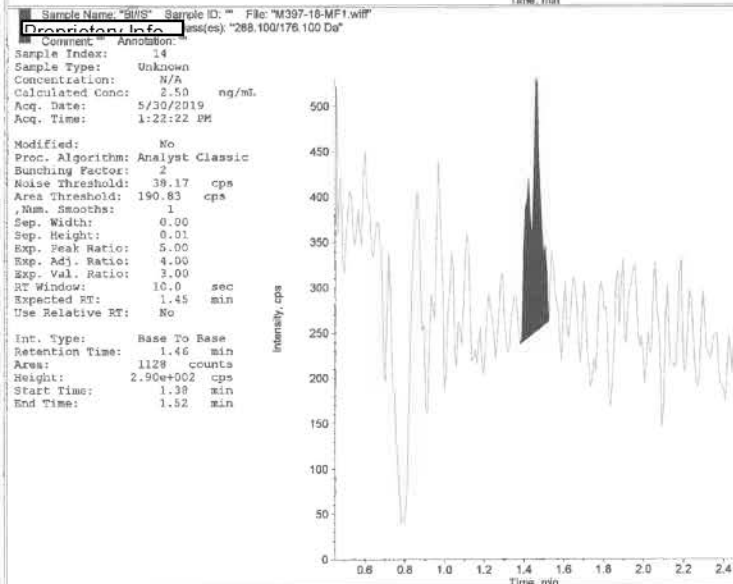
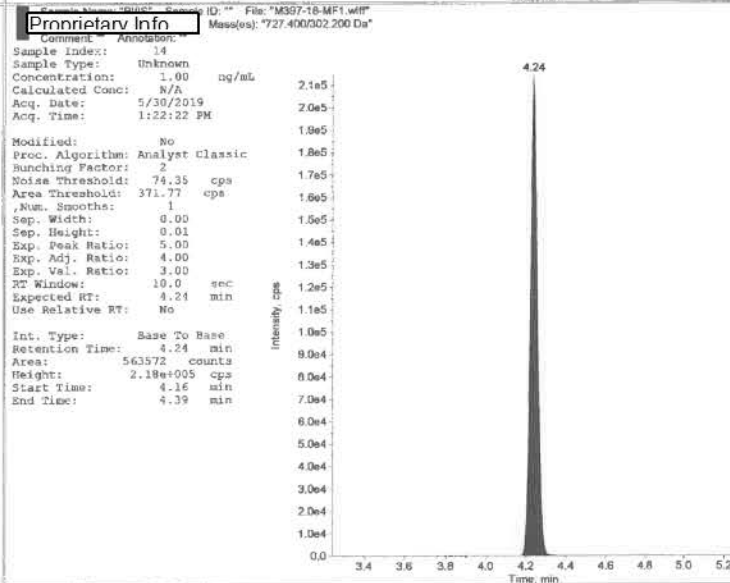
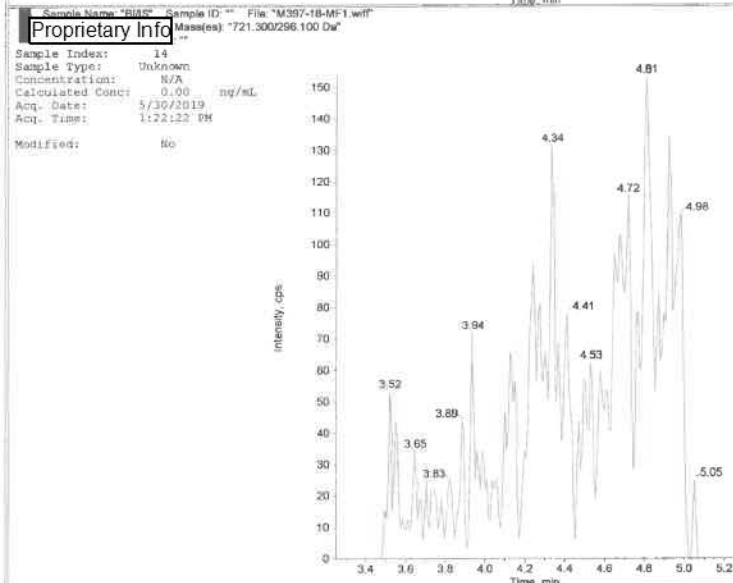
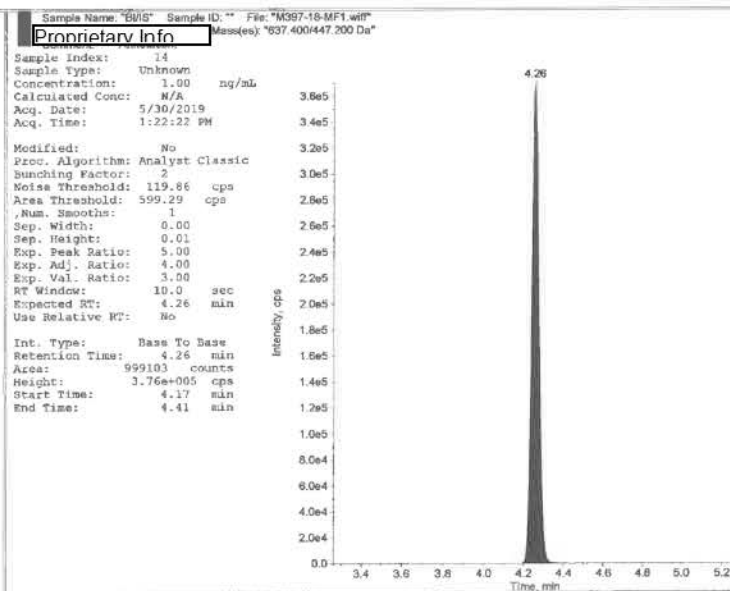
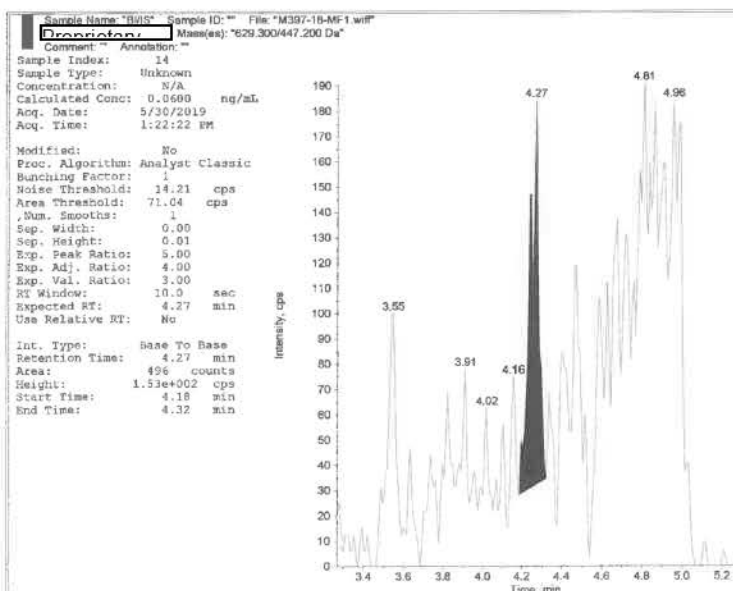


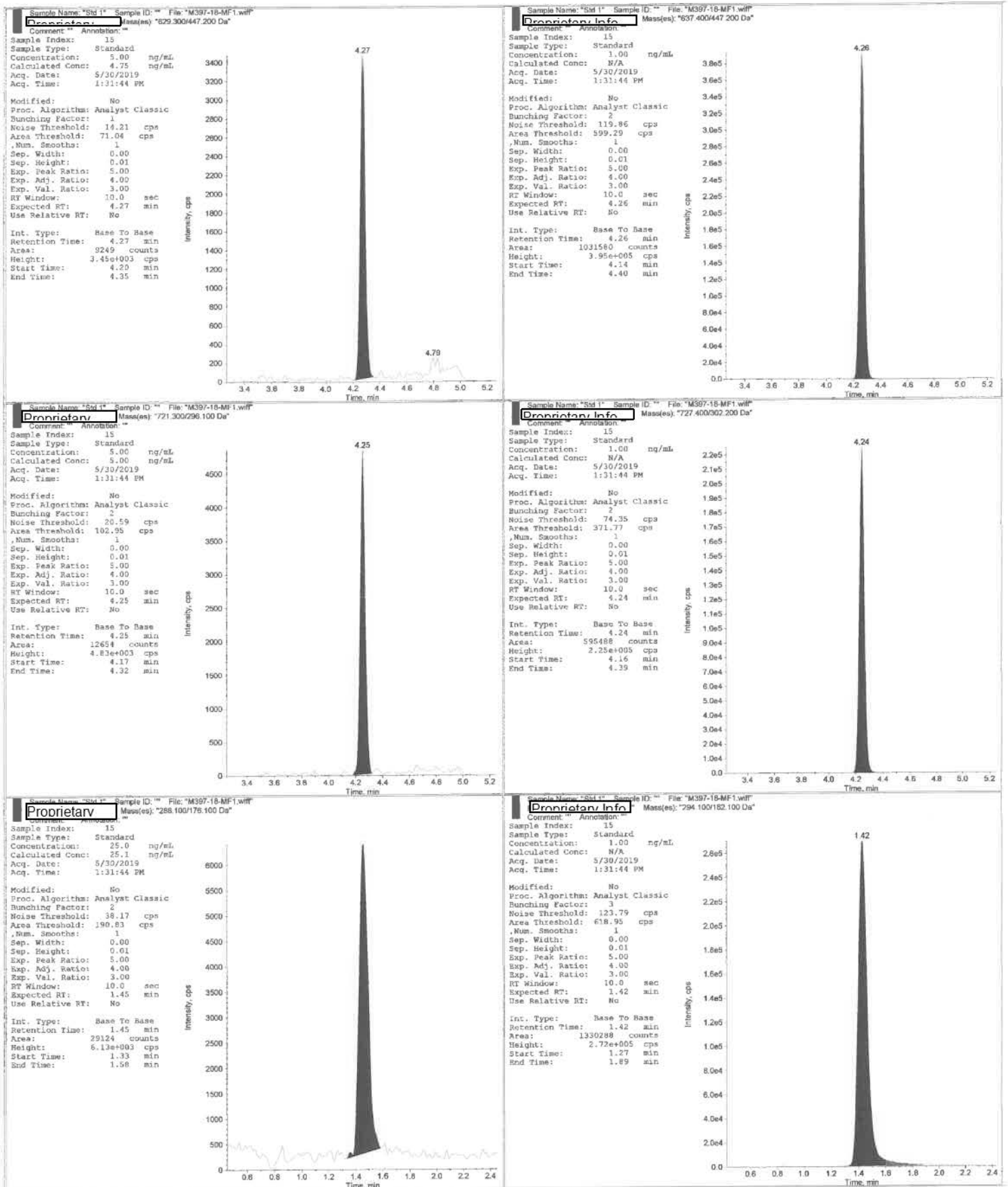






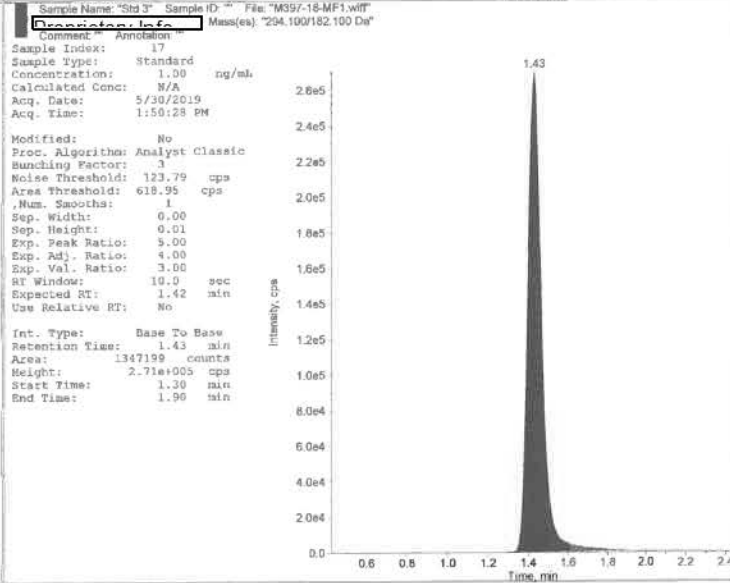
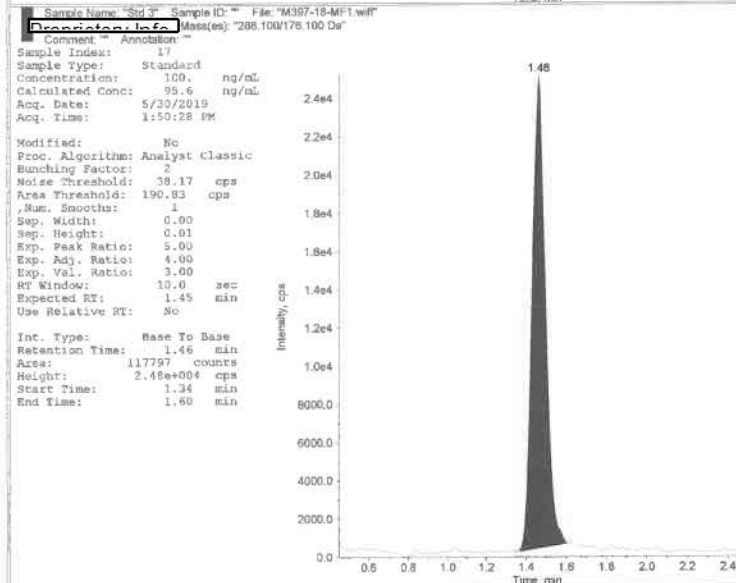
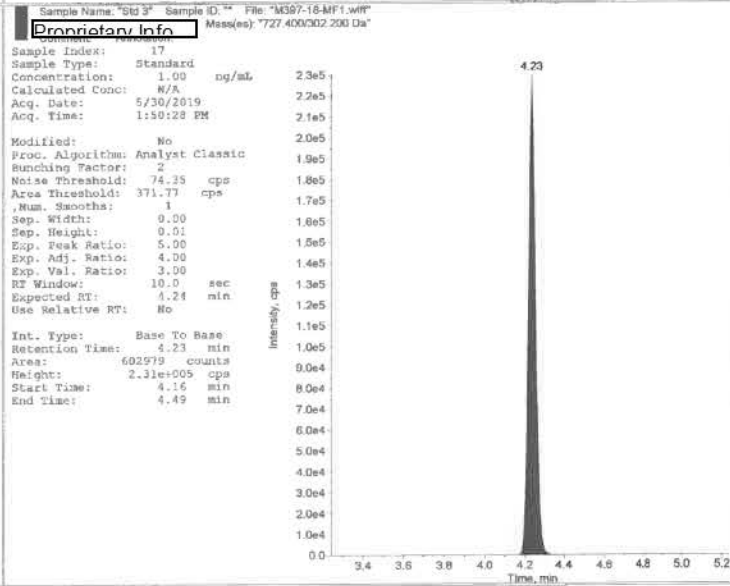
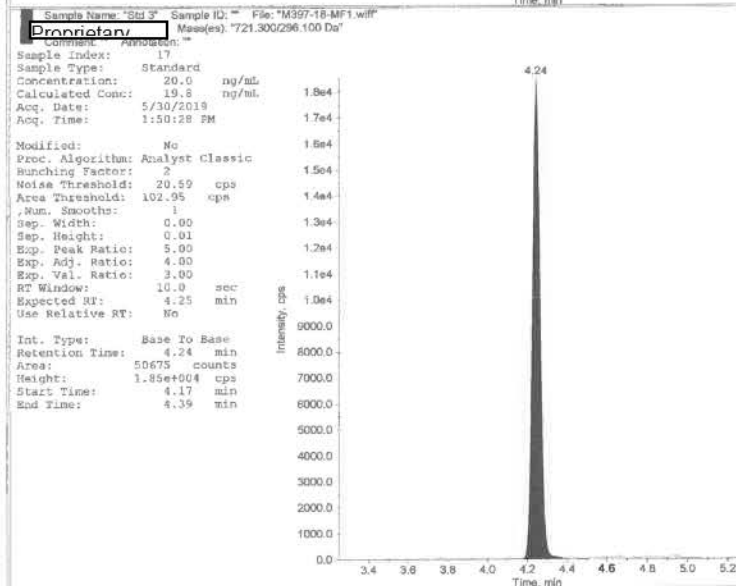
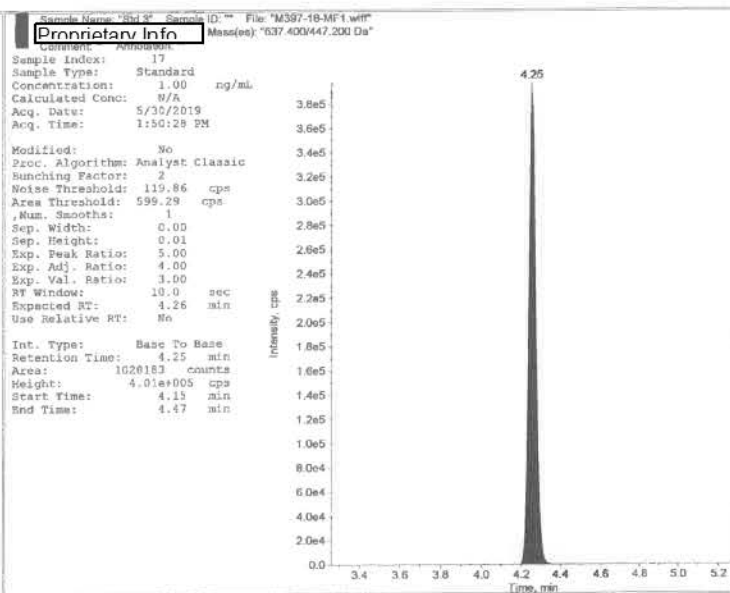
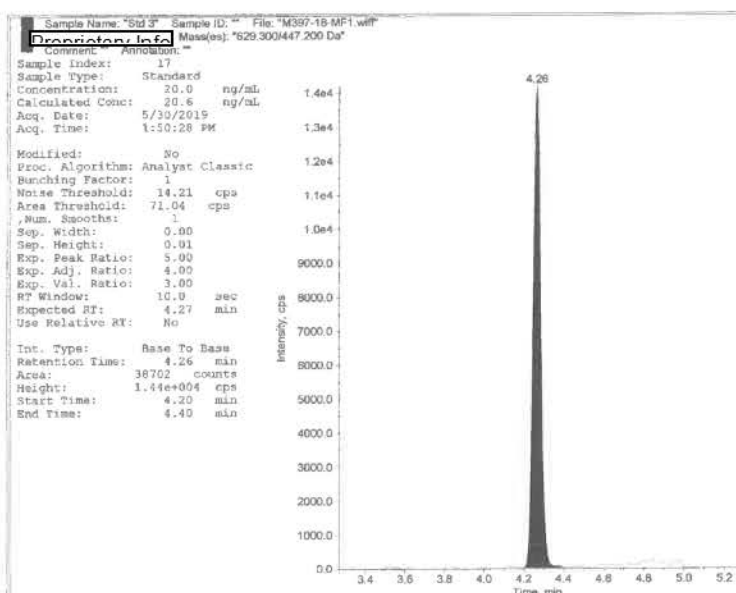






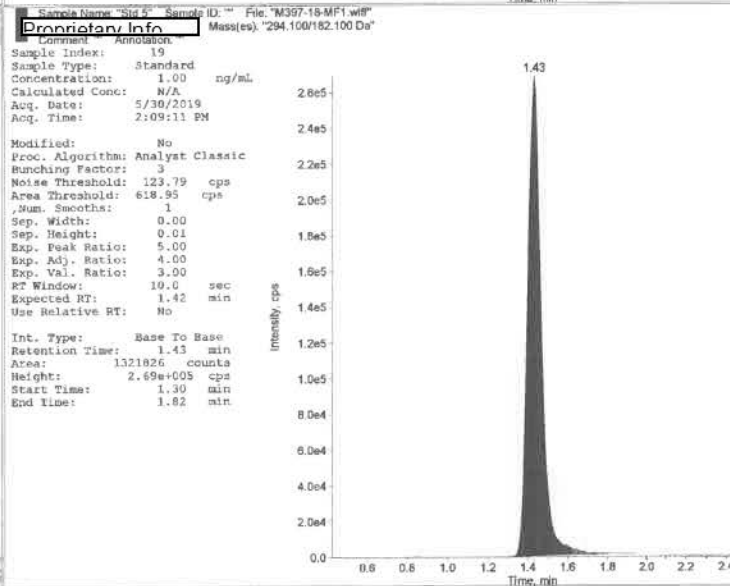
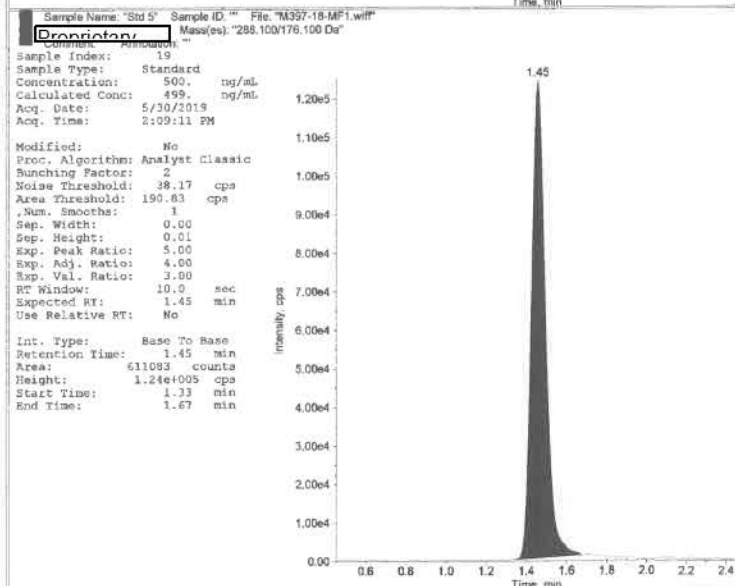
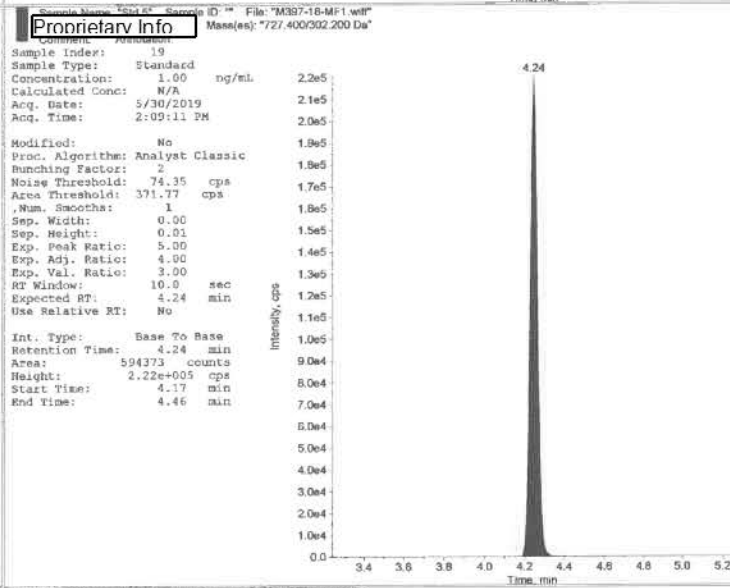
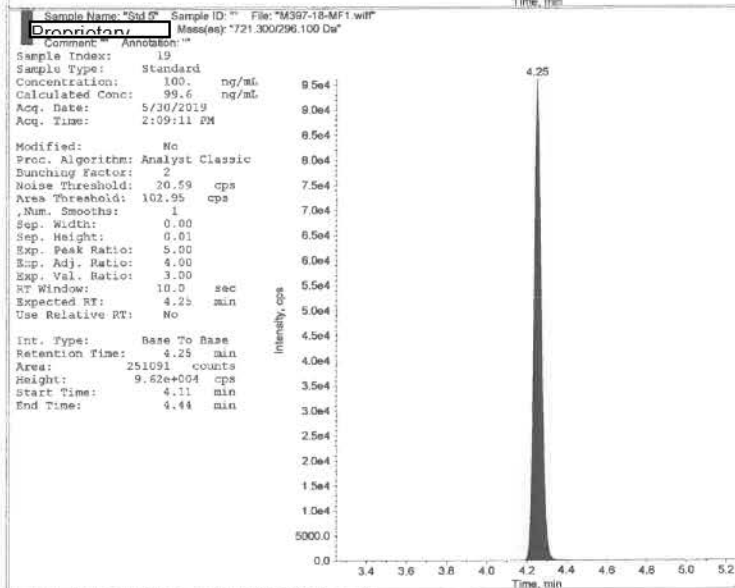
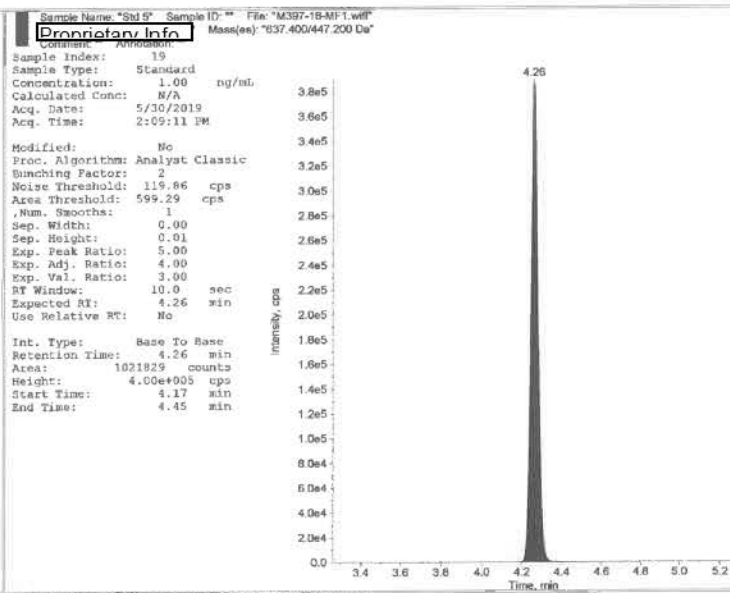
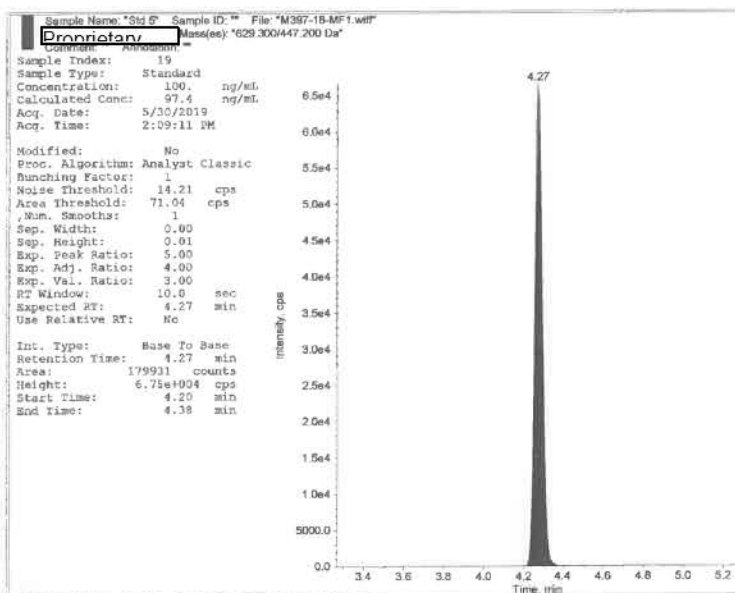


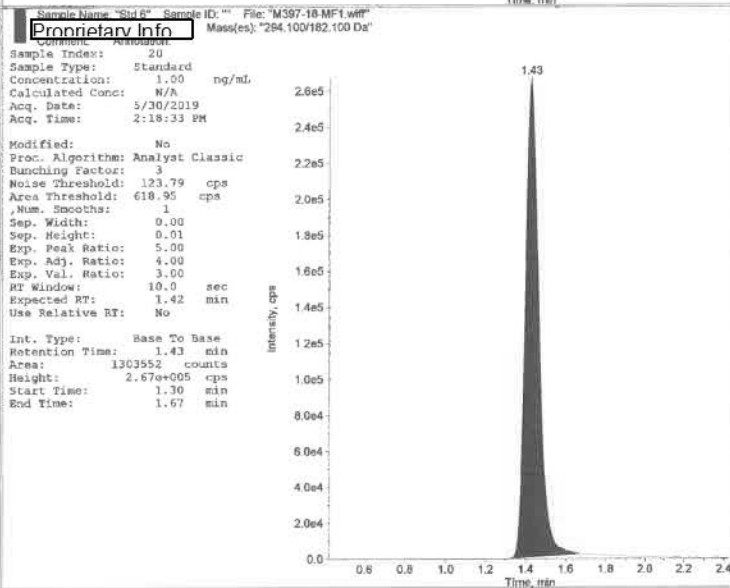
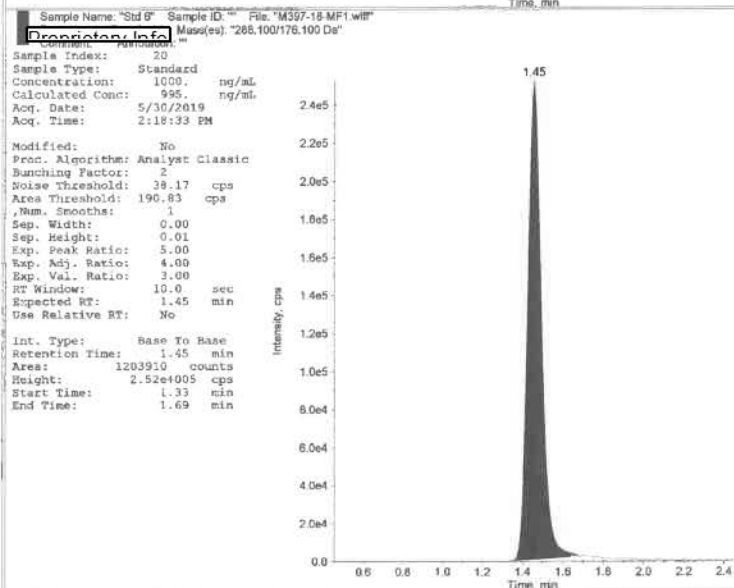
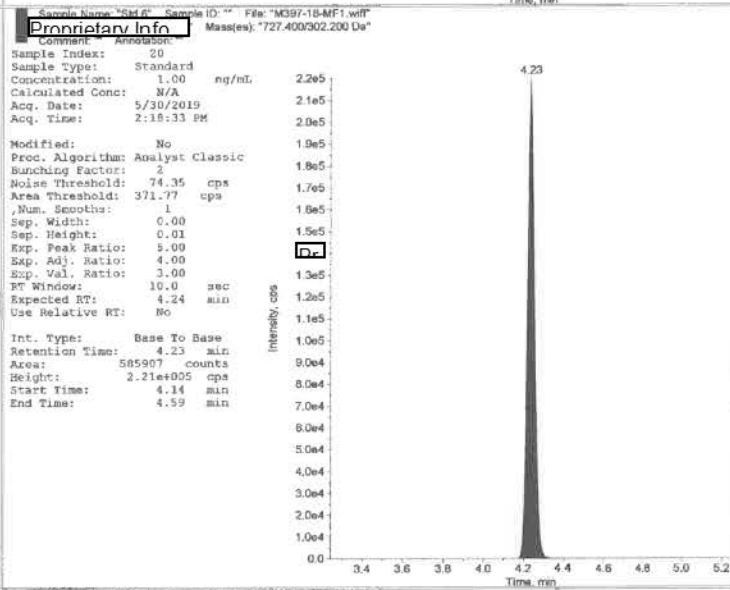
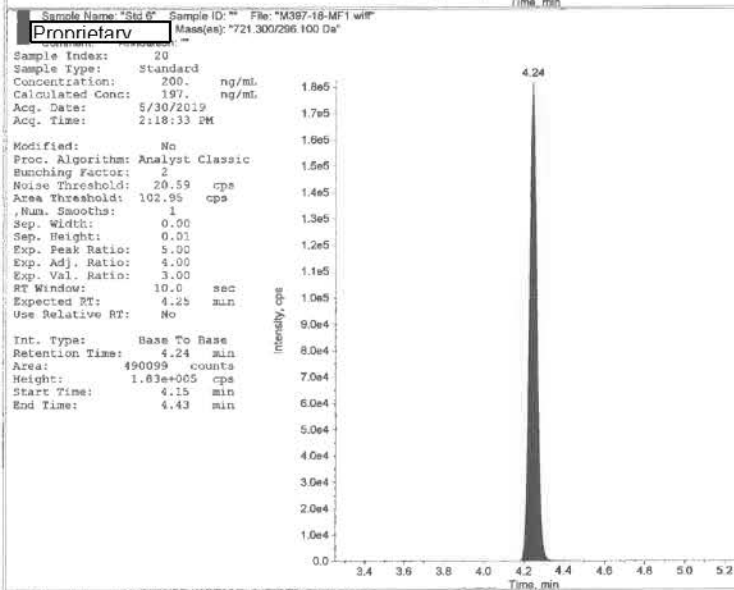
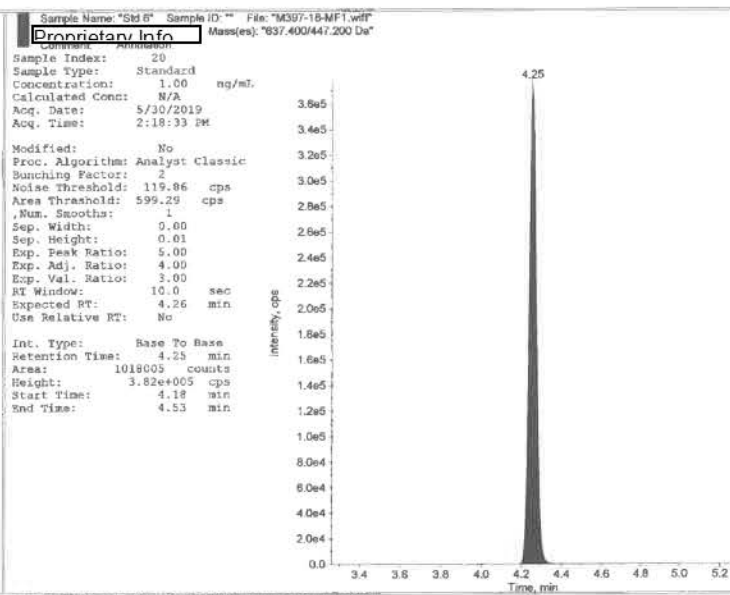
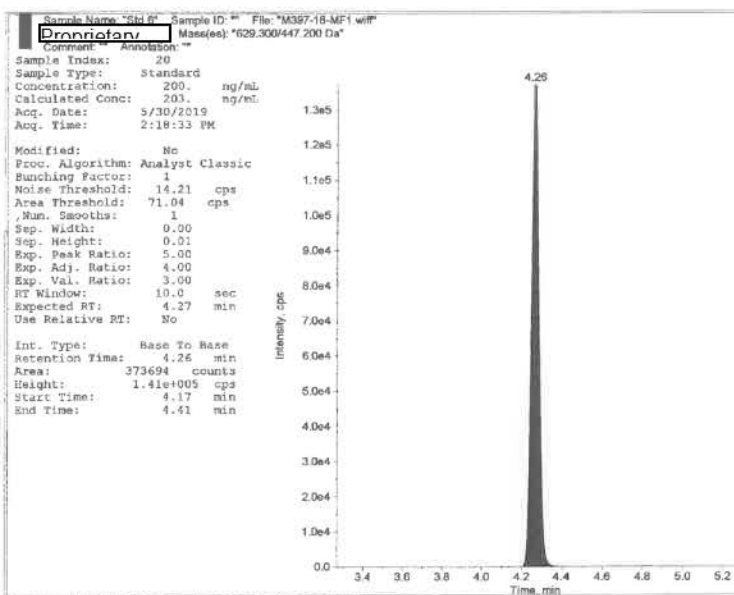










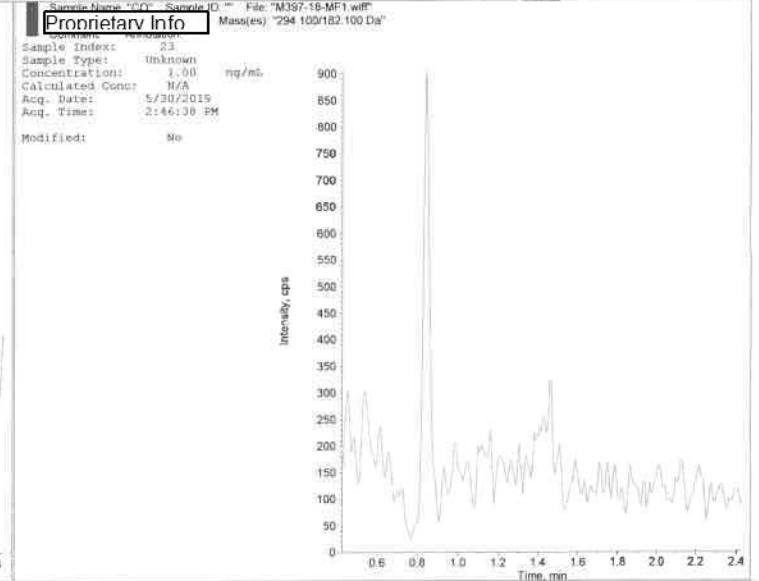
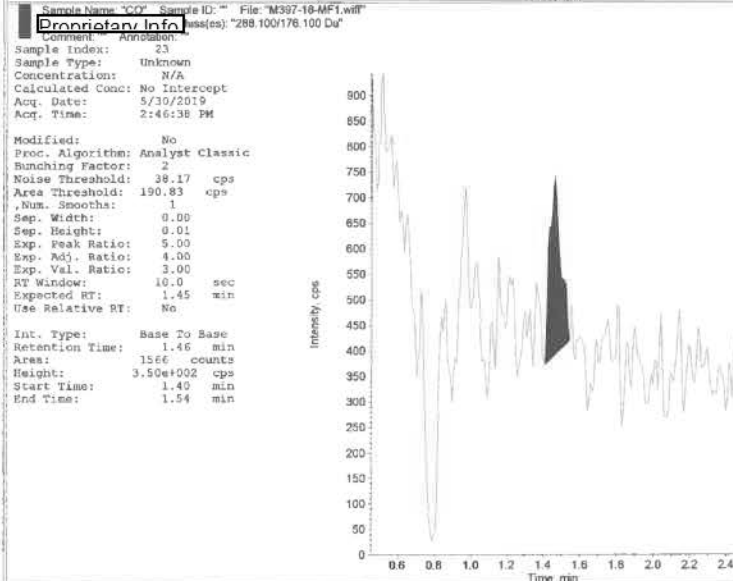
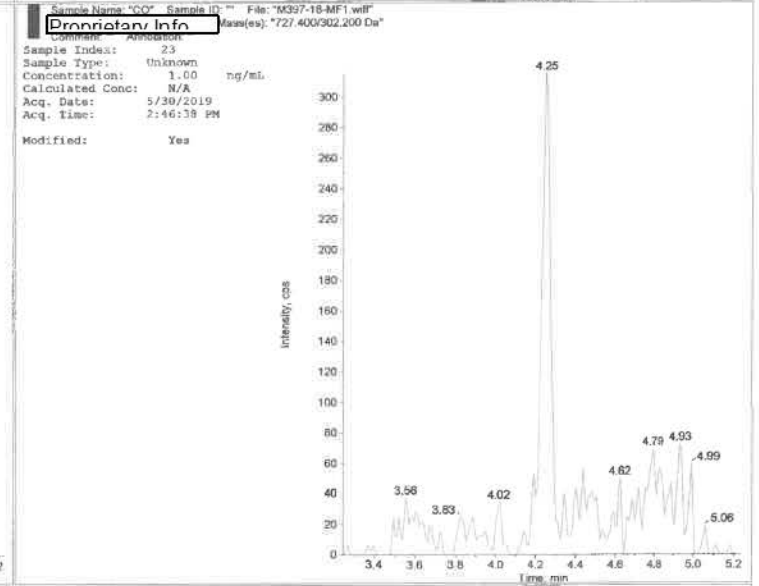
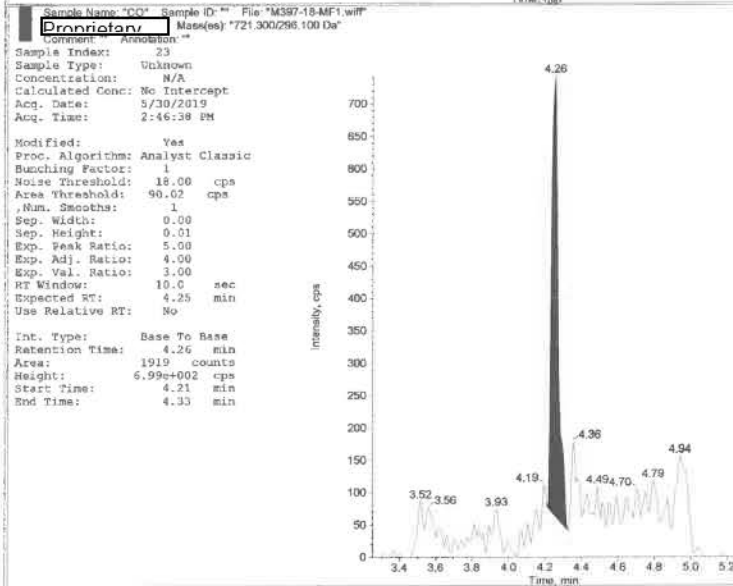
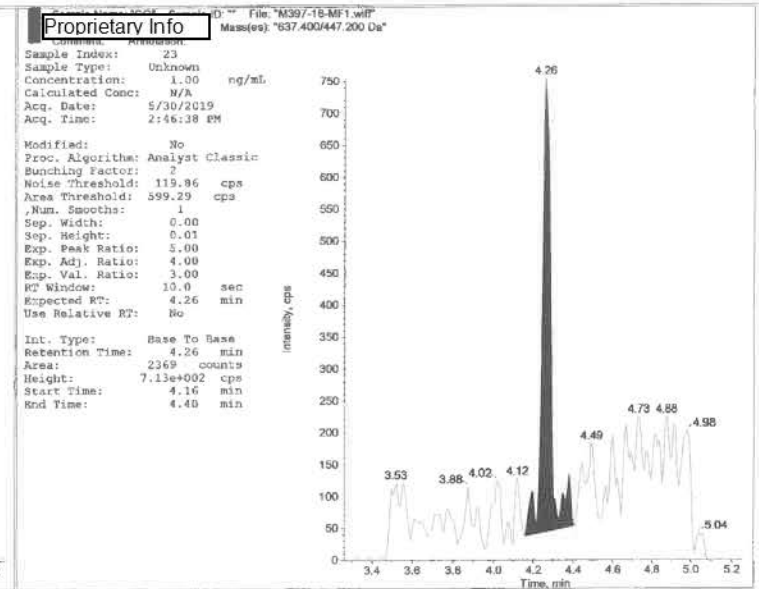
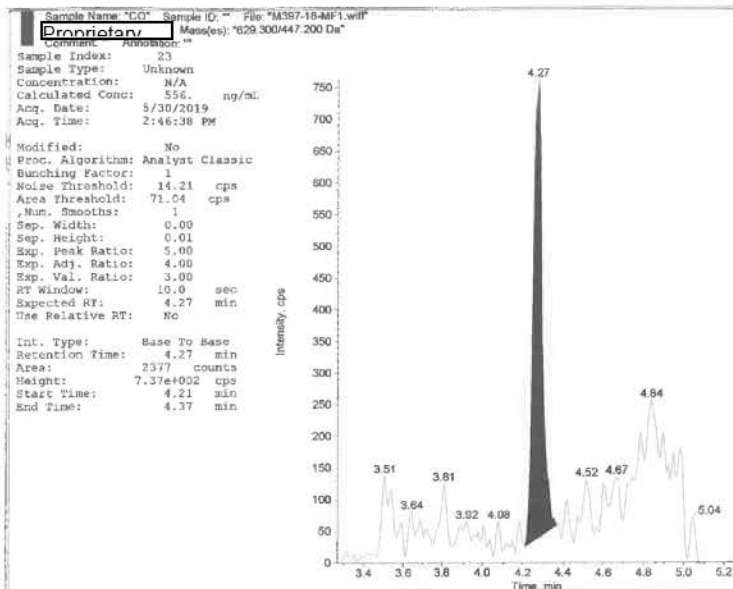




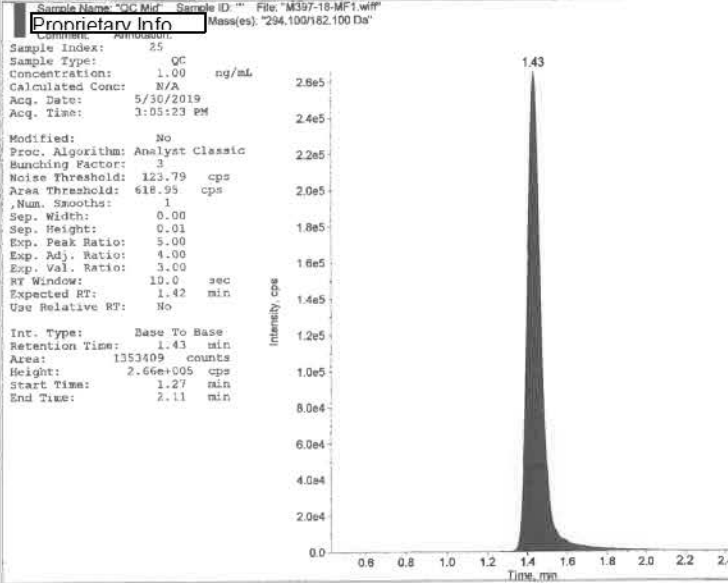
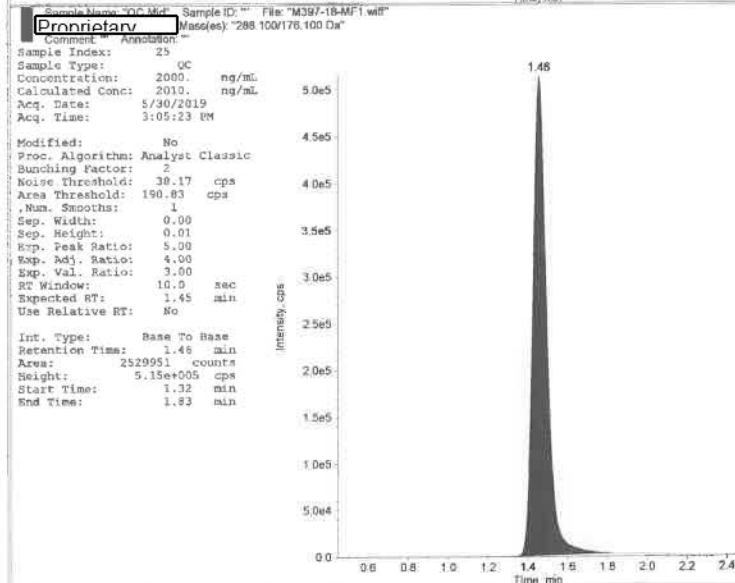
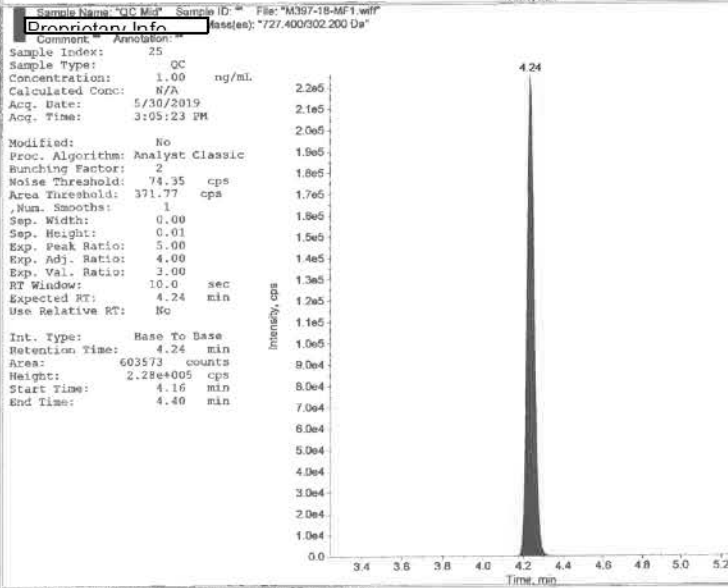
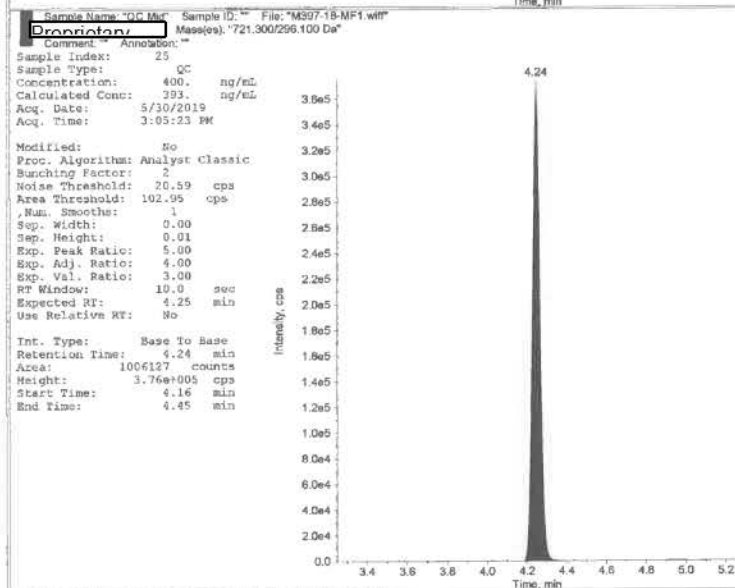
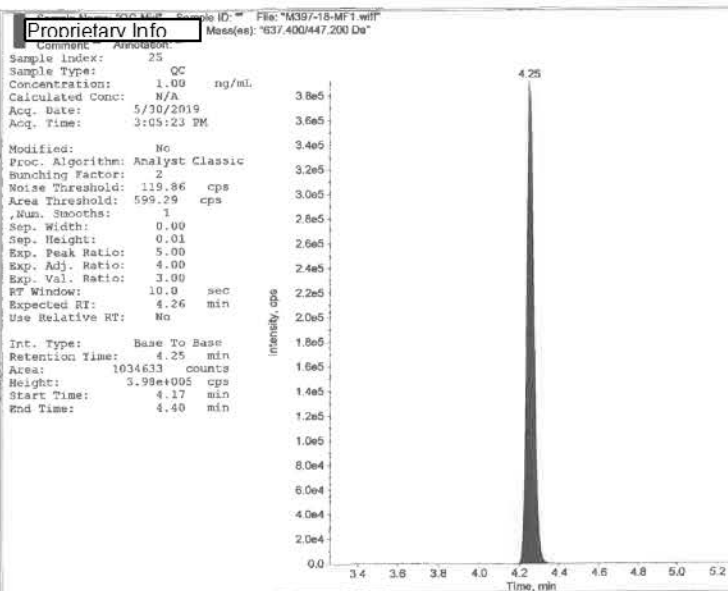
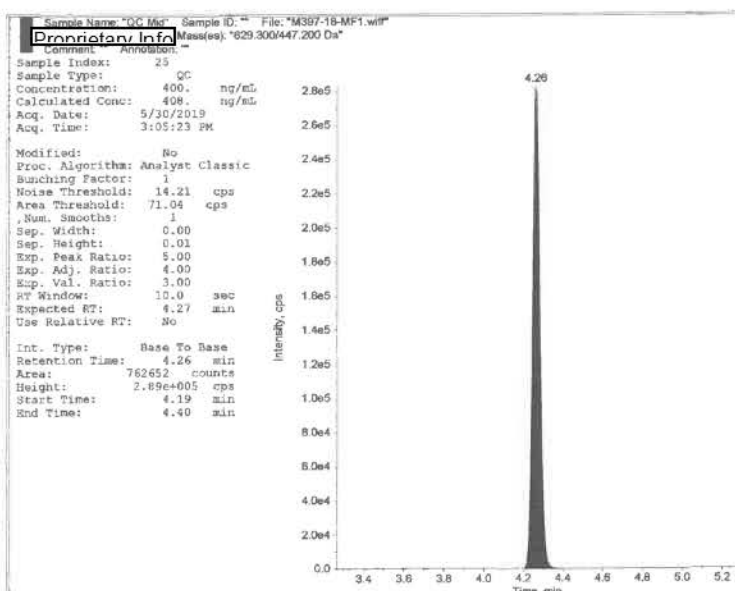




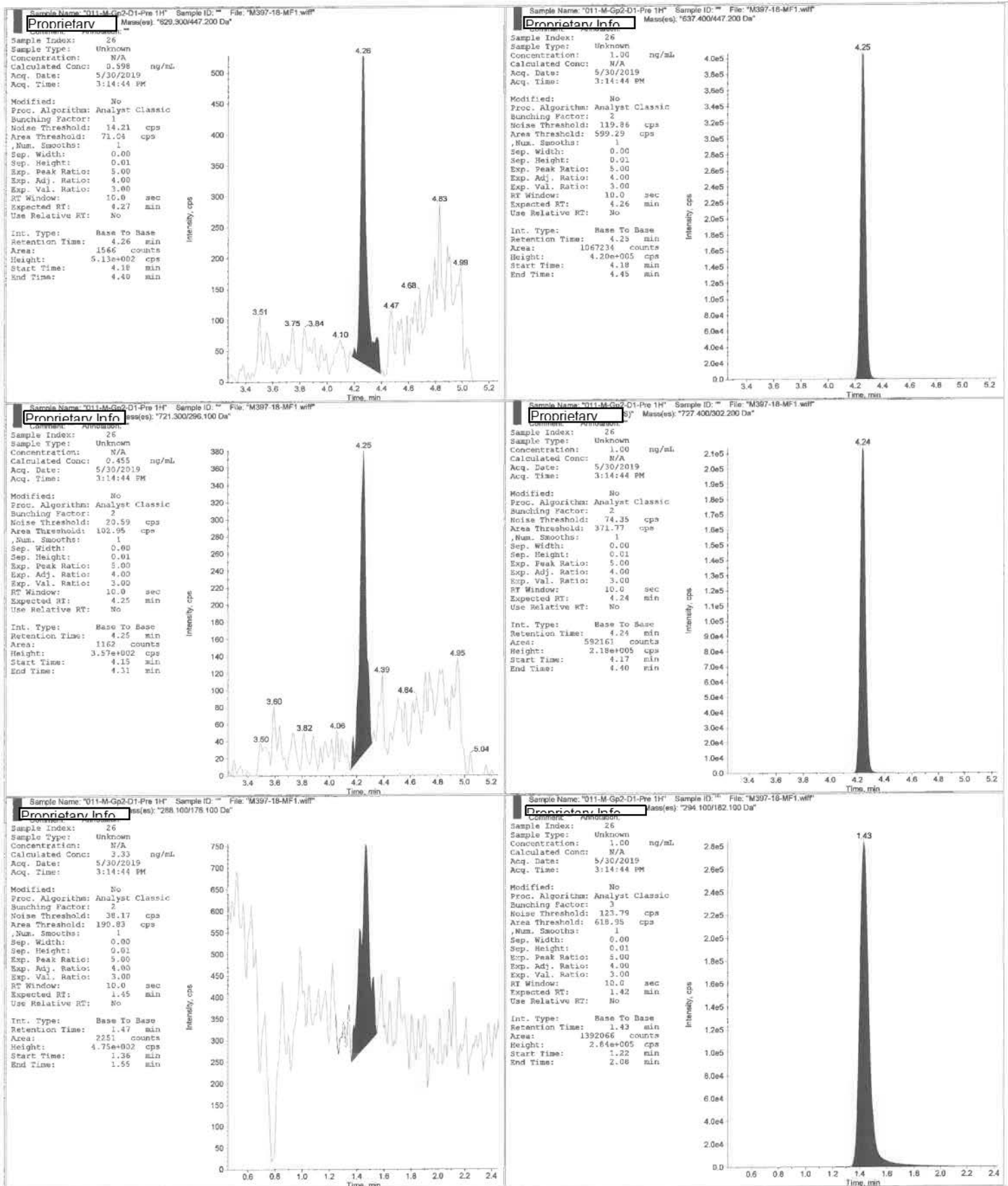


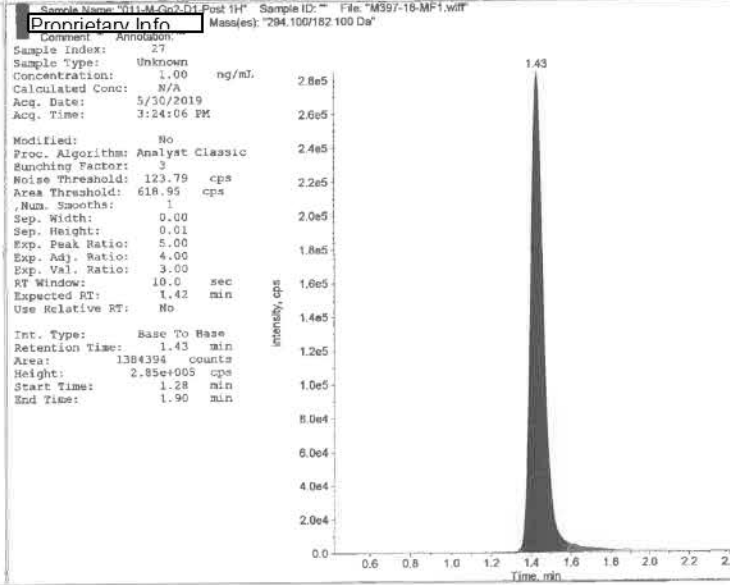
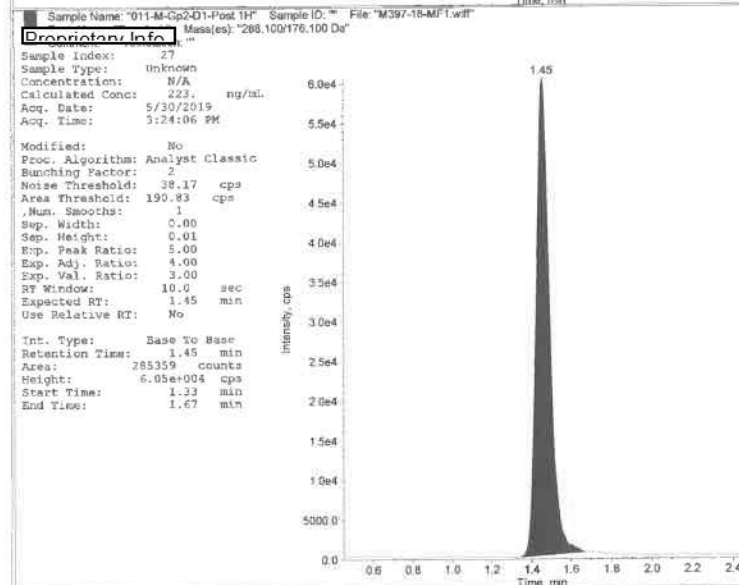
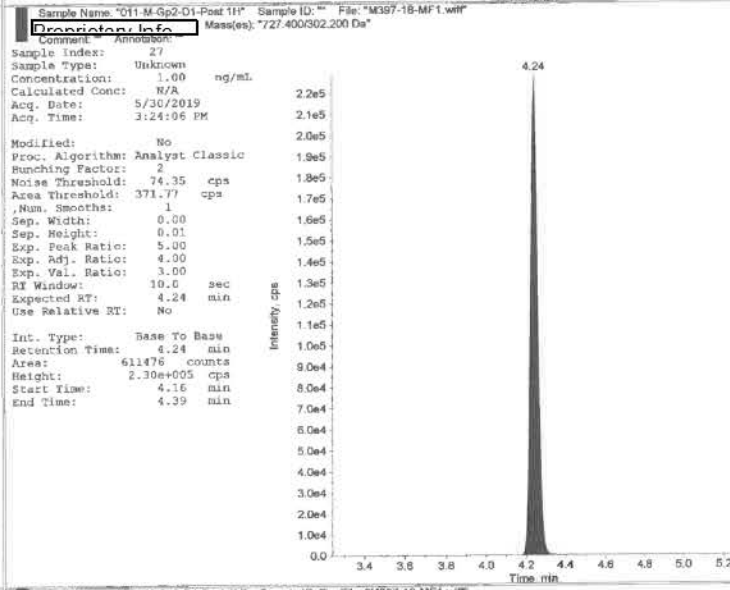
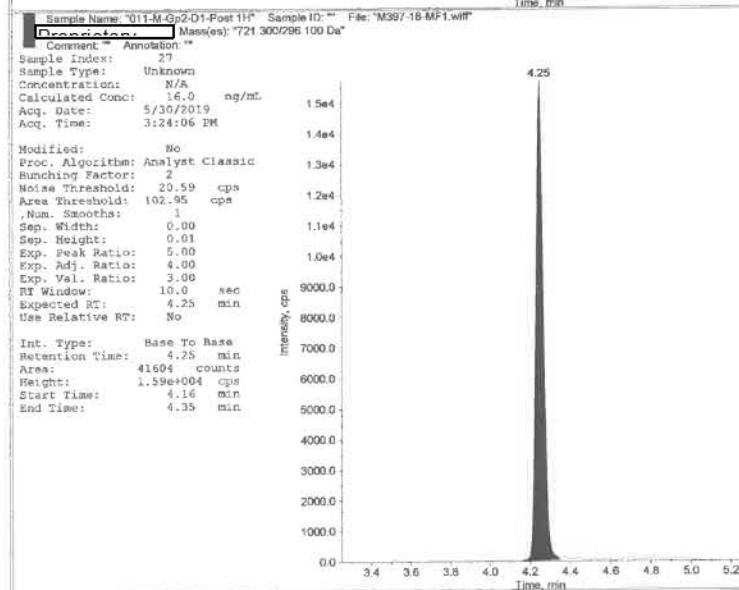
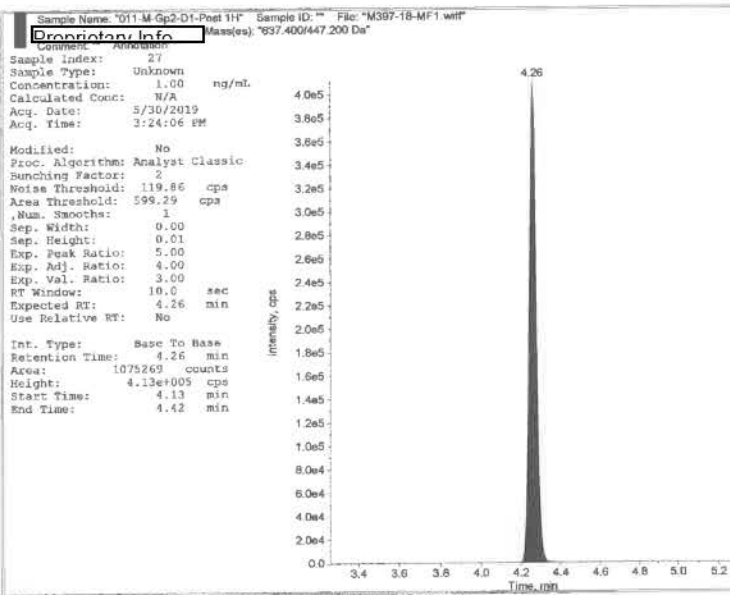
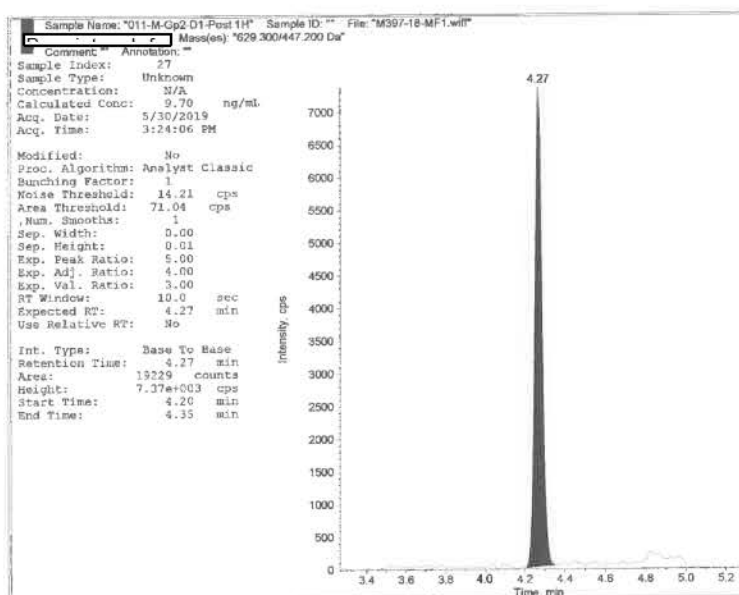




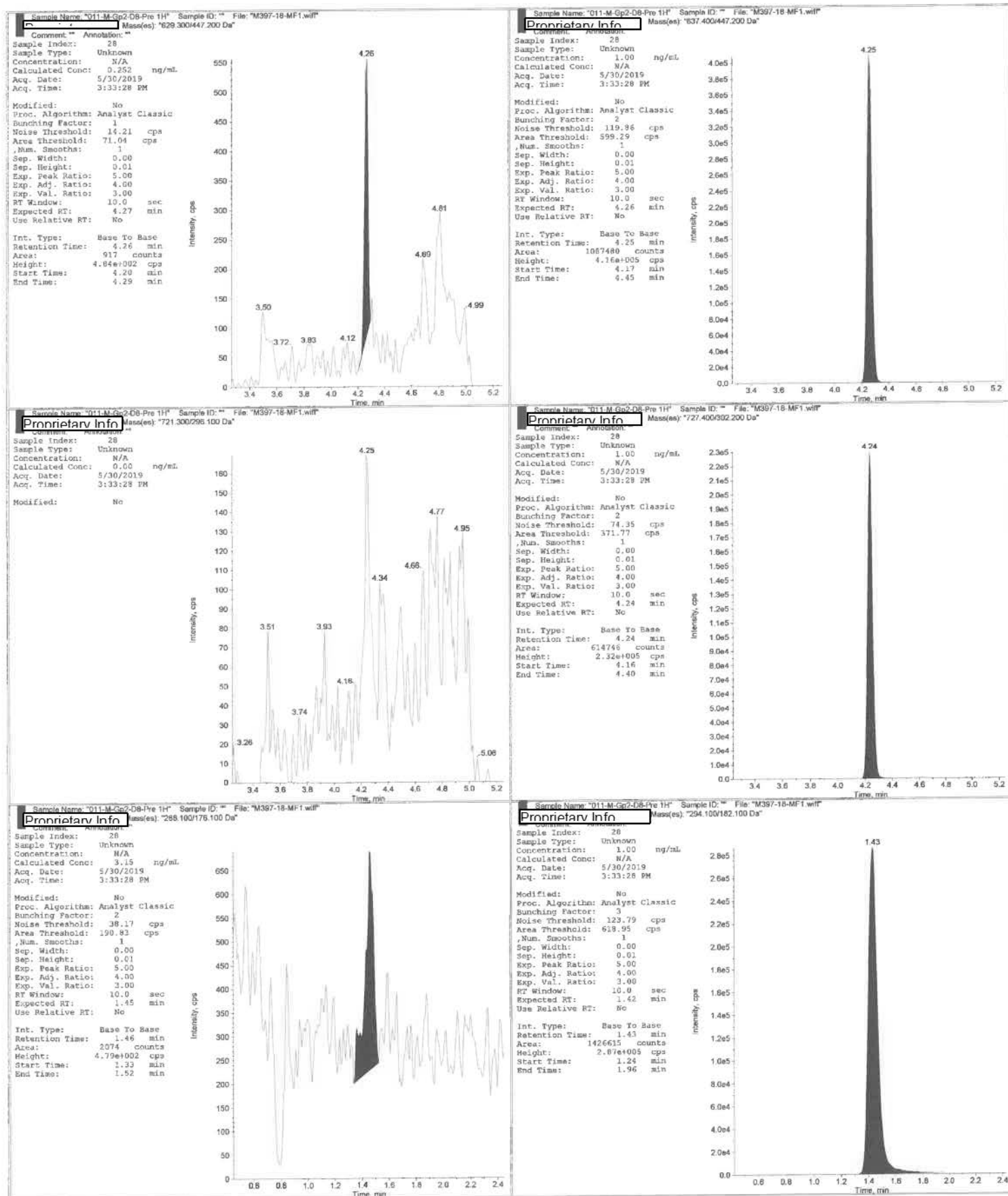


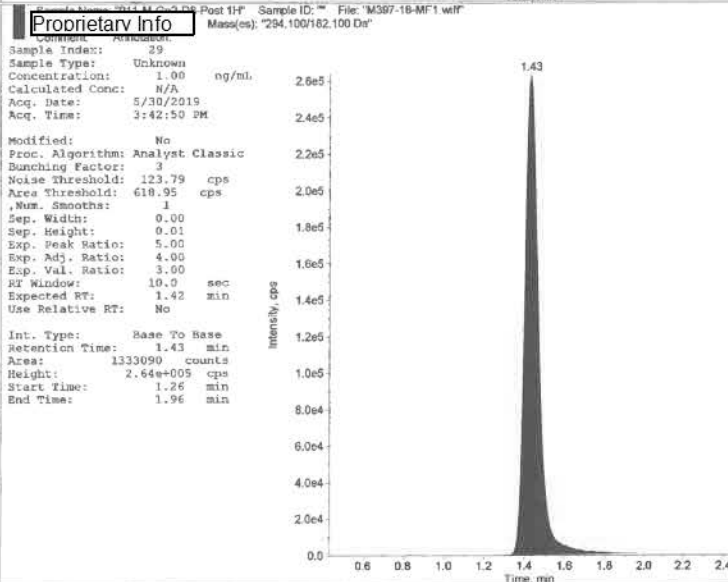
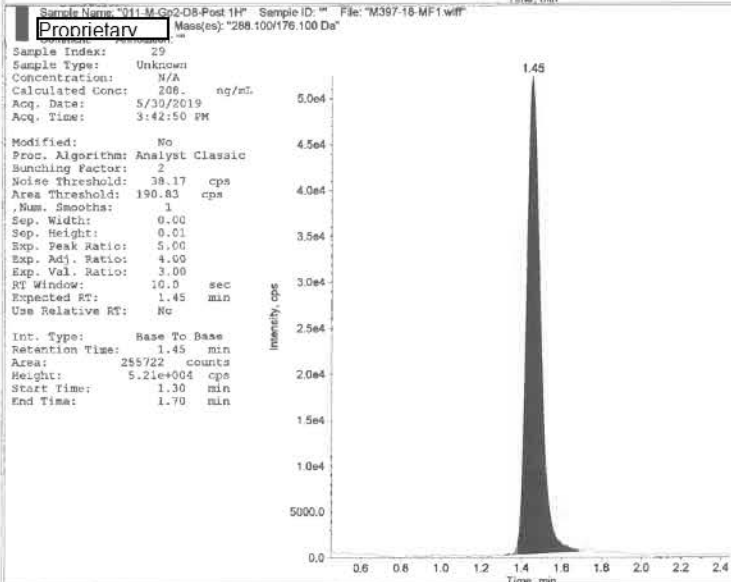
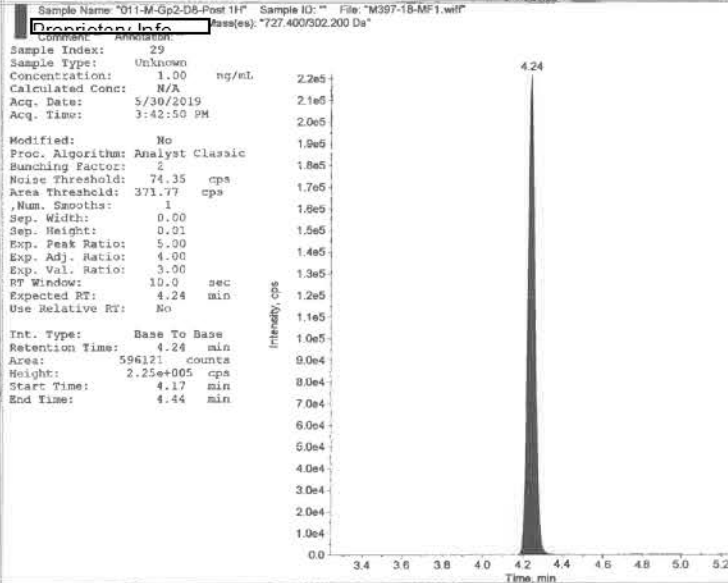
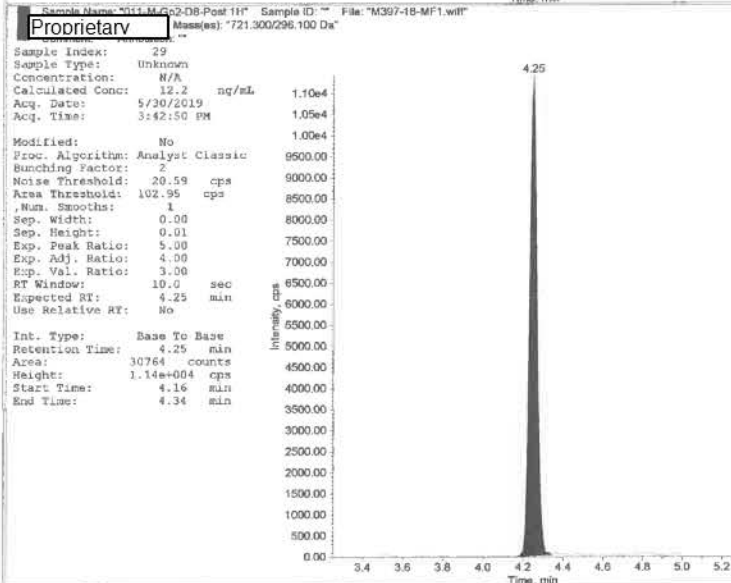
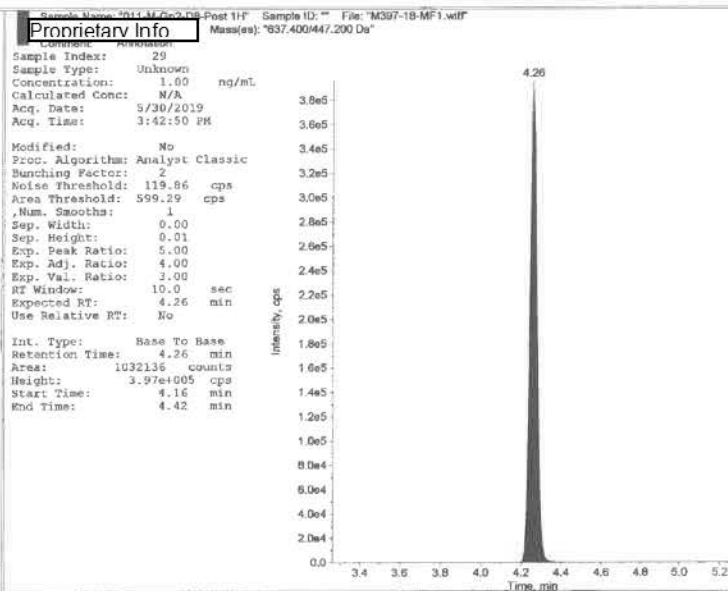
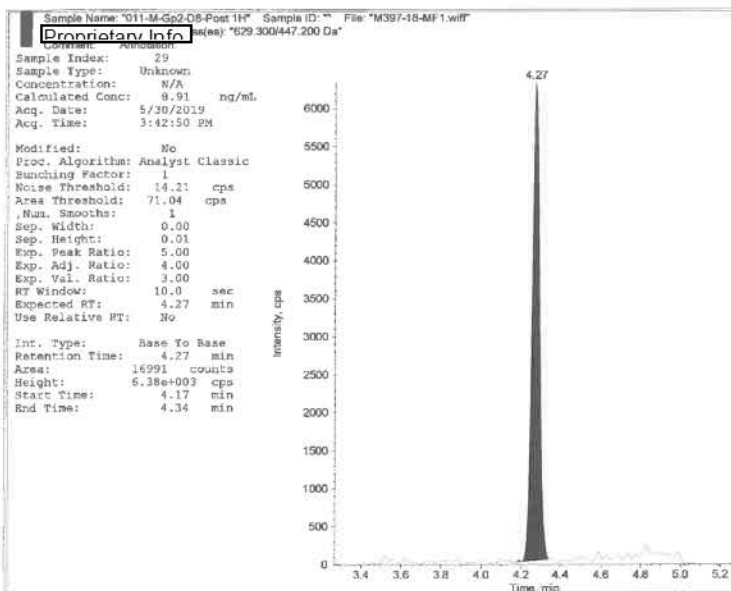


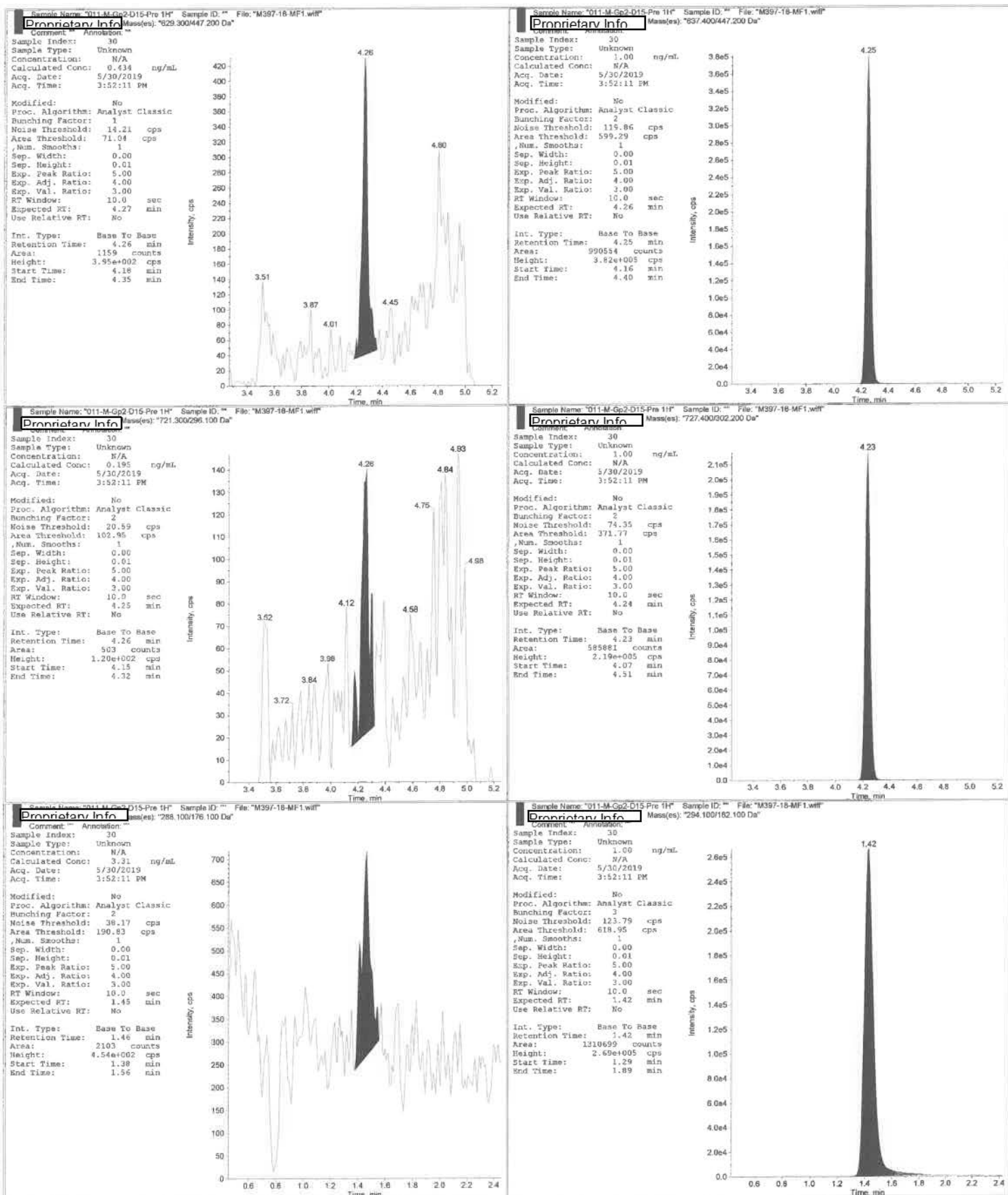






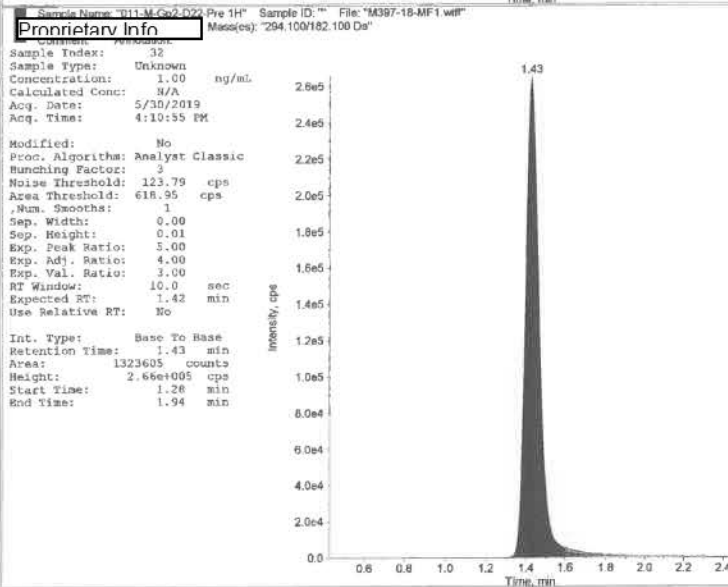
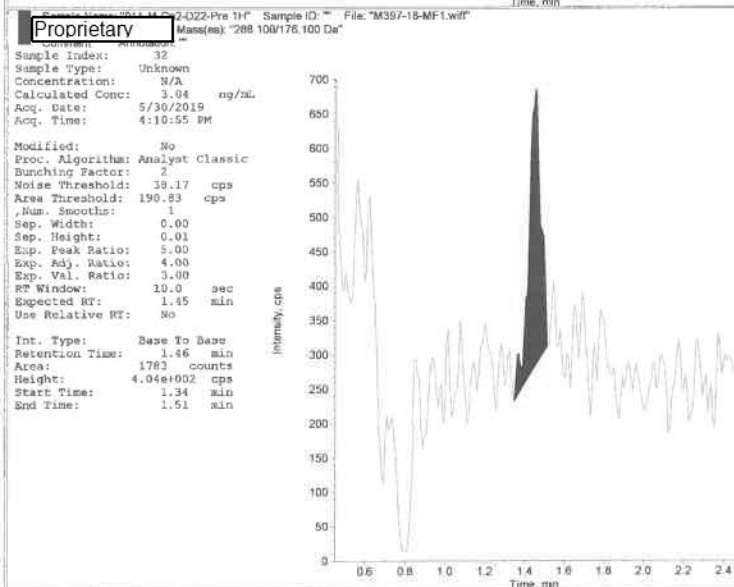
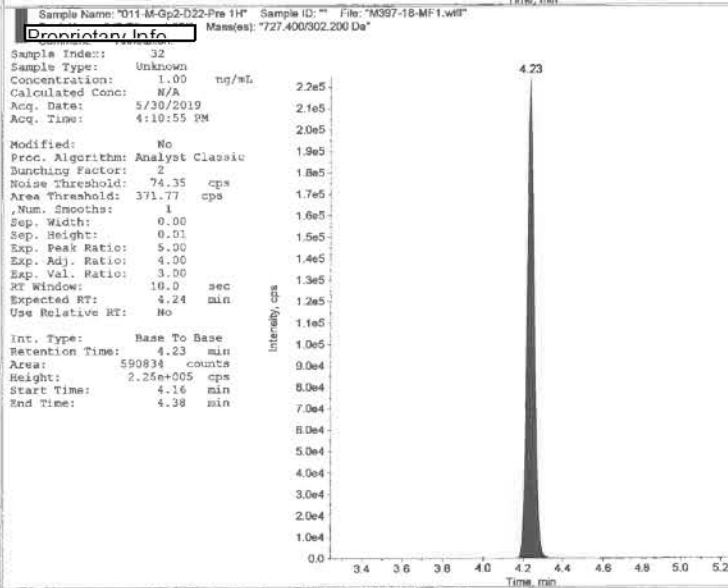
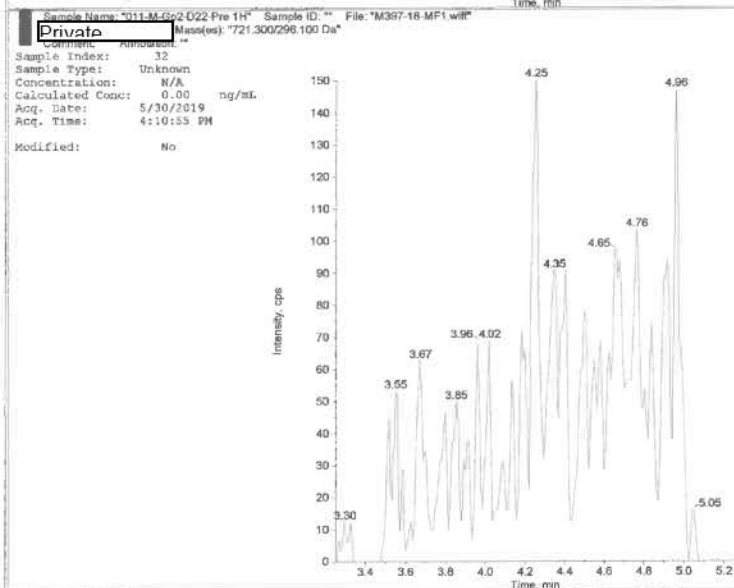
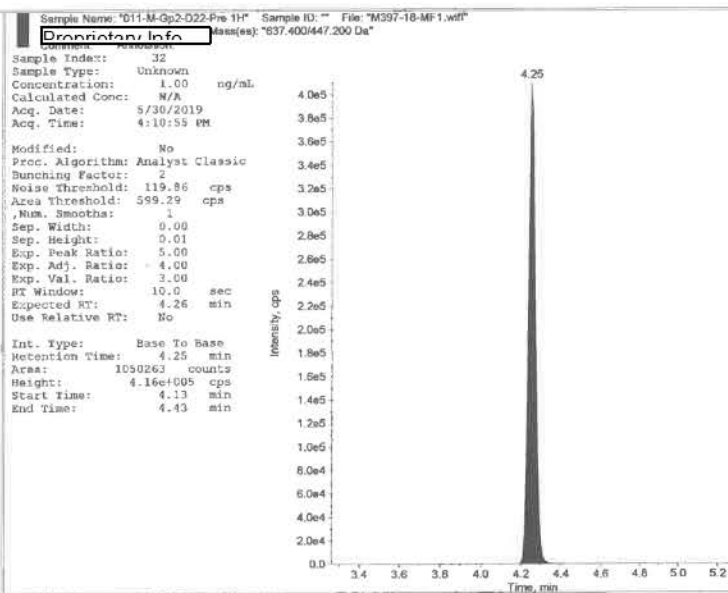
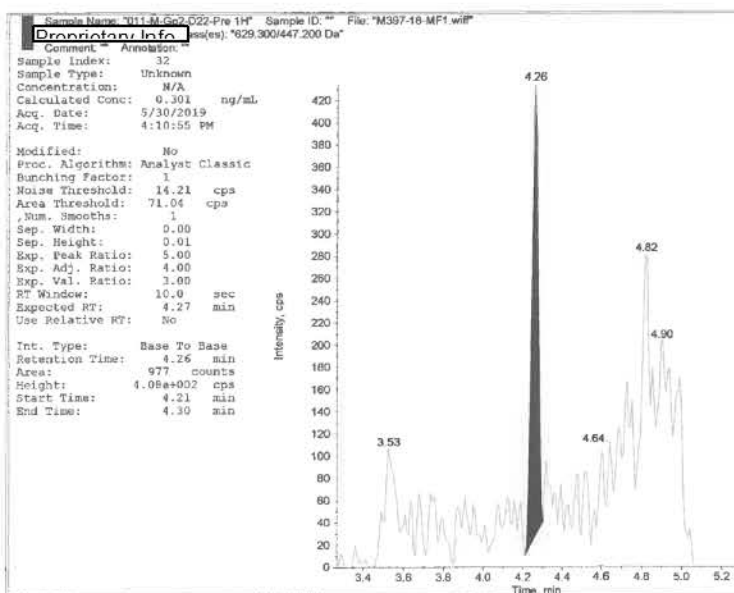




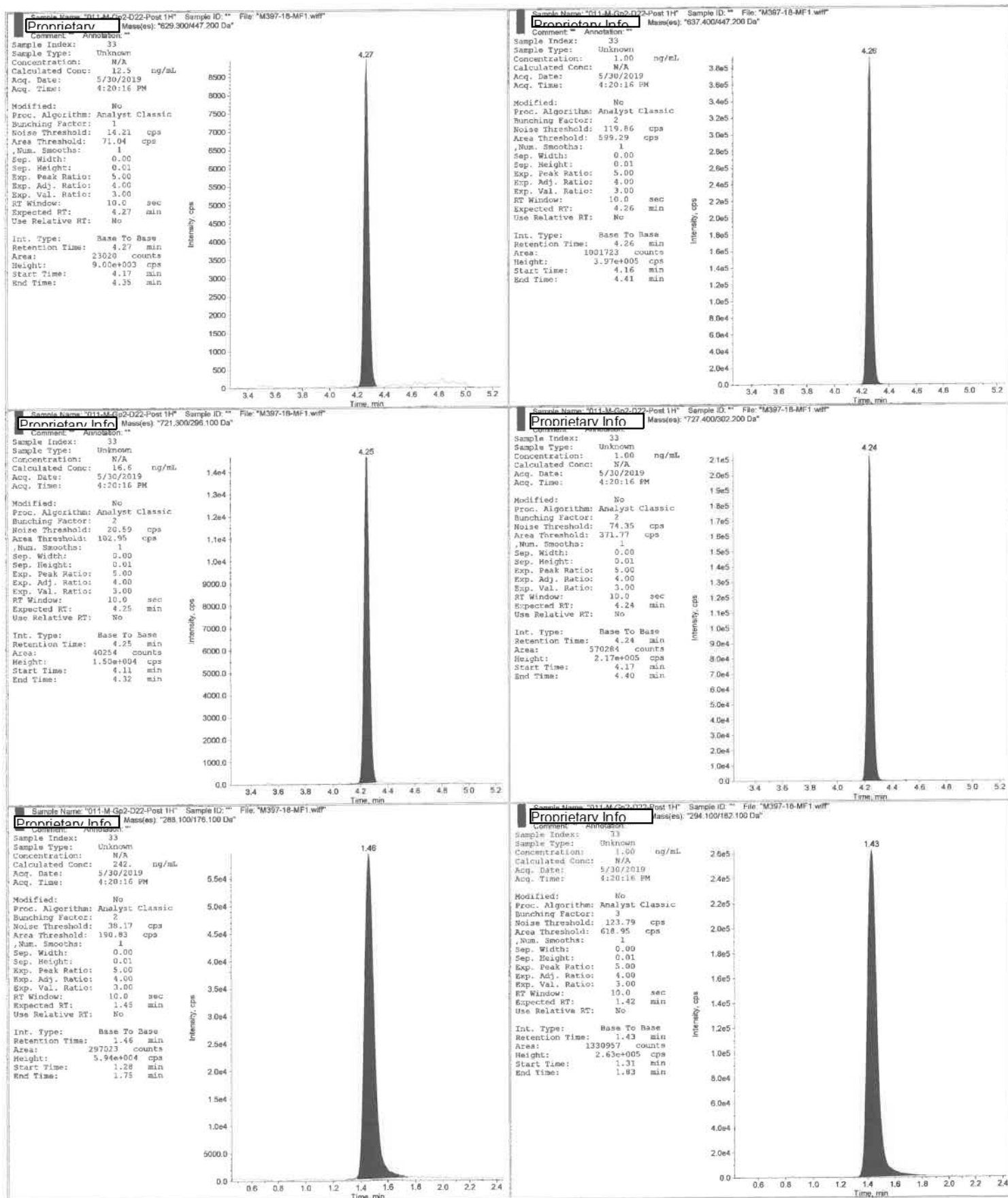


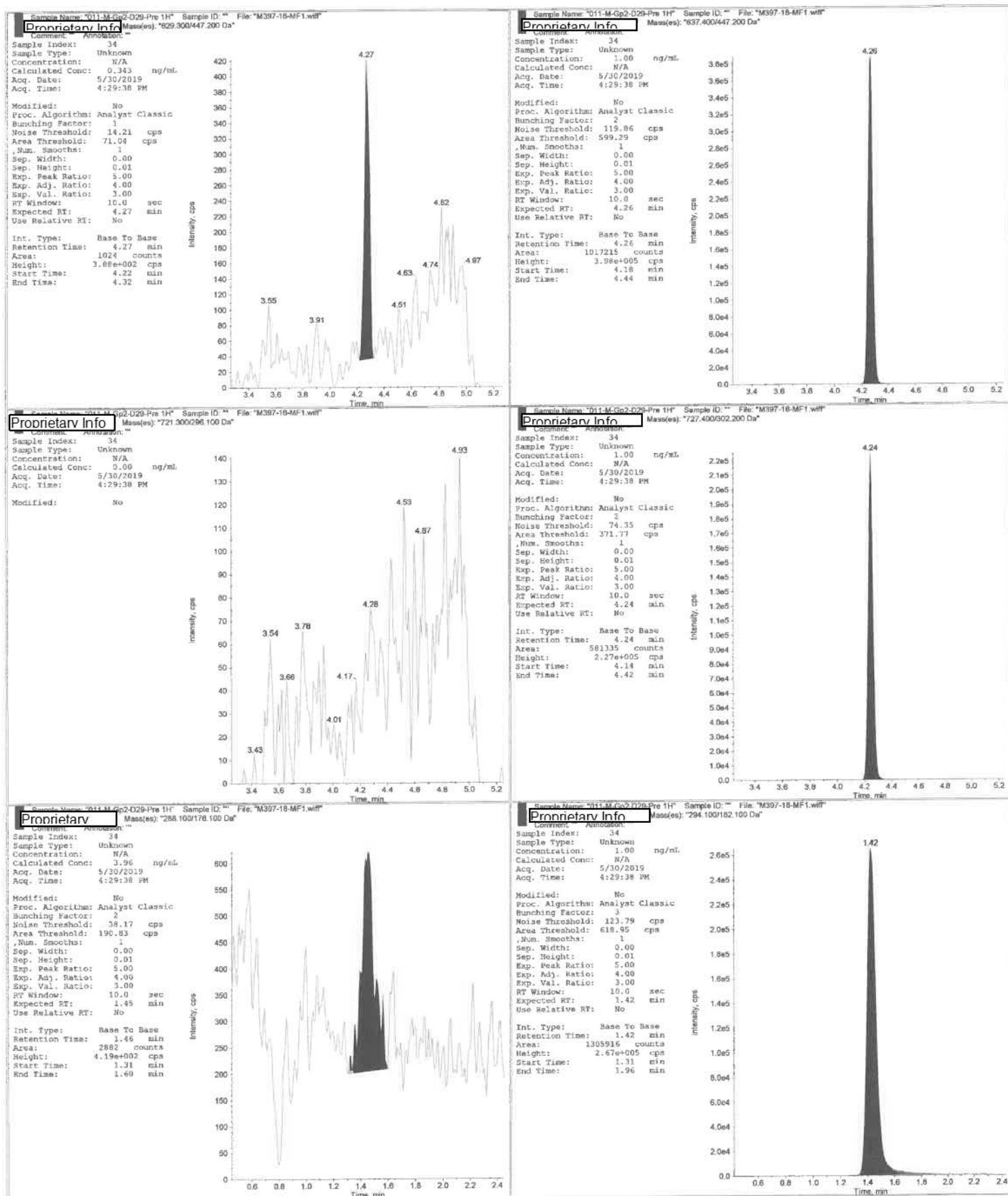






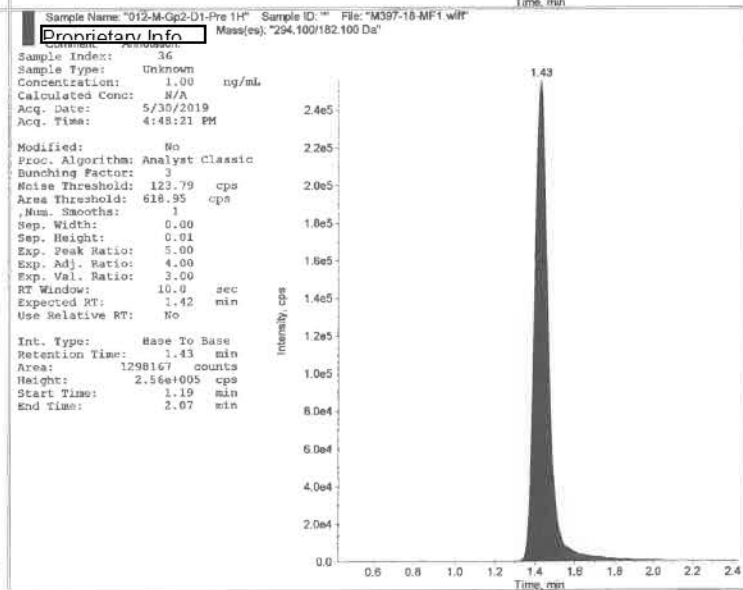
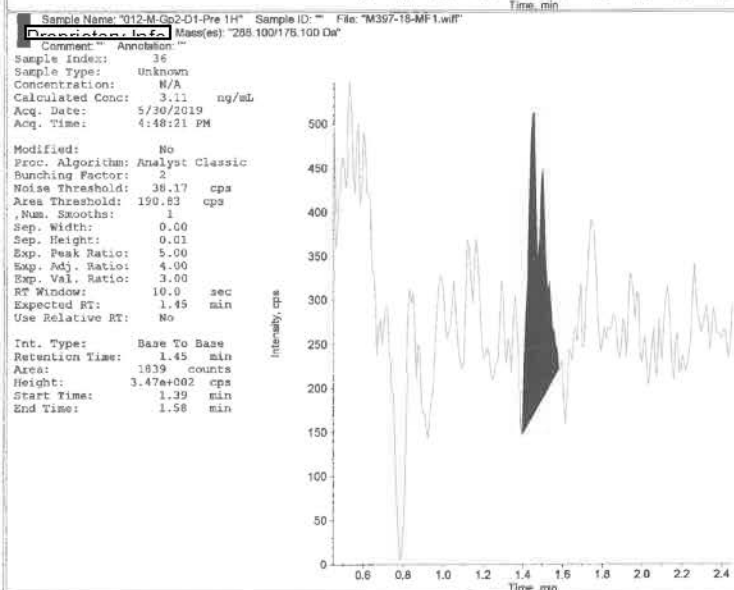
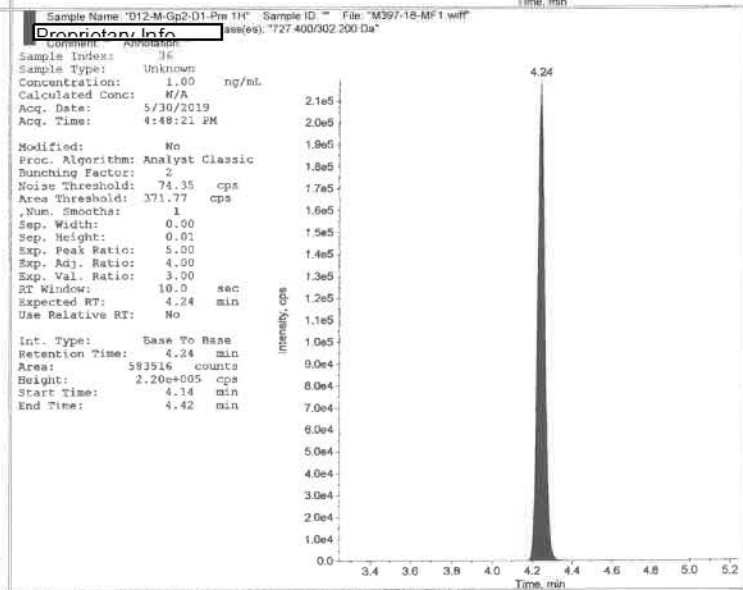
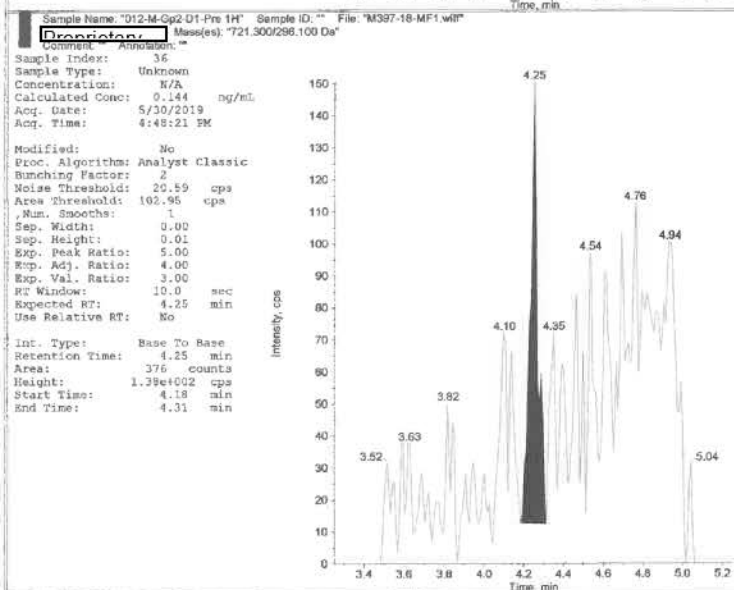
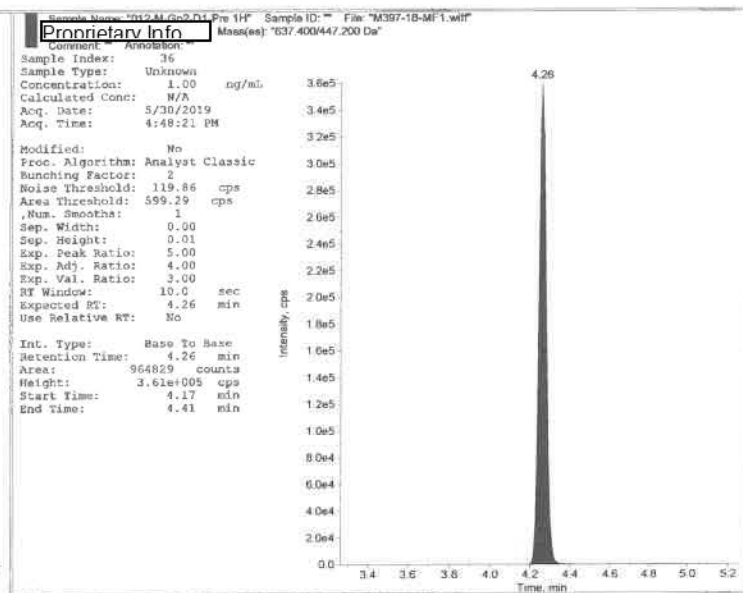
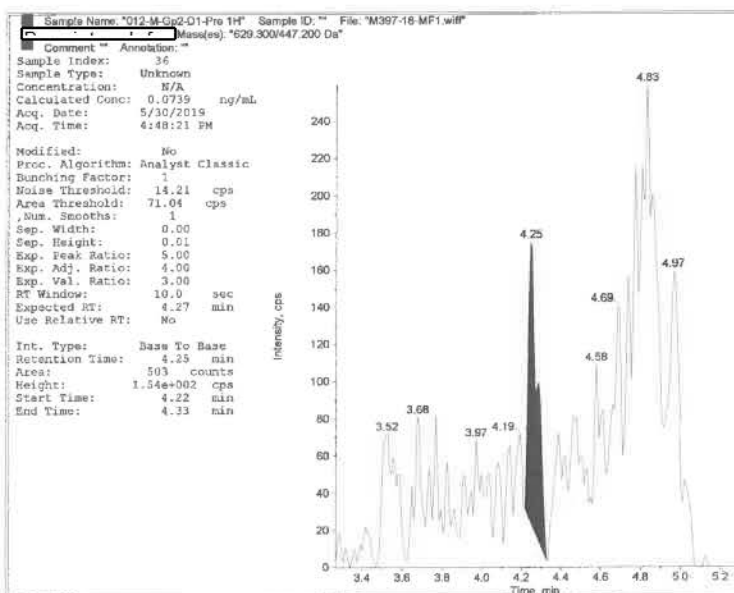


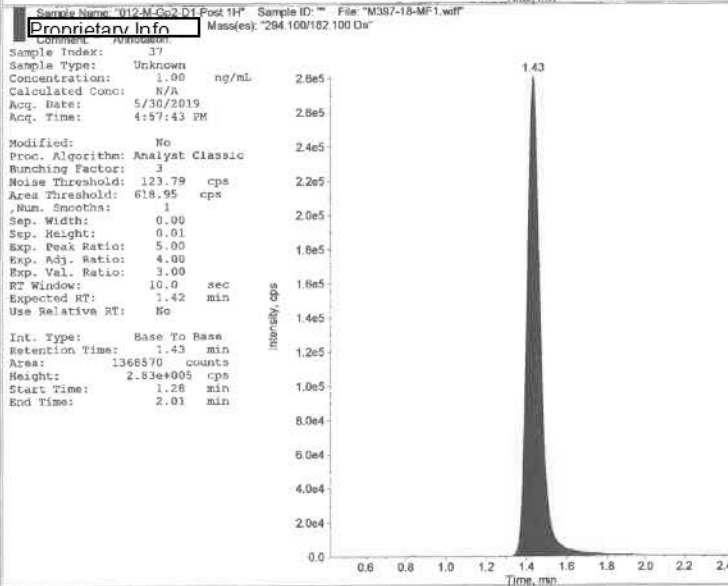
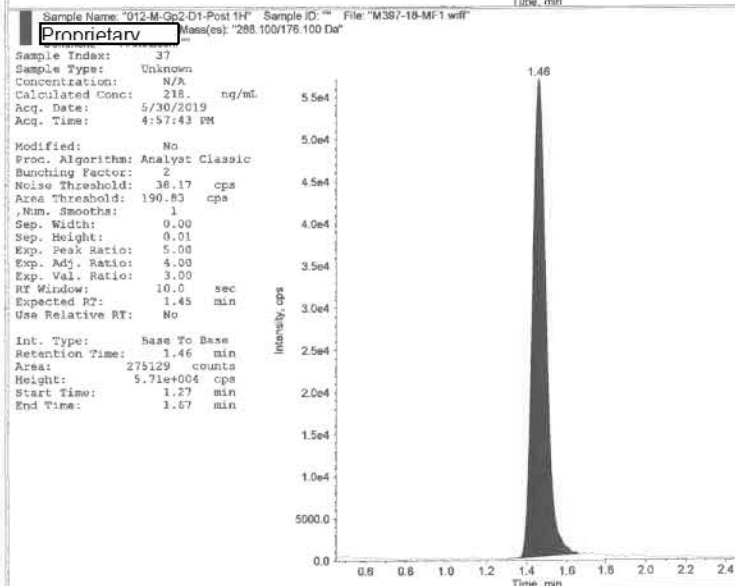
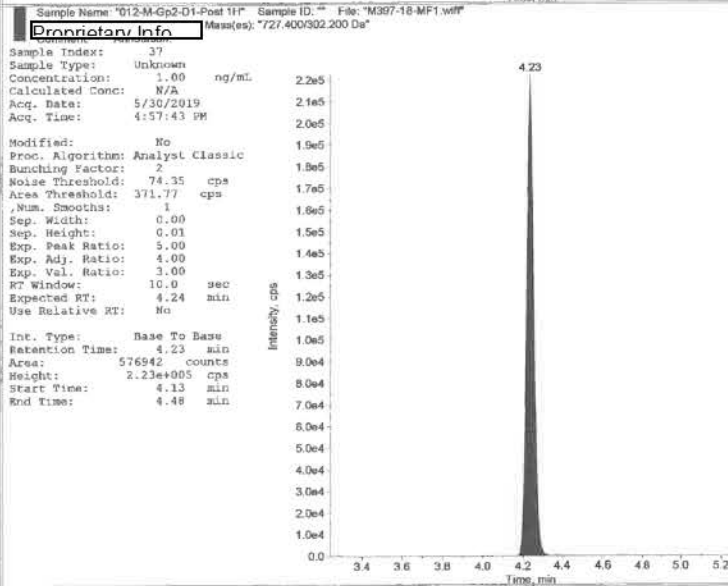
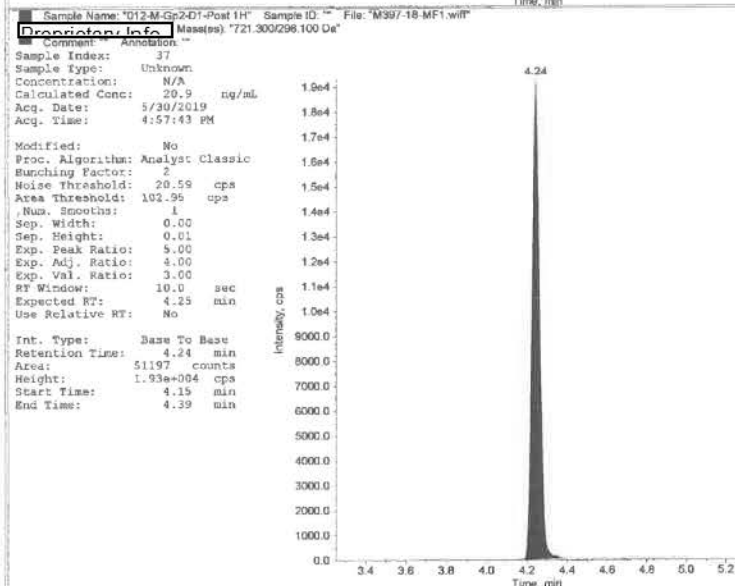
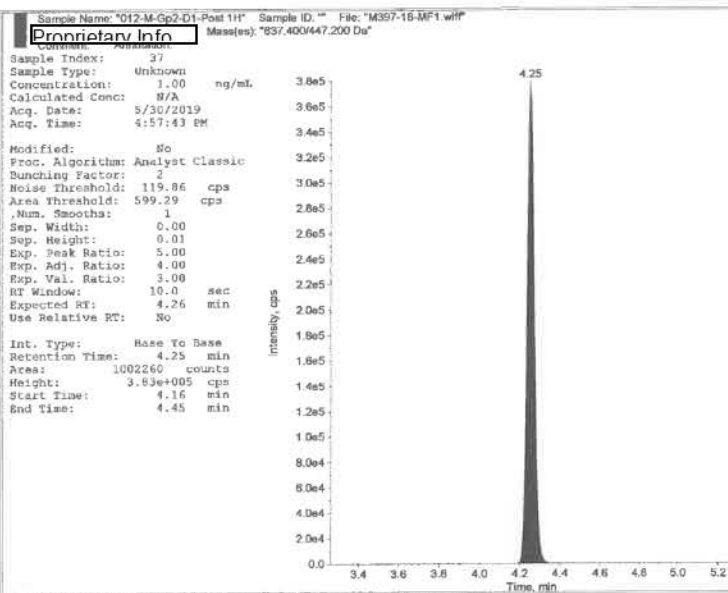
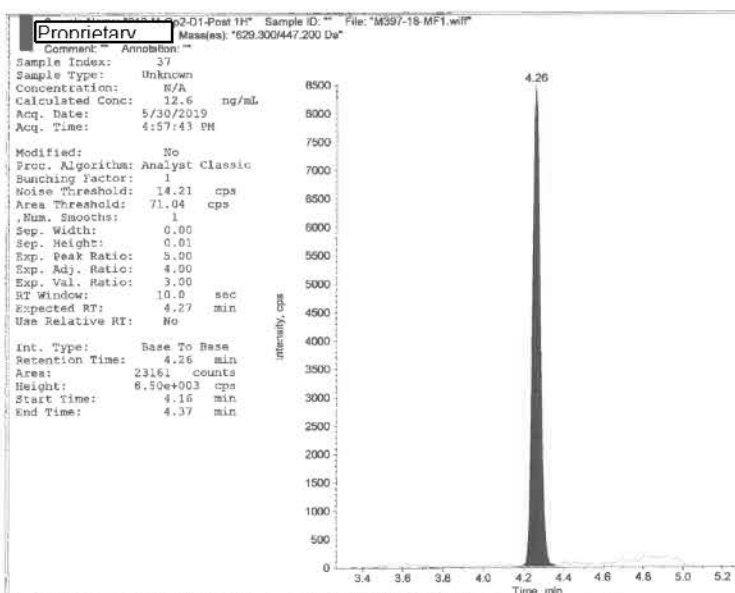




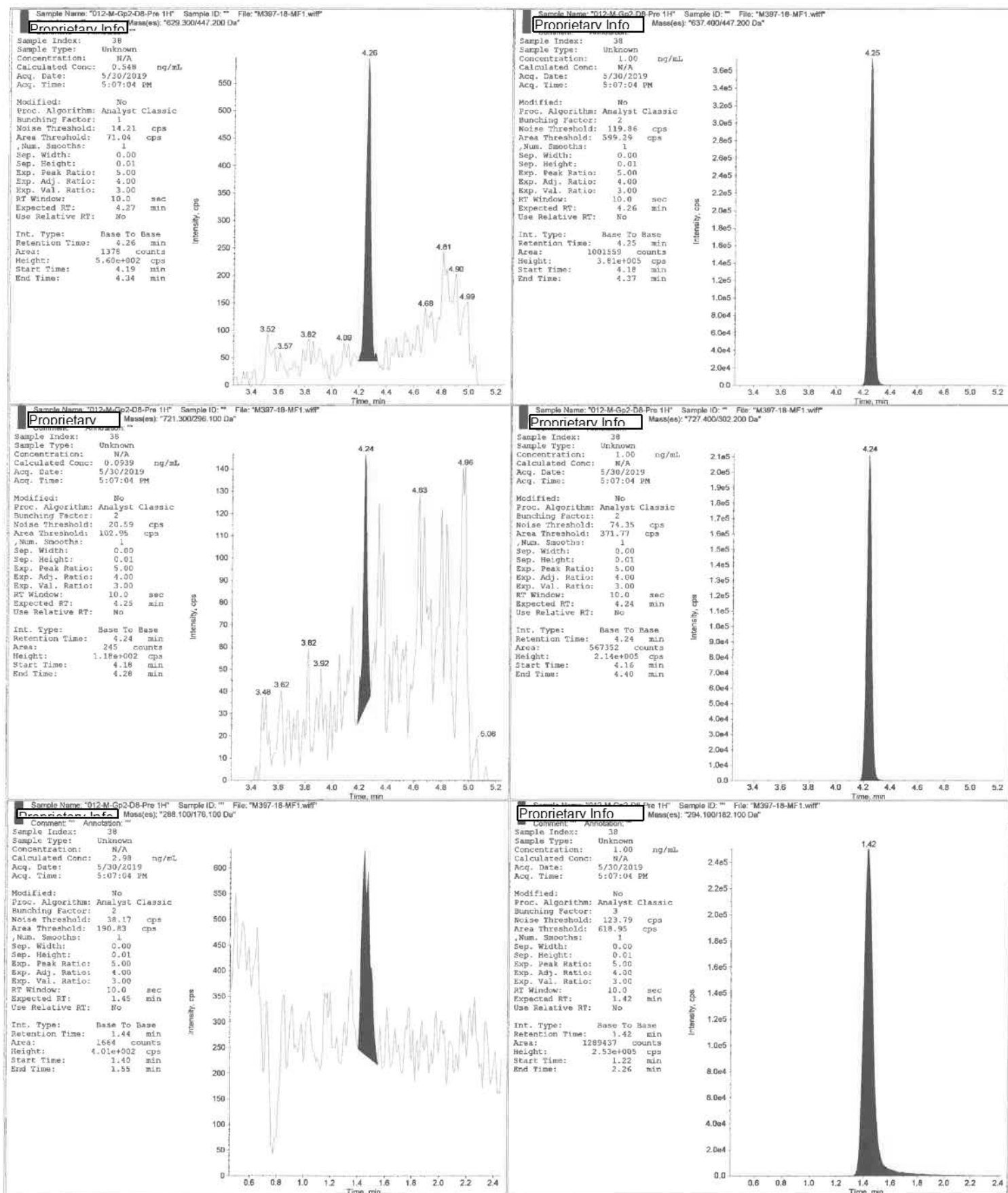


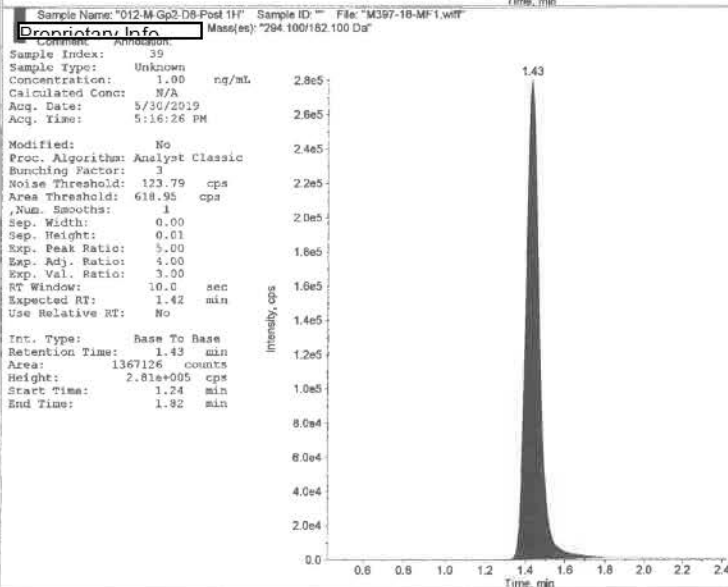
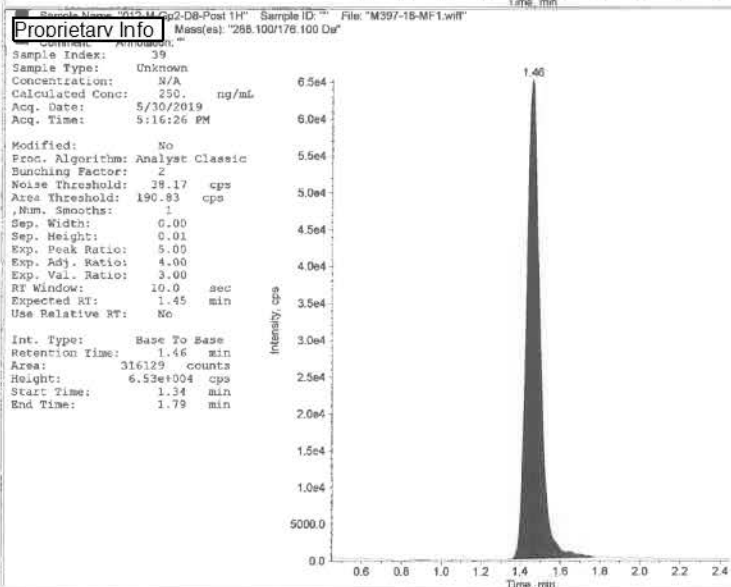
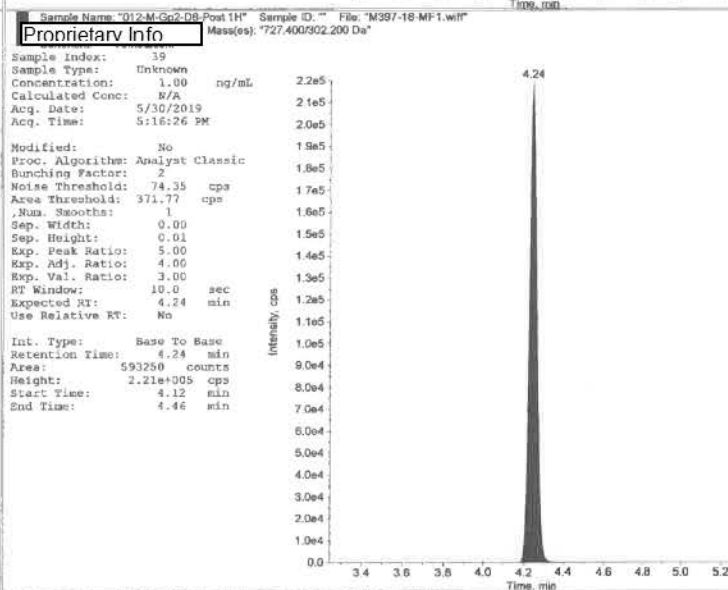
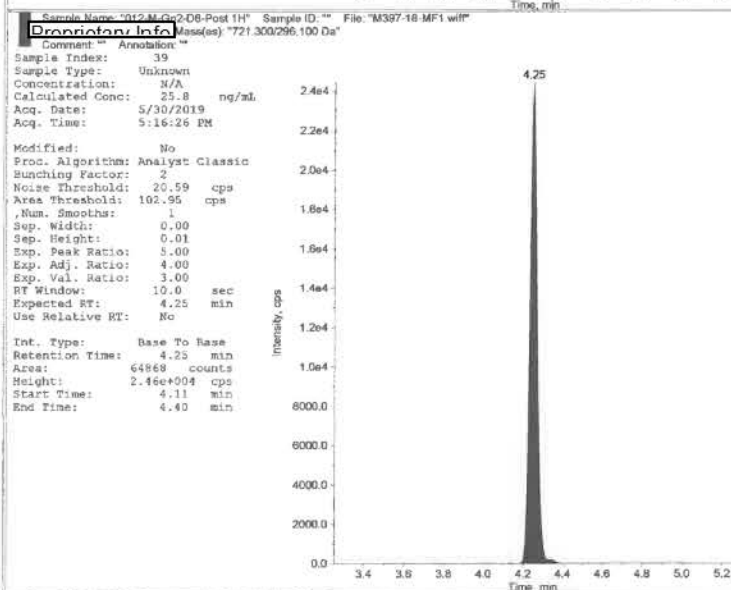
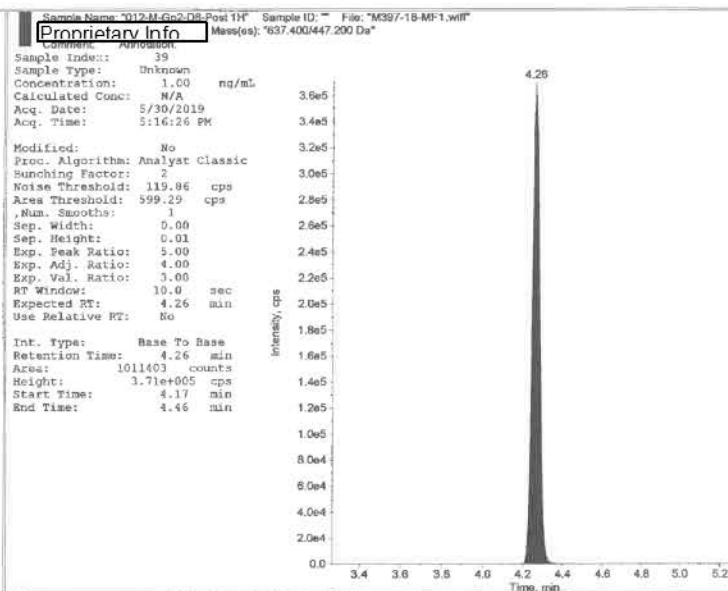
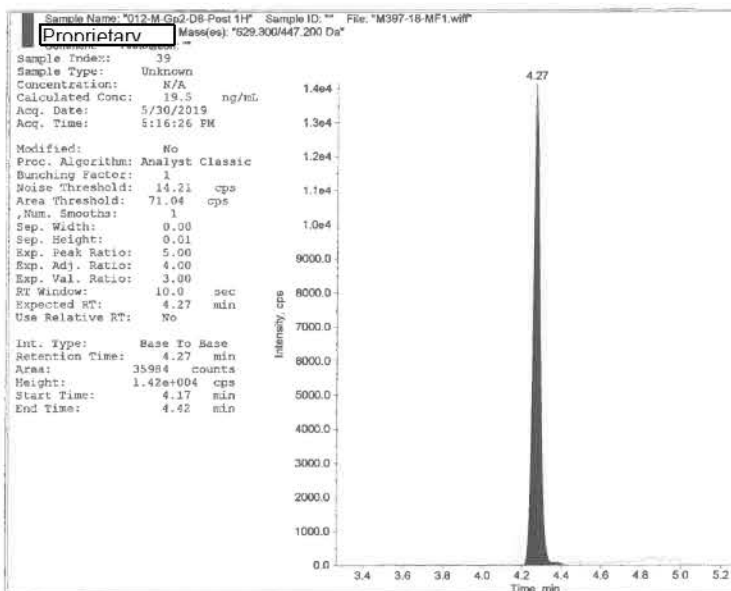


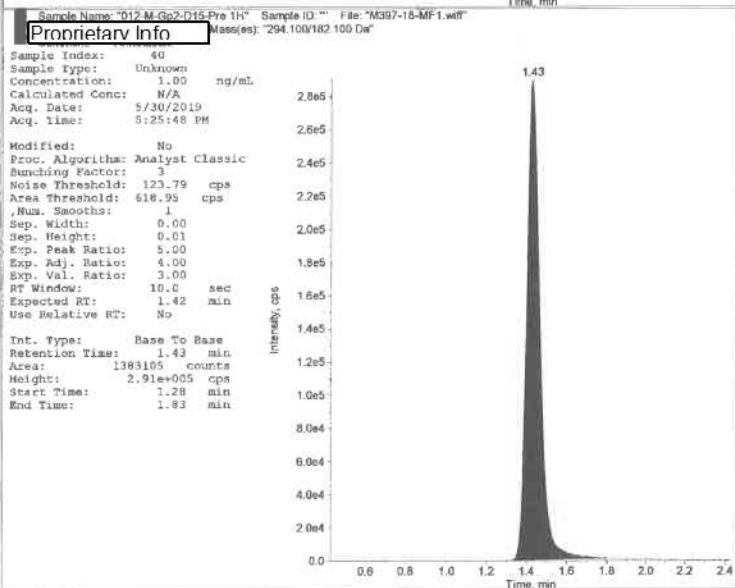
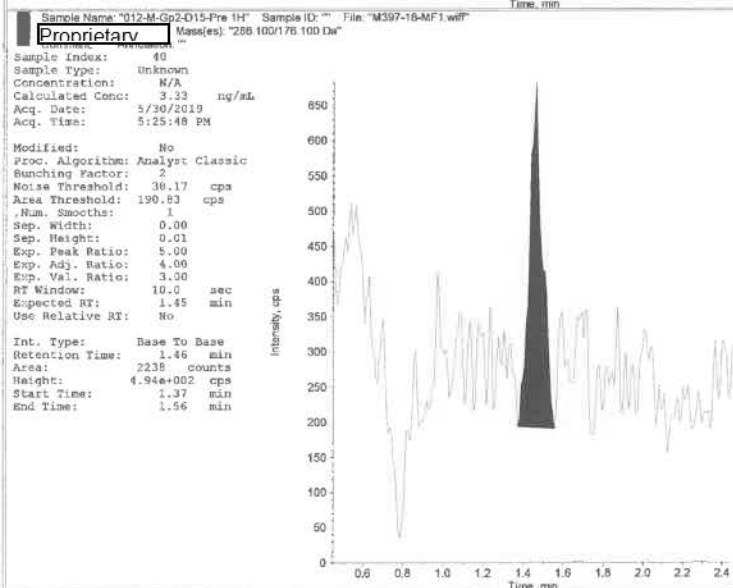
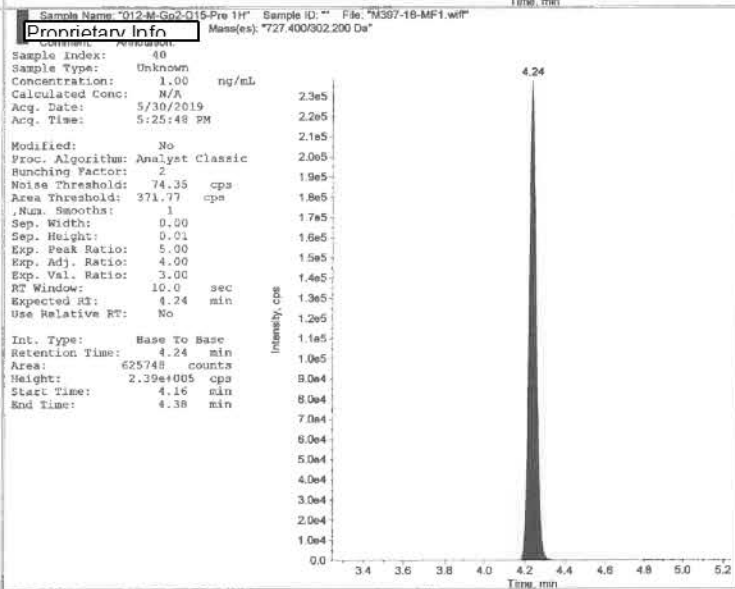
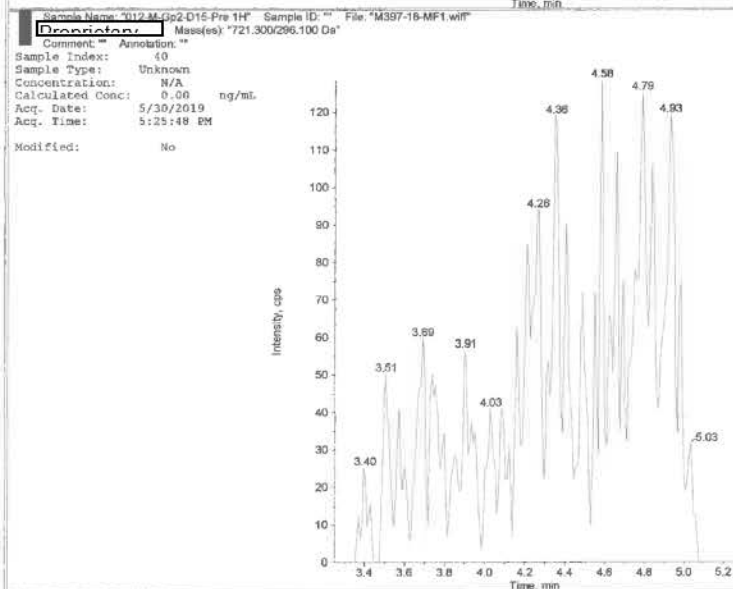
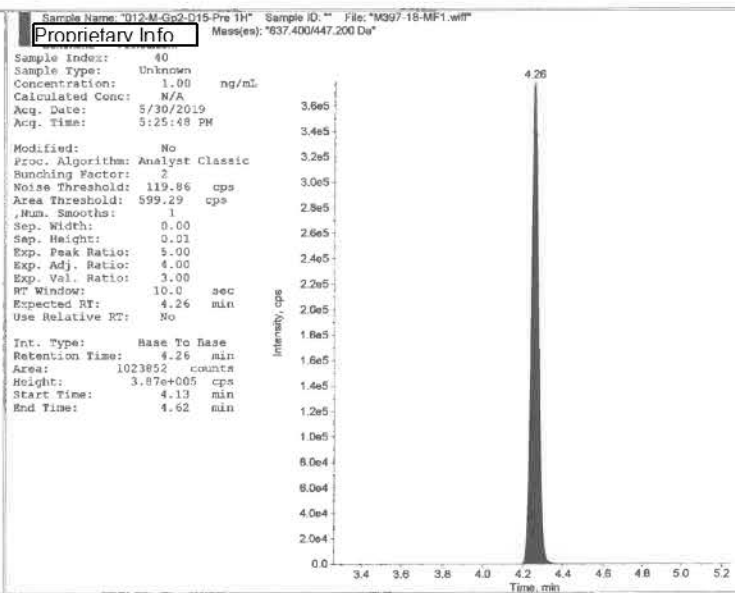
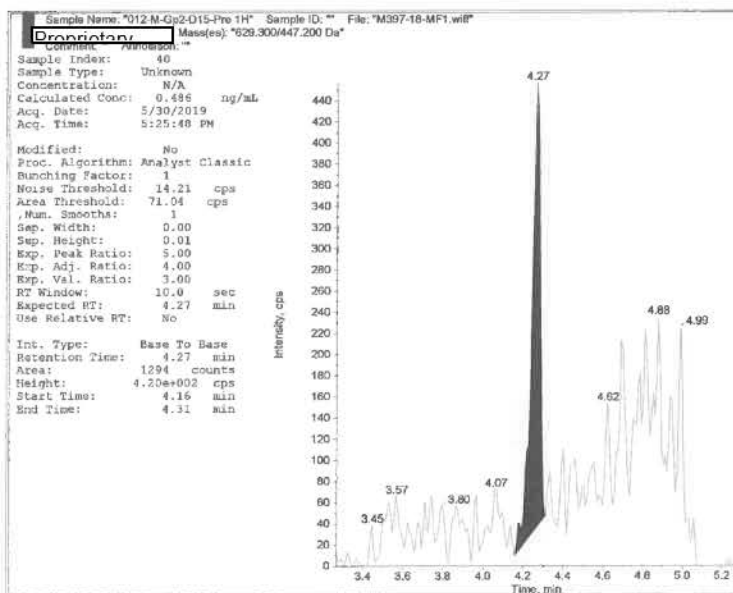




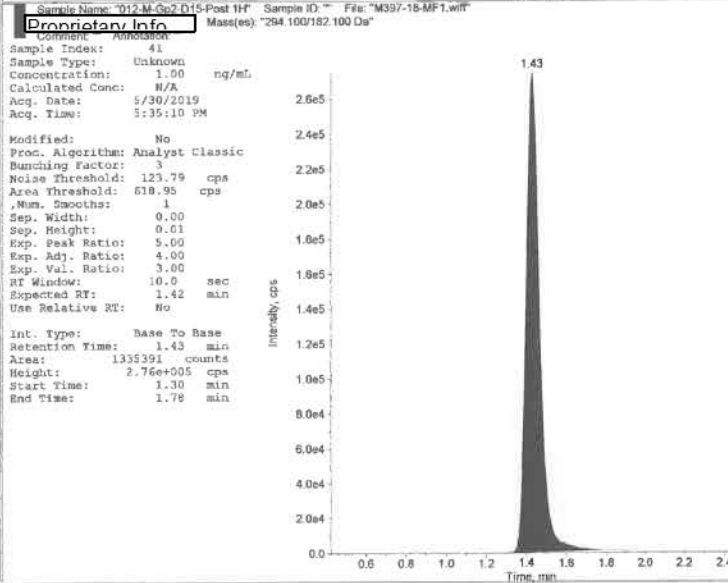
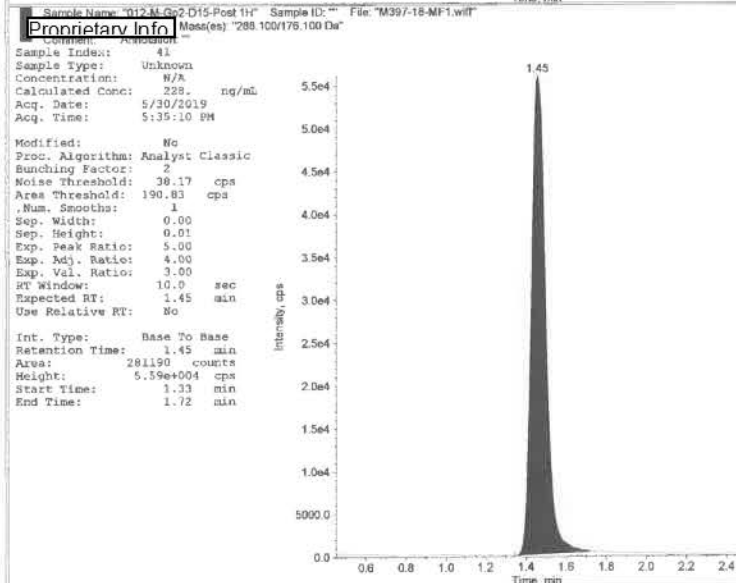
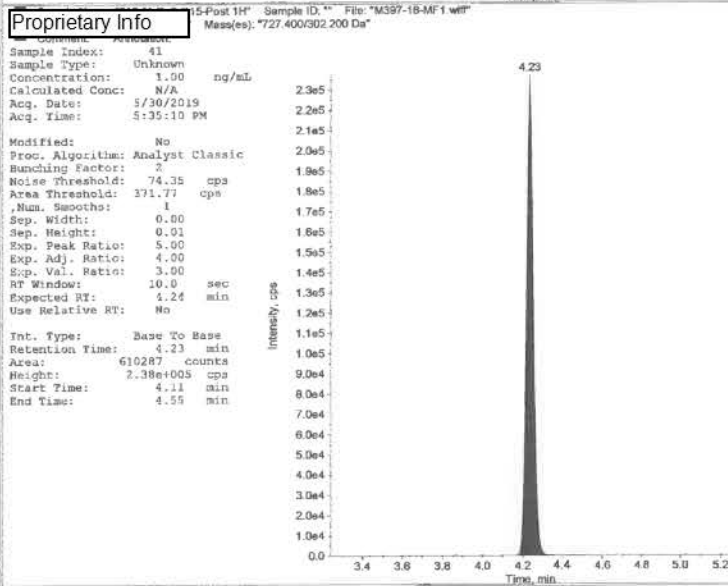
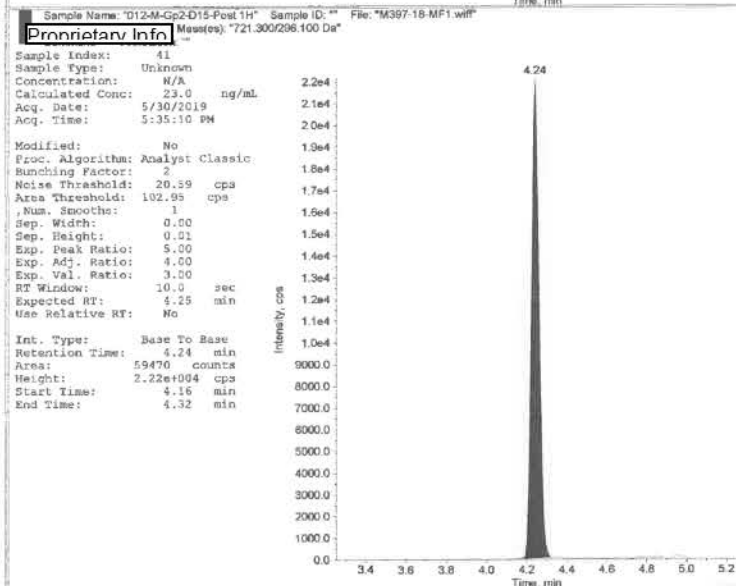
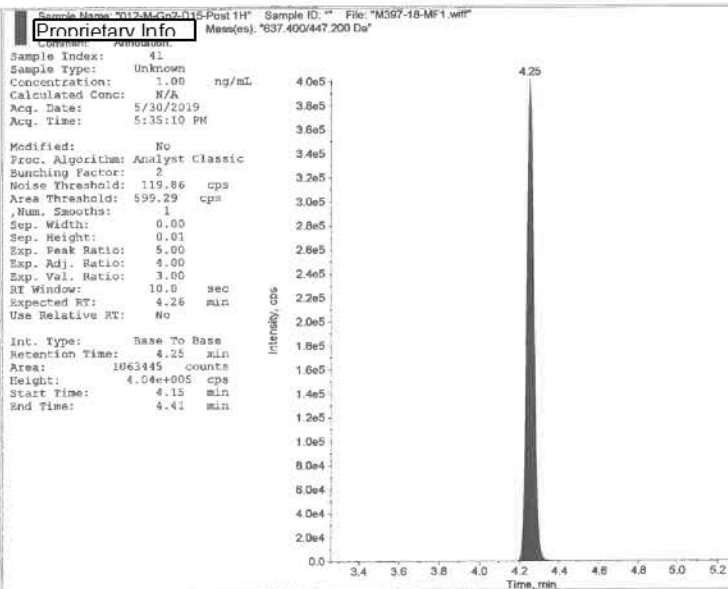
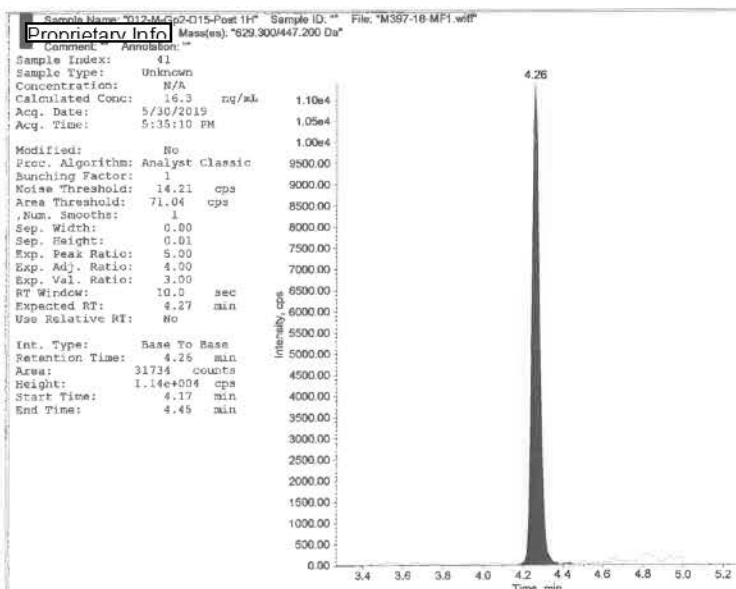


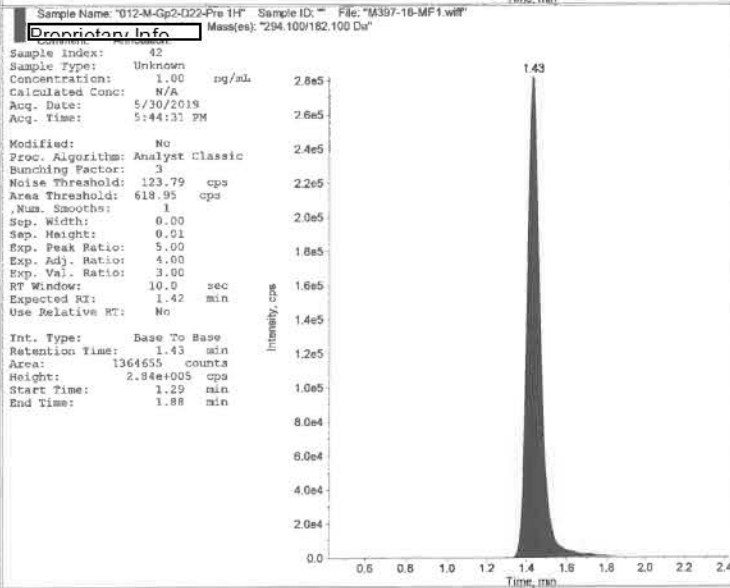
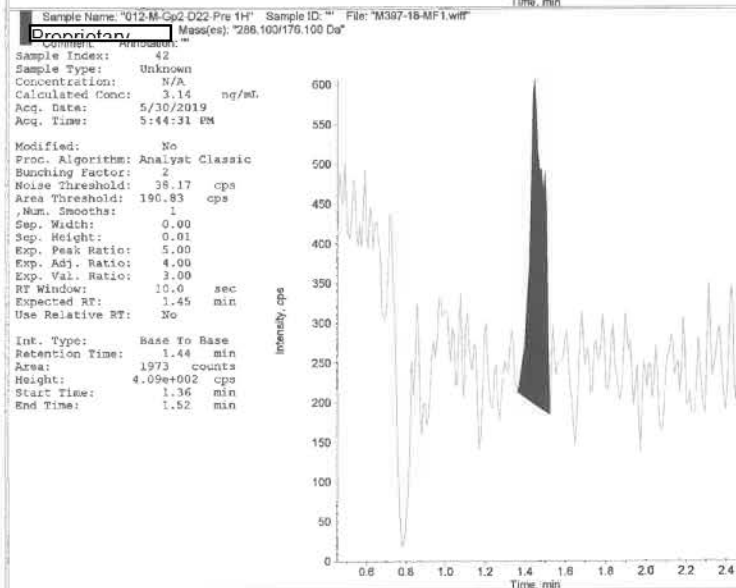
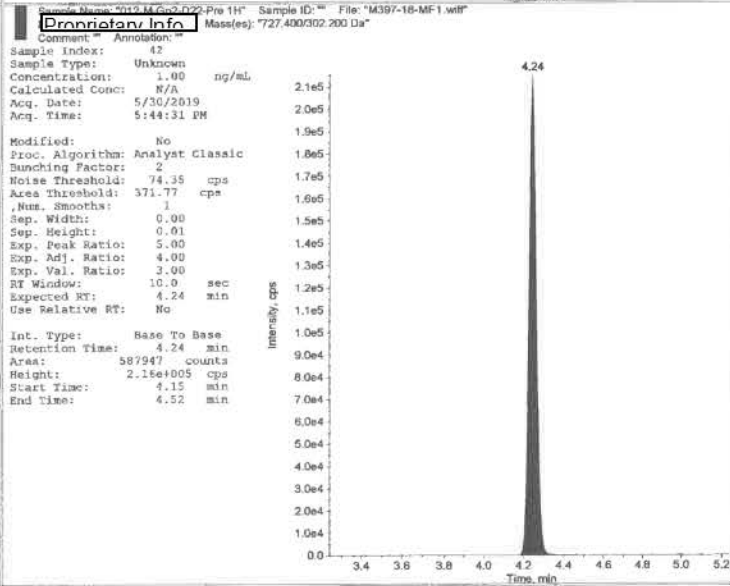
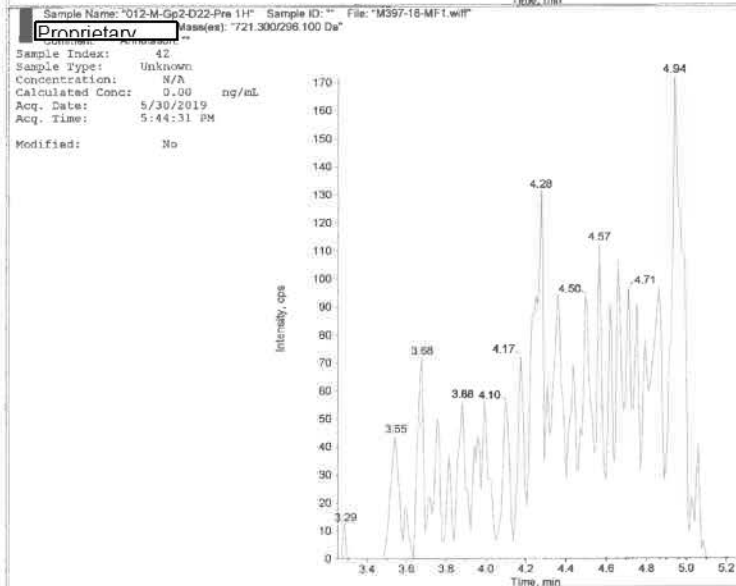
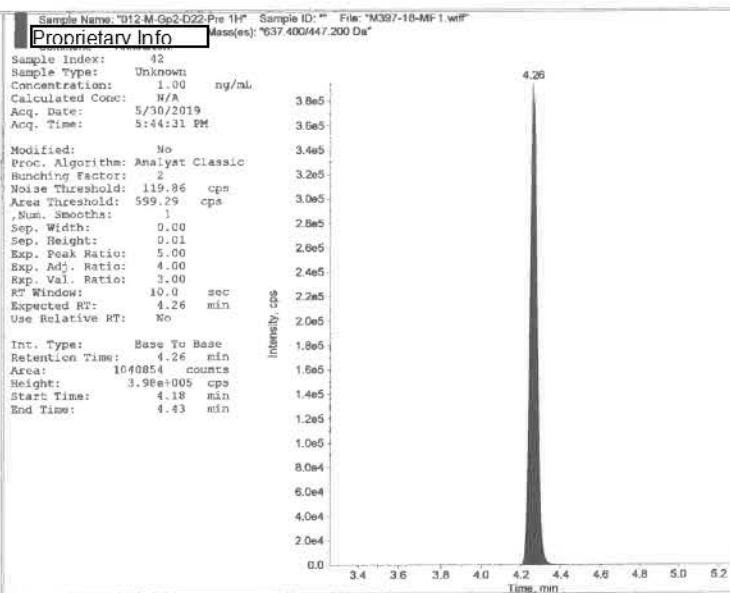
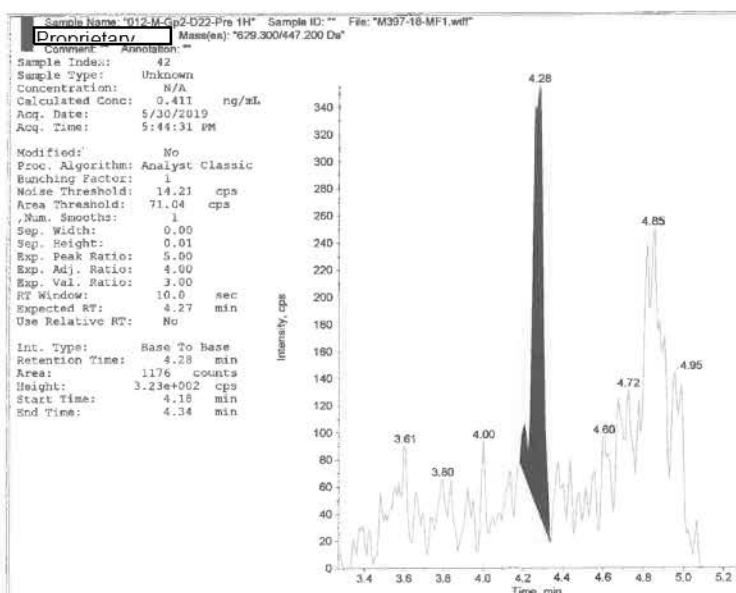




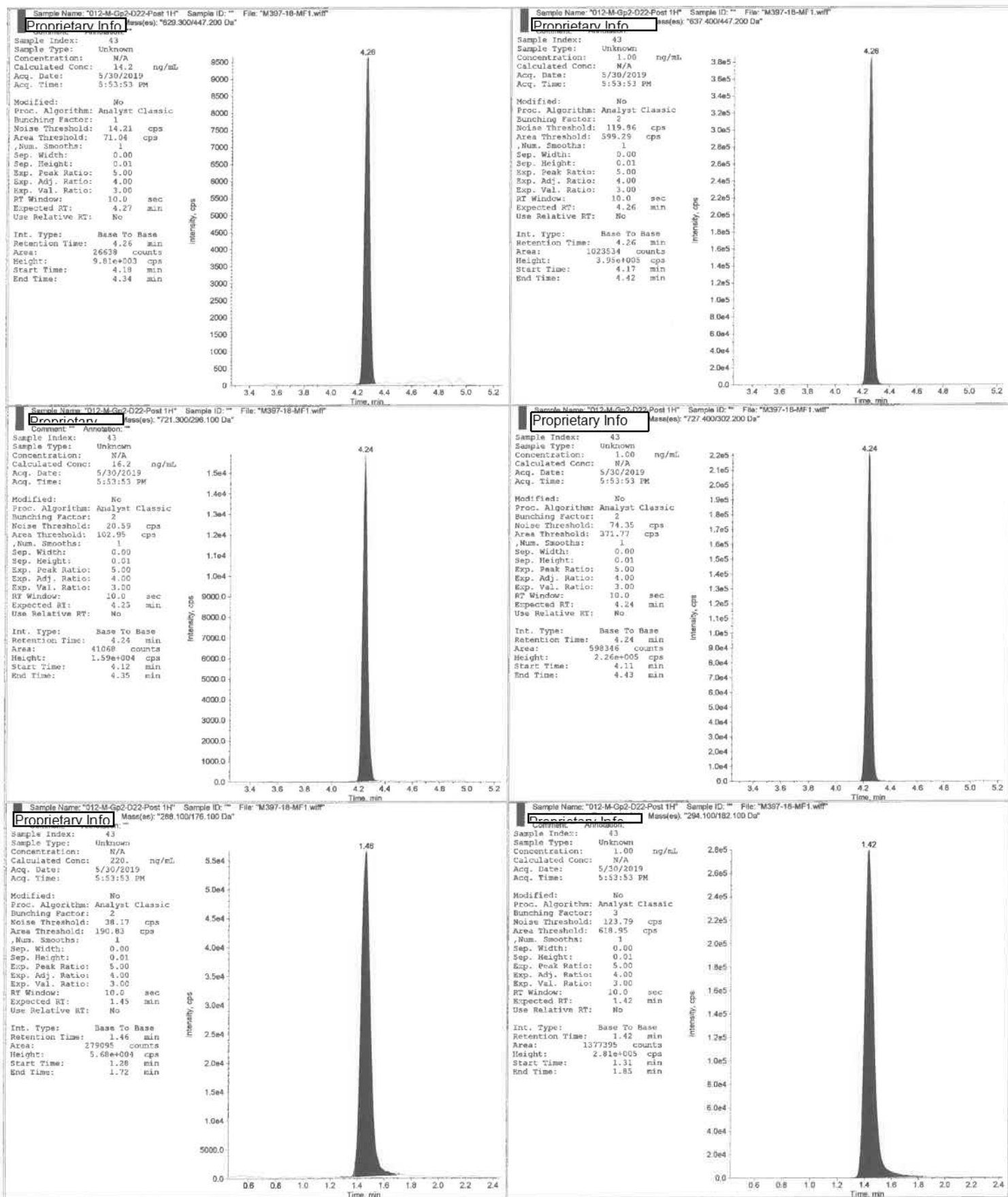


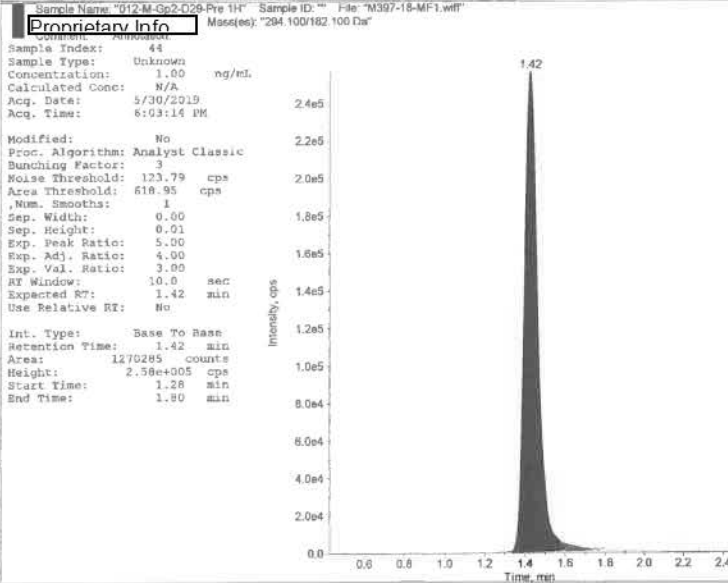
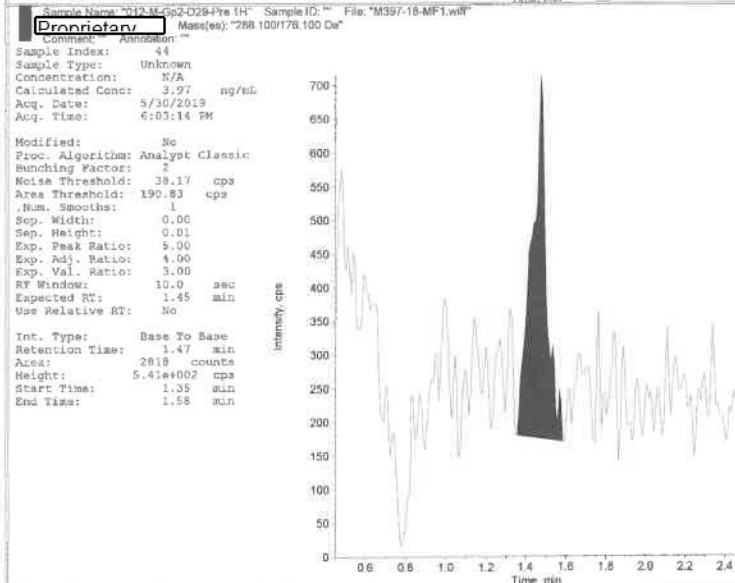
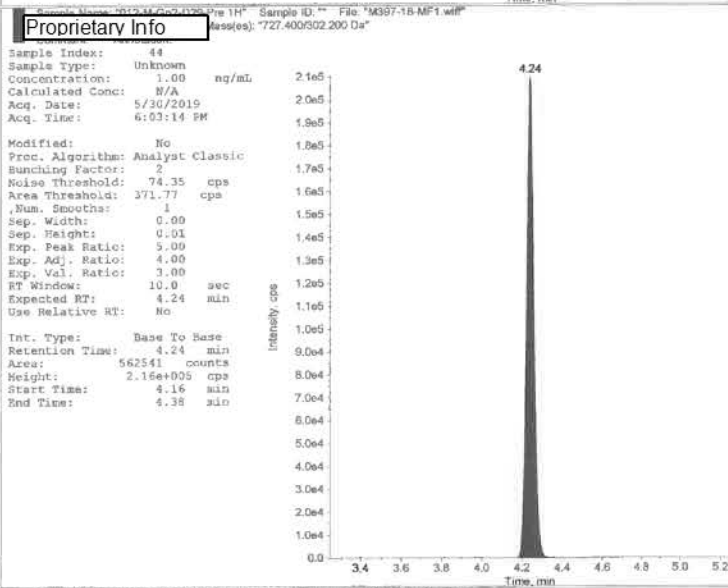
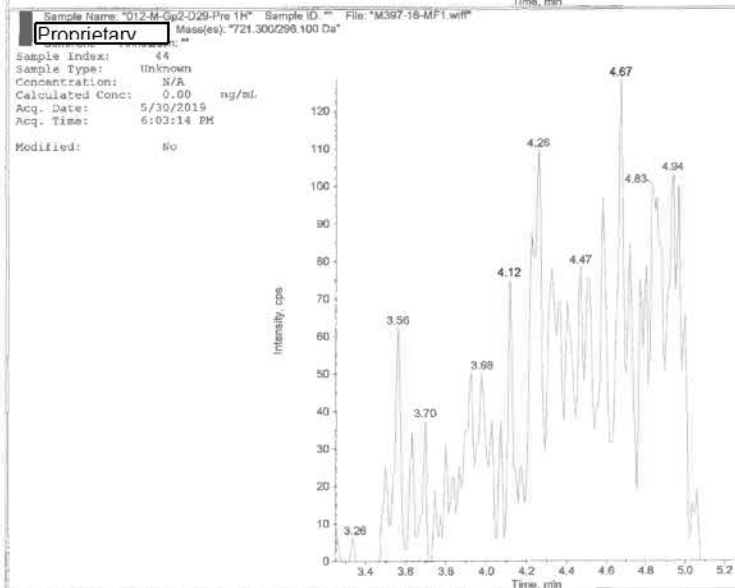
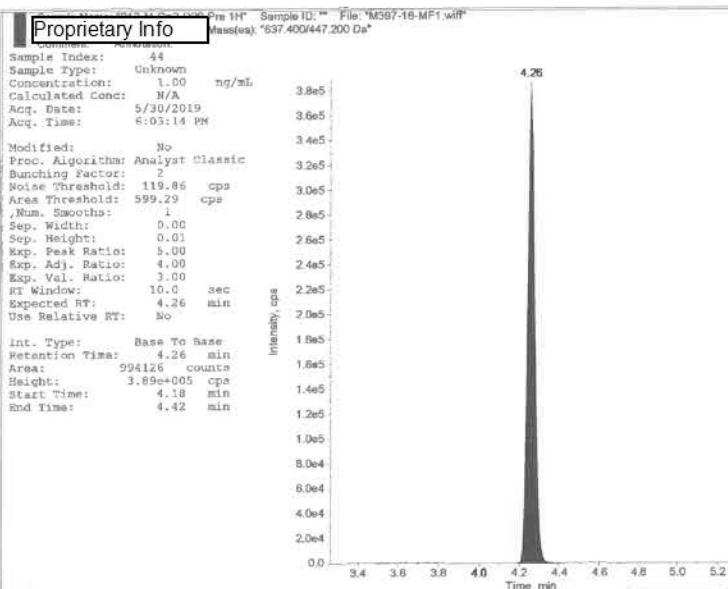
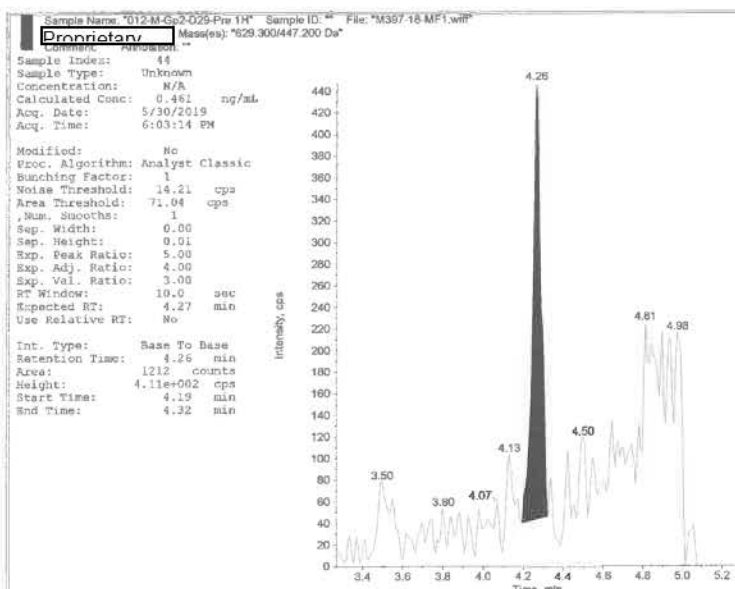






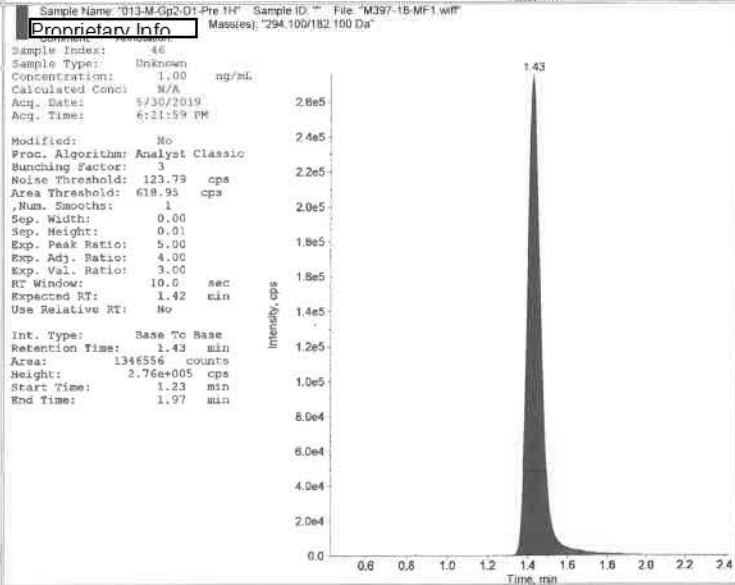
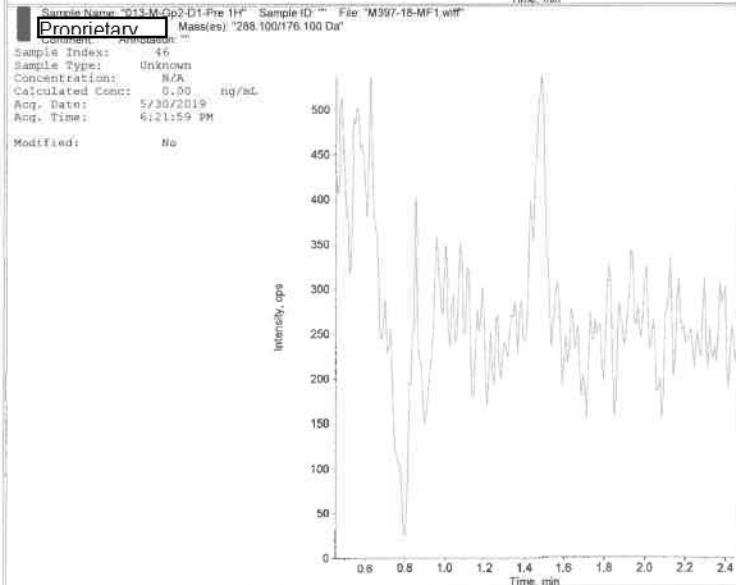
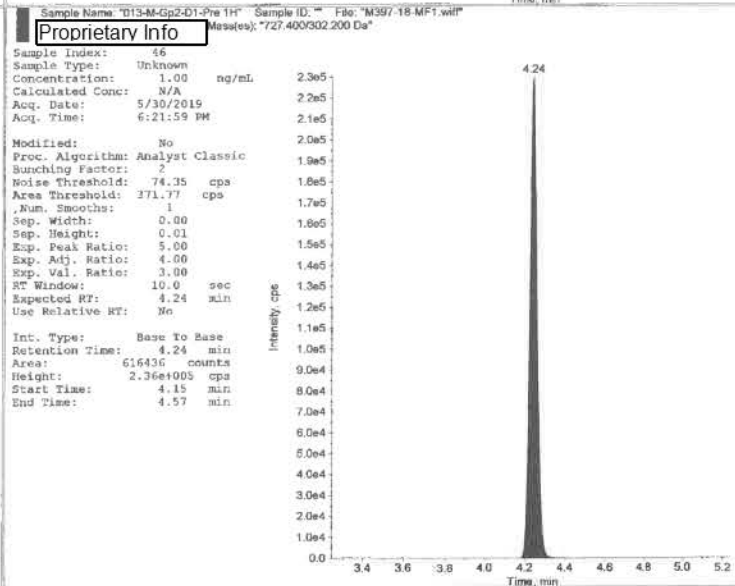
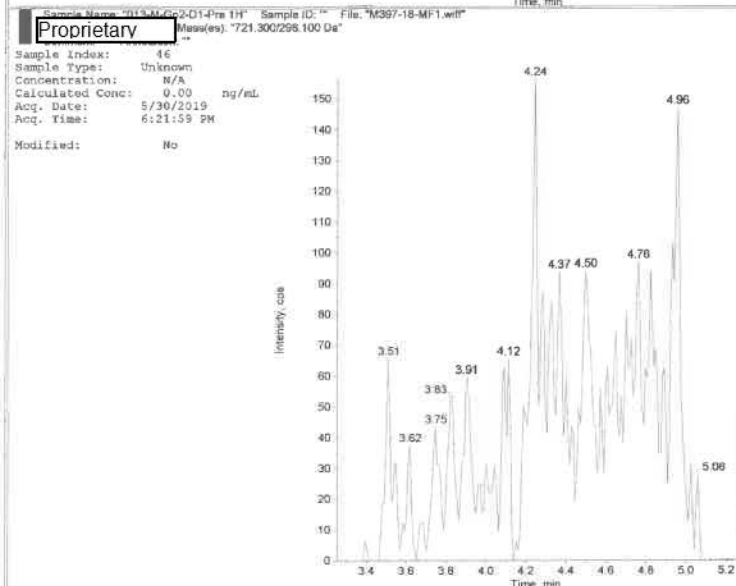
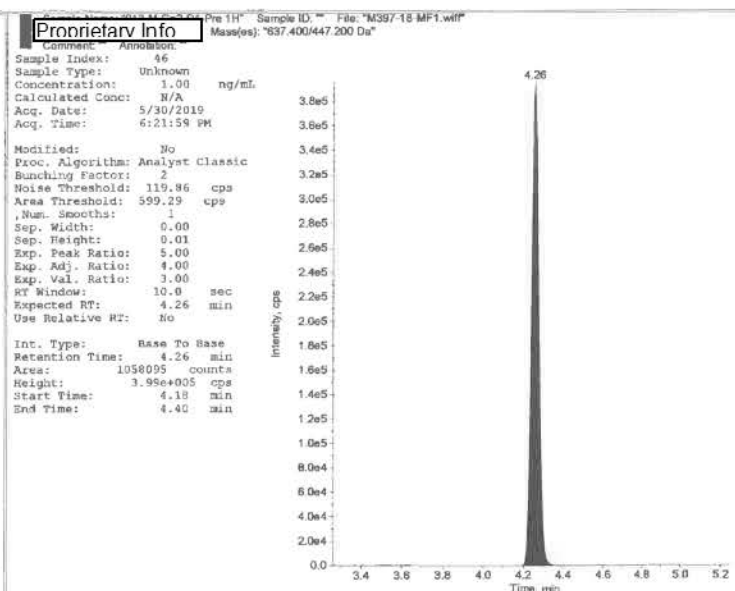
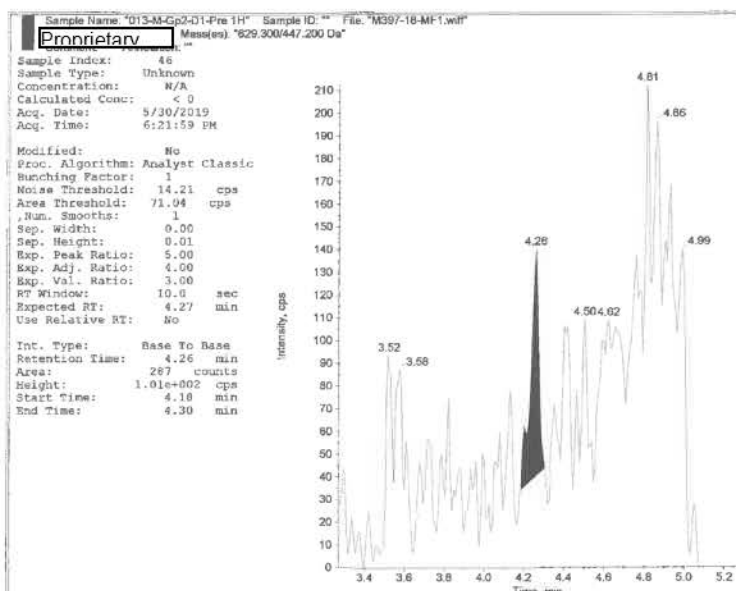


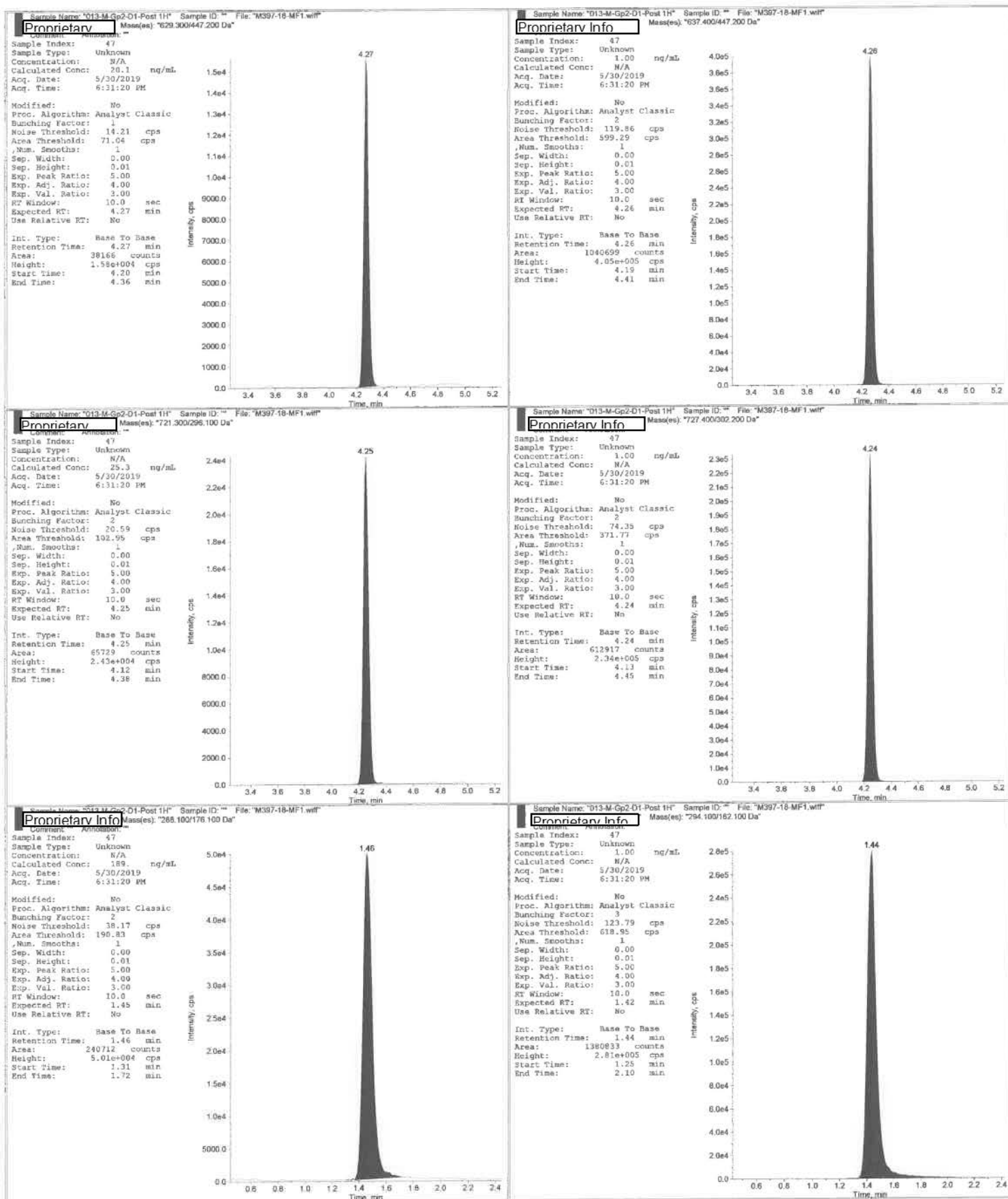




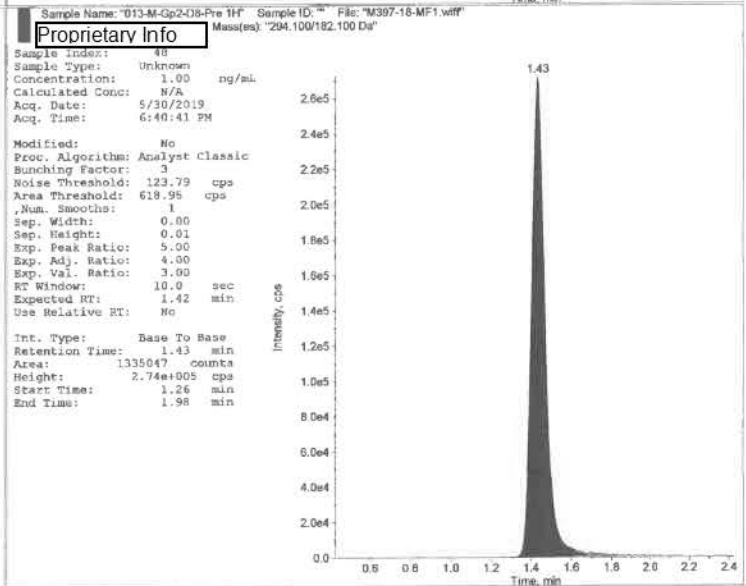
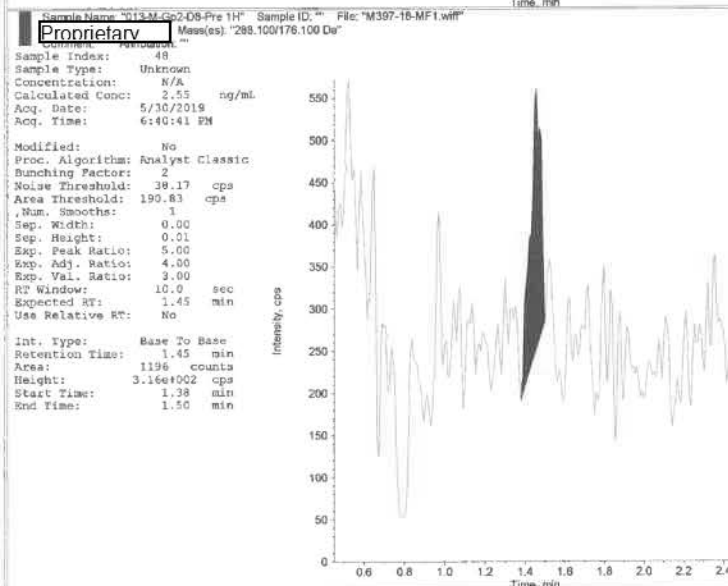
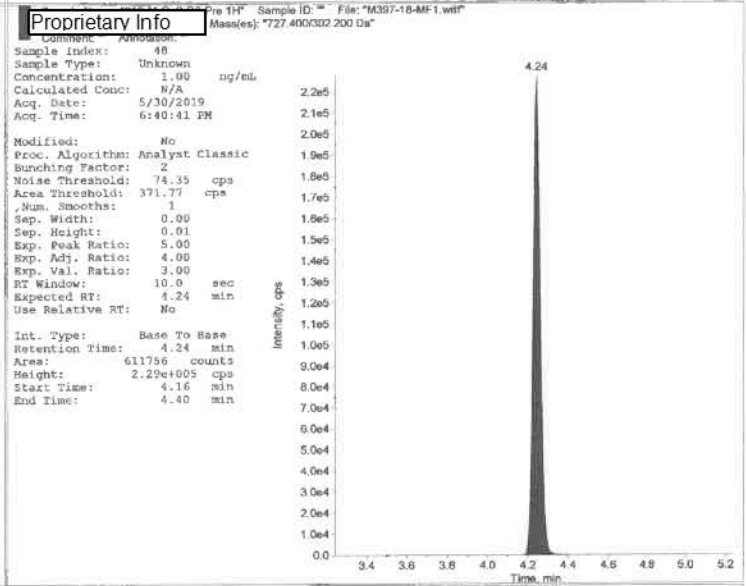
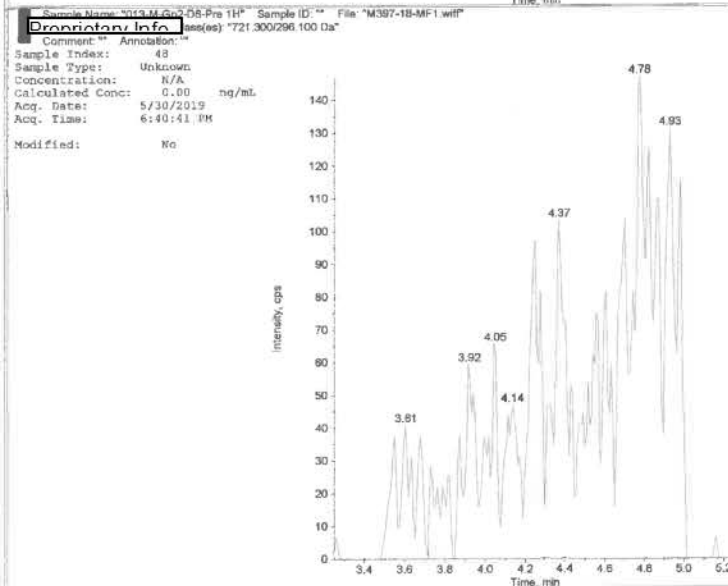
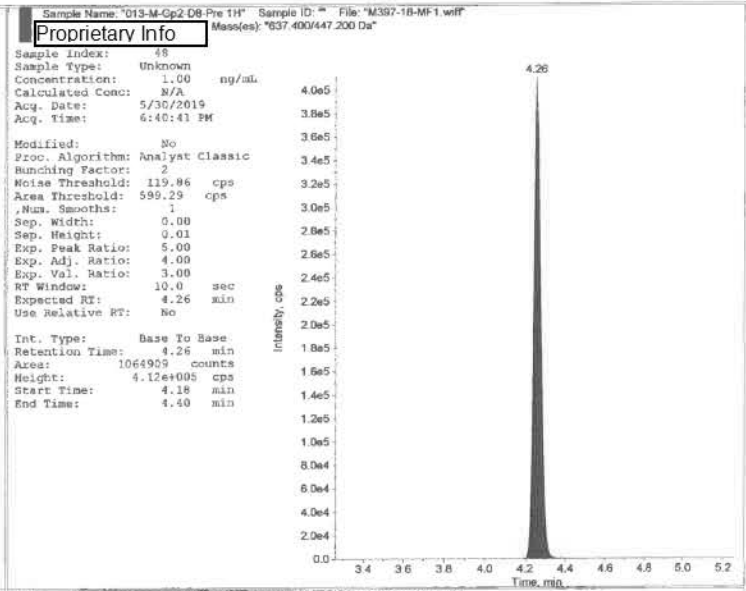
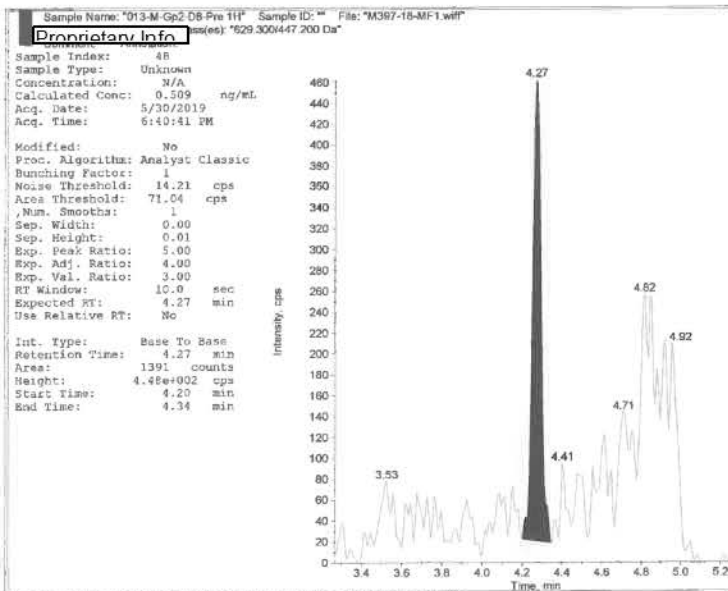




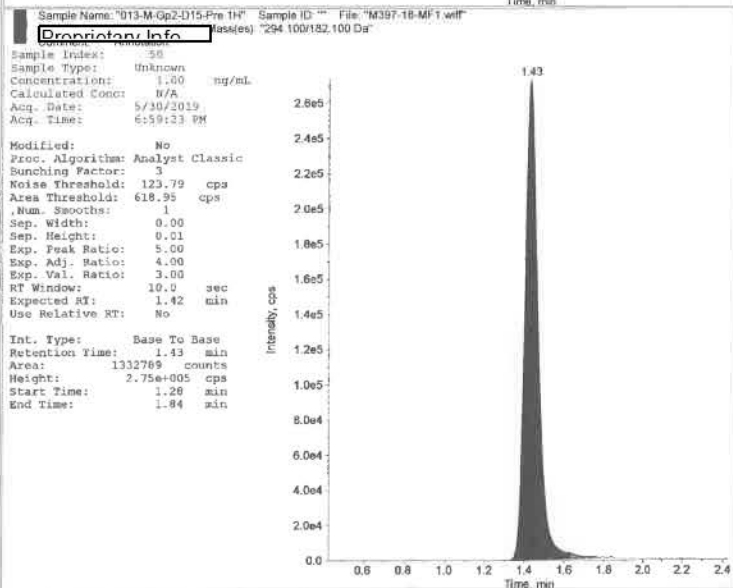
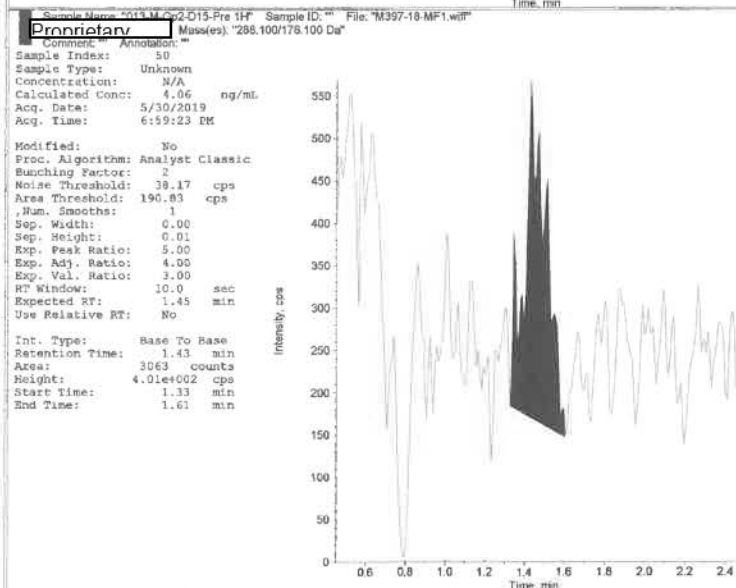
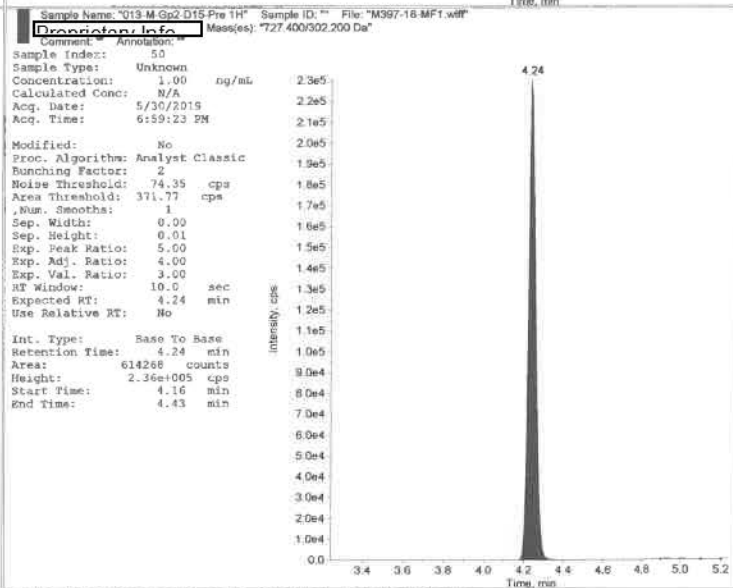
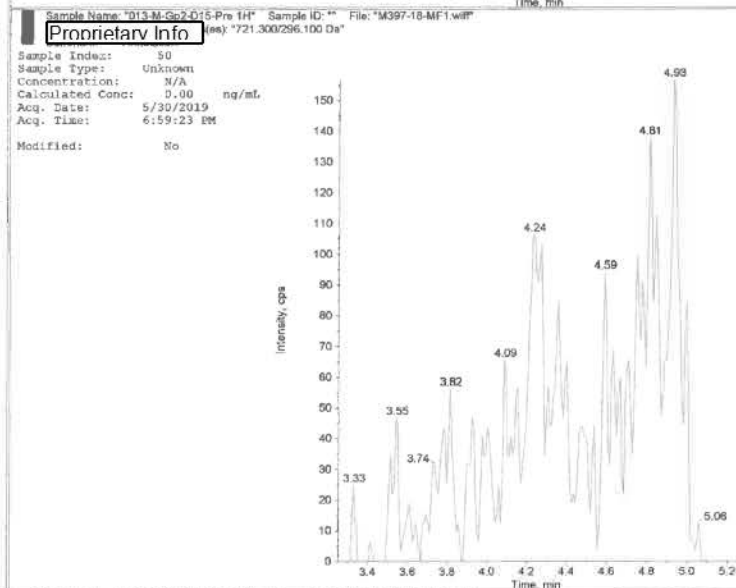
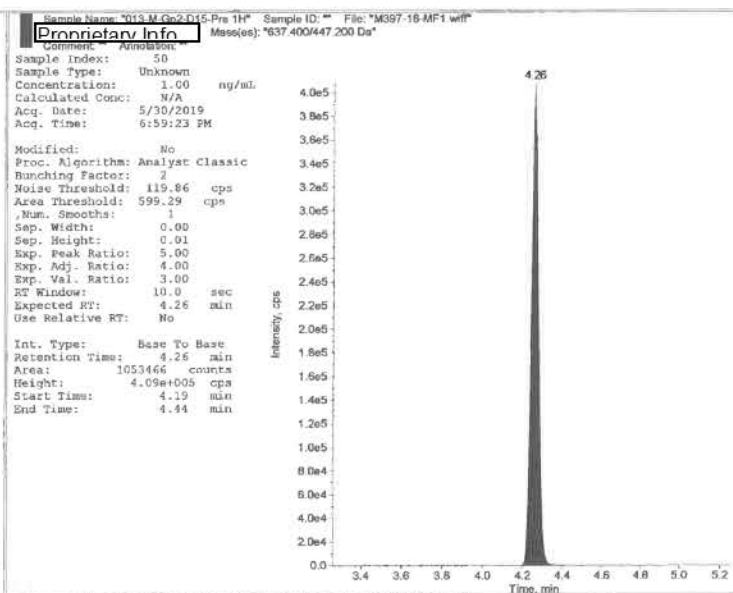
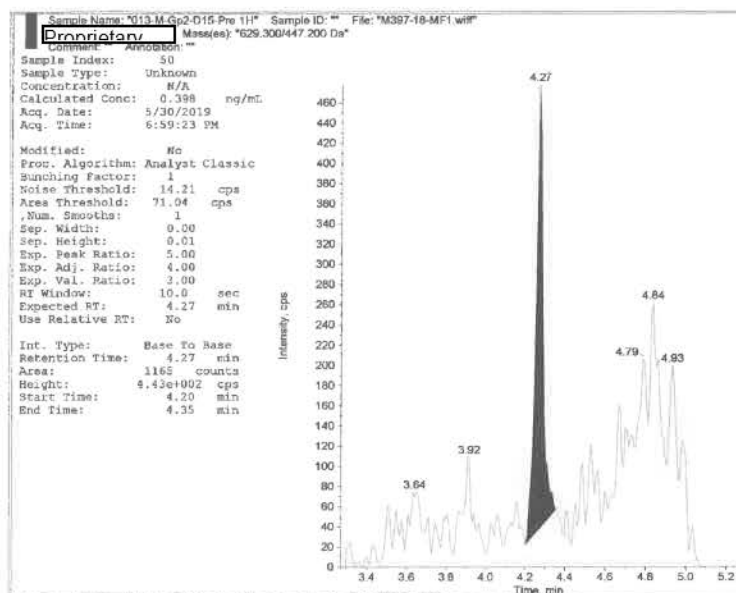






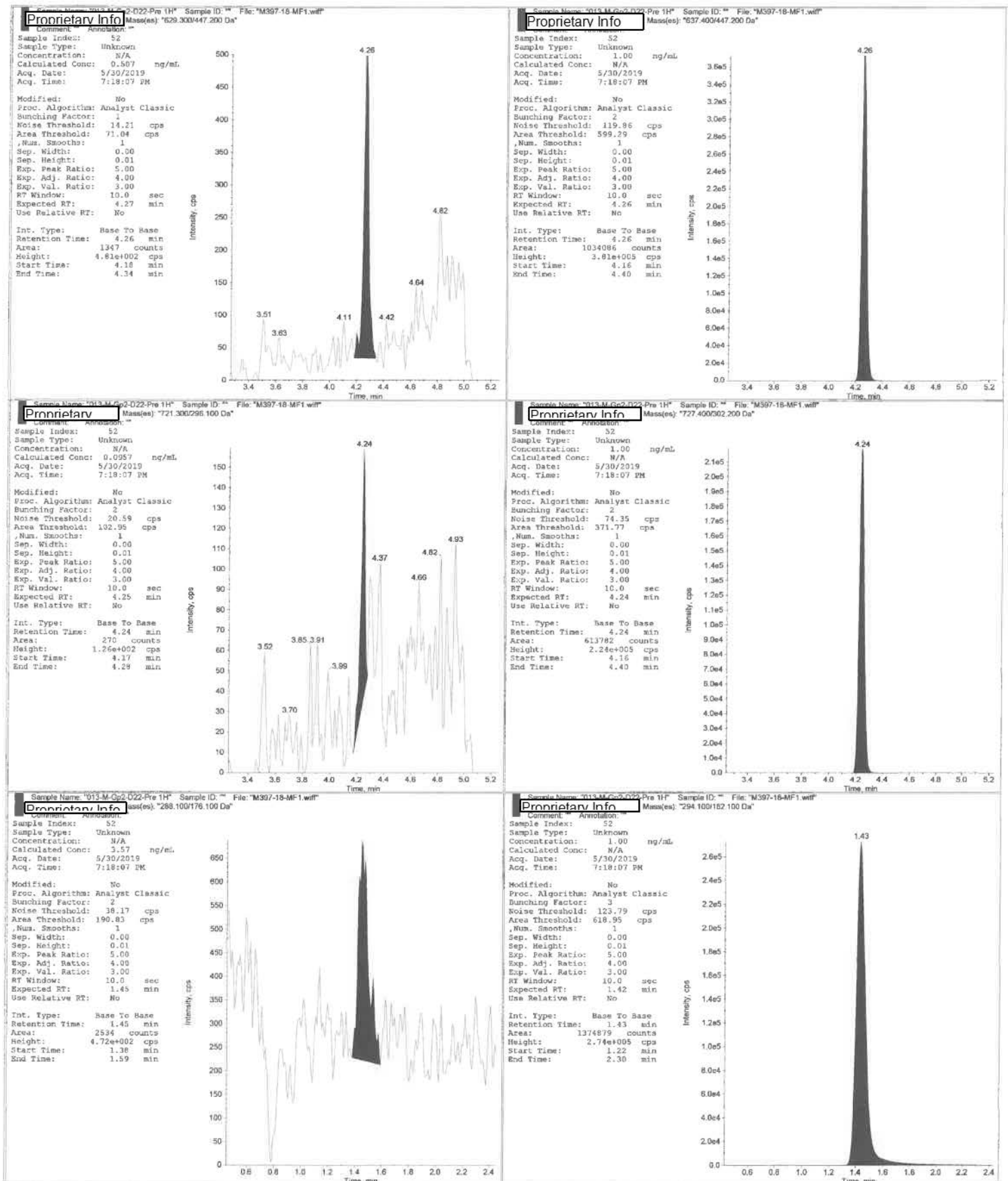






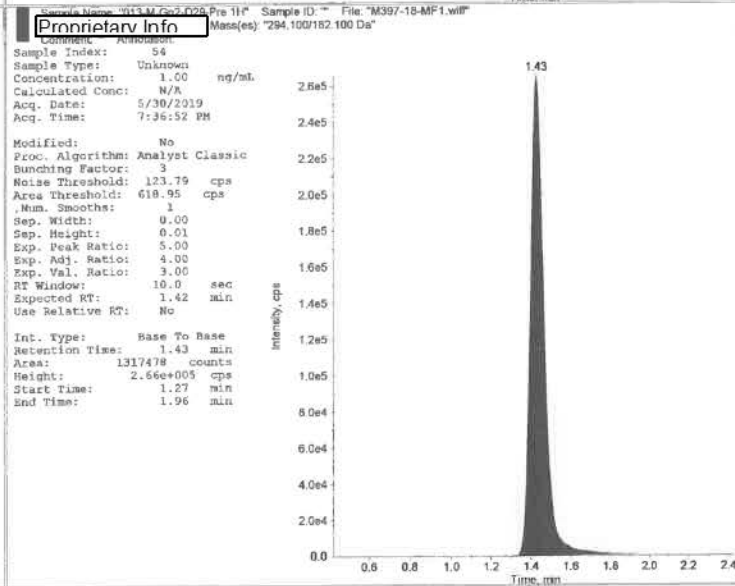
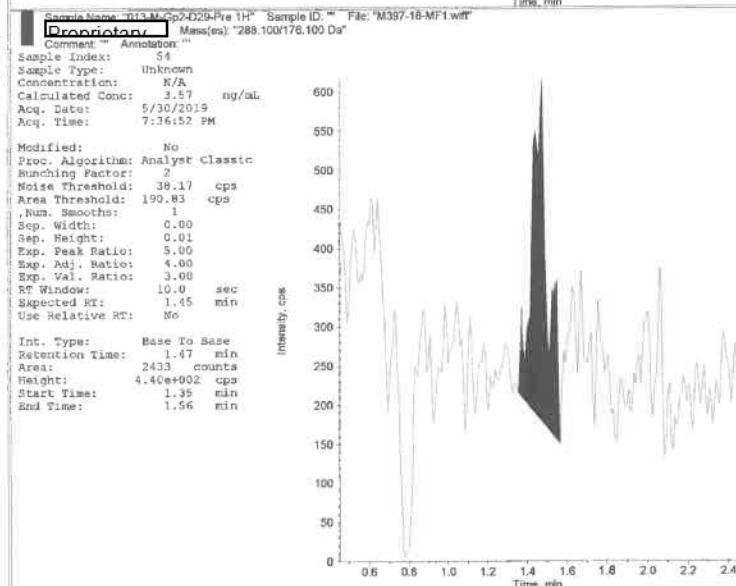
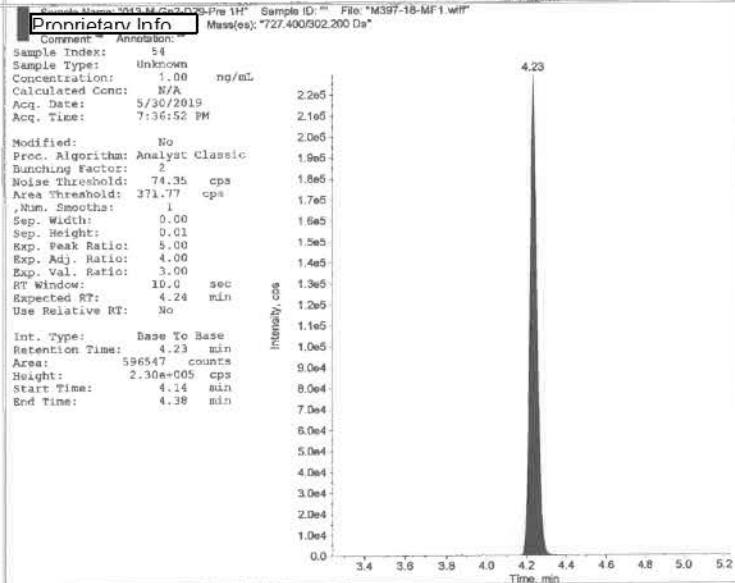
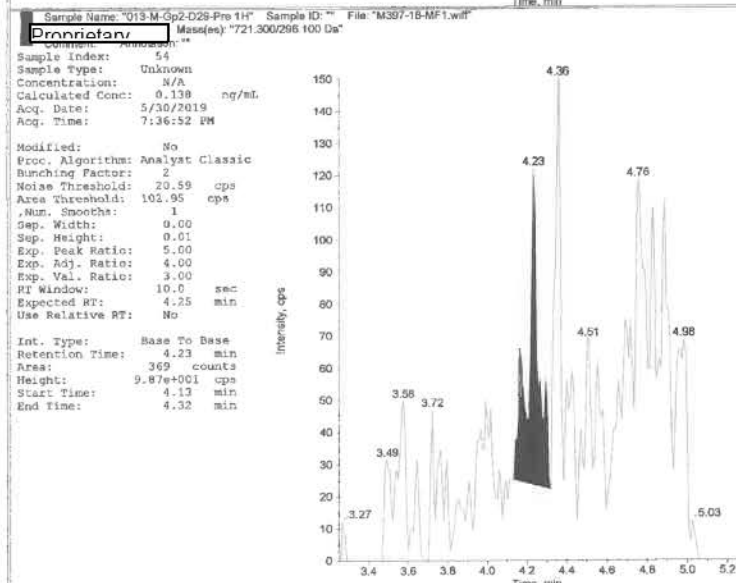
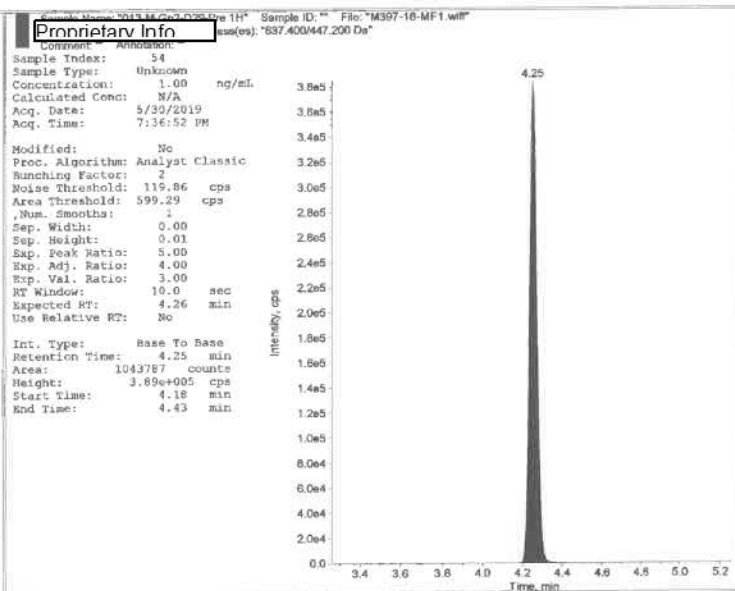
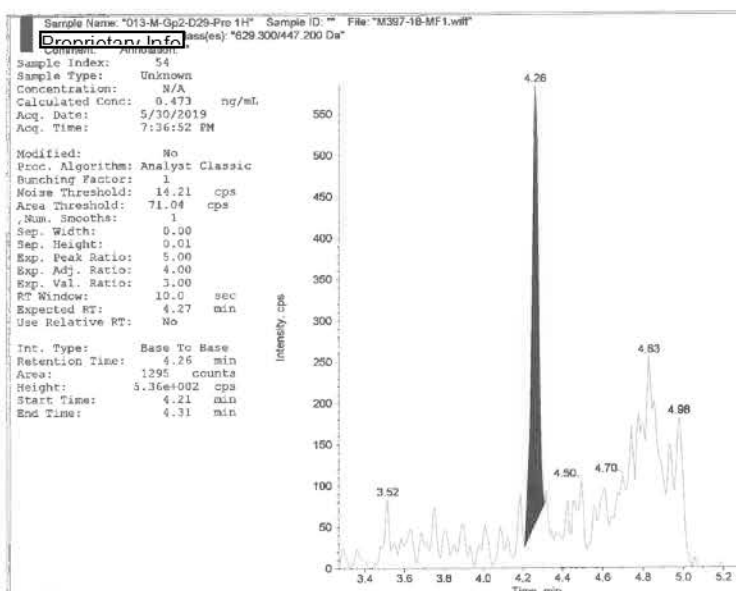


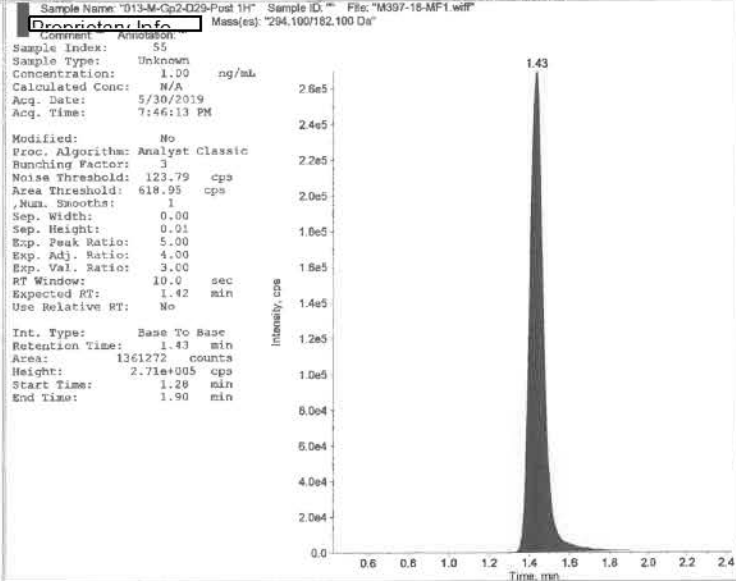
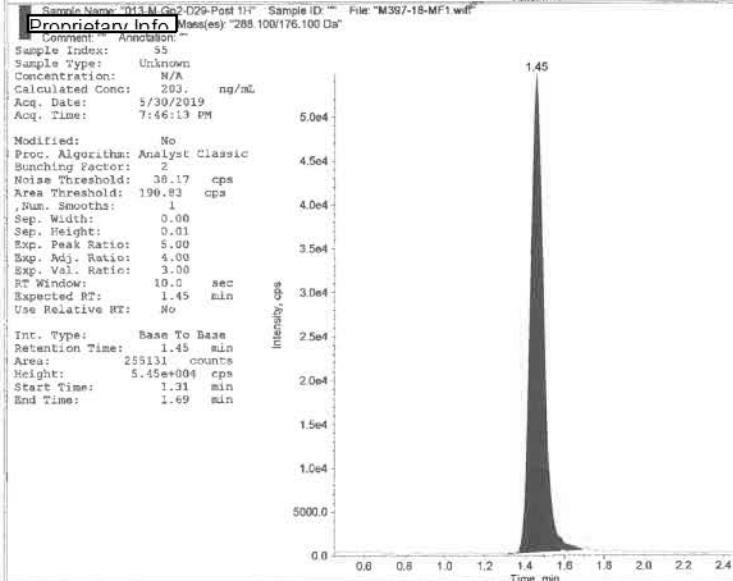
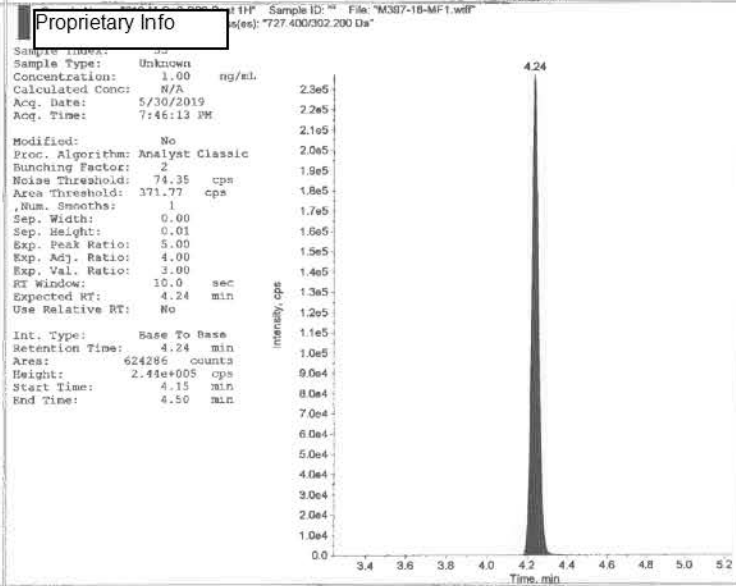
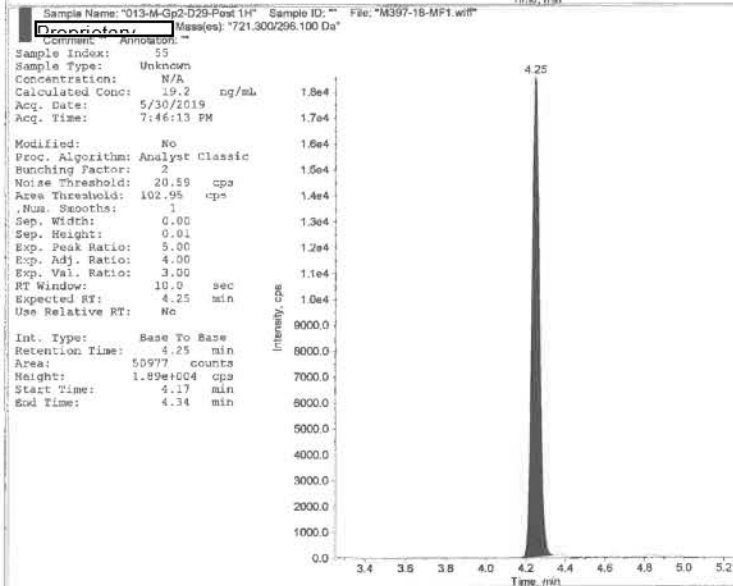
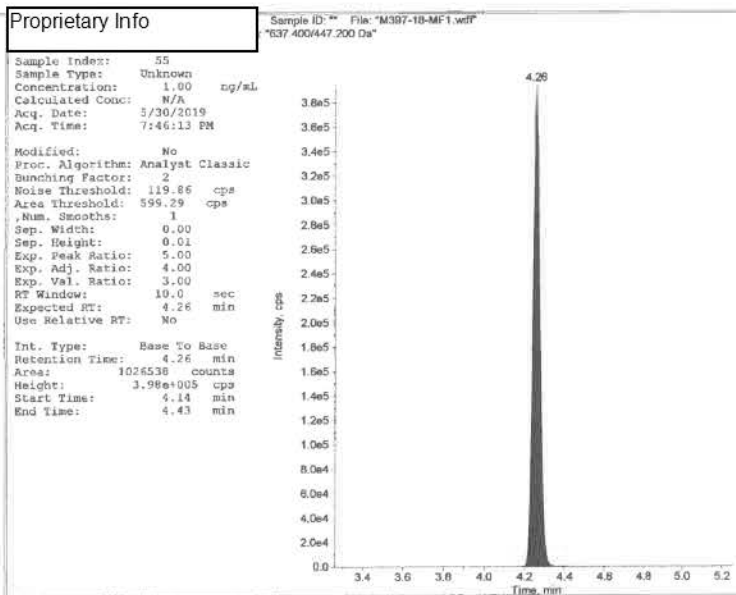
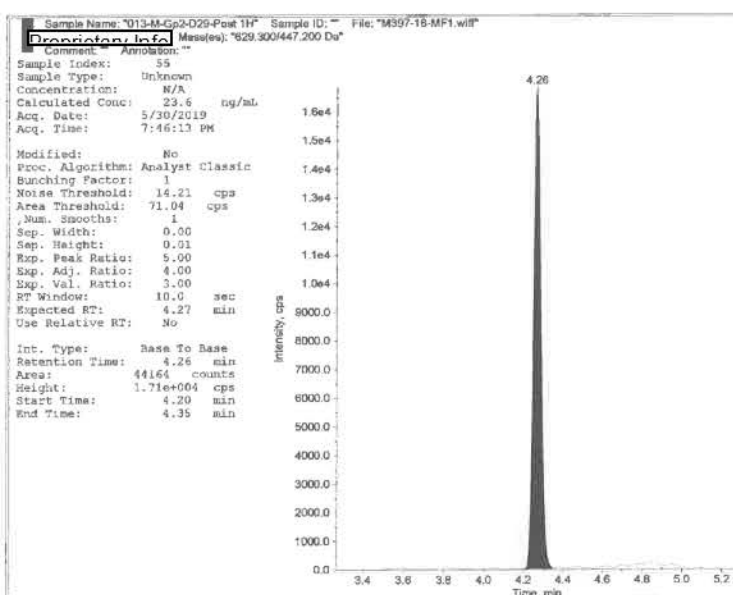




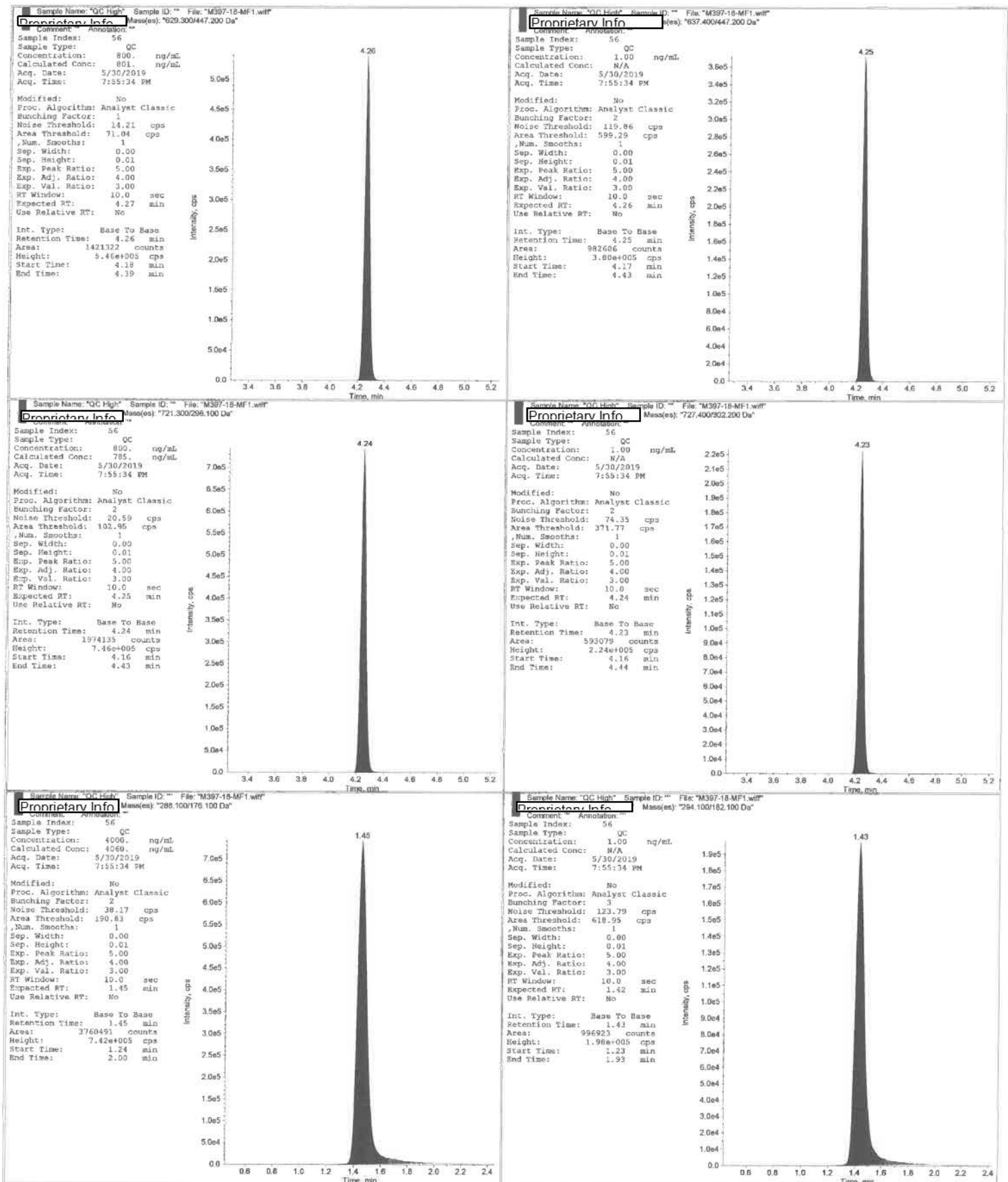








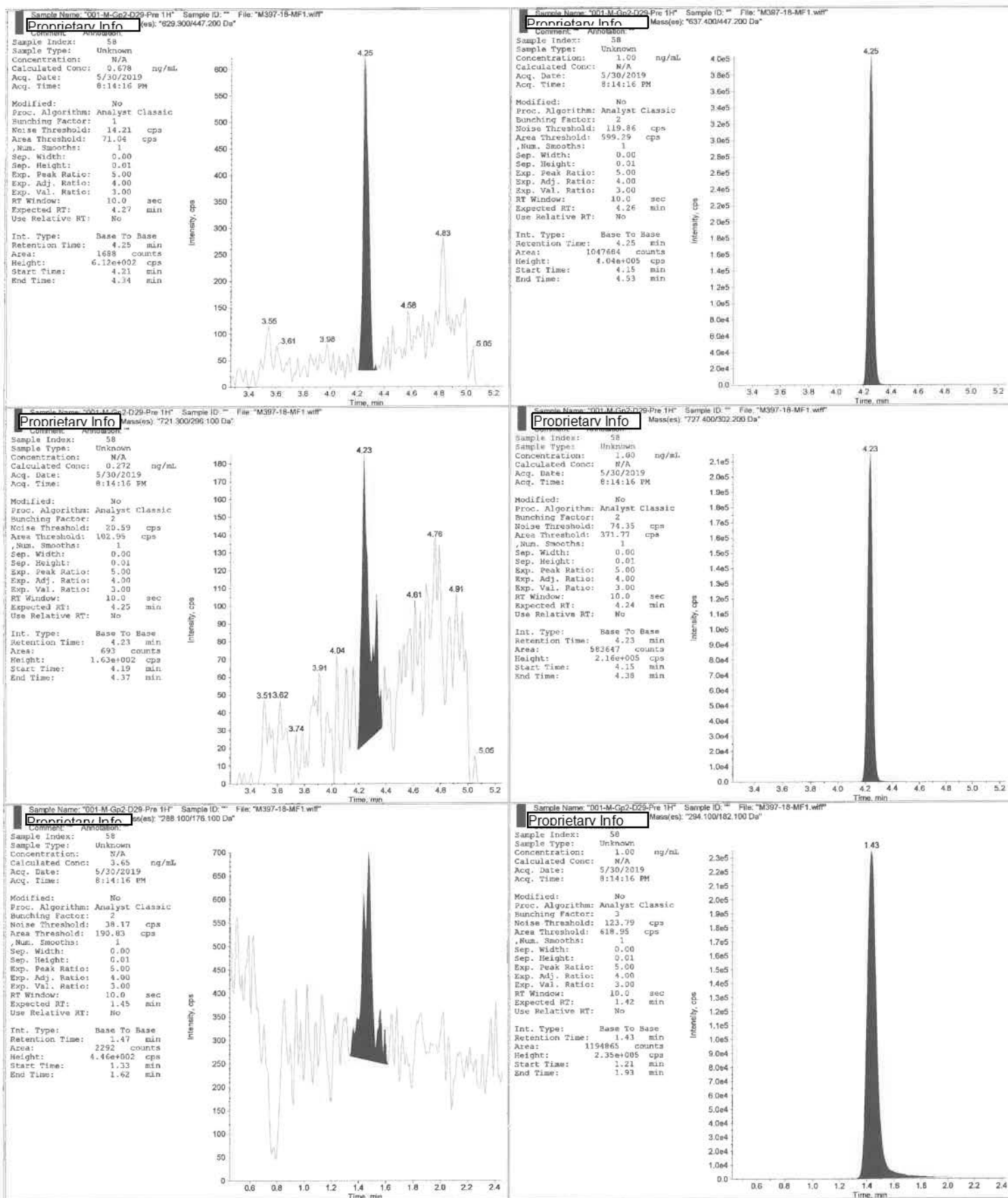




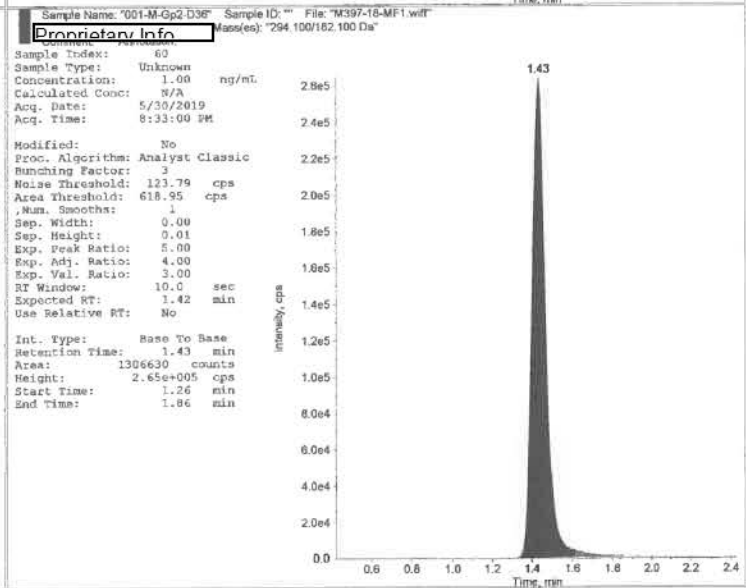
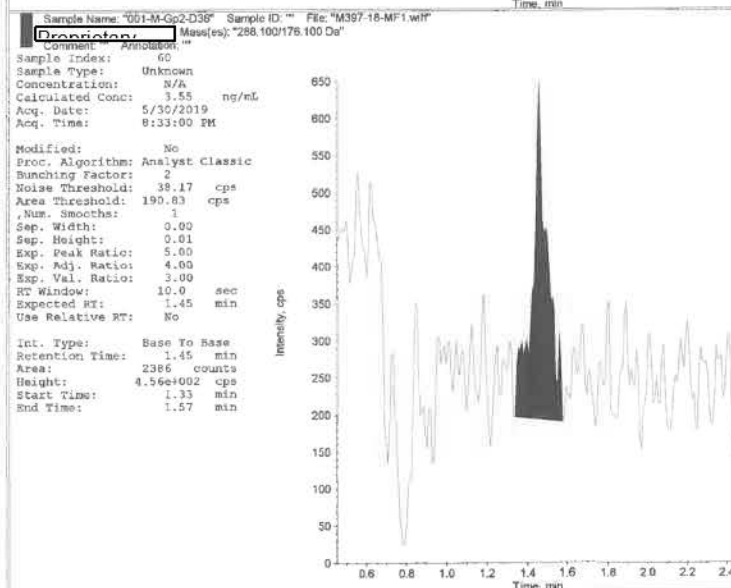
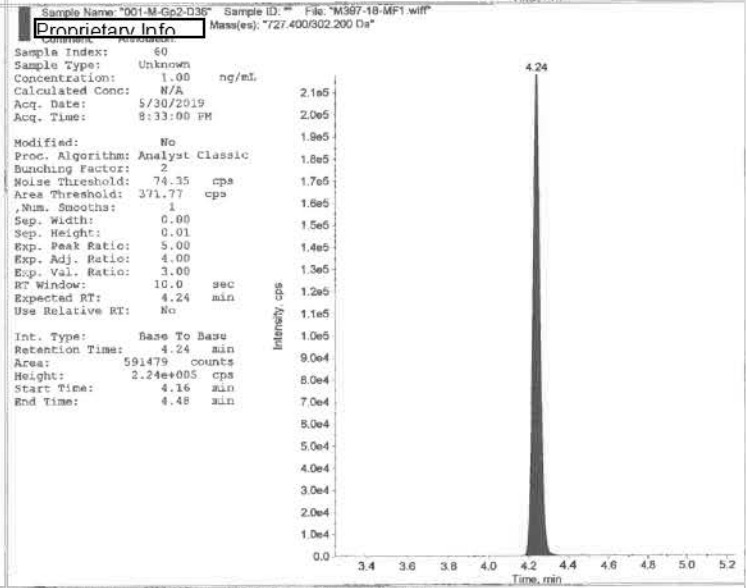
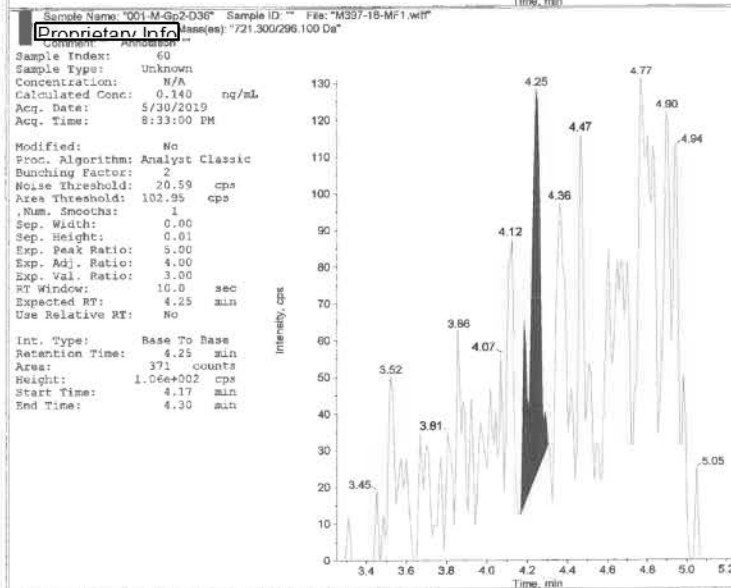
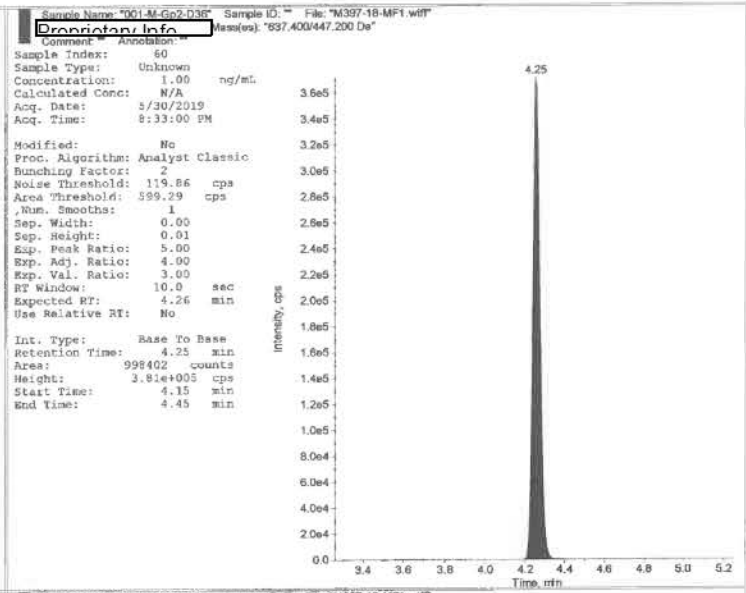
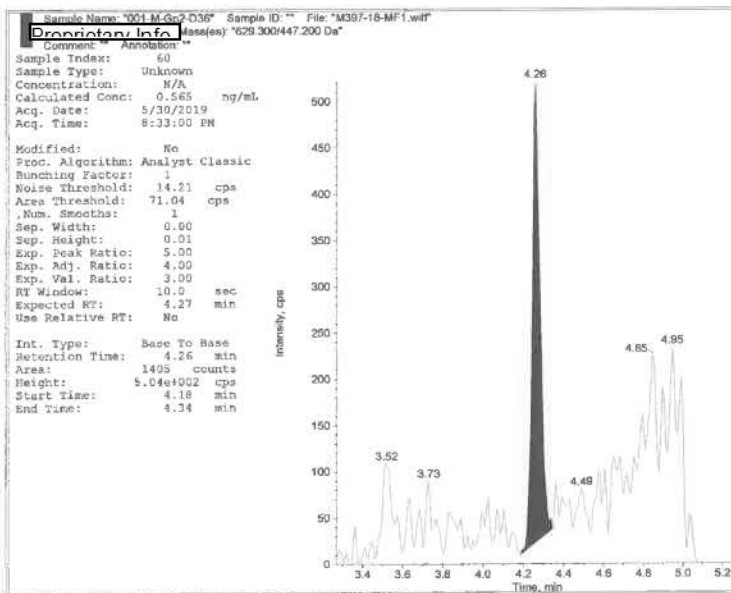




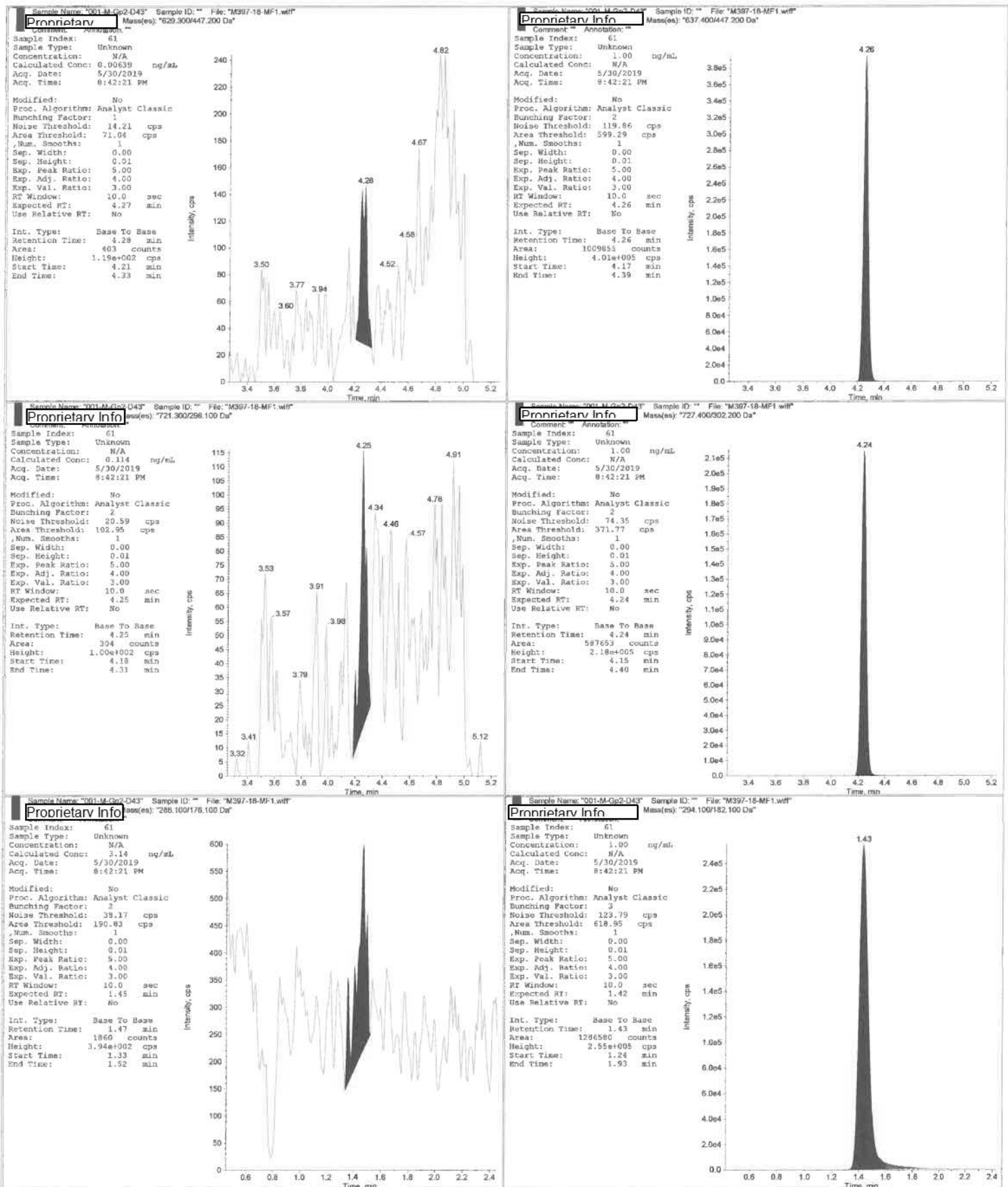
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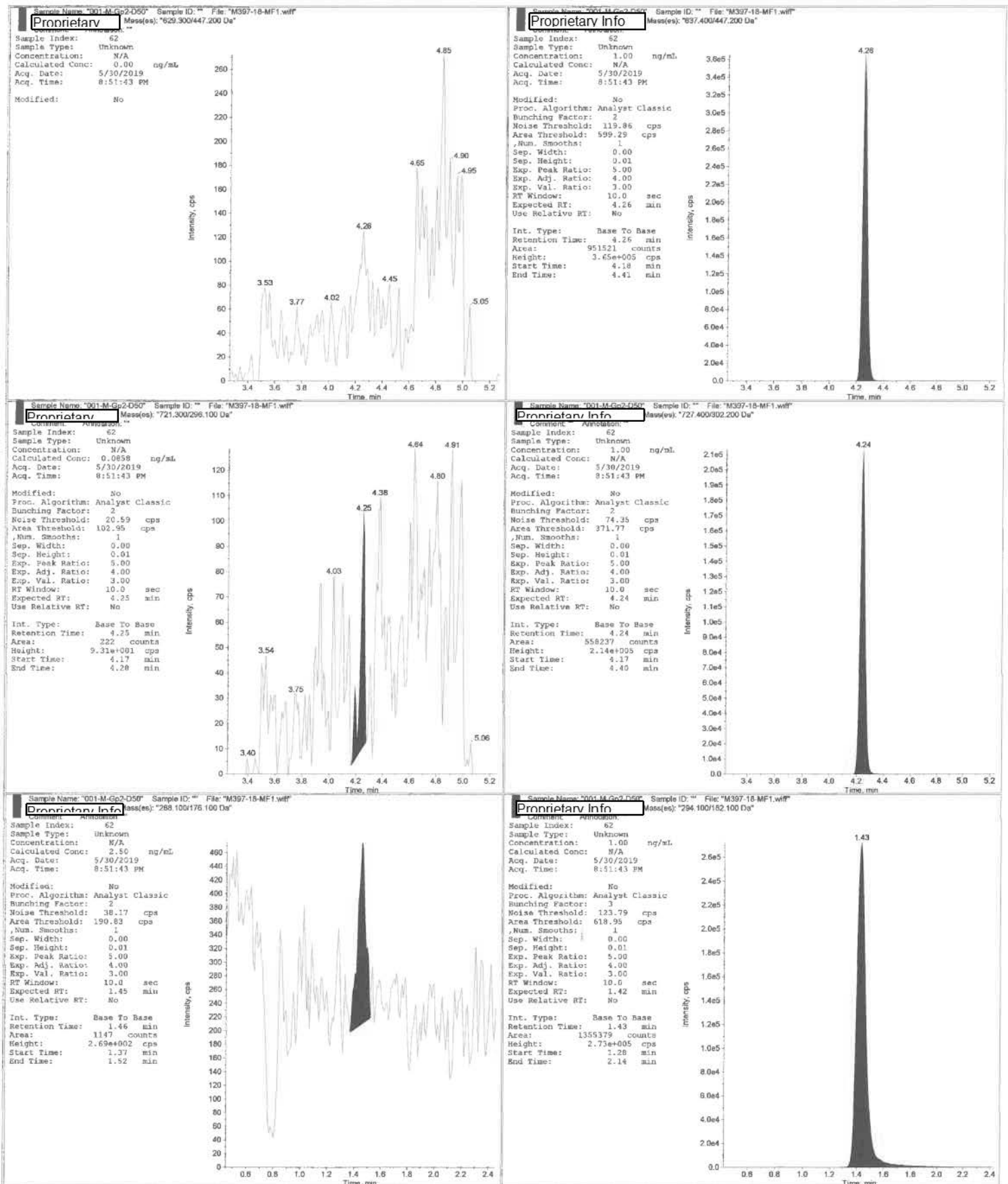




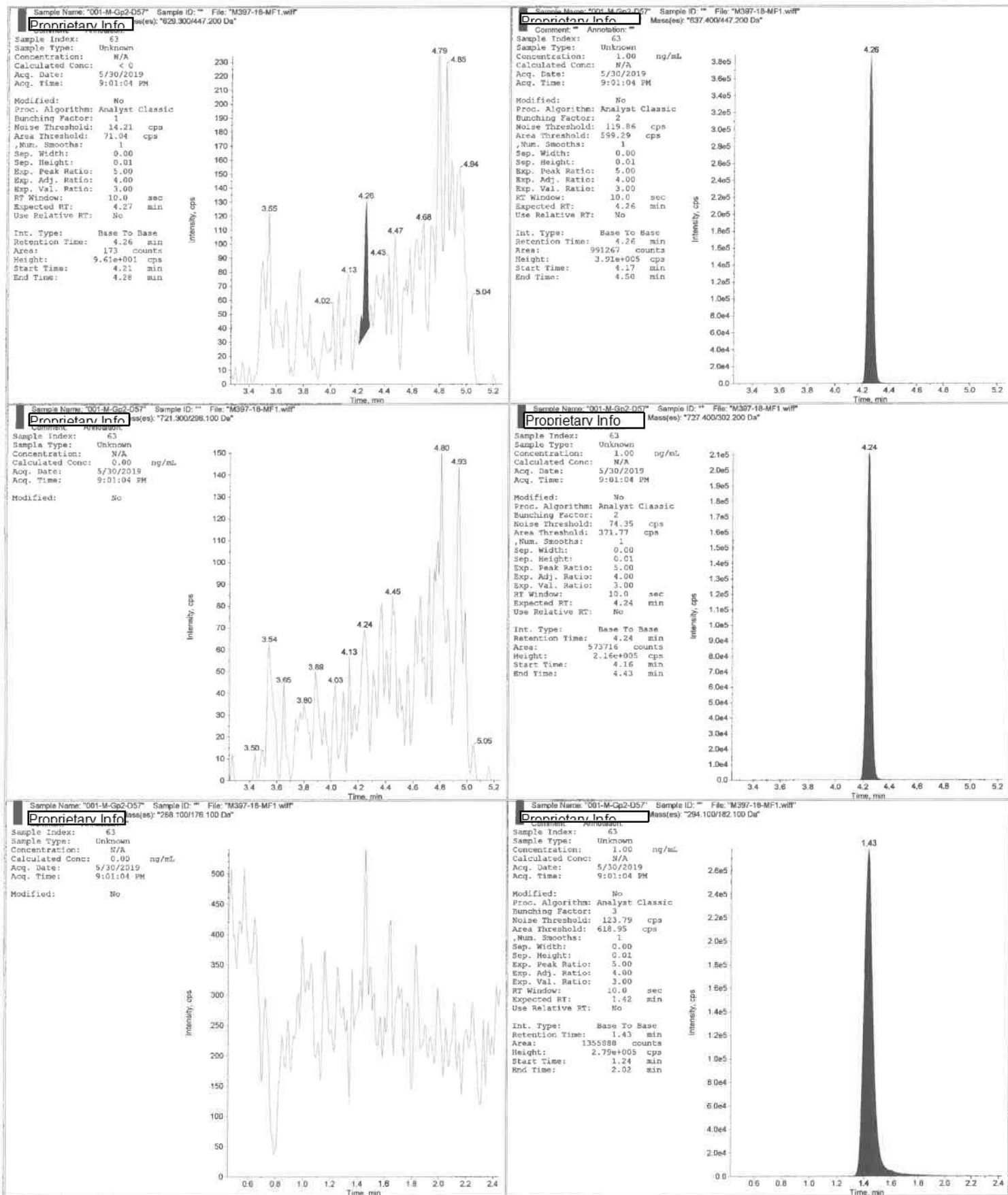


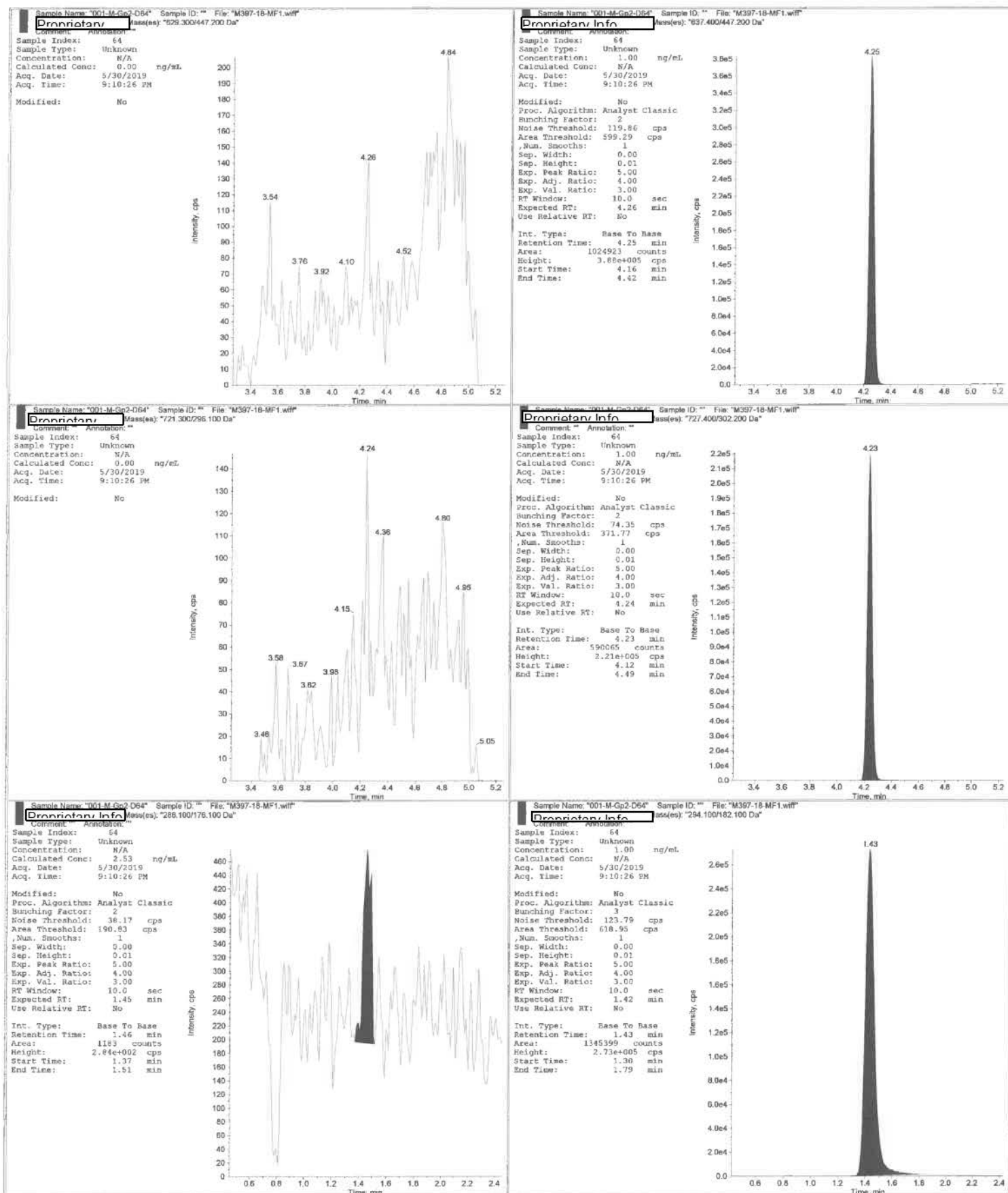


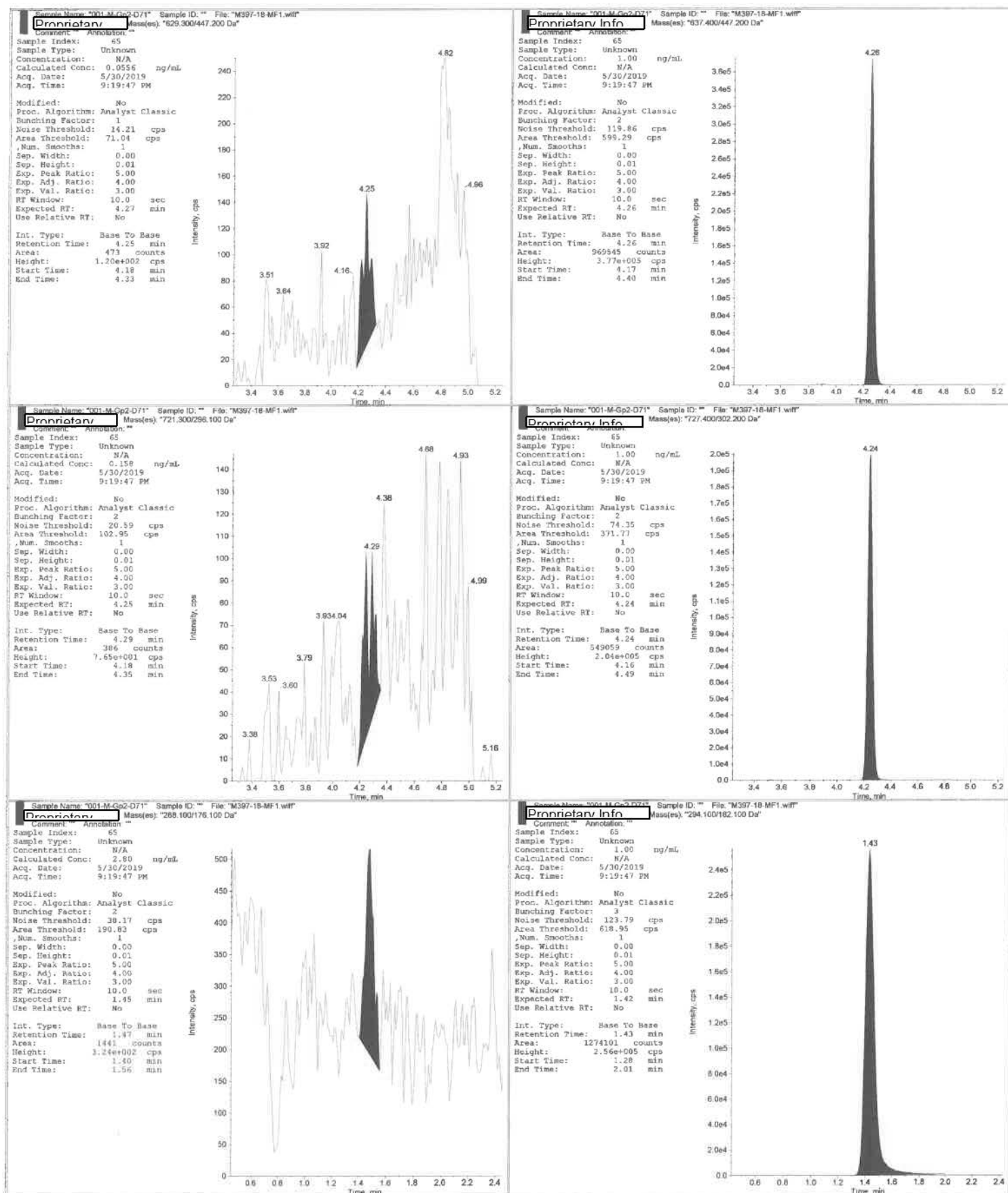




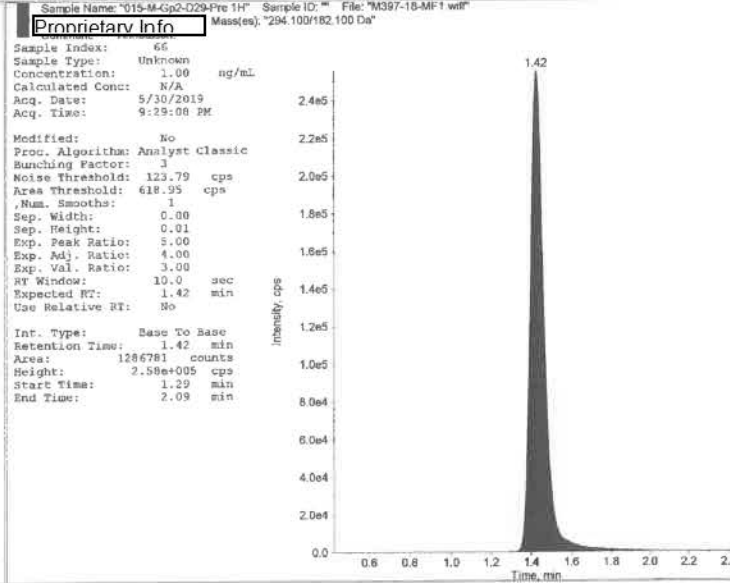
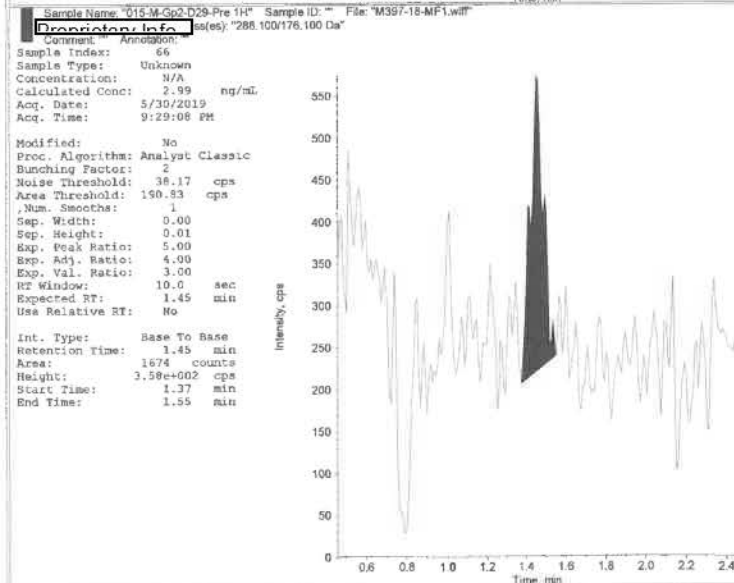
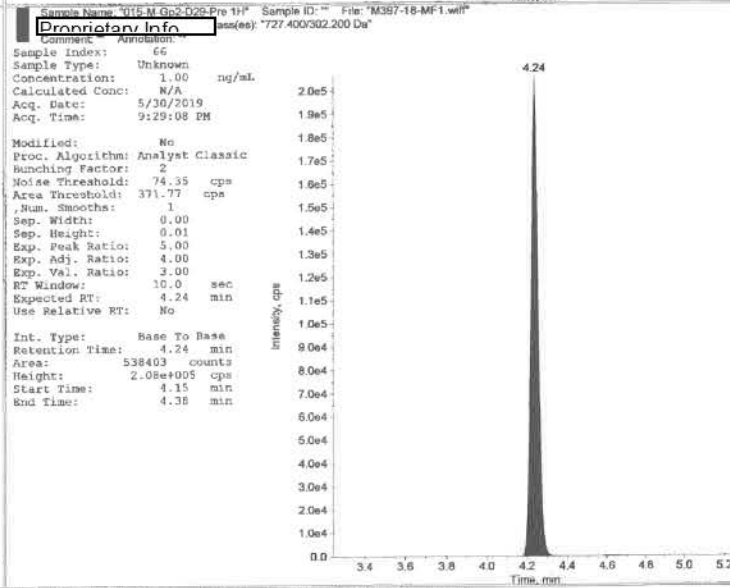
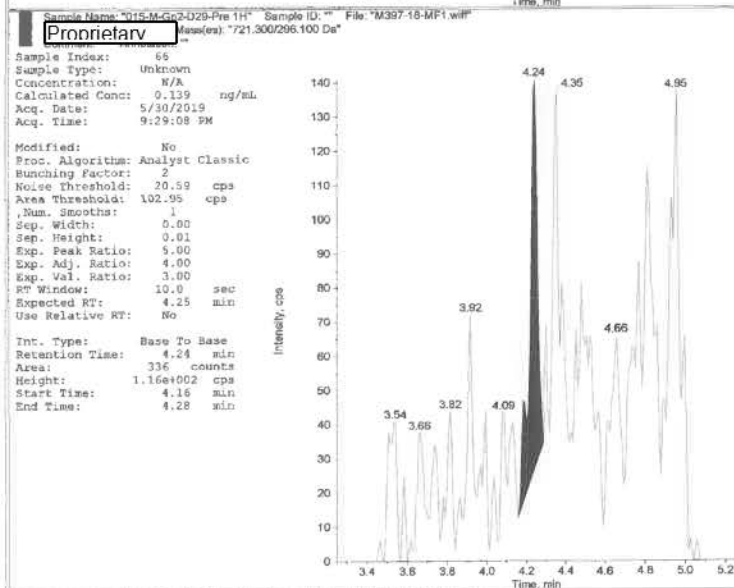
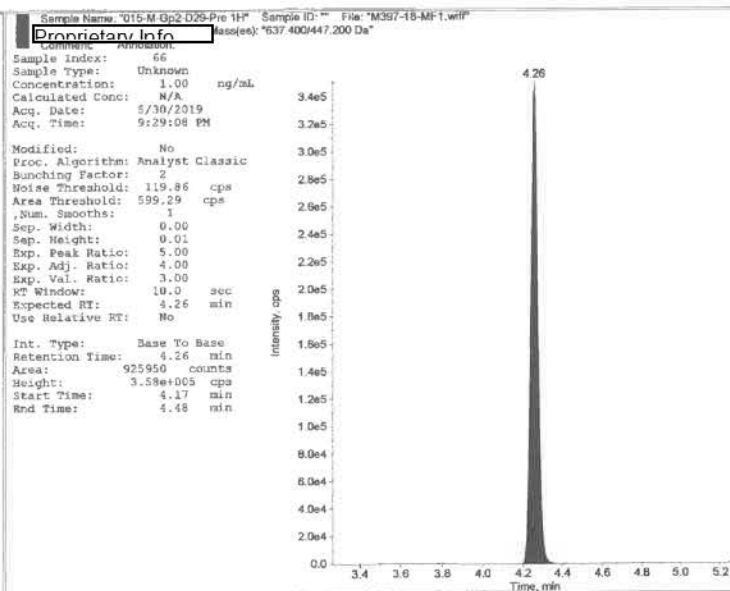
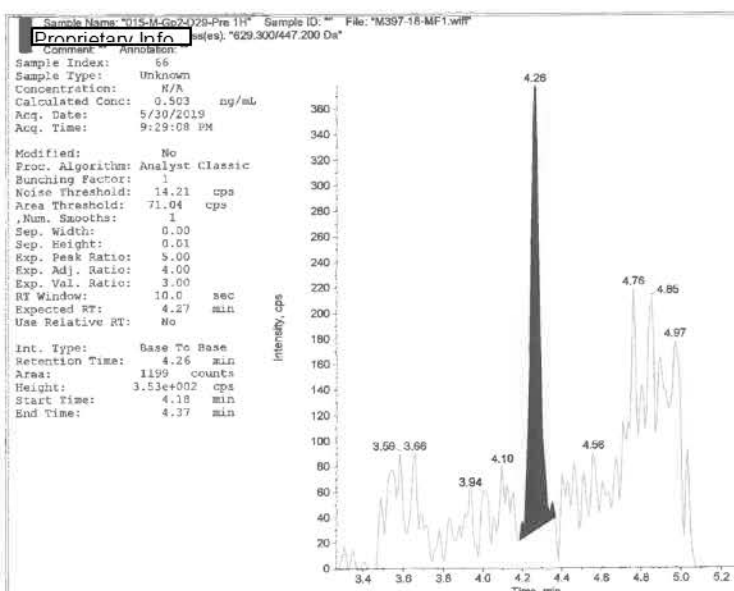






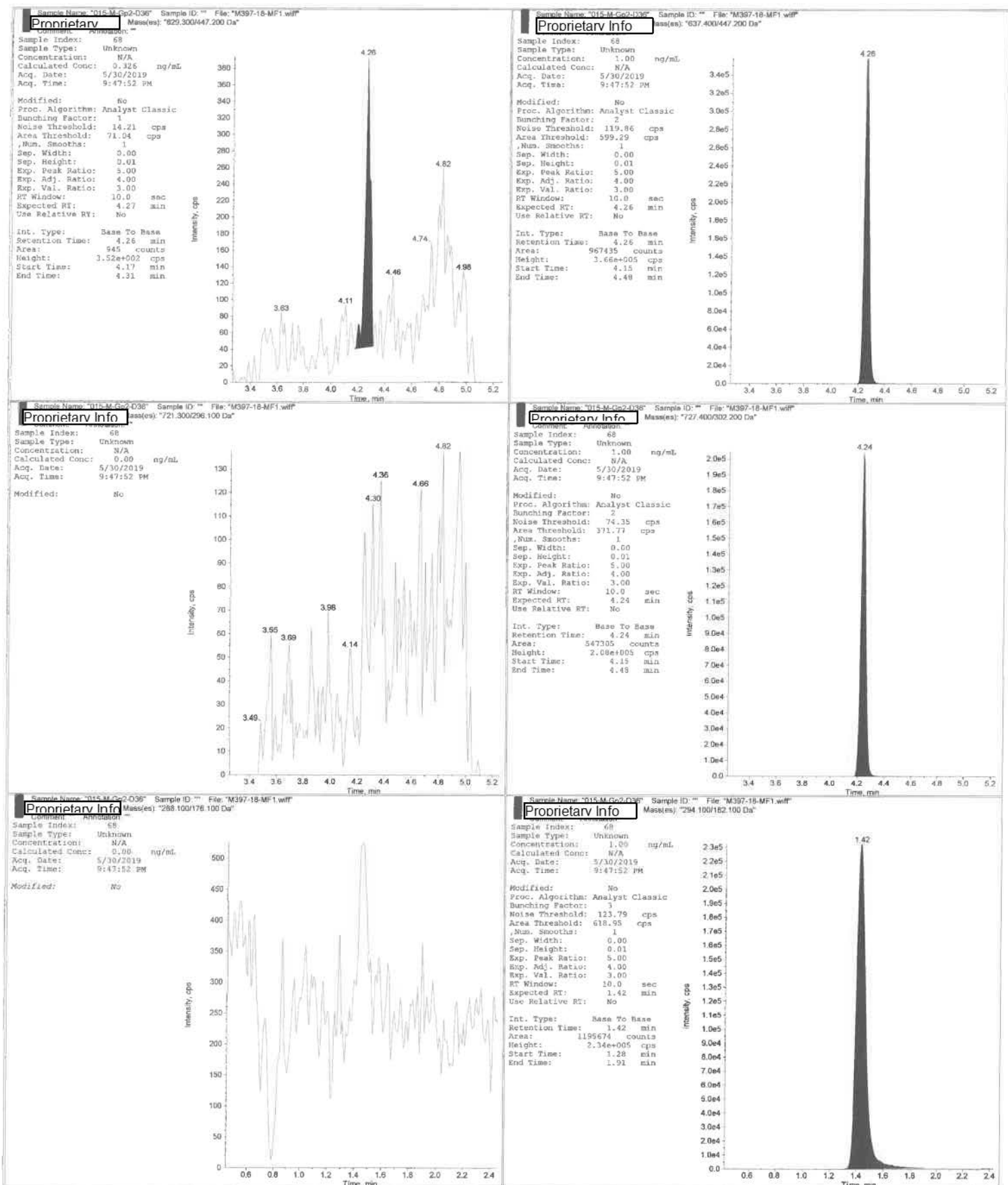


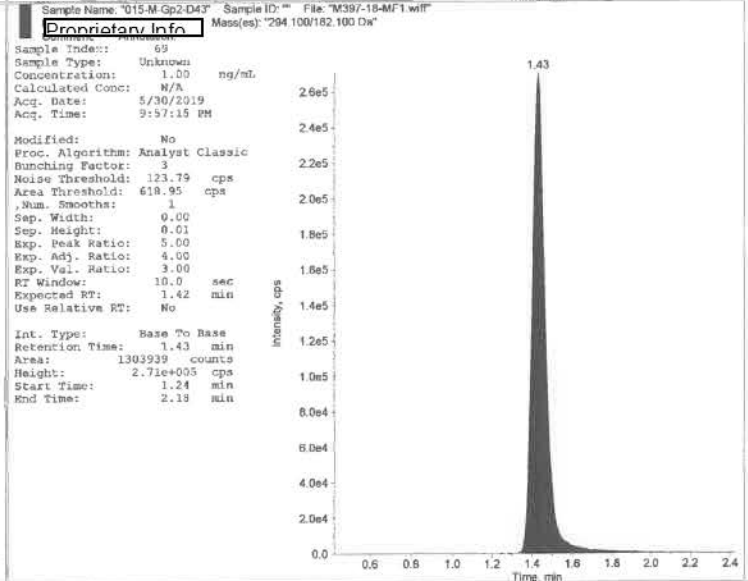
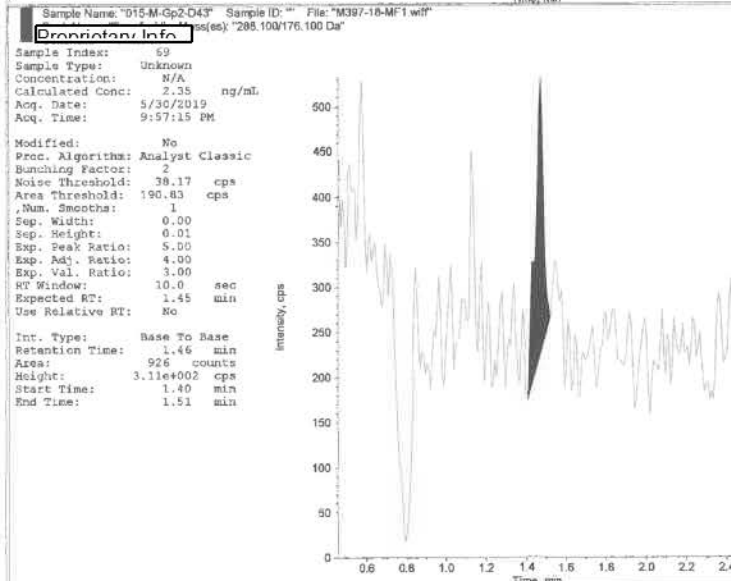
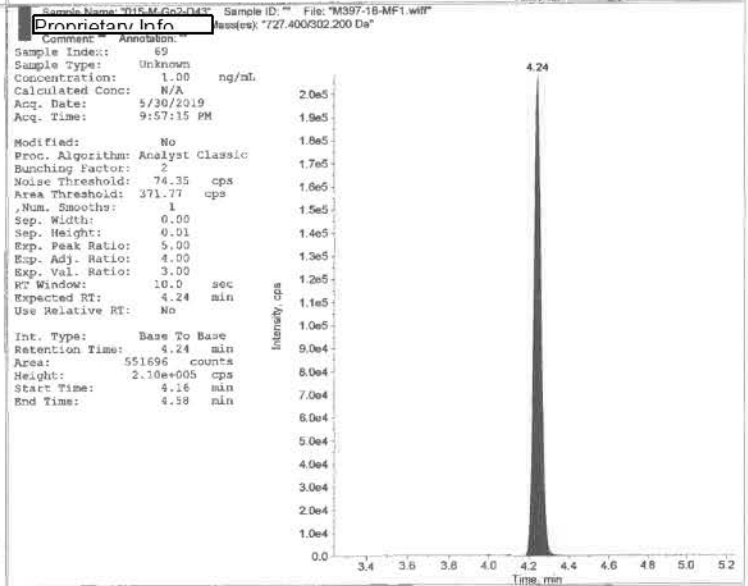
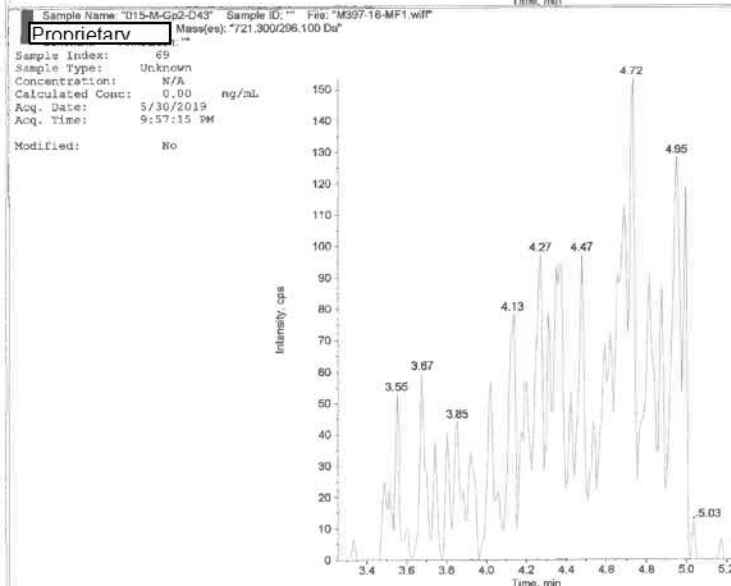
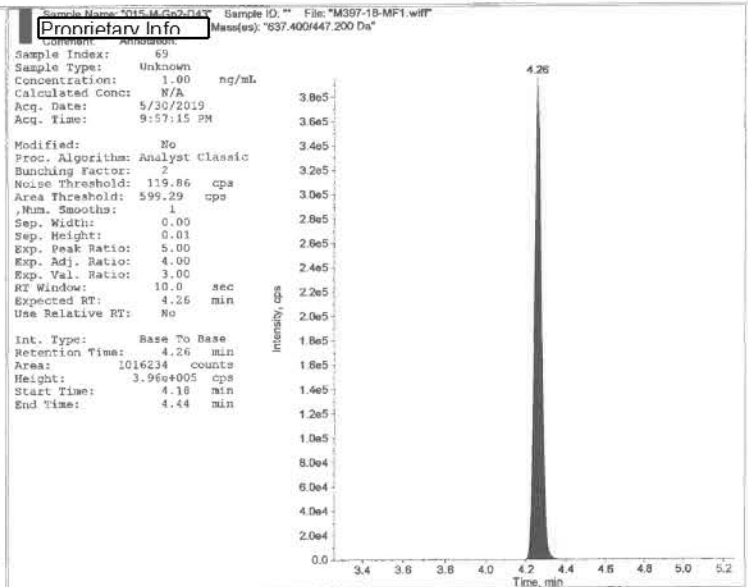
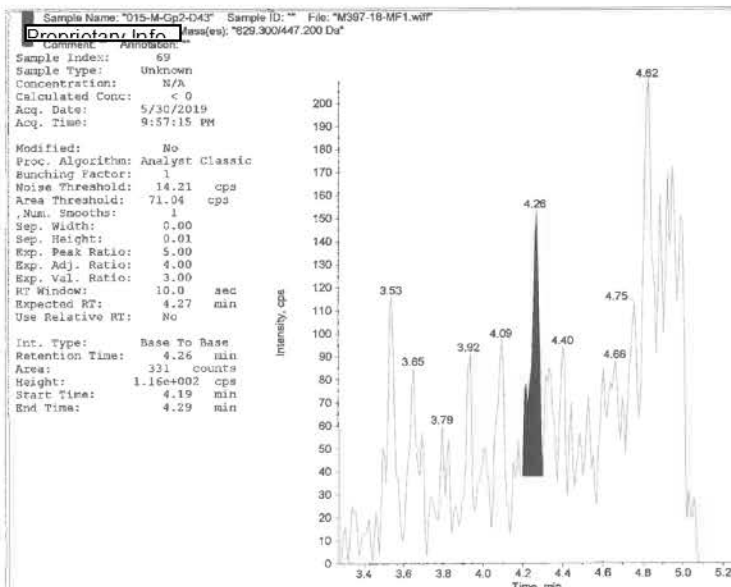


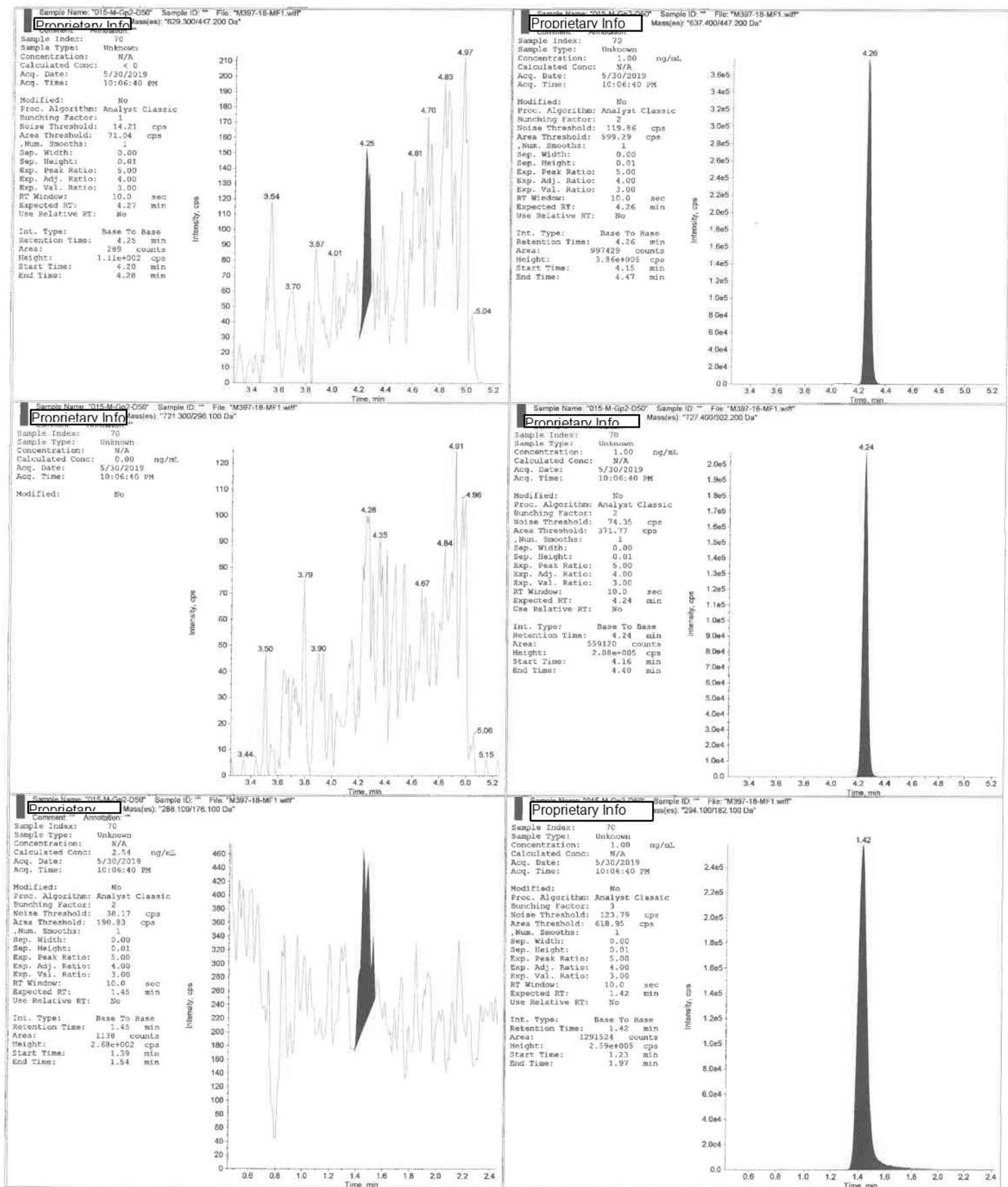




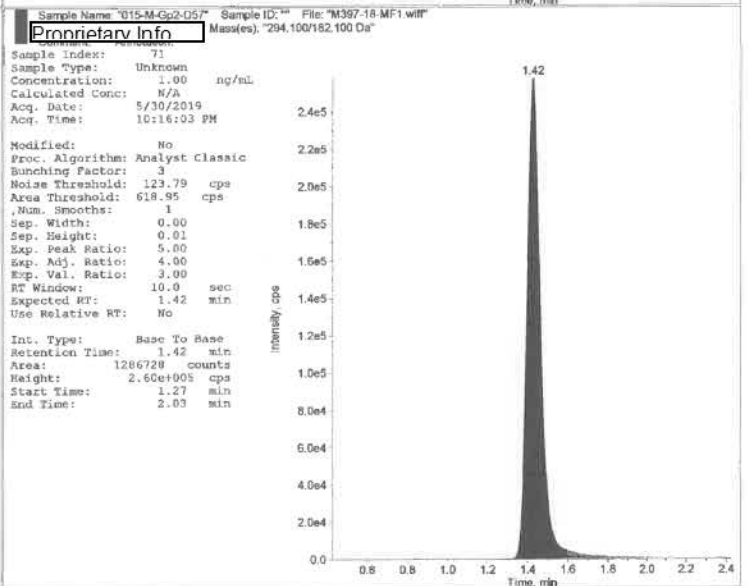
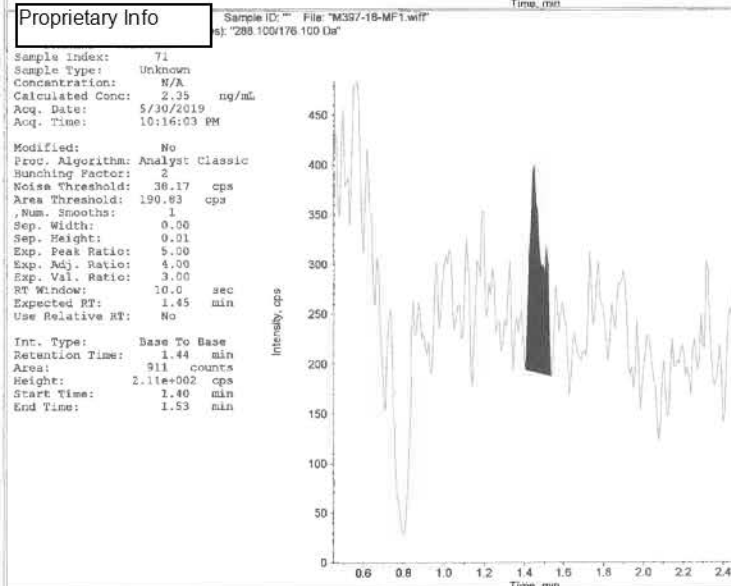
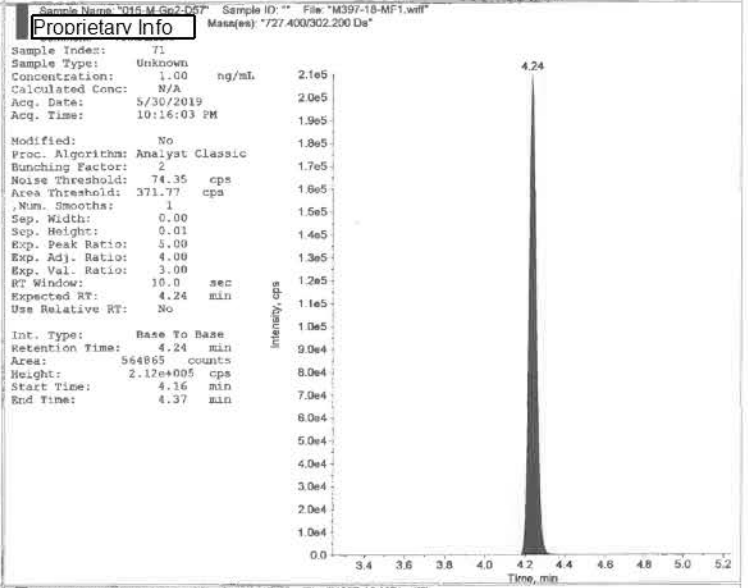
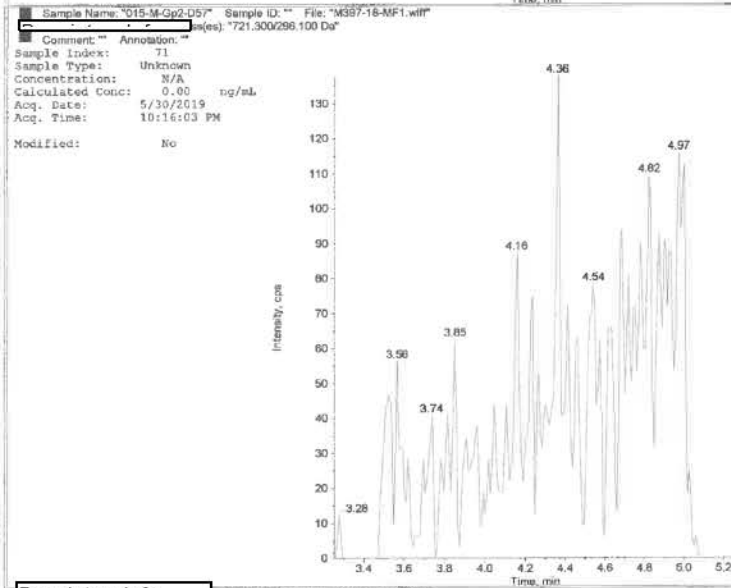
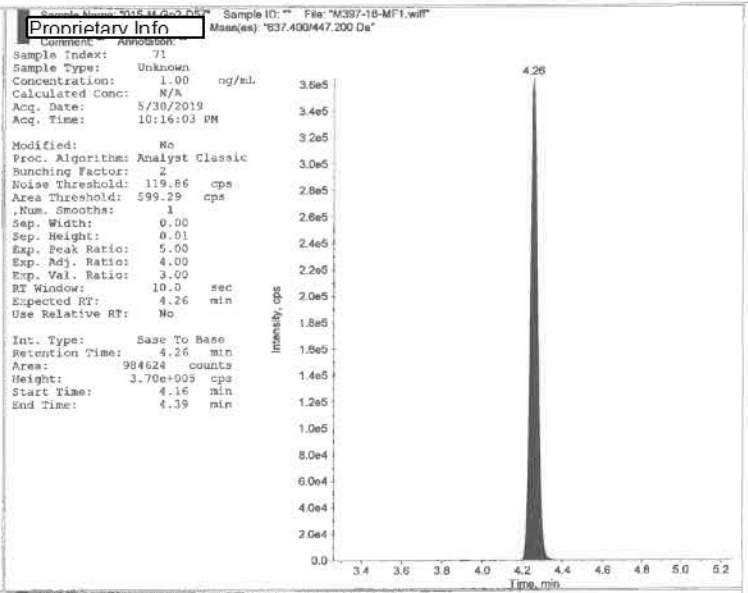
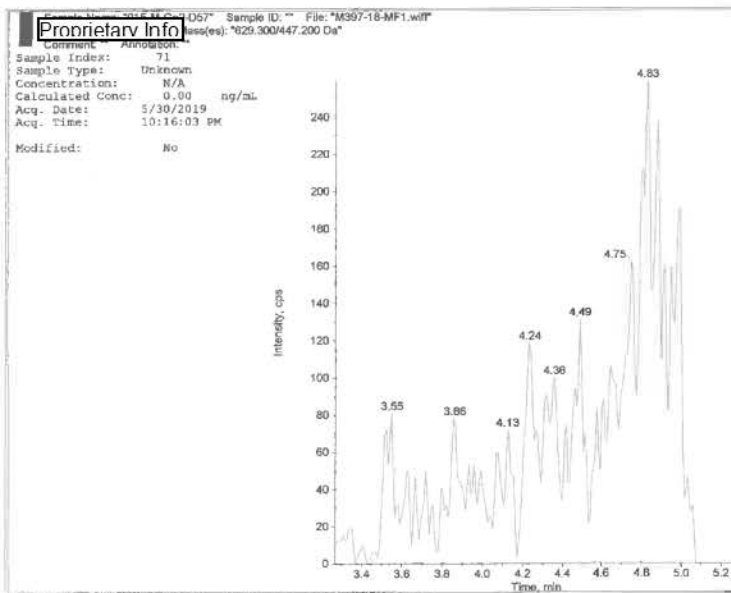


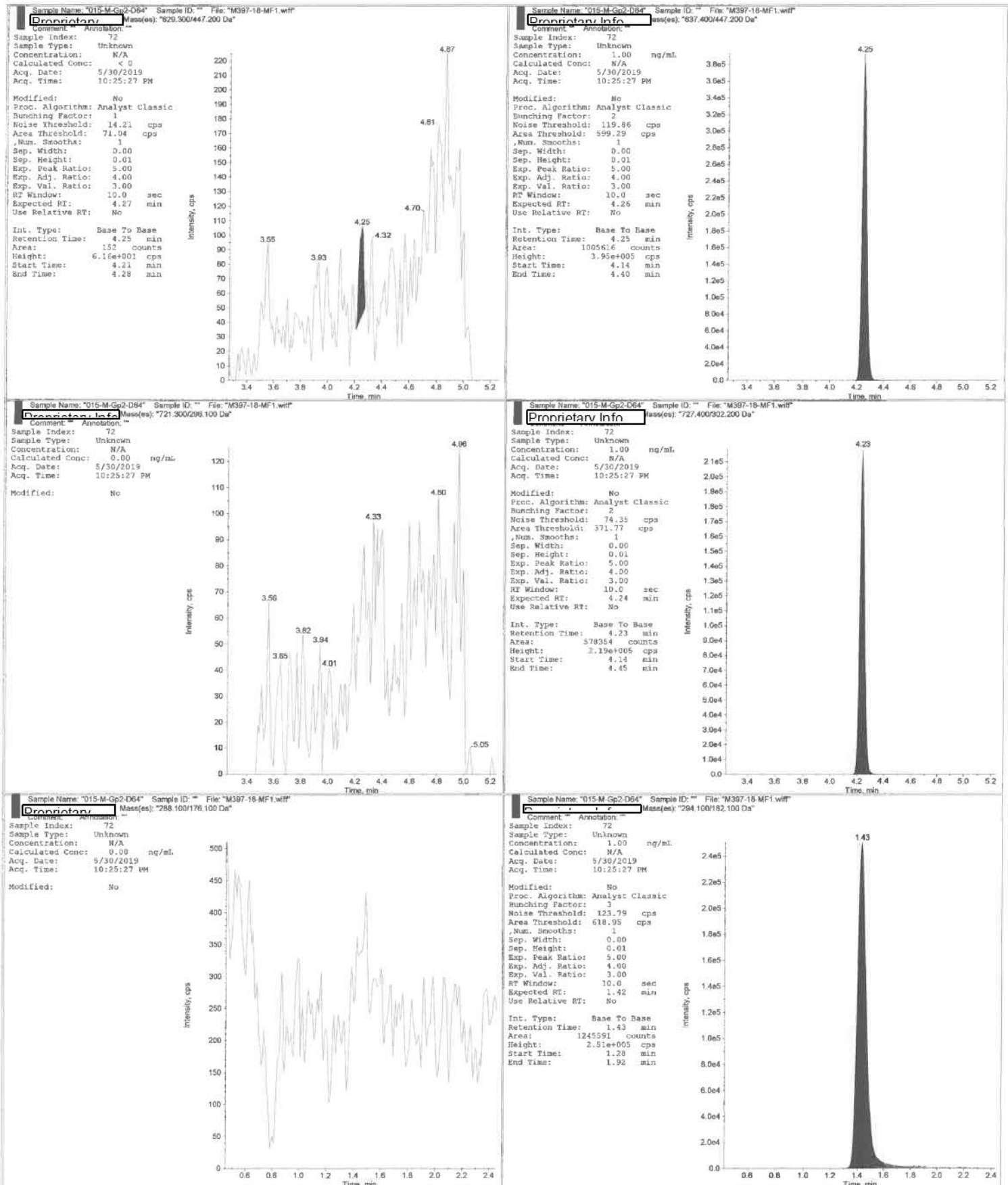




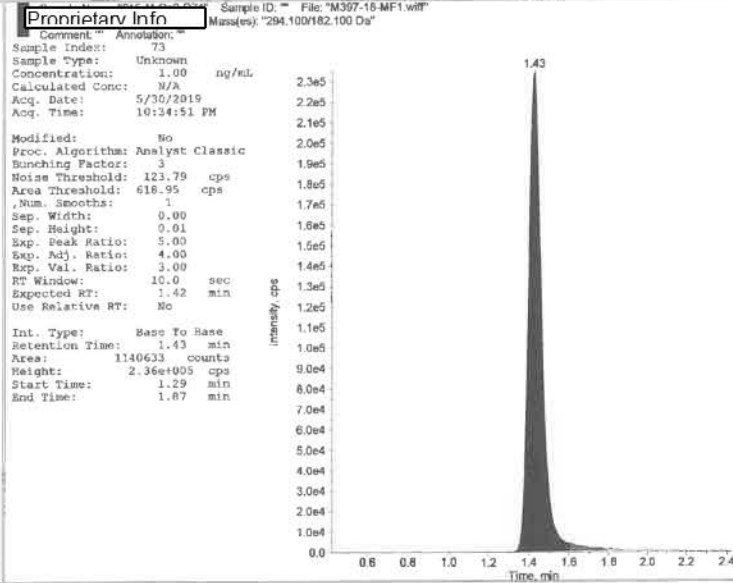
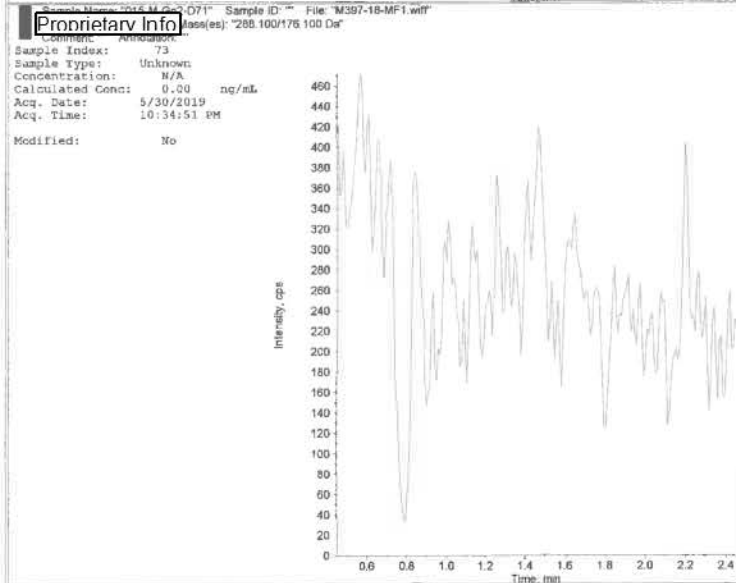
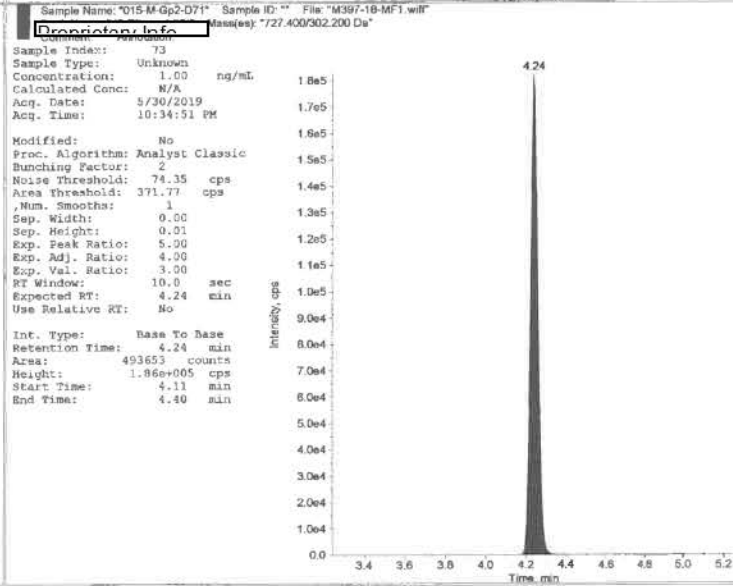
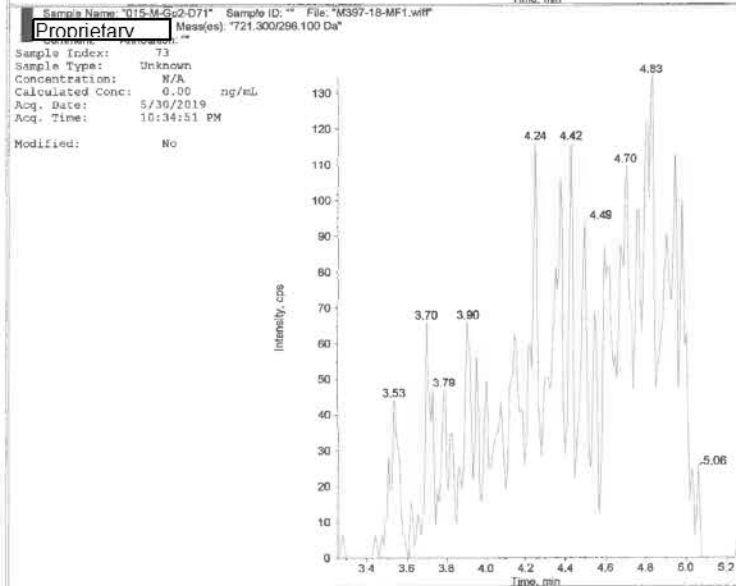
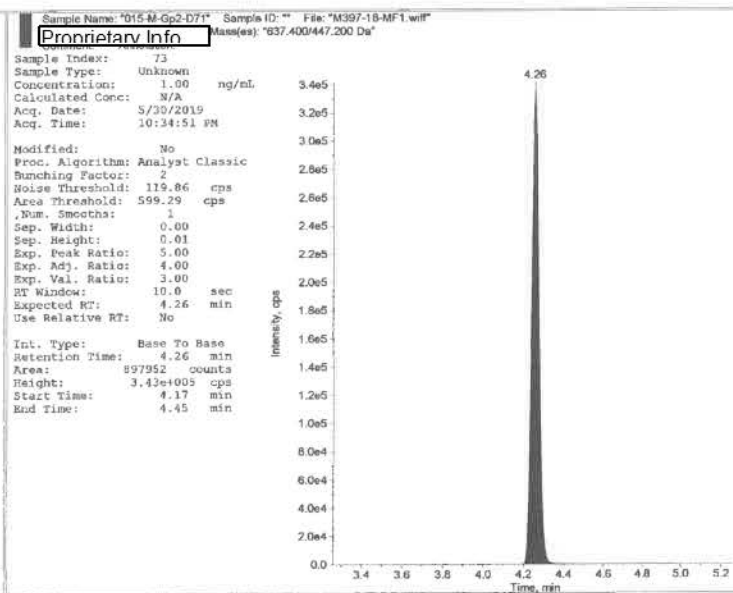
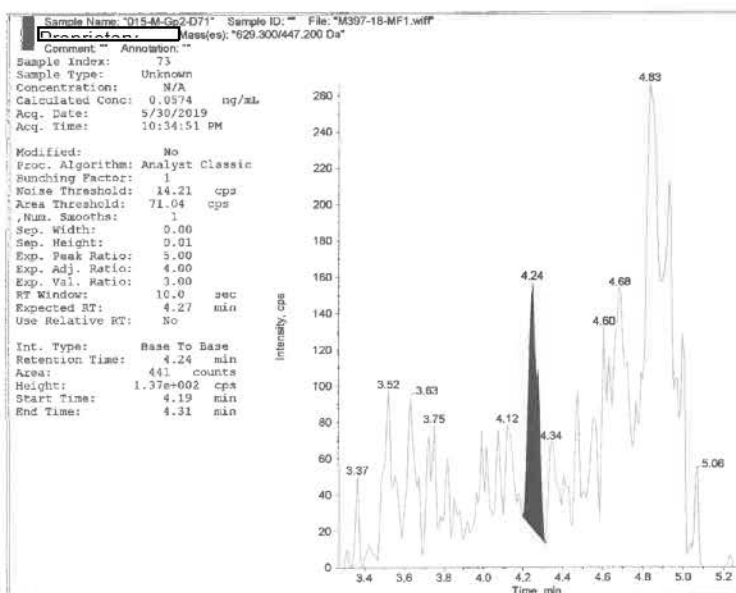


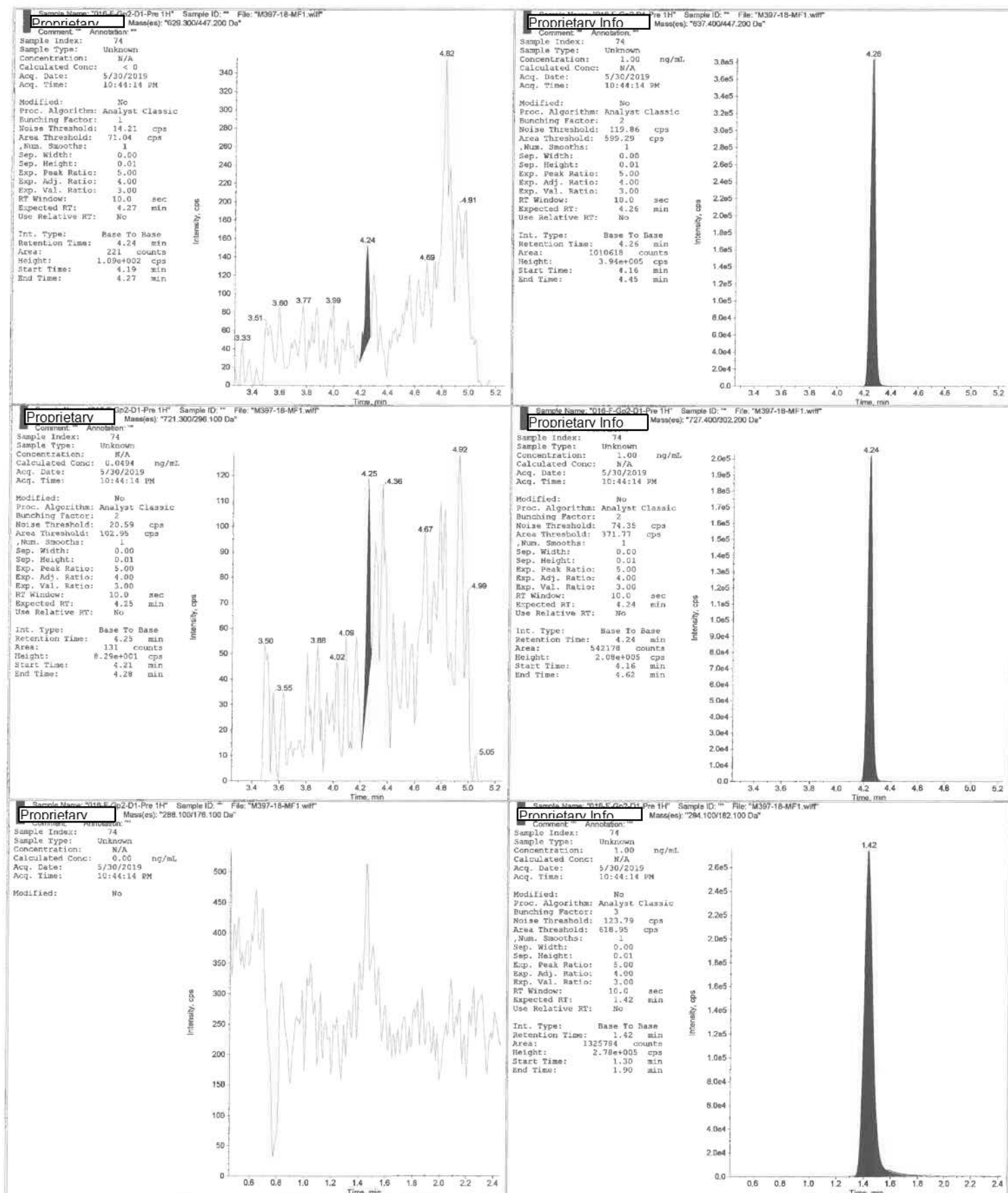


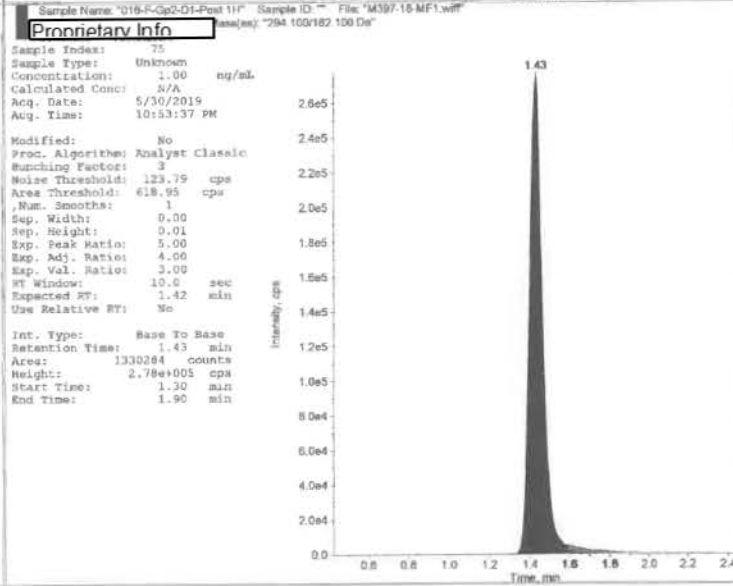
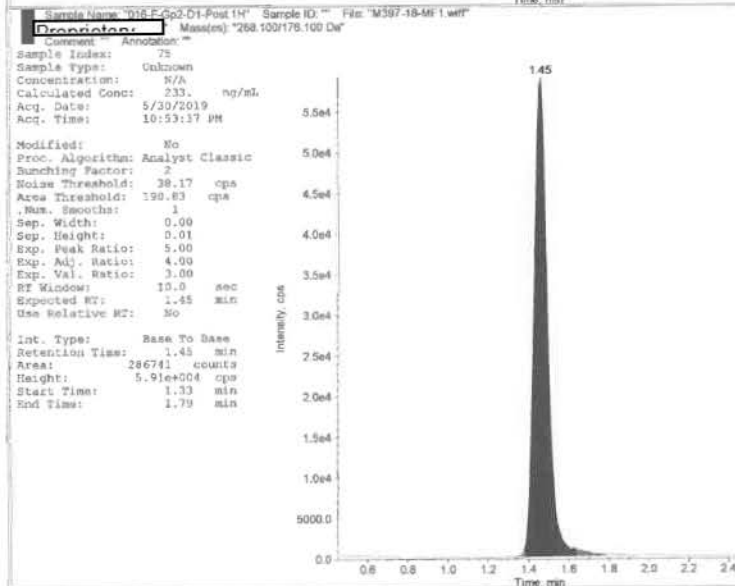
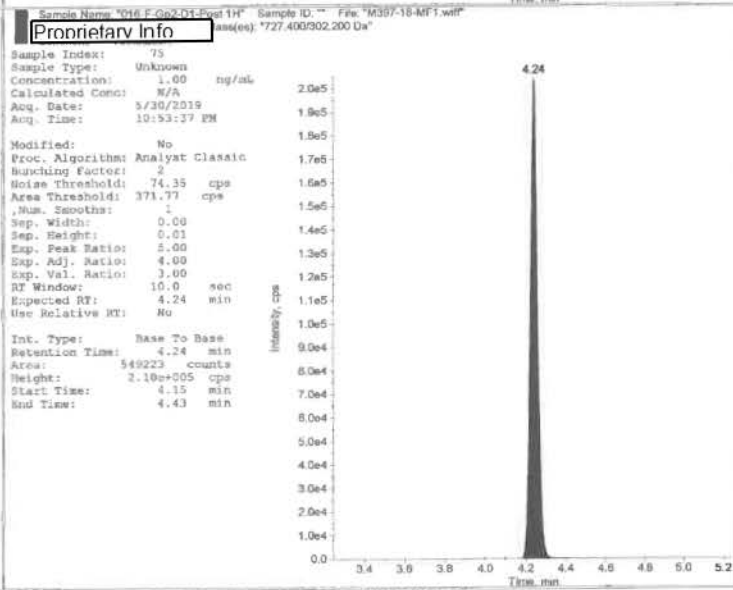
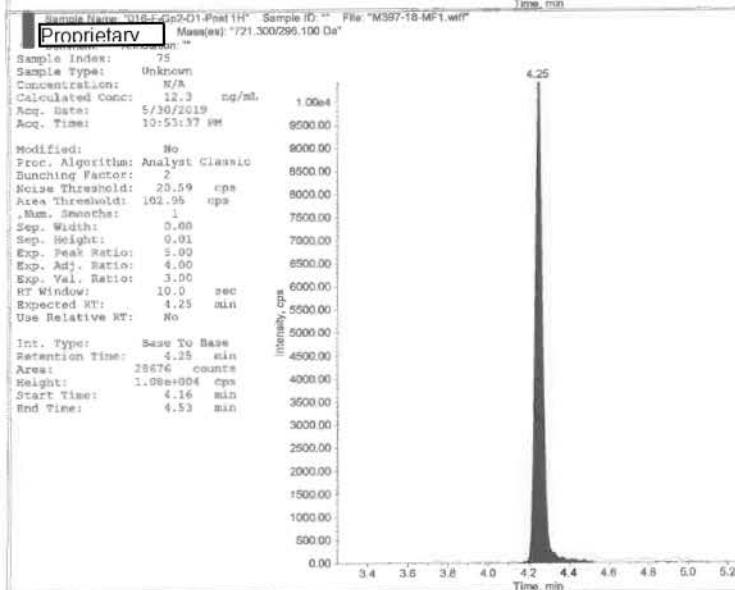
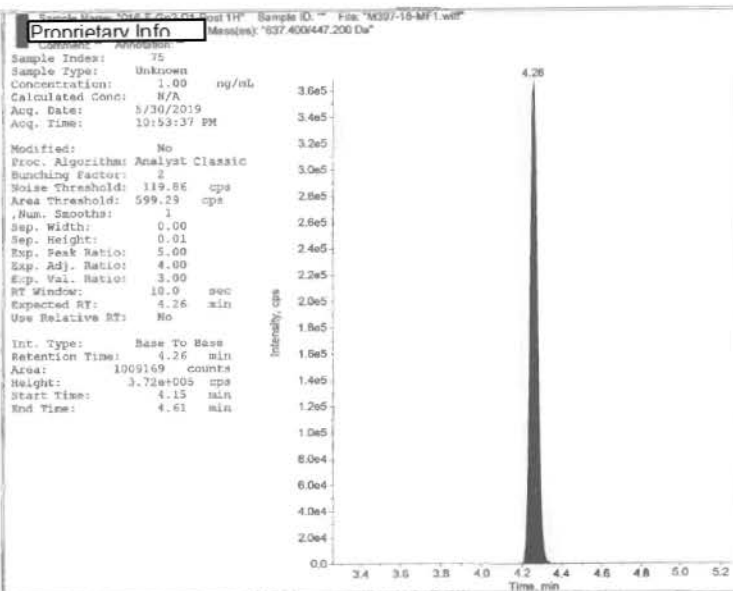
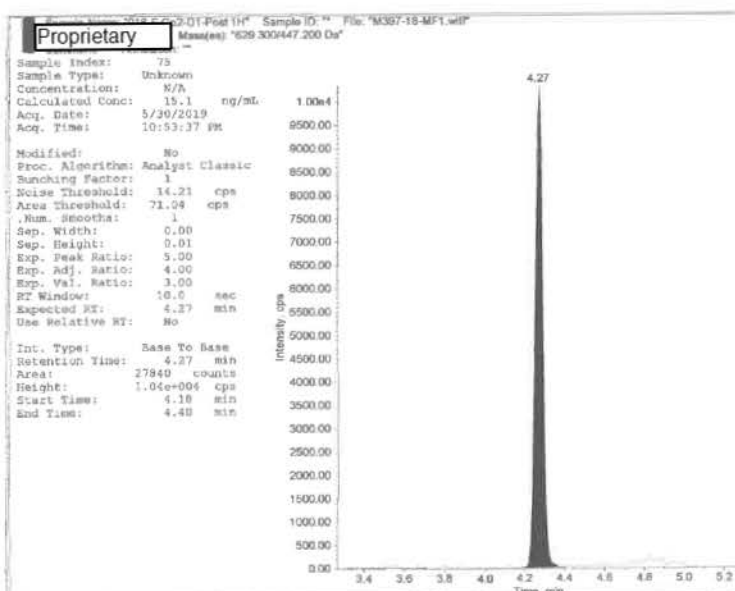




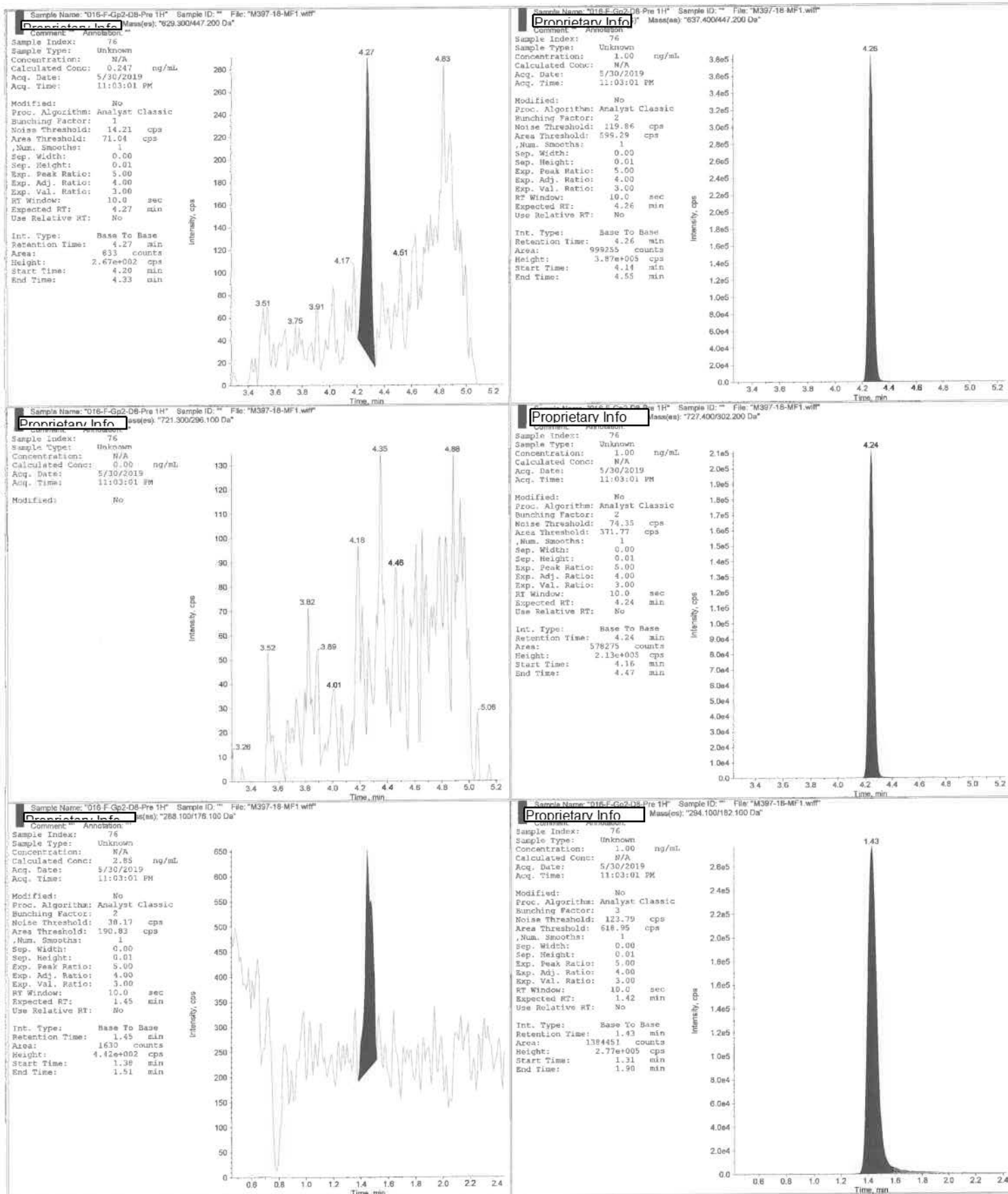


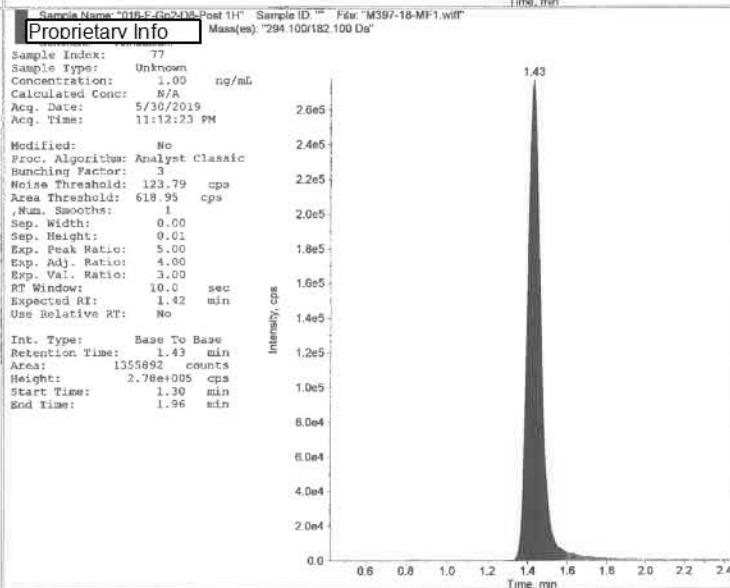
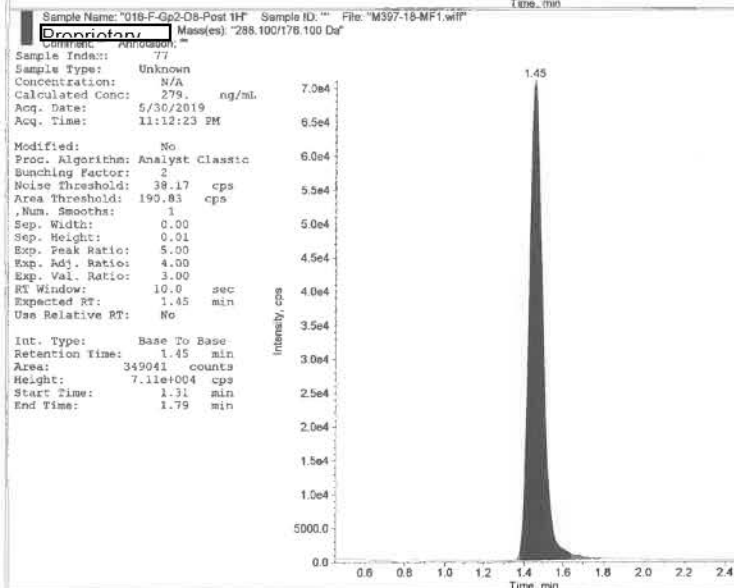
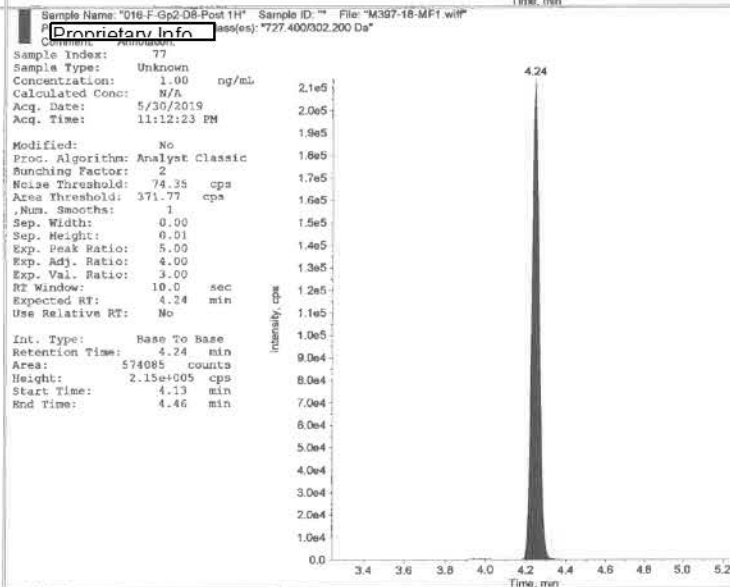
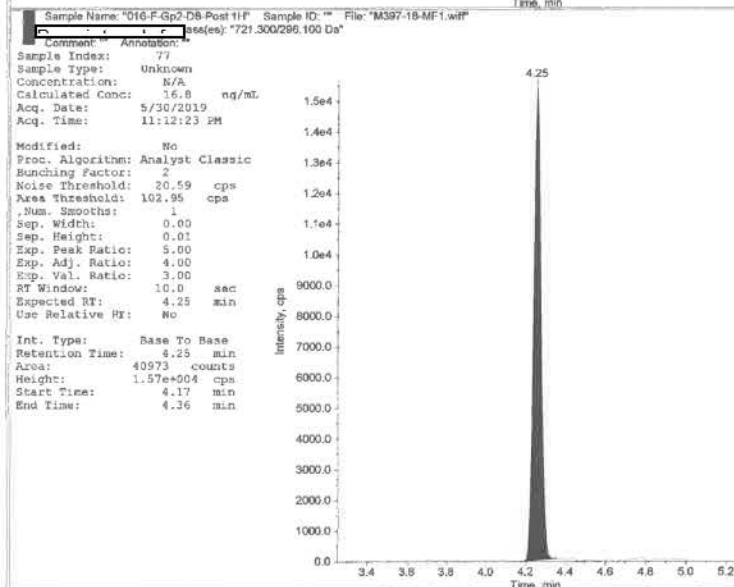
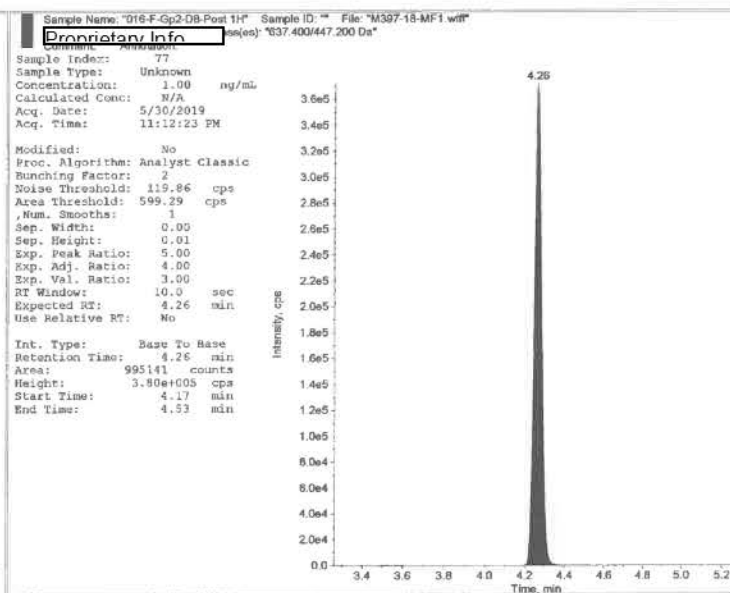
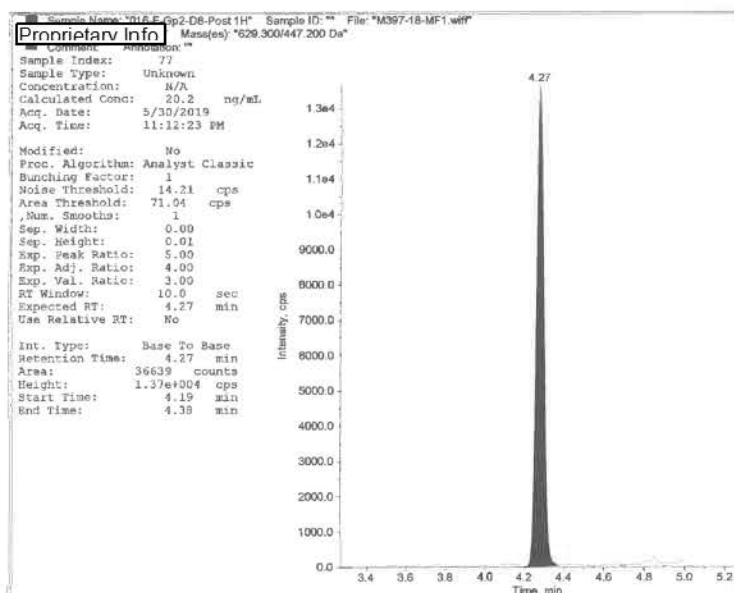




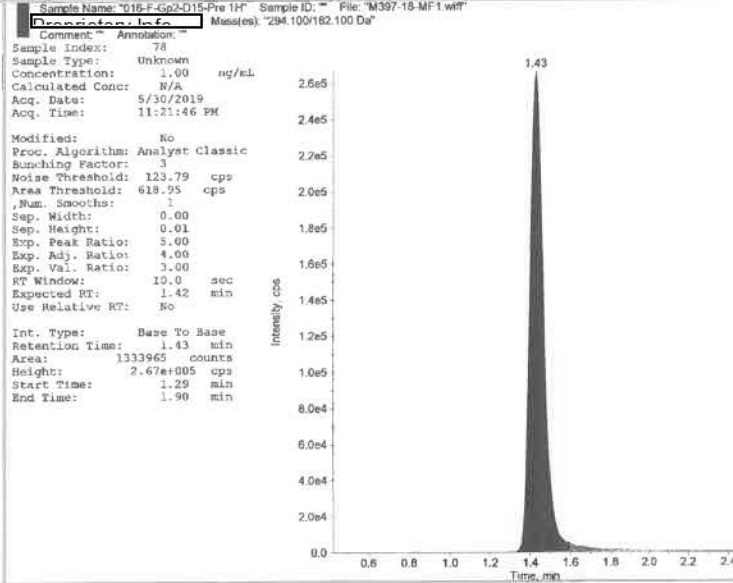
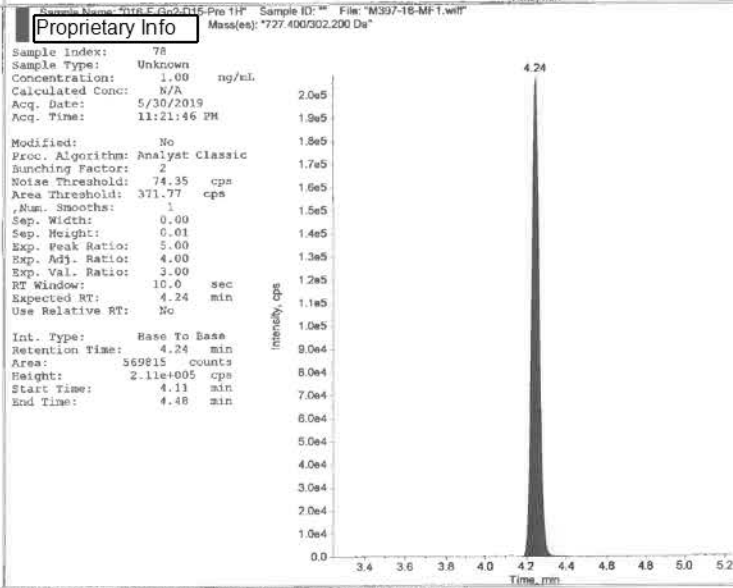
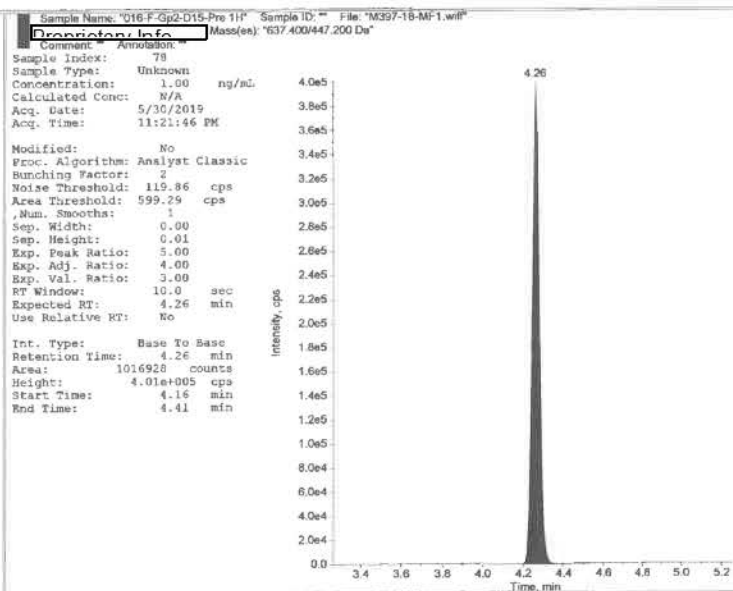


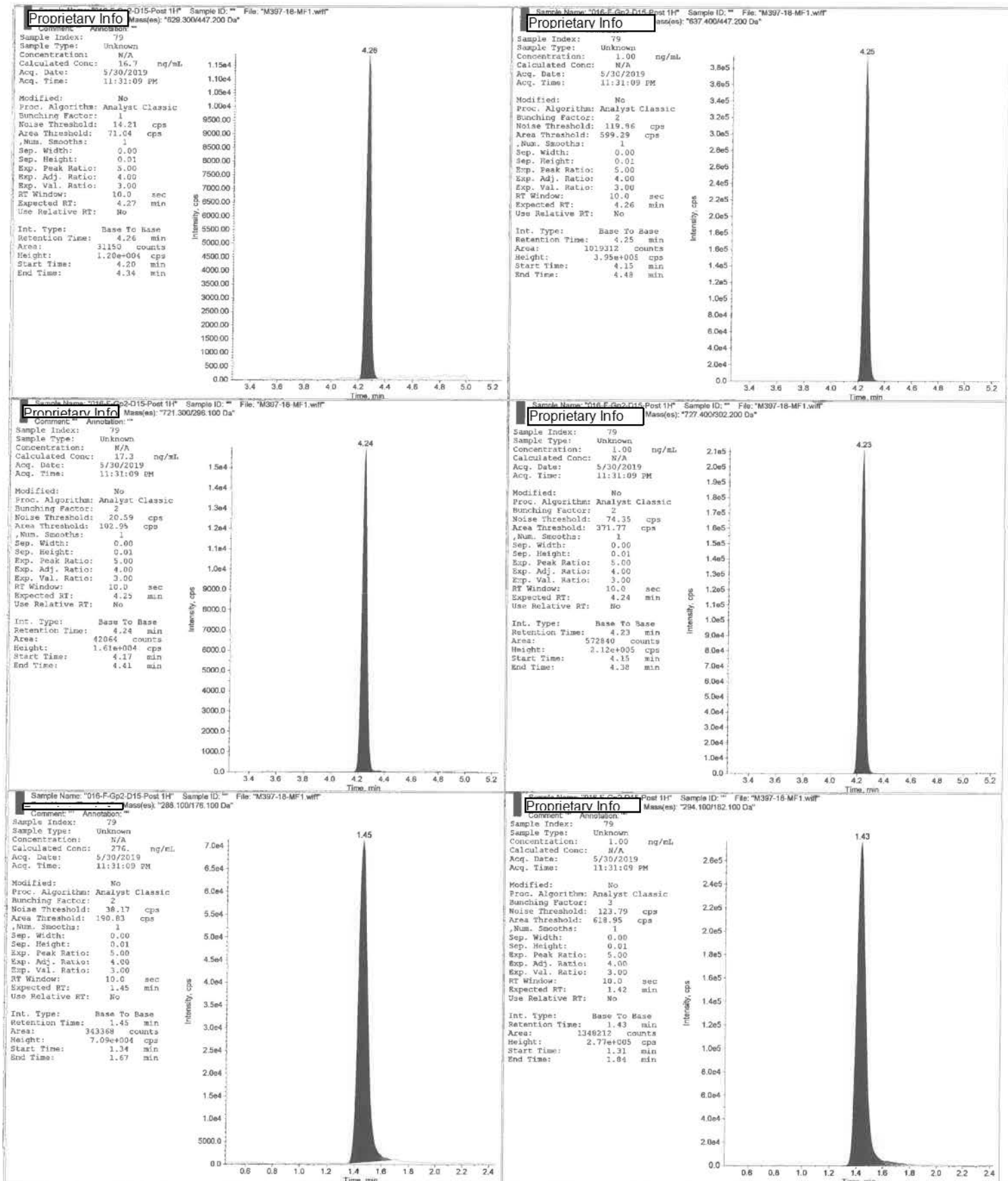


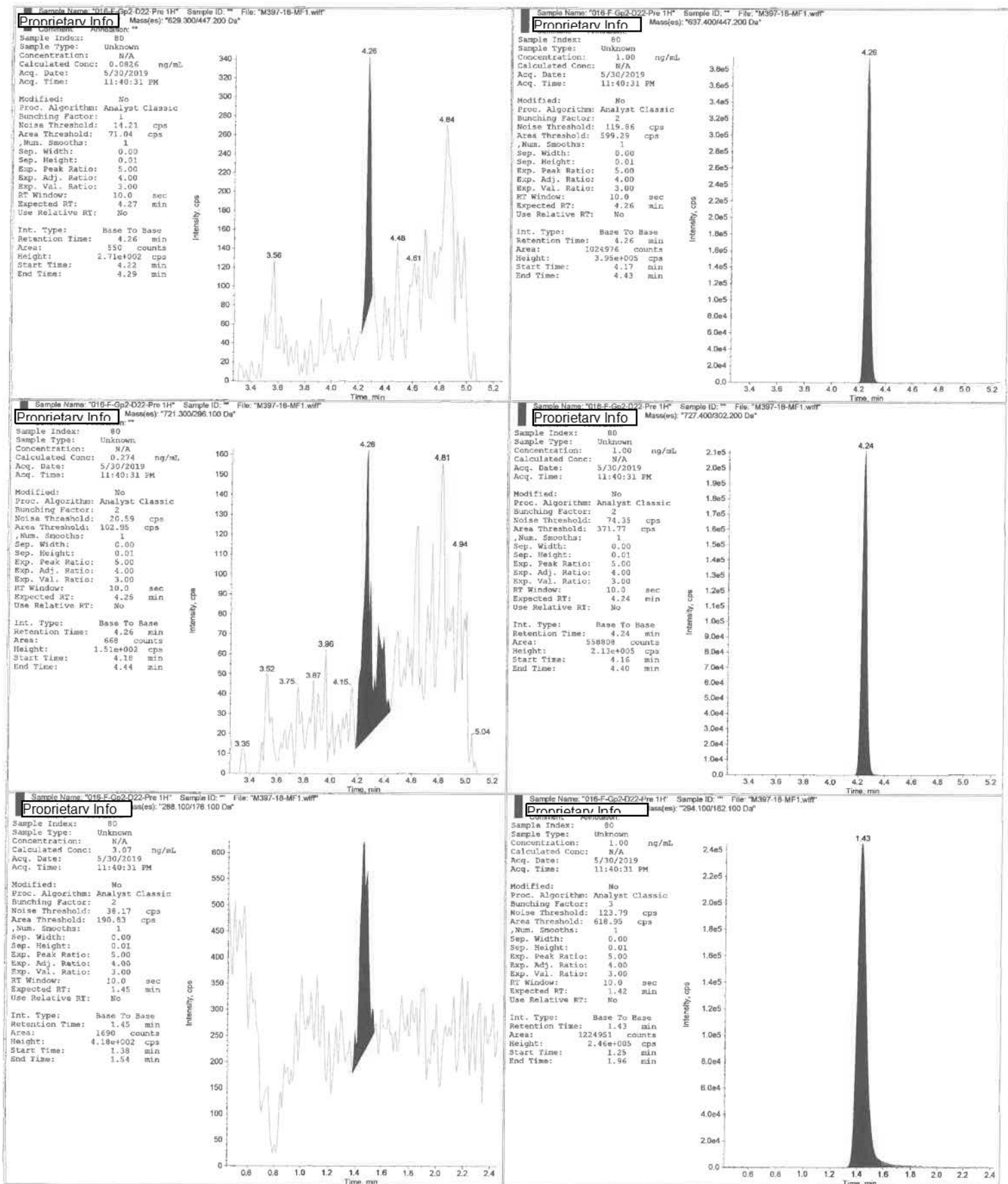




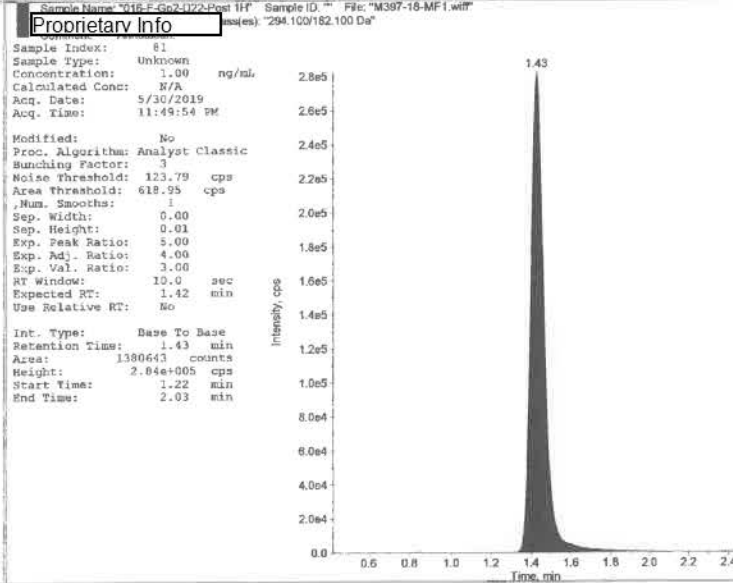
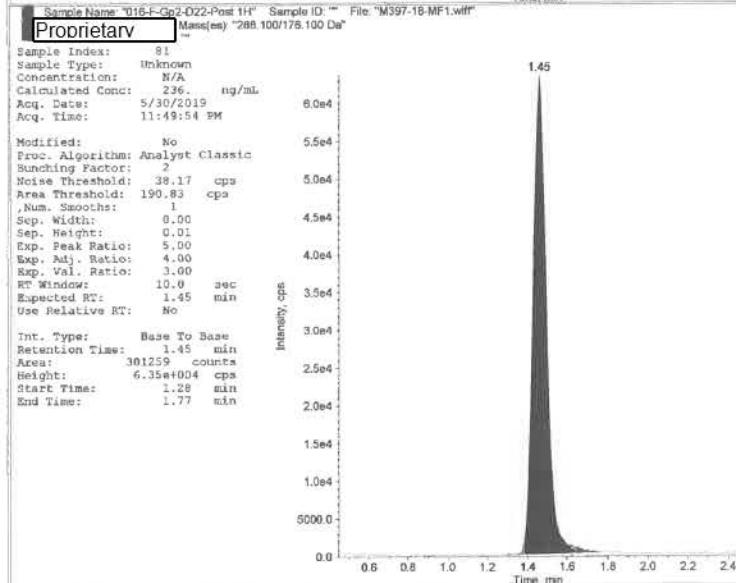
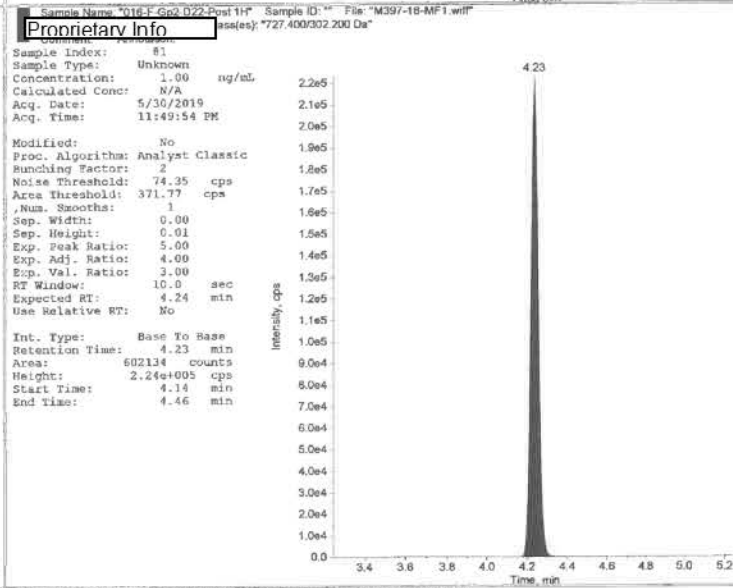
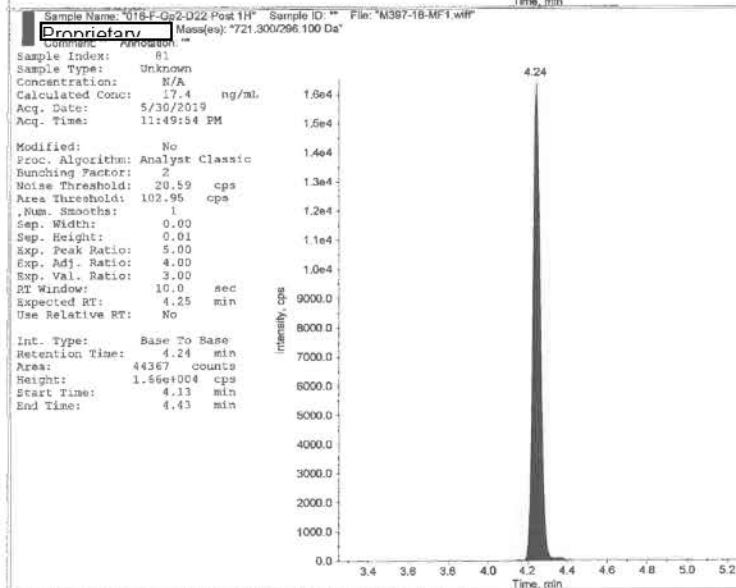
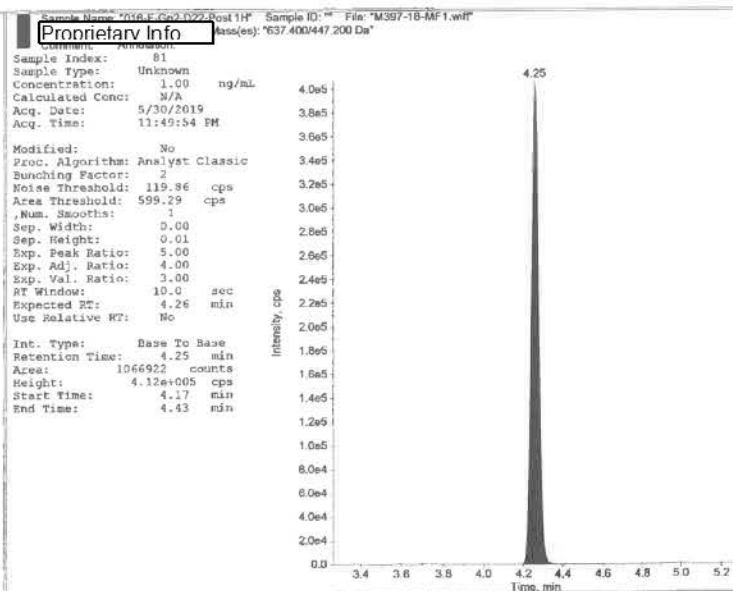
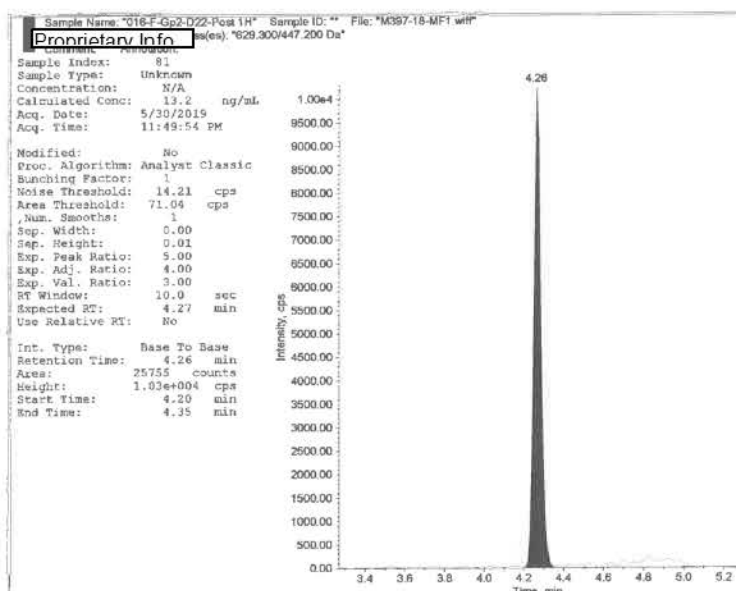


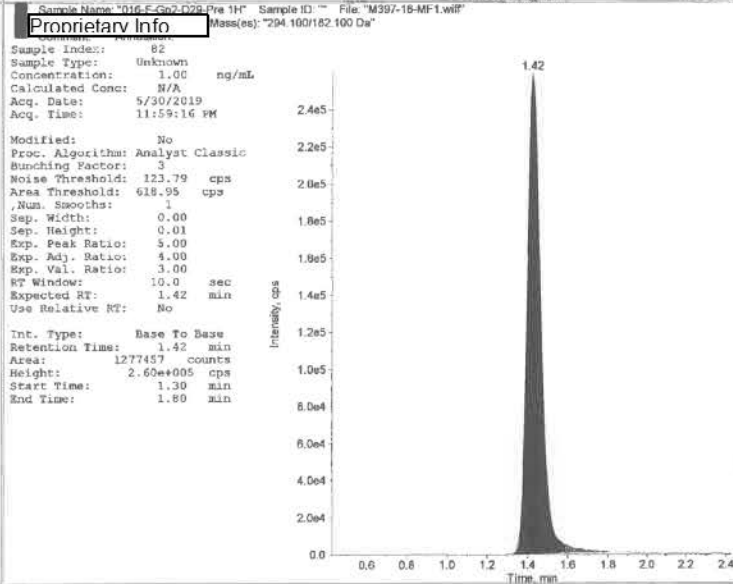
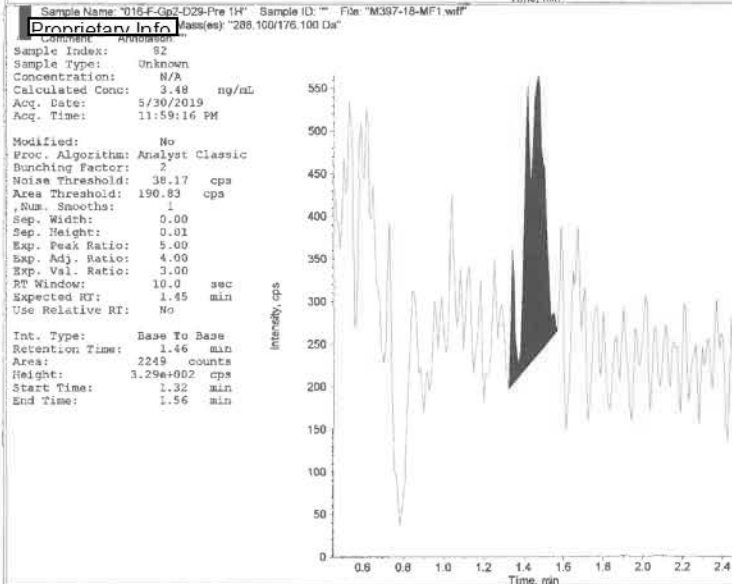
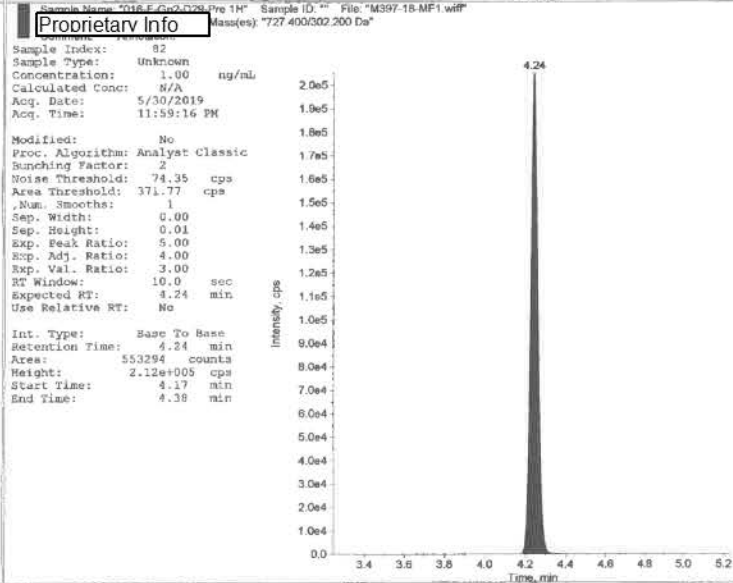
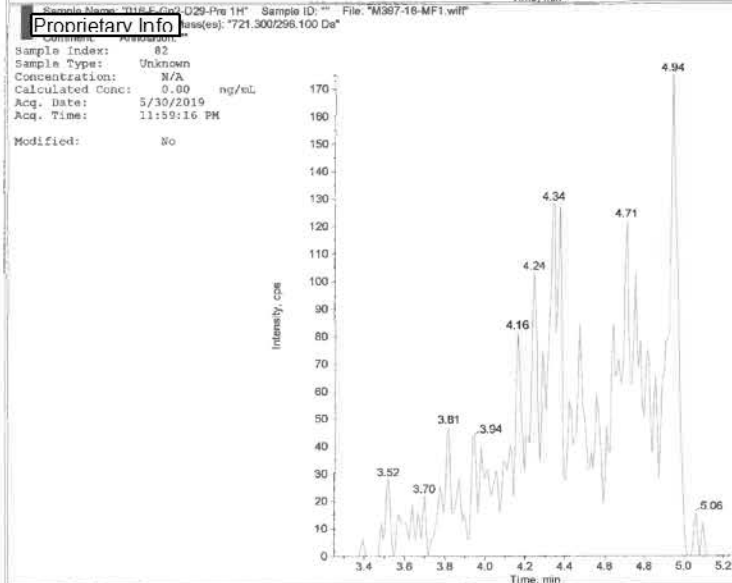
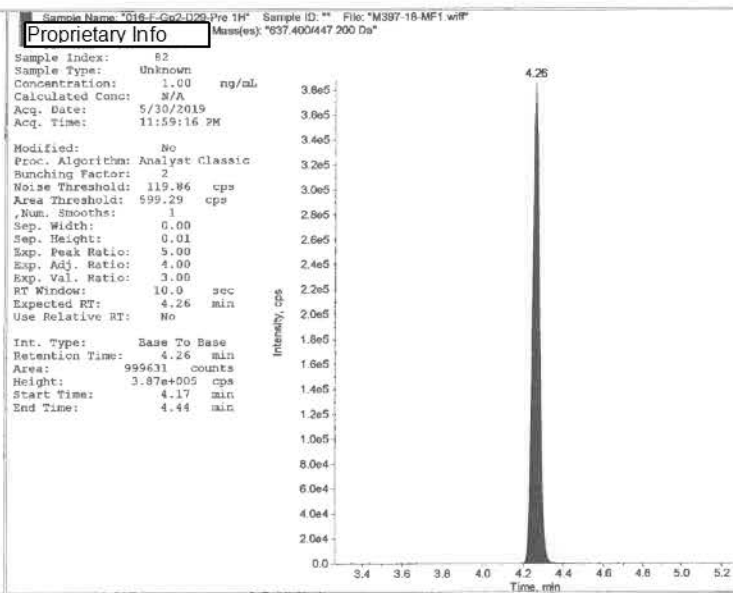
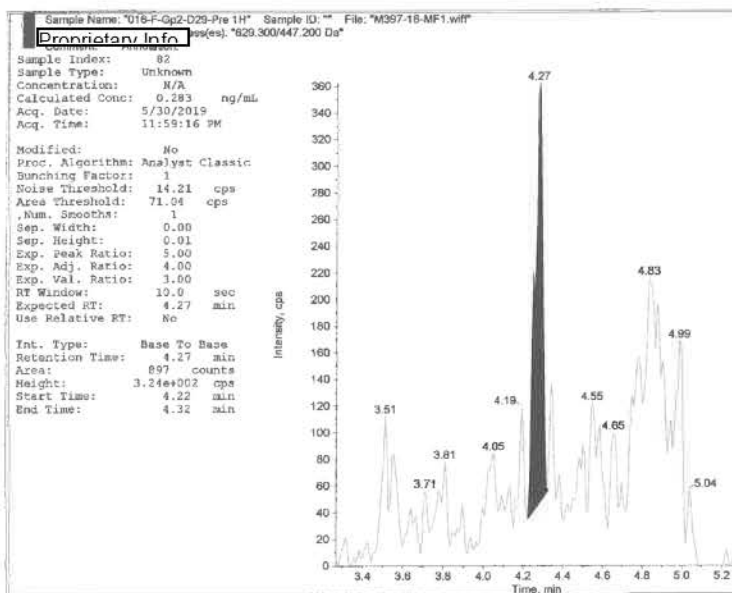








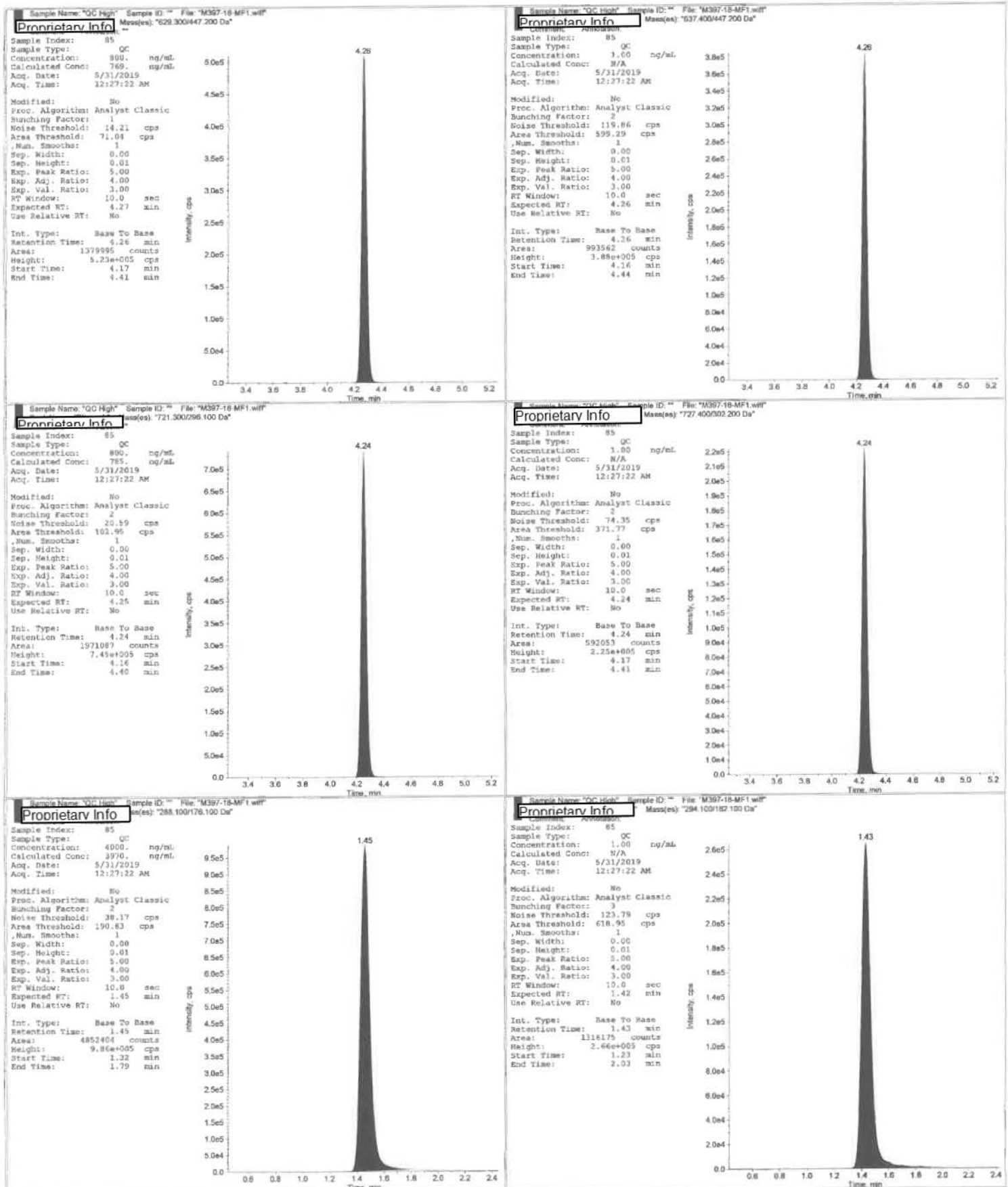




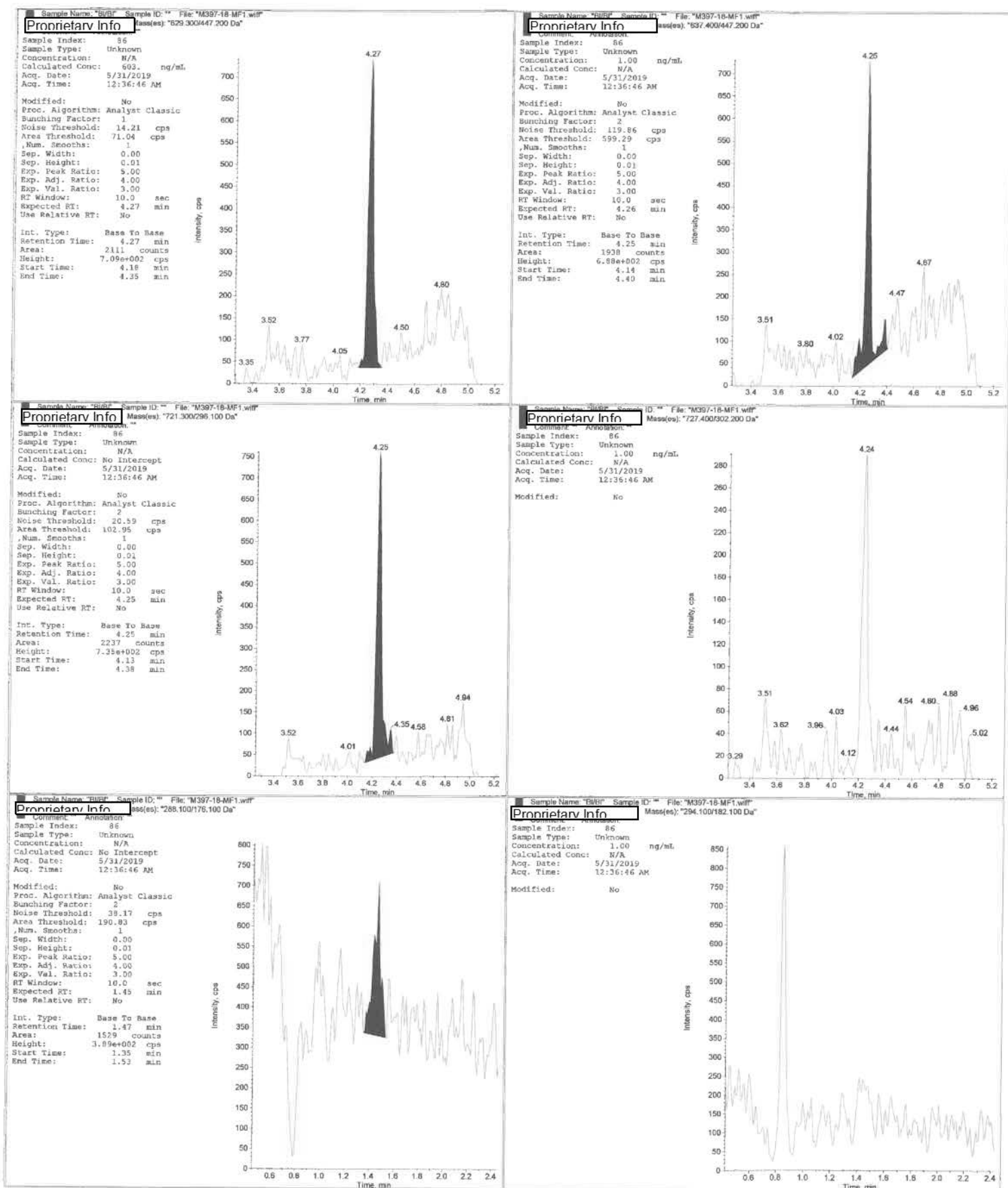


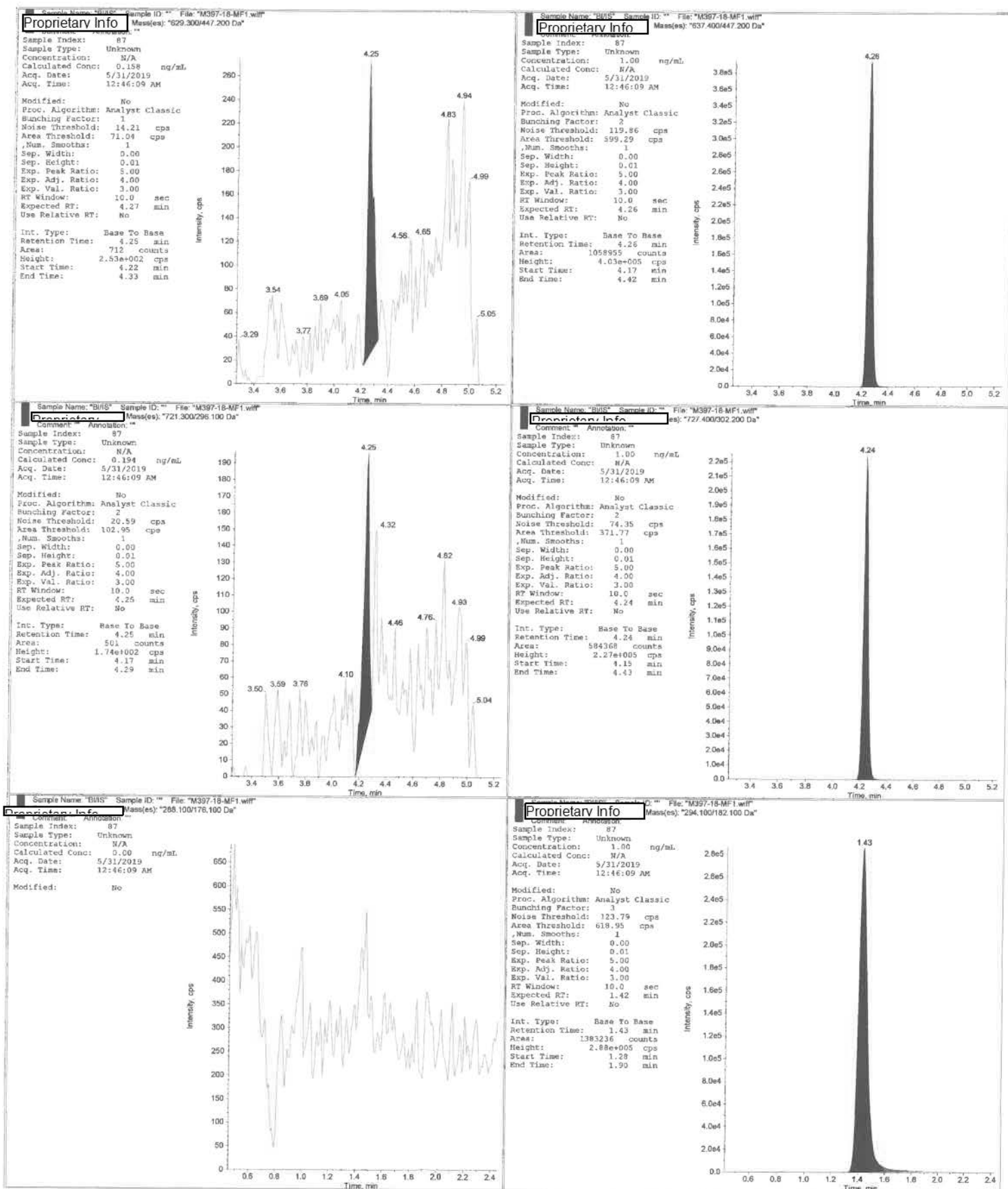






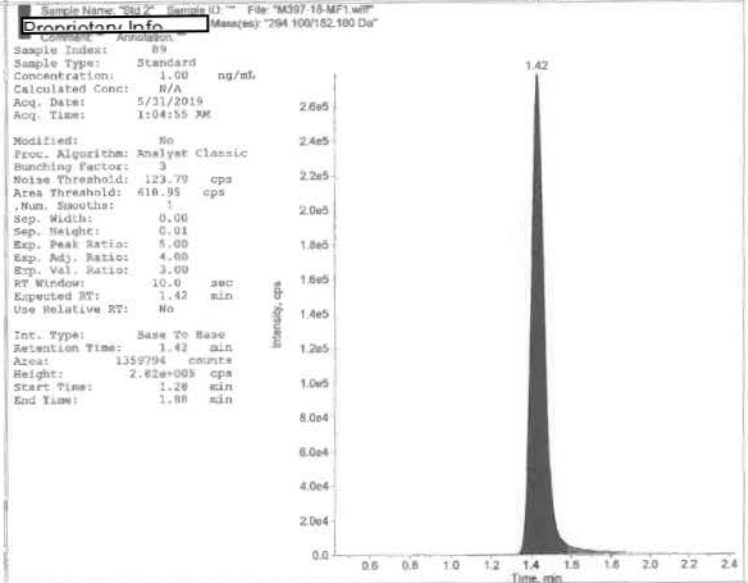
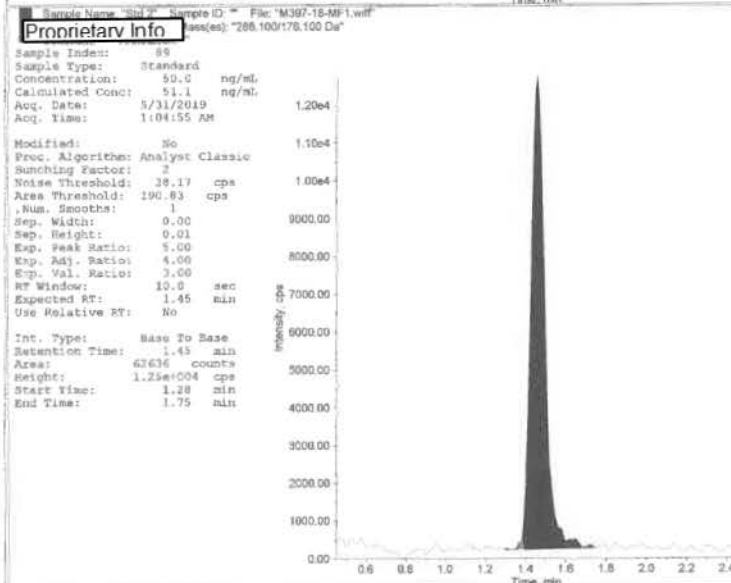
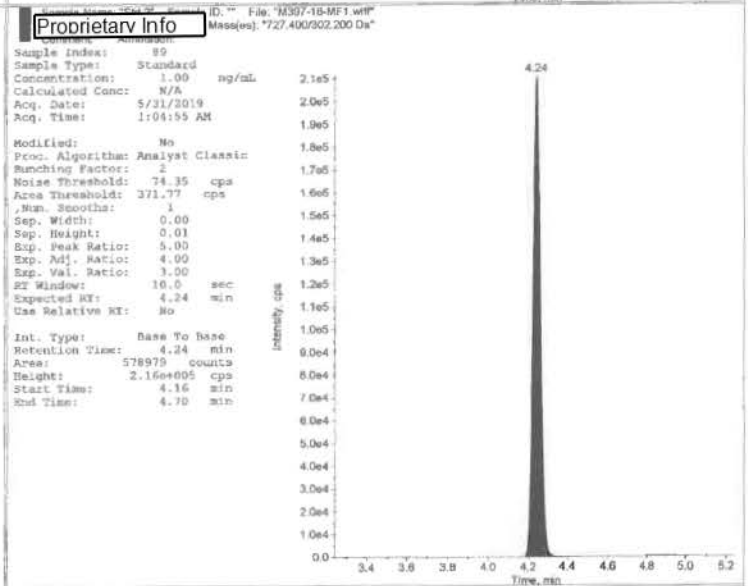
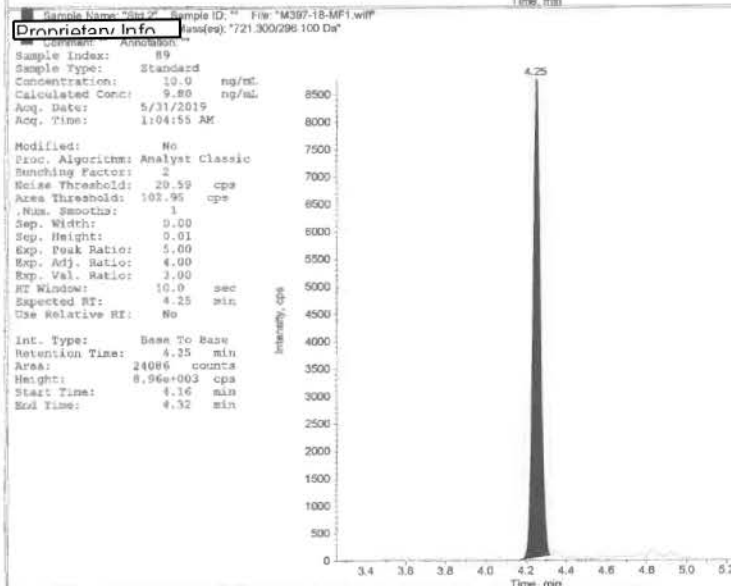
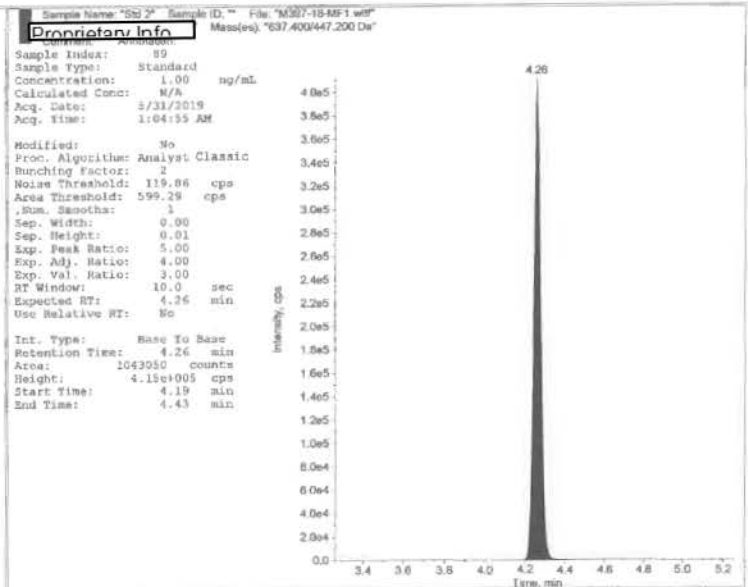
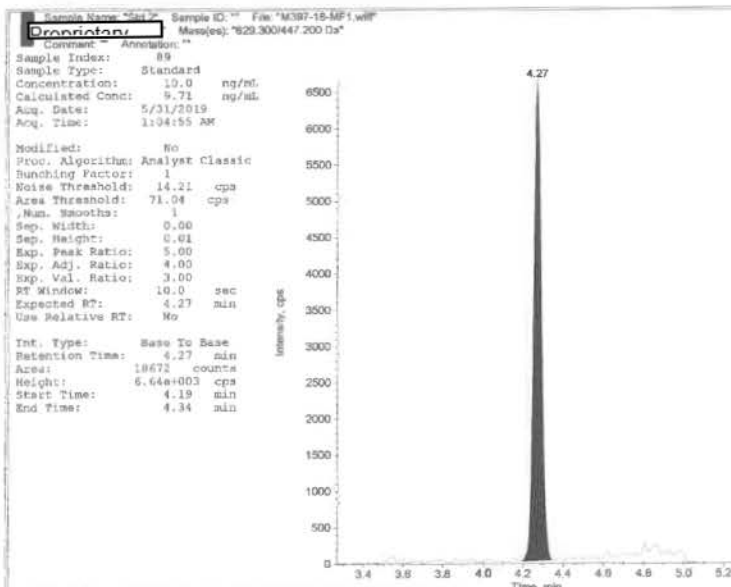


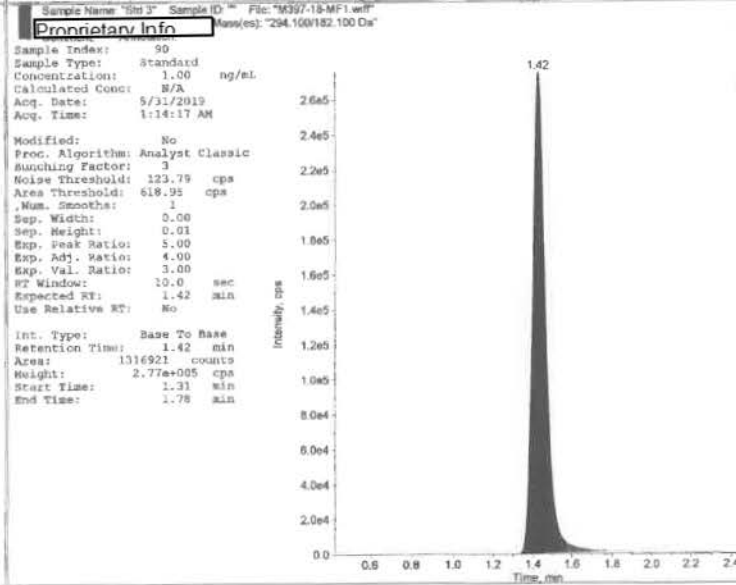
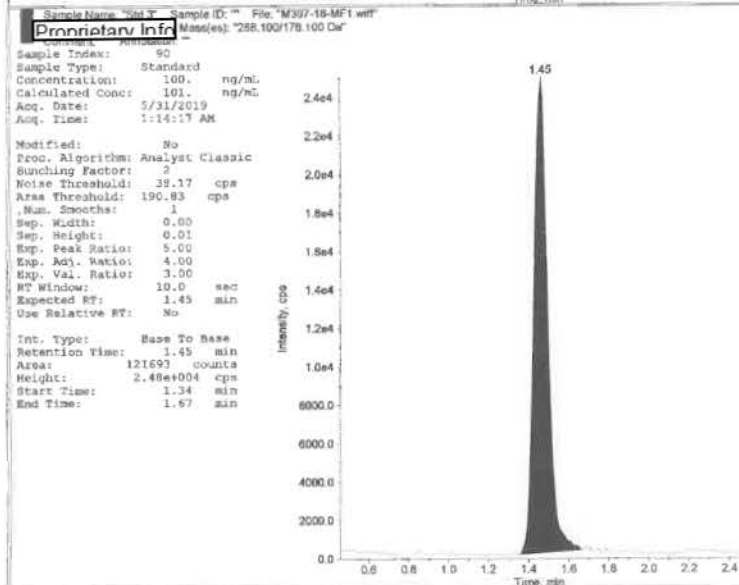
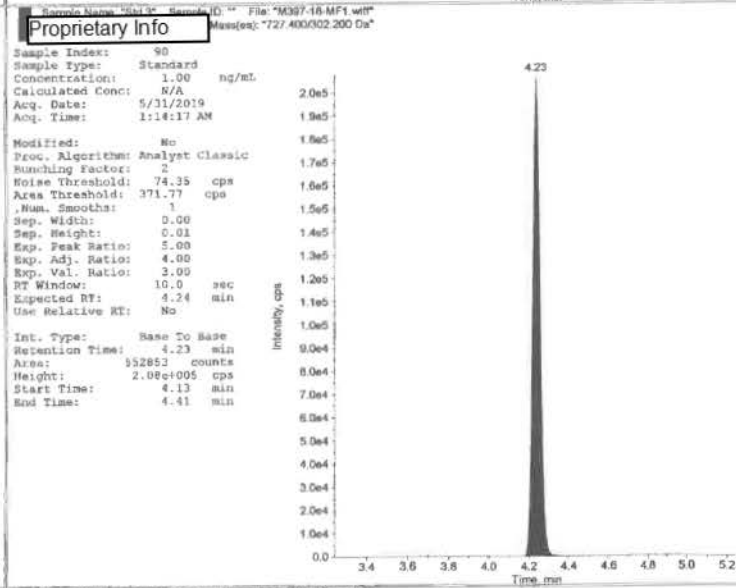
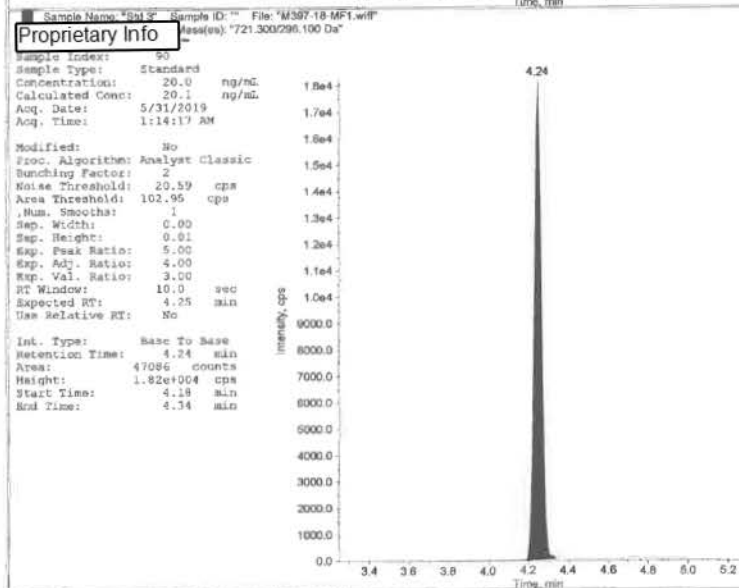
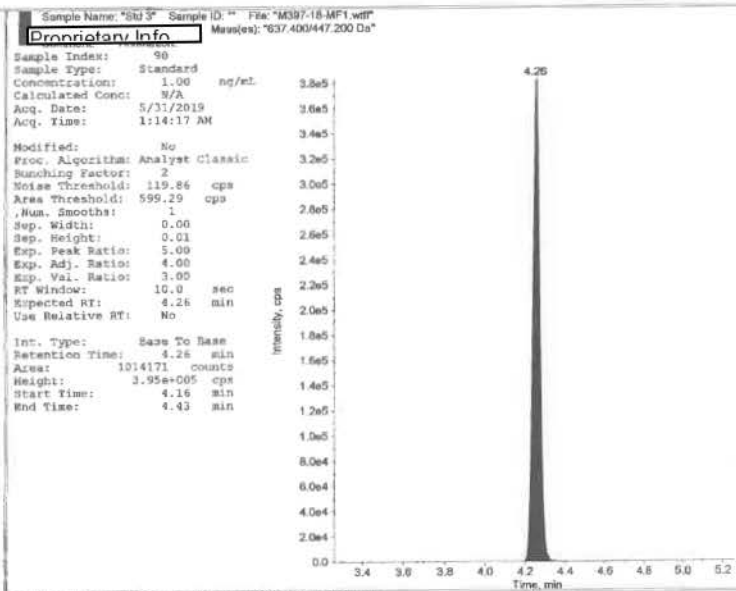
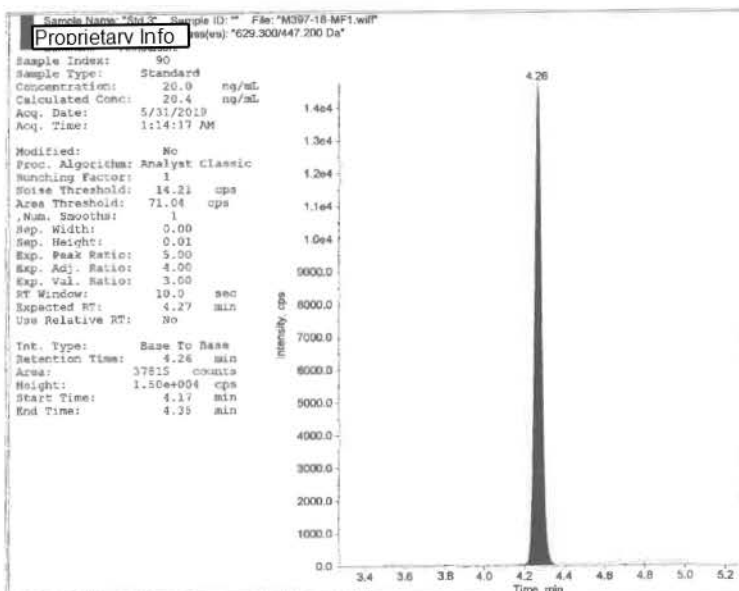




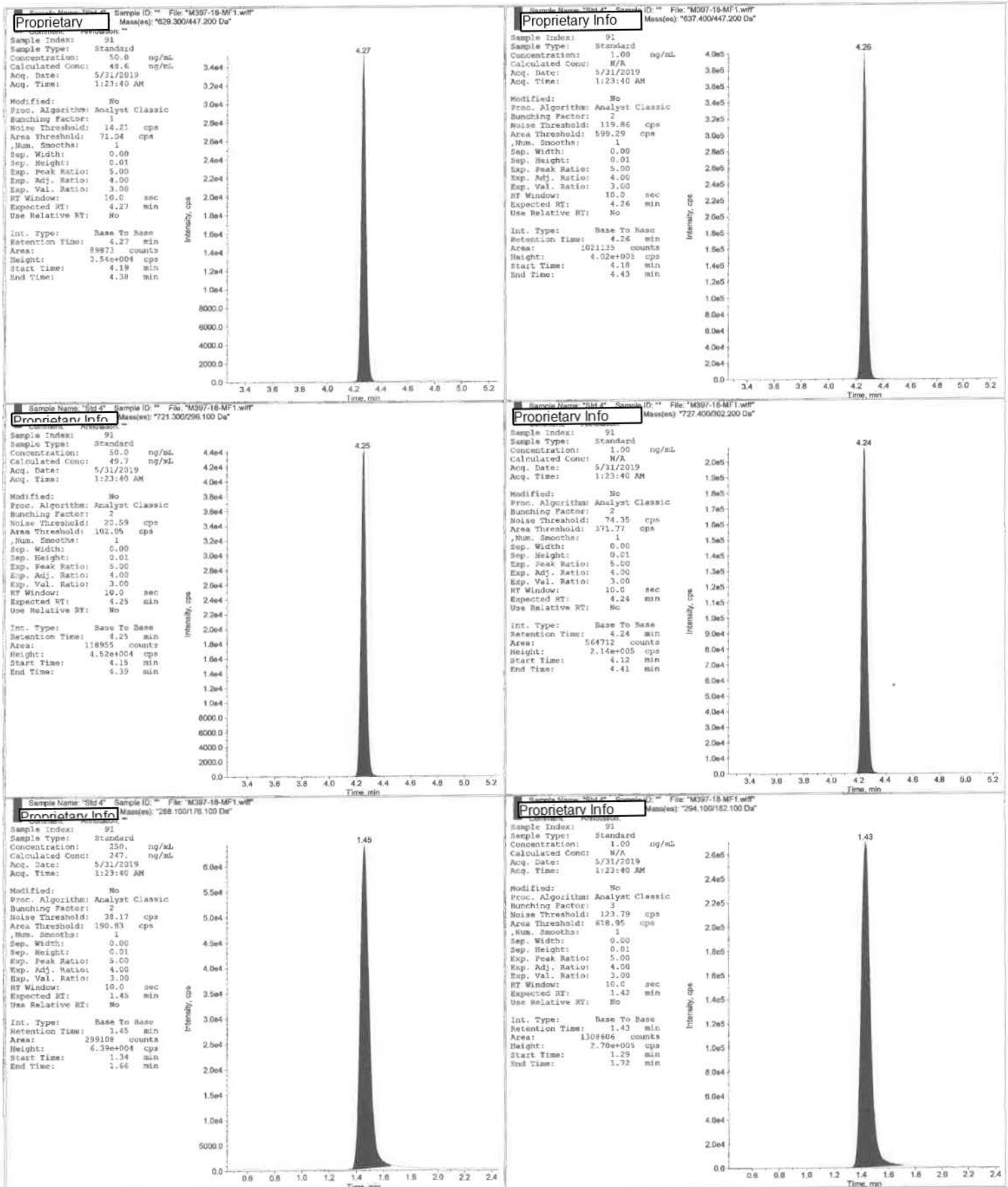


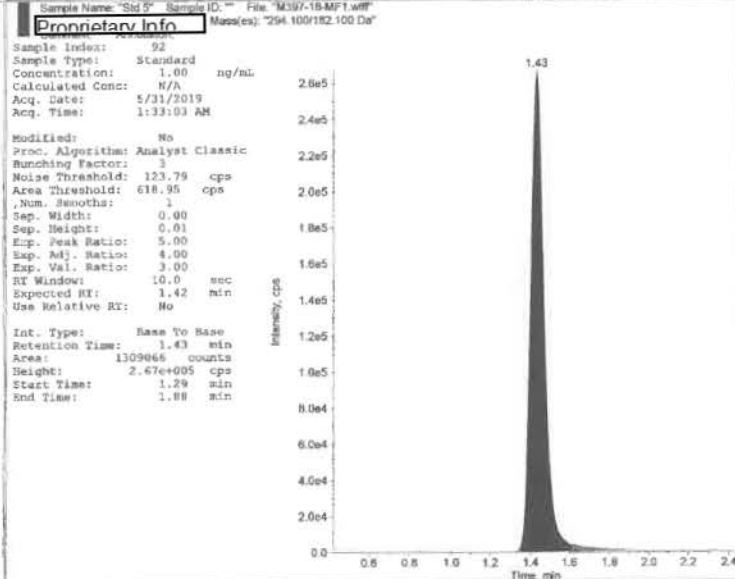
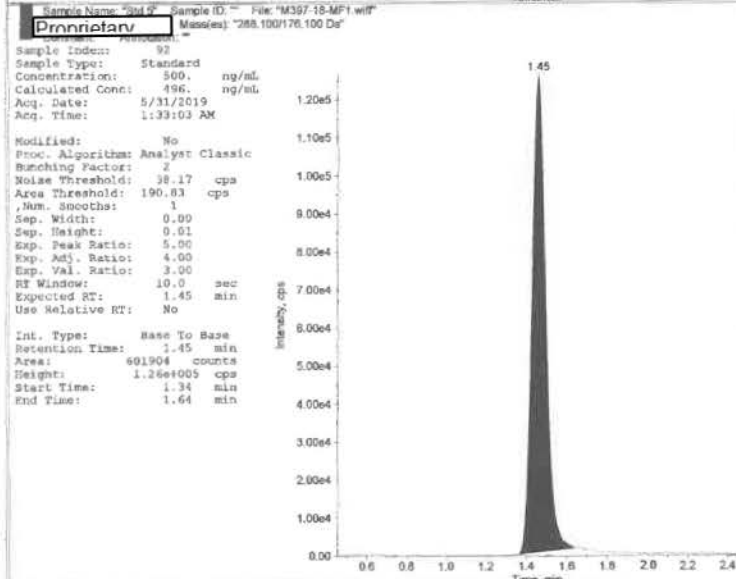
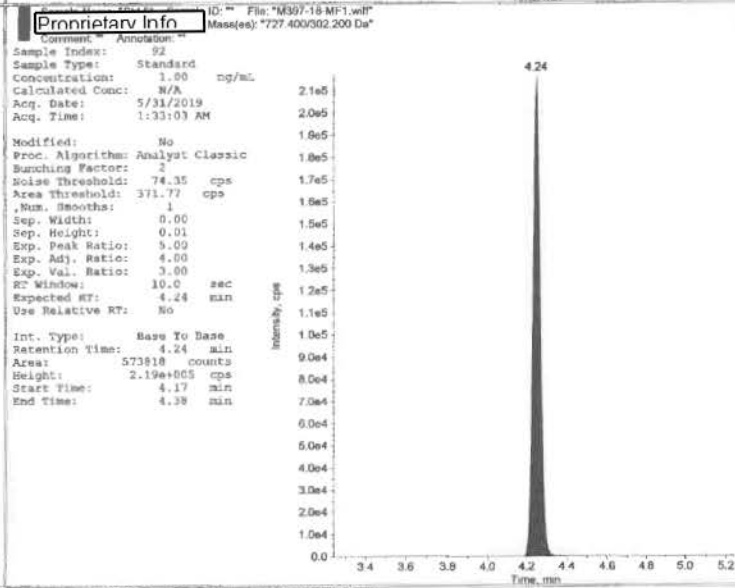
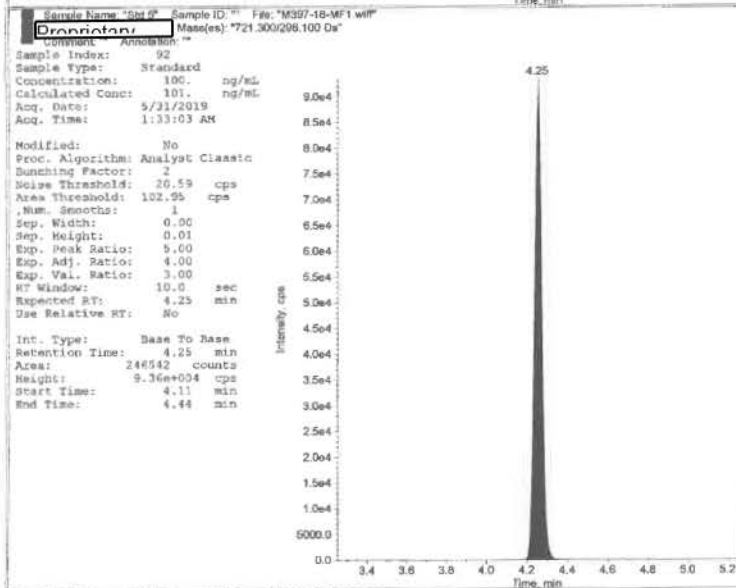
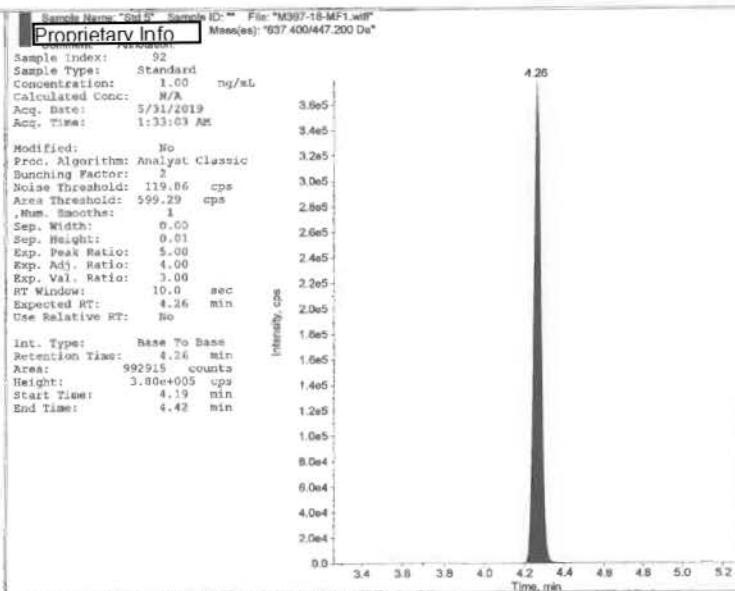
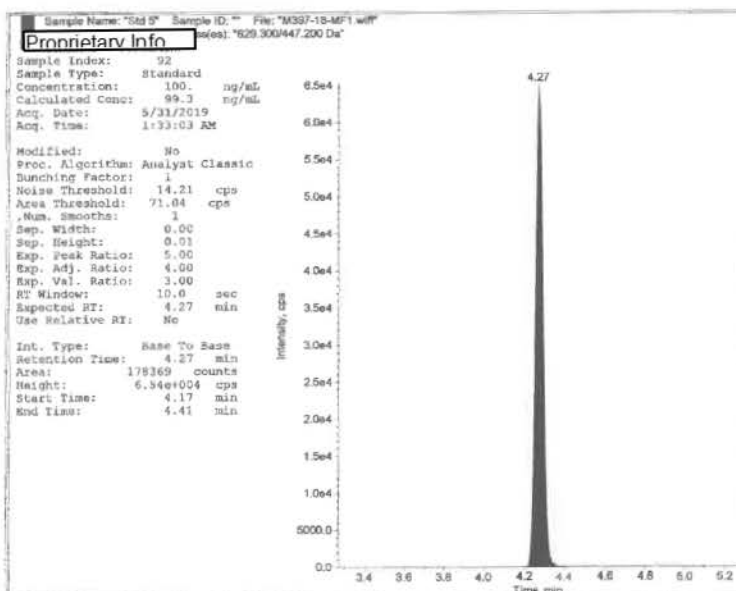




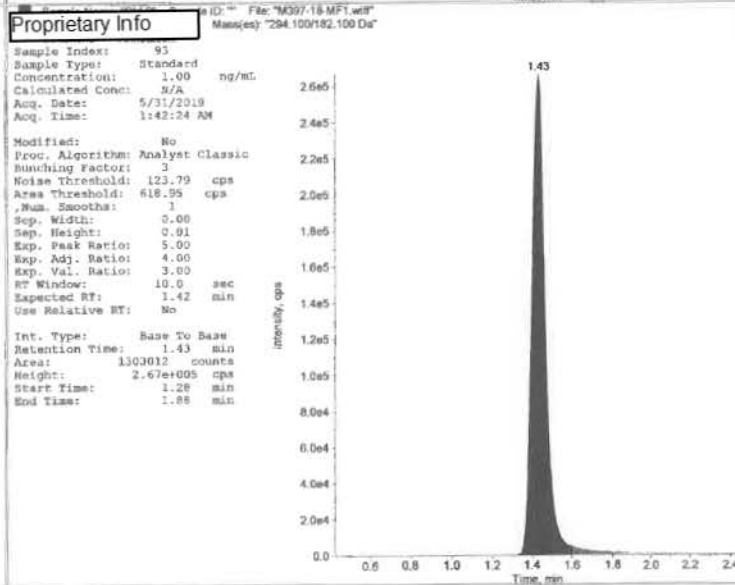
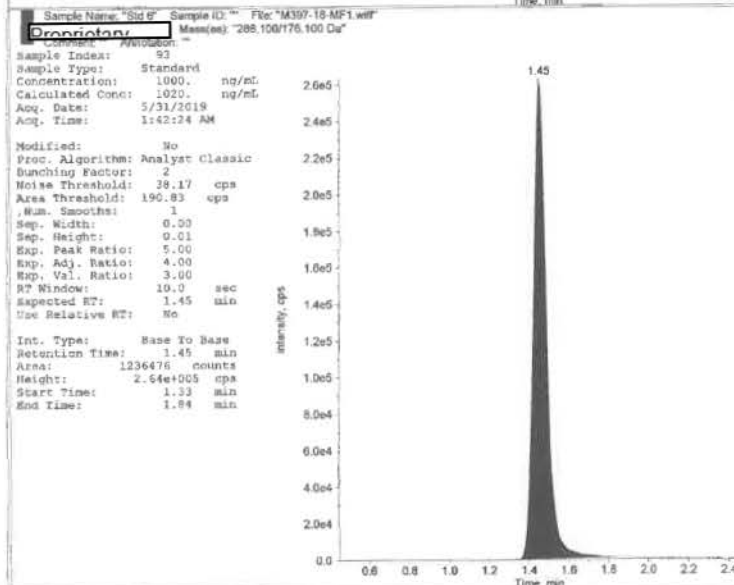
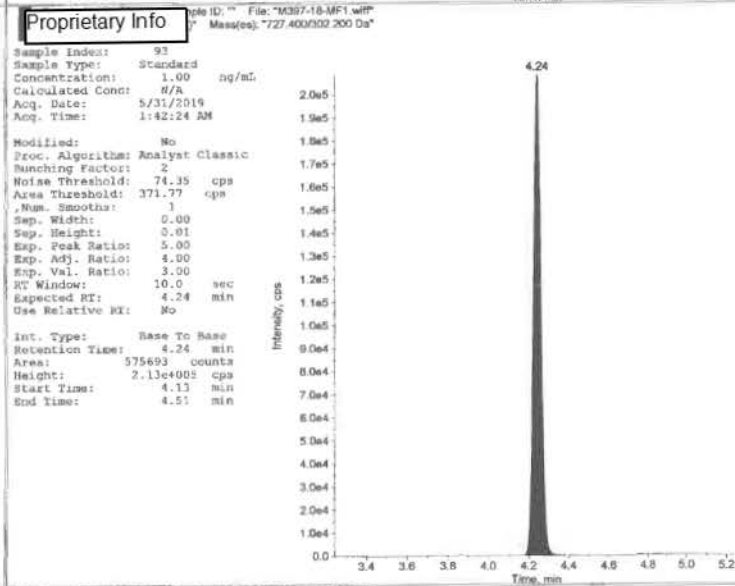
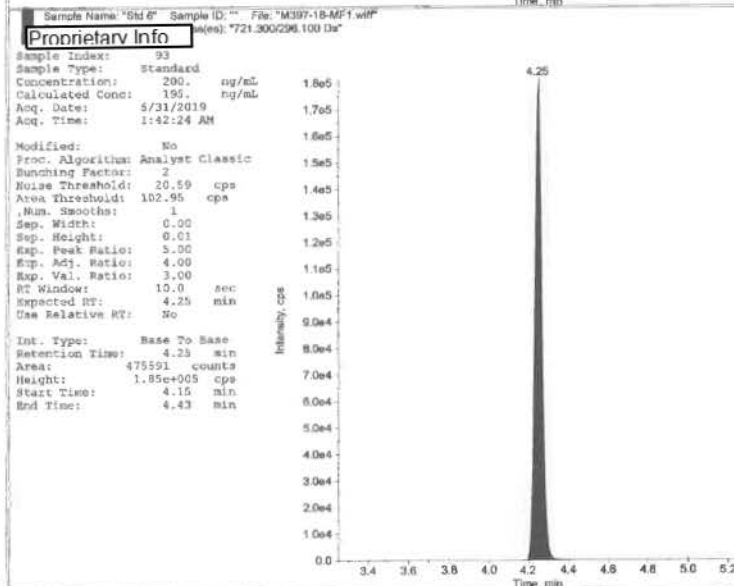
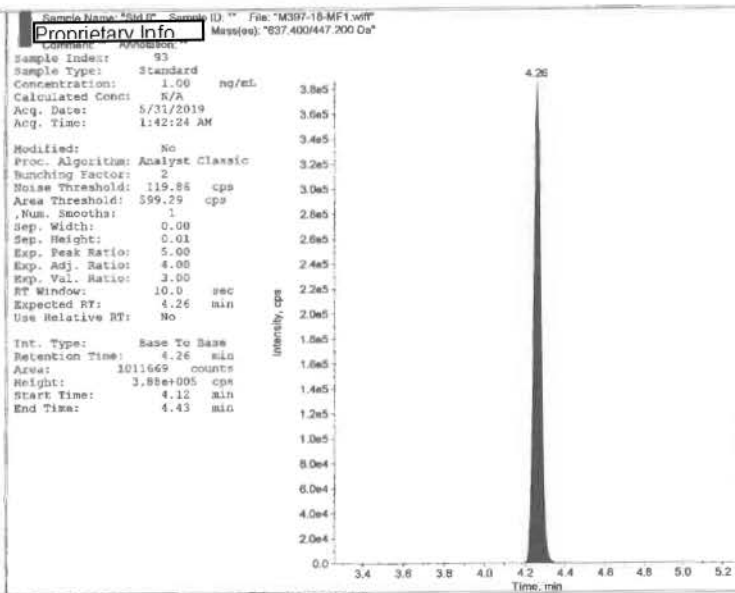
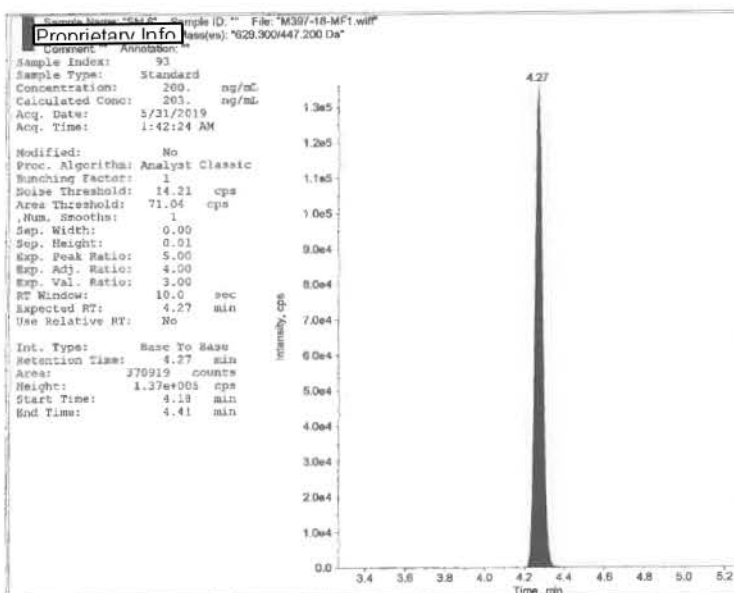


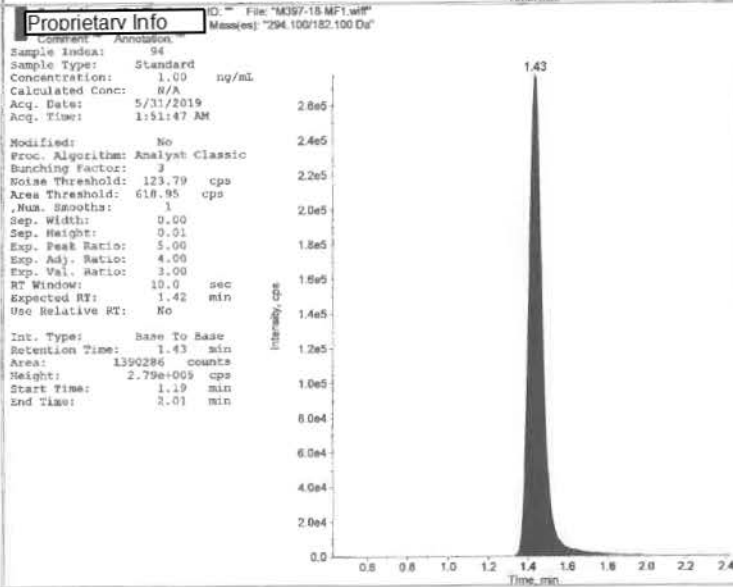
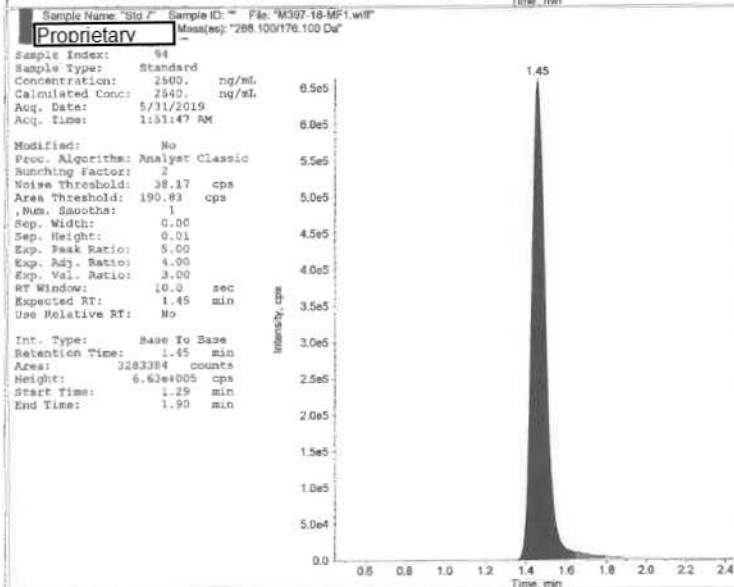
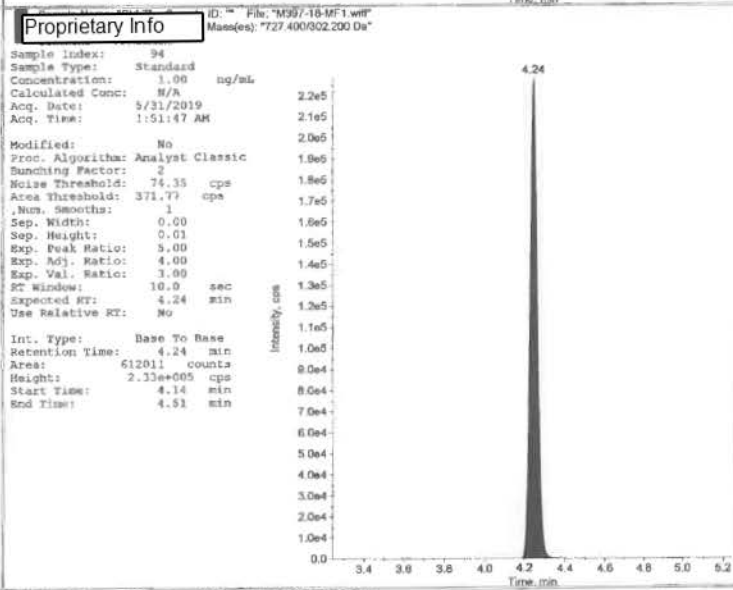
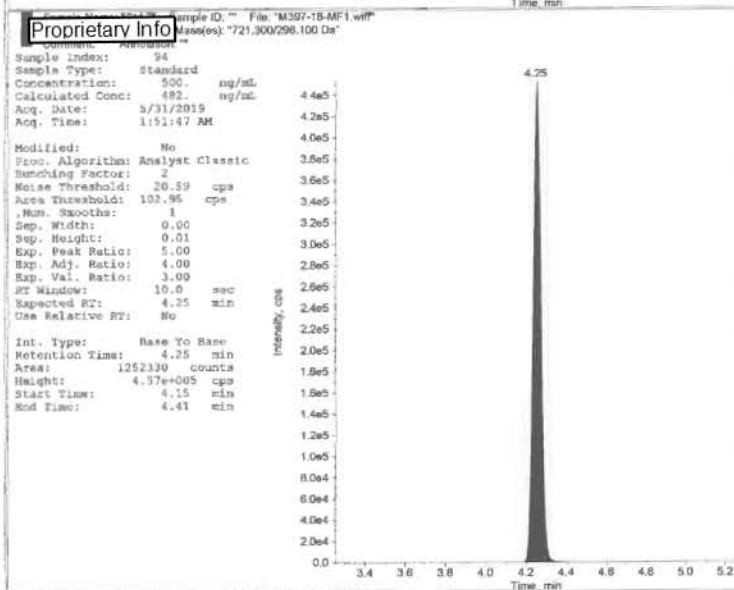
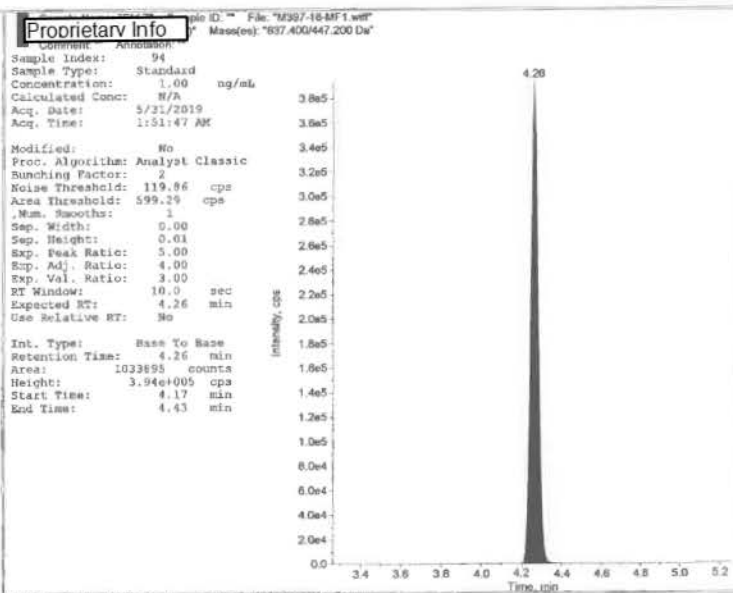
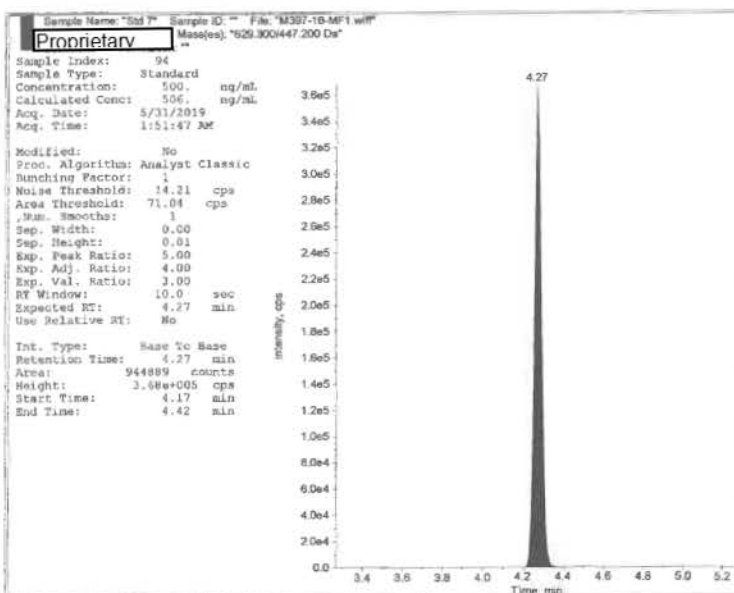






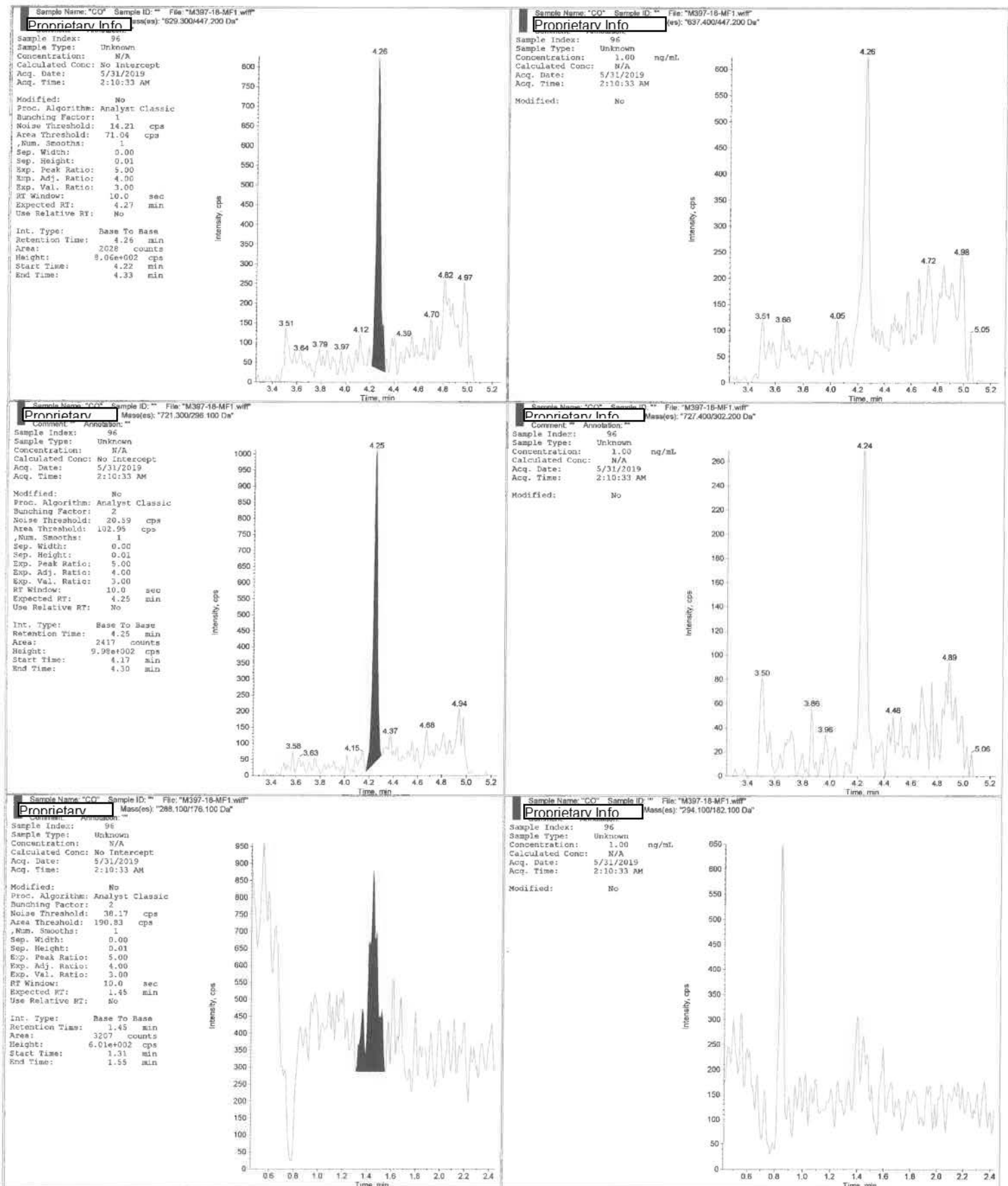


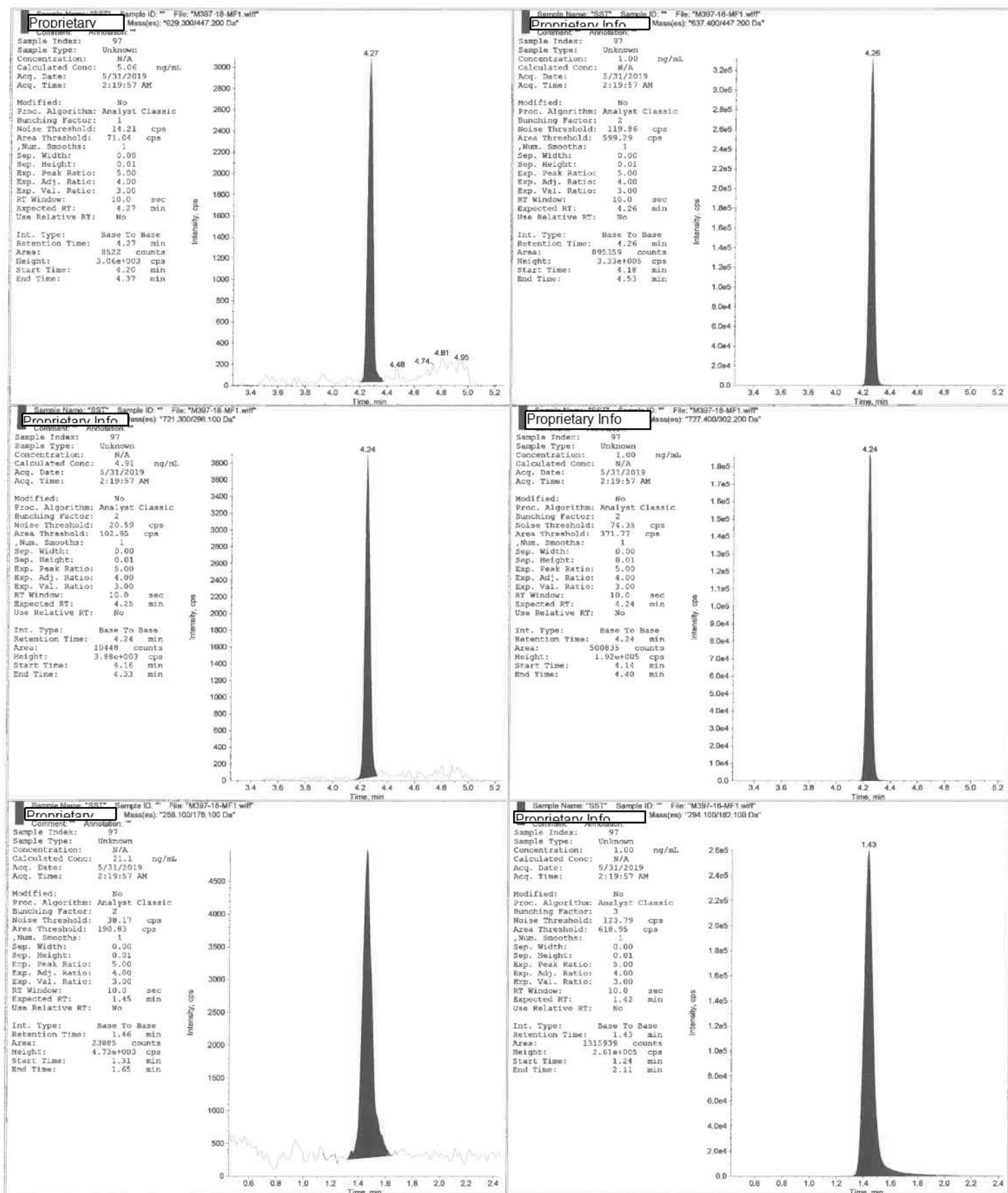












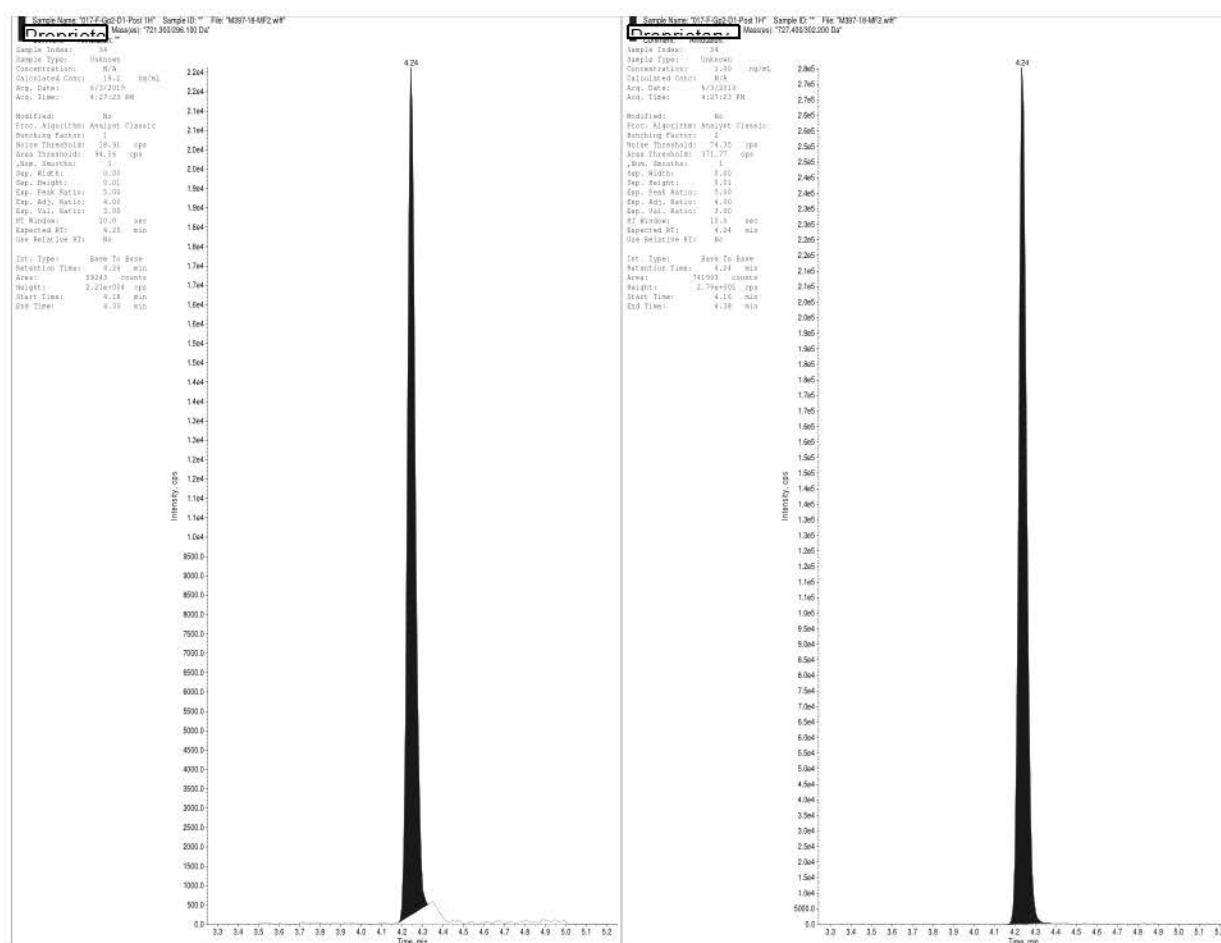


**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix G-7**

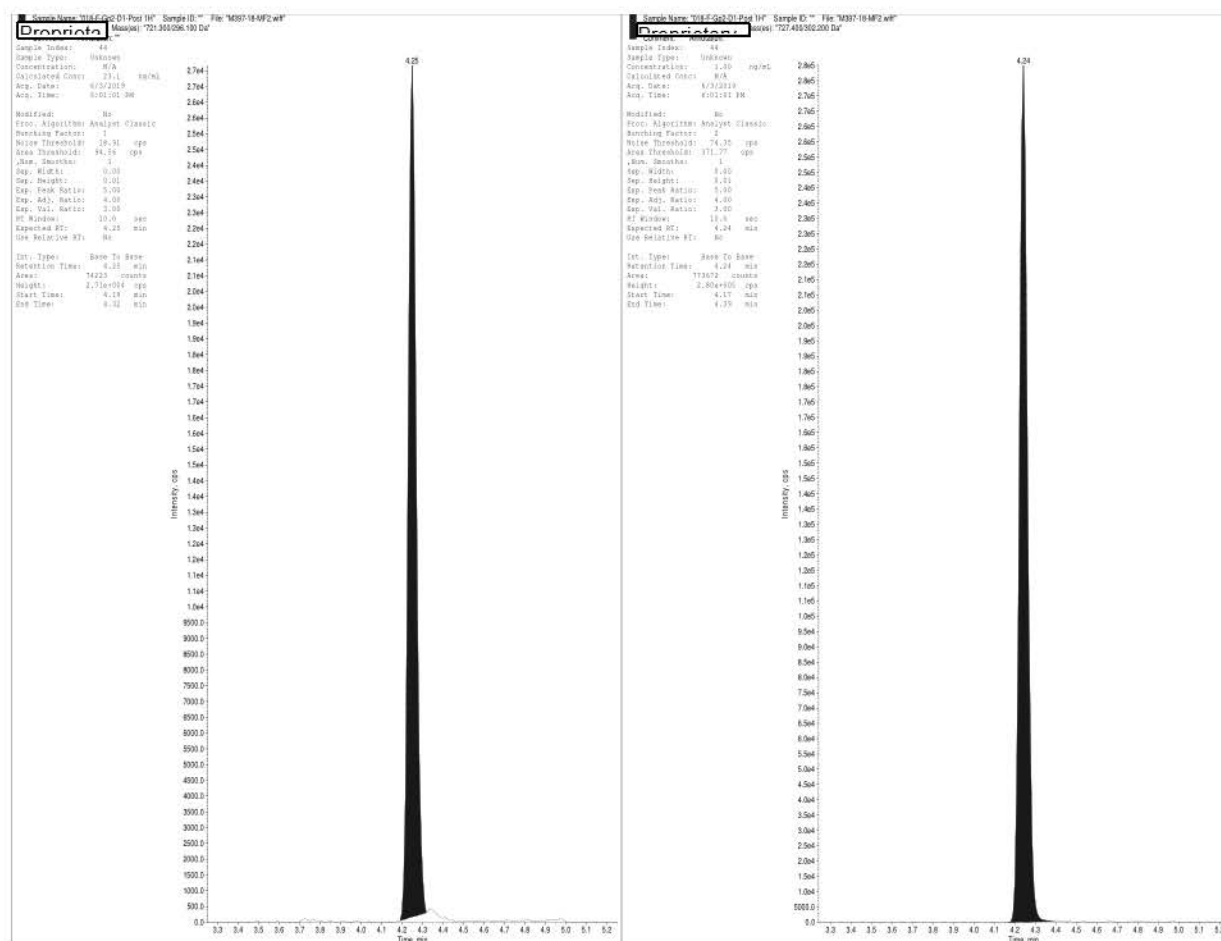
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# GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with [Proprietary Info] [Pro] in Male and Female Beagle Dogs SRI Study No. M397-18



Animal 017 Group 2 Day 1 1 hour postdose

# GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with [Proprietary Info] [Pro] in Male and Female Beagle Dogs SRI Study No. M397-18



Animal 018 Group 2 Day 1 1 hr postdose

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

**Appendix H**  
**TOXICOKINETICS**

Written by:

Redacted by agreement

Oct 25, 2019  
Date

Approved by:

10-25-19  
Date

Biosciences Division  
SRI International

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
**SRI Study No. M397-18**

## **I. METHODS**

Adult male and female Beagle dogs (5/sex; 3M/3F main animals and 2M/2F recovery animals) were administered weekly subcutaneous (sc) doses of [Proprietary] [Prop] a formulation of [Proprietary Info]. Individual test compound doses were as follows: [Proprietary] at 0.400 mg/kg (Group 2), 3.64 mg/kg (Group 3) and 14.55 mg/kg (Group 4); [Proprietary] at 0.230 mg/kg (Group 2), 2.09 mg/kg (Group 3) and 8.37 mg/kg (Group 4); and [Proprietary] at 0.110 mg/kg (Group 2), 1.0 mg/kg (Group 3) and 4.0 mg/kg (Group 4). [Proprietary] [Pro] was administered weekly for 5 weeks in Group 2 and for 3 weeks in Groups 3 and 4.

Blood samples for toxicokinetic (TK) analysis were collected from main animals at 1 hr predose and 1 hr postdose on dose administration days (Days 1, 8, 15, 22, and 29 in Group 2 and Days 1, 8, and 15 in Groups 3 and 4). Blood samples for TK analysis were collected from the recovery animals at 1 hr predose and 1 hr postdose on dose administration Day 29 and at a single time point on Days 36, 43, 50, 57, 64 and 71 in Group 2 and at a single time point on Days 22, 29, 36, 43, 50, 57, 64 and 71 in Groups 3 and 4.

TK data analysis was performed on the measurable plasma concentrations of each test compound from Appendix G (Bioanalytical Chemistry). The plasma concentration data of each test compound were analyzed using Phoenix<sup>®</sup> WinNonlin<sup>®</sup> (version 6.3) software to perform non-compartmental analysis. Individual animal plasma concentrations at each actual time of blood collection were used in TK data analysis. The dose administered in each dose group was entered into the program as mg/kg, and as a result no additional corrections for individual body weights of the animals were necessary.

Plasma concentrations that were less than the lower limit of quantitation (LLOQ of 5.00 ng/ml for [Proprietary Info] and 25.00 ng/ml for [Proprietary]) of the bioanalytical assay were not included in TK data analysis. TK parameters were determined only for the main animals and up to Day 15 (i.e. up to three doses), as all three dose groups had blood collections in the main animals up to Day 15. The following TK parameters were determined for each test compound in [Proprietary] [Pro] using the administration of the first dose on Day 1 as time zero: overall apparent maximal plasma concentration ( $C_{max}$ ) and area under the plasma concentration time curve up to the last blood collection time ( $AUC_{last}$ ). The time-course of mean plasma concentrations of each test compound was plotted for main and recovery animals at all time-points. Mean plasma concentrations that were less than the LLOQ of each compound were assigned a value of 2.00 ng/ml only to illustrate apparent troughs in the concentration vs. time profiles.

## **II. RESULTS**

### **A. Plasma Drug Levels**

The time-course of plasma concentrations of [Proprietary Info] from weekly [Proprietary] [Pro] after sc administration in main and recovery group animals is shown in Figures 1, 2, and 3, respectively (in the main study report). Measurable plasma concentrations were obtained for all three test compounds at the 1 hr postdose time



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points, with the highest concentrations noted for [Propri] (up to 11,000 ng/ml, Figure 2). Plasma concentrations declined to <LLOQ values for all three test compounds at almost all other time-points with a few exceptions. Measurable plasma concentrations were obtained for [Propri] (mean conc of 10.1 to 153 ng/ml) and [Propri] (210 to 331 ng/ml) in the recovery animals on Days 22 and 29 (Figures 1 and 2).

## **B. Toxicokinetic Parameters**

The individual and mean overall apparent  $C_{max}$  and  $AUC_{last}$  for [Propri] [Propri] and [Propri] are presented in Tables H-1 to H-3, respectively, in this Appendix. Mean  $C_{max}$  was 19.9, 197 and 704 ng/ml (males) and 20.0, 215, and 1,328 ng/ml (females) for [Propri] 237, 2,113 and 9,230 ng/ml (males) and 290, 2,713, and 11,000 ng/ml (females) for [Propri] and 23.3, 199 and 469 ng/ml (males) and 23.0, 266 and 1,663 ng/ml (females) for [Propri] in Groups 2, 3, and 4, respectively. Corresponding mean  $AUC_{last}$  was 236, 777, and 2,324 day\*ng/ml (males) and 240, 944 and 4,938 day\*ng/ml (females) for [Propri] 3,071, 26,949, and 75,047 day\*ng/ml (males) and 3,914, 31,017, and 87,408 day\*ng/ml (females) for [Propri] and 280, 2,427 and 4,989 day\*ng/ml (males) and 300, 2,747, and 11,956 day\*ng/ml (females) for [Propri] in Groups 2, 3, and 4, respectively.

Overall, the rank order of mean  $AUC_{last}$  between the three compounds was [Propri] >>> [Propri] > [Propri] at the dose ranges administered. Plasma exposure of each test compound increased disproportionately with dose increments from Group 2 to Groups 3 and 4. The mean  $AUC_{last}$  of [Propri] in Group 4 was ~10-fold (males) and ~21-fold (females) greater than the  $AUC_{last}$  in Group 2, for a ~36-fold increment from 0.400 to 14.55 mg/kg (Table H-1). Similar disproportionate increments were noted in the mean  $AUC_{last}$  from Group 2 to Group 4 for [Propri] (~24-fold in males and ~22-fold in females, Table H-2) and [Propri] (~18-fold in males and ~40-fold in females, Table H-3). No major sex differences were noted in the plasma exposure of all three test compounds, except for ~2-fold greater  $AUC_{last}$  in females in Group 4 when compared with males for [Propri] (Table H-1) and [Propri] (Table H-3).

## **III. CONCLUSION**

Toxicokinetic data analysis was performed on the measurable plasma concentrations of [Propri] [Propri] and [Propri] from weekly sc administration of [Proprietary] [Propri]. Plasma exposure parameters (overall  $C_{max}$  and  $AUC_{last}$ ) indicated a much greater exposure for [Propri] when compared with [Propri] and [Propri] in the dose ranges studied. There was a disproportionate increase in plasma exposure of all three test compounds with dose increments from Group 2 to Group 4. No major sex differences were noted in the plasma exposure, except for a 2-fold greater exposure in females than males at the highest dose level of [Propri] and [Propri].

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
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**Table H-1**

**Individual and Mean Toxicokinetic Parameters of [Proprietary] after weekly SC Administration in Male and Female Beagle Dogs**

Compound	Sex	Group	Dose (mg/kg)	Animal #	<sup>a</sup> C <sub>max</sub> (ng/ml)	<sup>a</sup> AUC <sub>last</sub> (day*ng/ml)
[Proprietary]	M	2	0.400	11	13.4	143
[Proprietary]	M	2	0.400	12	19.5	237
[Proprietary]	M	2	0.400	13	26.9	327
				<b>Mean</b>	<b>19.9</b>	<b>236</b>
				<b>SD</b>	<b>6.76</b>	<b>92.0</b>
[Proprietary]	M	3	3.64	21	197	584
[Proprietary]	M	3	3.64	22	256	1,089
[Proprietary]	M	3	3.64	23	138	659
				<b>Mean</b>	<b>197</b>	<b>777</b>
				<b>SD</b>	<b>59.0</b>	<b>273</b>
[Proprietary]	M	4	14.55	31	685	2,378
[Proprietary]	M	4	14.55	32	522	1,342
[Proprietary]	M	4	14.55	33	905	3,252
				<b>Mean</b>	<b>704</b>	<b>2,324</b>
				<b>SD</b>	<b>192</b>	<b>956</b>
[Proprietary]	F	2	0.400	16	20.2	253
[Proprietary]	F	2	0.400	17	18.9	234
[Proprietary]	F	2	0.400	18	20.9	233
				<b>Mean</b>	<b>20.0</b>	<b>240</b>
				<b>SD</b>	<b>1.01</b>	<b>11.3</b>
[Proprietary]	F	3	3.64	26	126	823
[Proprietary]	F	3	3.64	27	182	931
[Proprietary]	F	3	3.64	28	338	1,079
				<b>Mean</b>	<b>215</b>	<b>944</b>
				<b>SD</b>	<b>110</b>	<b>129</b>
[Proprietary]	F	4	14.55	36	1,240	4,874
[Proprietary]	F	4	14.55	37	1,830	5,553
[Proprietary]	F	4	14.55	38	913	4,387
				<b>Mean</b>	<b>1,328</b>	<b>4,938</b>
				<b>SD</b>	<b>465</b>	<b>586</b>

<sup>a</sup> TK parameters calculated from plasma concentration data from main group animals only and up to study Day 15 (i.e. up to administration of first 3 doses)

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study**  
**with [Proprietary Info] [Pro] in Male and Female Beagle Dogs**  
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**Table H-2**

**Individual and Mean Toxicokinetic Parameters of [Proprietary] after Weekly SC Administration in Male and Female Beagle Dogs**

<b>Compound</b>	<b>Sex</b>	<b>Group</b>	<b>Dose (mg/kg)</b>	<b>Animal #</b>	<b><sup>a</sup>C<sub>max</sub> (ng/ml)</b>	<b><sup>a</sup>AUC<sub>last</sub> (day*ng/ml)</b>
[Proprietary]	M	2	0.230	11	245	3,092
[Proprietary]	M	2	0.230	12	250	3,308
[Proprietary]	M	2	0.230	13	215	2,813
				<b>Mean</b>	<b>237</b>	<b>3,071</b>
				<b>SD</b>	<b>18.9</b>	<b>248</b>
[Proprietary]	M	3	2.09	21	1,970	26,731
[Proprietary]	M	3	2.09	22	2,240	30,802
[Proprietary]	M	3	2.09	23	2,130	23,313
				<b>Mean</b>	<b>2,113</b>	<b>26,949</b>
				<b>SD</b>	<b>136</b>	<b>3,749</b>
[Proprietary]	M	4	8.37	31	8,760	71,254
[Proprietary]	M	4	8.37	32	9,290	76,287
[Proprietary]	M	4	8.37	33	9,640	77,599
				<b>Mean</b>	<b>9,230</b>	<b>75,047</b>
				<b>SD</b>	<b>443</b>	<b>3,349</b>
[Proprietary]	F	2	0.230	16	279	3,744
[Proprietary]	F	2	0.230	17	291	3,894
[Proprietary]	F	2	0.230	18	300	4,105
				<b>Mean</b>	<b>290</b>	<b>3,914</b>
				<b>SD</b>	<b>10.5</b>	<b>181</b>
[Proprietary]	F	3	2.09	26	2,770	26,862
[Proprietary]	F	3	2.09	27	2,680	34,967
[Proprietary]	F	3	2.09	28	2,690	31,223
				<b>Mean</b>	<b>2,713</b>	<b>31,017</b>
				<b>SD</b>	<b>49.3</b>	<b>4,056</b>
[Proprietary]	F	4	8.37	36	11,200	84,588
[Proprietary]	F	4	8.37	37	11,400	91,514
[Proprietary]	F	4	8.37	38	10,400	86,121
				<b>Mean</b>	<b>11,000</b>	<b>87,408</b>
				<b>SD</b>	<b>529</b>	<b>3,638</b>

<sup>a</sup> TK parameters calculated from plasma concentration data from main group animals only and up to study Day 15 (i.e. up to administration of first 3 doses)



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**SRI Study No. M397-18**

**Table H-3**

**Individual and Mean Toxicokinetic Parameters of [Proprietary] after Weekly SC Administration in Male and Female Beagle Dogs**

<b>Compound</b>	<b>Sex</b>	<b>Group</b>	<b>Dose (mg/kg)</b>	<b>Animal #</b>	<b><sup>a</sup>C<sub>max</sub> (ng/ml)</b>	<b><sup>a</sup>AUC<sub>last</sub> (day*ng/ml)</b>
[Proprietary]	M	2	0.110	11	18.7	206
[Proprietary]	M	2	0.110	12	25.8	334
[Proprietary]	M	2	0.110	13	25.3	299
				<b>Mean</b>	<b>23.3</b>	<b>280</b>
				<b>SD</b>	<b>3.96</b>	<b>66.2</b>
[Proprietary]	M	3	1.0	21	185	2,166
[Proprietary]	M	3	1.0	22	230	2,779
[Proprietary]	M	3	1.0	23	182	2,337
				<b>Mean</b>	<b>199</b>	<b>2,427</b>
				<b>SD</b>	<b>26.9</b>	<b>316</b>
[Proprietary]	M	4	4.0	31	479	5,638
[Proprietary]	M	4	4.0	32	344	3,223
[Proprietary]	M	4	4.0	33	585	6,107
				<b>Mean</b>	<b>469</b>	<b>4,989</b>
				<b>SD</b>	<b>121</b>	<b>1,548</b>
[Proprietary]	F	2	0.110	16	17.3	222
[Proprietary]	F	2	0.110	17	19.6	262
[Proprietary]	F	2	0.110	18	32.1	416
				<b>Mean</b>	<b>23.0</b>	<b>300</b>
				<b>SD</b>	<b>7.96</b>	<b>102</b>
[Proprietary]	F	3	1.0	26	261	2,372
[Proprietary]	F	3	1.0	27	244	2,713
[Proprietary]	F	3	1.0	28	292	3,157
				<b>Mean</b>	<b>266</b>	<b>2,747</b>
				<b>SD</b>	<b>24.3</b>	<b>394</b>
[Proprietary]	F	4	4.0	36	2,650	14,408
[Proprietary]	F	4	4.0	37	1,280	13,191
[Proprietary]	F	4	4.0	38	1,060	8,270
				<b>Mean</b>	<b>1,663</b>	<b>11,956</b>
				<b>SD</b>	<b>862</b>	<b>3,250</b>

<sup>a</sup> TK parameters calculated from plasma concentration data from main group animals only and up to study Day 15 (i.e. up to administration of first 3 doses)

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix I**

**INDIVIDUAL ANIMAL CLINICAL PATHOLOGY**



**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix I-1**

**INDIVIDUAL HEMATOLOGY**

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Withheld pursuant to exemption

Proprietary Info

of the Freedom of Information and Privacy Act

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
1	-4	1	Male	003	White Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
1	-4	1	Male	003	Red Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
1	-4	1	Male	014	White Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
1	-4	1	Male	014	Red Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
1	72	1	Male	005	White Blood Cells	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
2	-4	1	Male	003	Hemoglobin	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
2	-4	1	Male	014	Hemoglobin	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
2	72	1	Male	005	Red Blood Cells	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
3	-4	1	Male	003	Hematocrit	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
3	-4	1	Male	003	MCV	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
3	-4	1	Male	014	Hematocrit	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
3	-4	1	Male	014	MCV	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
3	72	1	Male	005	Hemoglobin	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
3	72	1	Male	005	Hematocrit	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
4	-4	1	Male	003	MCH	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
4	-4	1	Male	014	MCH	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
4	72	1	Male	005	MCV	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
5	-4	1	Male	003	MCHC	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
5	-4	1	Male	014	MCHC	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
5	72	1	Male	005	MCH	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
5	72	1	Male	005	MCHC	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
6	-4	1	Male	003	RDW	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
6	-4	1	Male	003	Platelet Count	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
6	-4	1	Male	014	RDW	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
6	-4	1	Male	014	Platelet Count	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
6	72	1	Male	005	RDW	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
7	-4	1	Male	003	MeanPlatelet Volume	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
7	-4	1	Male	014	MeanPlatelet Volume	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
7	72	1	Male	005	Platelet Count	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
8	-4	1	Male	003	Percent Neutrophils	Result	
				<i>Comment: results obtained by manual differential.</i>			
8	-4	1	Male	003	Percent Lymphocytes	Result	
				<i>Comment: results obtained by manual differential.</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
8	-4	1	Male	014	Percent Neutrophils	Result	
				<i>Comment: results obtained by manual differential.</i>			
8	-4	1	Male	014	Percent Lymphocytes	Result	
				<i>Comment: results obtained by manual differential.</i>			
8	72	1	Male	005	MeanPlatelet Volume	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
8	72	1	Male	005	Percent Neutrophils	Result	
				<i>Comment: Result obtained by manual differential</i>			
9	-4	1	Male	003	Percent Monocytes	Result	
				<i>Comment: results obtained by manual differential.</i>			
9	-4	1	Male	014	Percent Monocytes	Result	
				<i>Comment: results obtained by manual differential.</i>			
9	72	1	Male	005	Percent Lymphocytes	Result	
				<i>Comment: Result obtained by manual differential</i>			
10	-4	1	Male	003	Percent Eosinophils	Result	
				<i>Comment: results obtained by manual differential.</i>			
10	-4	1	Male	014	Percent Eosinophils	Result	
				<i>Comment: results obtained by manual differential.</i>			
10	72	1	Male	005	Percent Monocytes	Result	
				<i>Comment: Result obtained by manual differential</i>			
10	72	1	Male	005	Percent Eosinophils	Result	
				<i>Comment: Result obtained by manual differential</i>			
11	-4	1	Male	003	Percent Basophils	Result	
				<i>Comment: results obtained by manual differential.</i>			
11	-4	1	Male	014	Percent Basophils	Result	
				<i>Comment: results obtained by manual differential.</i>			
11	72	1	Male	005	Percent Basophils	Result	
				<i>Comment: Result obtained by manual differential</i>			
12	72	1	Male	005	Percent Band	Result	
				<i>Comment: Result obtained by manual differential</i>			
13	-4	1	Male	003	Neutrophils (Absolute)	Result	
				<i>Comment: results obtained by manual differential.</i>			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
13	-4	1	Male	003	Lymphocytes (Absolute)	Result	
				<i>Comment: results obtained by manual differential.</i>			
13	-4	1	Male	014	Neutrophils (Absolute)	Result	
				<i>Comment: results obtained by manual differential.</i>			
13	-4	1	Male	014	Lymphocytes (Absolute)	Result	
				<i>Comment: results obtained by manual differential.</i>			
13	72	1	Male	005	Percent Atyp Lymphocytes	Result	
				<i>Comment: Result obtained by manual differential</i>			
13	72	1	Male	005	Neutrophils (Absolute)	Result	
				<i>Comment: Result obtained by manual differential</i>			
14	-4	1	Male	003	Monocytes (Absolute)	Result	
				<i>Comment: results obtained by manual differential.</i>			
14	-4	1	Male	014	Monocytes (Absolute)	Result	
				<i>Comment: results obtained by manual differential.</i>			
14	72	1	Male	005	Lymphocytes (Absolute)	Result	
				<i>Comment: Result obtained by manual differential</i>			
15	-4	1	Male	003	Eosinophils (Absolute)	Result	
				<i>Comment: results obtained by manual differential.</i>			
15	-4	1	Male	014	Eosinophils (Absolute)	Result	
				<i>Comment: results obtained by manual differential.</i>			
15	72	1	Male	005	Monocytes (Absolute)	Result	
				<i>Comment: Result obtained by manual differential</i>			
15	72	1	Male	005	Eosinophils (Absolute)	Result	
				<i>Comment: Result obtained by manual differential</i>			
16	-4	1	Male	003	Basophils (Absolute)	Result	
				<i>Comment: results obtained by manual differential.</i>			
16	-4	1	Male	014	Basophils (Absolute)	Result	
				<i>Comment: results obtained by manual differential.</i>			
16	72	1	Male	005	Basophils (Absolute)	Result	
				<i>Comment: Result obtained by manual differential</i>			
17	72	1	Male	005	Band (Absolute)	Result	
				<i>Comment: Result obtained by manual differential</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
18	-4	1	Male	003	Percent Reticulocyte	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
18	-4	1	Male	003	Reticulocyte (Absolute)	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
18	-4	1	Male	014	Percent Reticulocyte	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
18	-4	1	Male	014	Reticulocyte (Absolute)	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
18	72	1	Male	005	Atyp Lymph (Absolute)	Result	
				<i>Comment: Result obtained by manual differential</i>			
18	72	1	Male	005	Percent Reticulocyte	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
19	72	1	Male	005	Reticulocyte (Absolute)	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
20	-4	2	Male	001	White Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
20	-4	2	Male	001	Red Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
20	-4	2	Male	015	White Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
20	-4	2	Male	015	Red Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
21	-4	2	Male	001	Hemoglobin	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
21	-4	2	Male	015	Hemoglobin	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
22	-4	2	Male	001	Hematocrit	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
22	-4	2	Male	001	MCV	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
22	-4	2	Male	015	Hematocrit	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
22	-4	2	Male	015	MCV	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
23	-4	2	Male	001	MCH	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
23	-4	2	Male	015	MCH	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
24	-4	2	Male	001	MCHC	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
24	-4	2	Male	015	MCHC	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
25	-4	2	Male	001	RDW	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
25	-4	2	Male	001	Platelet Count	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
25	-4	2	Male	015	RDW	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
25	-4	2	Male	015	Platelet Count	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
26	-4	2	Male	001	MeanPlatelet Volume	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
26	-4	2	Male	015	MeanPlatelet Volume	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
27	-4	2	Male	001	Percent Neutrophils	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
27	-4	2	Male	001	Percent Lymphocytes	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
27	-4	2	Male	015	Percent Neutrophils	Result	
				<i>Comment: results obtained by manual differential.</i>			
27	-4	2	Male	015	Percent Lymphocytes	Result	
				<i>Comment: results obtained by manual differential.</i>			
27	72	2	Male	001	Percent Neutrophils	Quality Flag	I (Include)

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
28	-4	2	Male	001	Percent Monocytes	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
28	-4	2	Male	015	Percent Monocytes	Result	
				<i>Comment: results obtained by manual differential.</i>			
28	72	2	Male	001	Percent Lymphocytes	Quality Flag	I (Include)
29	-4	2	Male	001	Percent Eosinophils	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
29	-4	2	Male	015	Percent Eosinophils	Result	
				<i>Comment: results obtained by manual differential.</i>			
29	72	2	Male	001	Percent Monocytes	Quality Flag	I (Include)
29	72	2	Male	001	Percent Eosinophils	Quality Flag	I (Include)
30	-4	2	Male	001	Percent Basophils	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
30	-4	2	Male	015	Percent Basophils	Result	
				<i>Comment: results obtained by manual differential.</i>			
30	72	2	Male	001	Percent Basophils	Quality Flag	I (Include)
32	-4	2	Male	001	Neutrophils (Absolute)	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
32	-4	2	Male	001	Lymphocytes (Absolute)	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
32	-4	2	Male	015	Neutrophils (Absolute)	Result	
				<i>Comment: results obtained by manual differential.</i>			
32	-4	2	Male	015	Lymphocytes (Absolute)	Result	
				<i>Comment: results obtained by manual differential.</i>			
32	72	2	Male	001	Neutrophils (Absolute)	Quality Flag	I (Include)
33	-4	2	Male	001	Monocytes (Absolute)	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
33	-4	2	Male	015	Monocytes (Absolute)	Result	
				<i>Comment: results obtained by manual differential.</i>			
33	72	2	Male	001	Lymphocytes (Absolute)	Quality Flag	I (Include)
34	-4	2	Male	001	Eosinophils (Absolute)	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
34	-4	2	Male	015	Eosinophils (Absolute)	Result	
				<i>Comment: results obtained by manual differential.</i>			
34	72	2	Male	001	Monocytes (Absolute)	Quality Flag	I (Include)
34	72	2	Male	001	Eosinophils (Absolute)	Quality Flag	I (Include)
35	-4	2	Male	001	Basophils (Absolute)	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
35	-4	2	Male	015	Basophils (Absolute)	Result	
				<i>Comment: results obtained by manual differential.</i>			
35	72	2	Male	001	Basophils (Absolute)	Quality Flag	I (Include)
37	-4	2	Male	001	Percent Reticulocyte	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
37	-4	2	Male	001	Reticulocyte (Absolute)	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
37	-4	2	Male	015	Percent Reticulocyte	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
37	-4	2	Male	015	Reticulocyte (Absolute)	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
39	-4	3	Male	021	White Blood Cells	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
39	-4	3	Male	021	Red Blood Cells	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
39	17	3	Male	021	White Blood Cells	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
39	17	3	Male	023	White Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
40	-4	3	Male	021	Hemoglobin	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
40	17	3	Male	021	Red Blood Cells	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
40	17	3	Male	021	Hemoglobin	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
40	17	3	Male	023	Red Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
40	17	3	Male	023	Hemoglobin	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
41	-4	3	Male	021	Hematocrit	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
41	-4	3	Male	021	MCV	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
41	17	3	Male	021	Hematocrit	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
41	17	3	Male	023	Hematocrit	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
42	-4	3	Male	021	MCH	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
42	17	3	Male	021	MCV	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
42	17	3	Male	021	MCH	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
42	17	3	Male	023	MCV	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
42	17	3	Male	023	MCH	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
43	-4	3	Male	021	MCHC	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
43	17	3	Male	021	MCHC	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
43	17	3	Male	023	MCHC	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
44	-4	3	Male	021	RDW	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
44	-4	3	Male	021	Platelet Count	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
44	17	3	Male	021	RDW	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
44	17	3	Male	023	RDW	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
45	-4	3	Male	021	MeanPlatelet Volume	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
45	17	3	Male	021	Platelet Count	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
45	17	3	Male	021	MeanPlatelet Volume	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
45	17	3	Male	023	Platelet Count	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
45	17	3	Male	023	MeanPlatelet Volume	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
46	-4	3	Male	021	Percent Neutrophils	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
46	-4	3	Male	021	Percent Lymphocytes	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
46	17	3	Male	021	Percent Neutrophils	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
46	17	3	Male	023	Percent Neutrophils	Result	
				<i>Comment: Result obtained by manual differential.</i>			
47	-4	3	Male	021	Percent Monocytes	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
47	17	3	Male	021	Percent Lymphocytes	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
47	17	3	Male	021	Percent Monocytes	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
47	17	3	Male	023	Percent Lymphocytes	Result	
				<i>Comment: Result obtained by manual differential.</i>			
47	17	3	Male	023	Percent Monocytes	Result	
				<i>Comment: Result obtained by manual differential.</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
48	-4	3	Male	021	Percent Eosinophils	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
48	17	3	Male	021	Percent Eosinophils	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
48	17	3	Male	023	Percent Eosinophils	Result	
				<i>Comment: Result obtained by manual differential.</i>			
49	-4	3	Male	021	Percent Basophils	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
49	17	3	Male	021	Percent Basophils	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
49	17	3	Male	023	Percent Basophils	Result	
				<i>Comment: Result obtained by manual differential.</i>			
50	17	3	Male	023	Percent Band	Result	
				<i>Comment: Result obtained by manual differential.</i>			
50	17	3	Male	023	Percent Atyp Lymphocytes	Result	
				<i>Comment: Result obtained by manual differential.</i>			
51	-4	3	Male	021	Neutrophils (Absolute)	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
51	-4	3	Male	021	Lymphocytes (Absolute)	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
51	17	3	Male	021	Neutrophils (Absolute)	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
51	17	3	Male	023	Neutrophils (Absolute)	Result	
				<i>Comment: Result obtained by manual differential.</i>			
52	-4	3	Male	021	Monocytes (Absolute)	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
52	17	3	Male	021	Lymphocytes (Absolute)	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
52	17	3	Male	021	Monocytes (Absolute)	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
52	17	3	Male	023	Lymphocytes (Absolute)	Result	
				<i>Comment: Result obtained by manual differential.</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
52	17	3	Male	023	Monocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
53	-4	3	Male	021	Eosinophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
53	17	3	Male	021	Eosinophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
53	17	3	Male	023	Eosinophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
54	-4	3	Male	021	Basophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
54	17	3	Male	021	Basophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
54	17	3	Male	023	Basophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
55	17	3	Male	023	Band (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
55	17	3	Male	023	Atyp Lymph (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
56	-4	3	Male	021	Percent Reticulocyte	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
56	-4	3	Male	021	Reticulocyte (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
56	17	3	Male	021	Percent Reticulocyte	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
56	17	3	Male	023	Percent Reticulocyte	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
57	17	3	Male	021	Reticulocyte (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
57	17	3	Male	023	Reticulocyte (Absolute)	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
77	-2	1	Female	007	White Blood Cells	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
77	-2	1	Female	007	Red Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
77	-2	1	Female	008	White Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
77	-2	1	Female	008	Red Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
77	-2	1	Female	010	White Blood Cells	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
77	-2	1	Female	010	Red Blood Cells	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
77	30	1	Female	008	White Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
77	72	1	Female	009	White Blood Cells	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
77	72	1	Female	010	White Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
78	-2	1	Female	007	Hemoglobin	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
78	-2	1	Female	008	Hemoglobin	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
78	-2	1	Female	010	Hemoglobin	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
78	30	1	Female	008	Red Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
78	30	1	Female	008	Hemoglobin	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
78	72	1	Female	009	Red Blood Cells	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
78	72	1	Female	010	Red Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
79	-2	1	Female	007	Hematocrit	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
79	-2	1	Female	008	Hematocrit	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
79	-2	1	Female	010	Hematocrit	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
79	30	1	Female	008	Hematocrit	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
79	72	1	Female	009	Hemoglobin	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
79	72	1	Female	009	Hematocrit	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
79	72	1	Female	010	Hemoglobin	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
79	72	1	Female	010	Hematocrit	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
80	-2	1	Female	007	MCV	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
80	-2	1	Female	007	MCH	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
80	-2	1	Female	008	MCV	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
80	-2	1	Female	008	MCH	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
80	-2	1	Female	010	MCV	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
80	-2	1	Female	010	MCH	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
80	30	1	Female	008	MCV	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
80	72	1	Female	009	MCV	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
80	72	1	Female	010	MCV	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
81	-2	1	Female	007	MCHC	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
81	-2	1	Female	008	MCHC	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
81	-2	1	Female	010	MCHC	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
81	30	1	Female	008	MCH	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
81	30	1	Female	008	MCHC	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
81	72	1	Female	009	MCH	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
81	72	1	Female	009	MCHC	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
81	72	1	Female	010	MCH	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
81	72	1	Female	010	MCHC	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
82	-2	1	Female	007	RDW	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
82	-2	1	Female	007	Platelet Count	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
82	-2	1	Female	008	RDW	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
82	-2	1	Female	008	Platelet Count	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
82	-2	1	Female	010	RDW	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
82	-2	1	Female	010	Platelet Count	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
82	30	1	Female	008	RDW	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
82	72	1	Female	009	RDW	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
82	72	1	Female	010	RDW	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
83	-2	1	Female	007	MeanPlatelet Volume	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
83	-2	1	Female	008	MeanPlatelet Volume	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
83	-2	1	Female	010	MeanPlatelet Volume	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
83	30	1	Female	008	Platelet Count	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
83	30	1	Female	008	MeanPlatelet Volume	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
83	72	1	Female	009	Platelet Count	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
83	72	1	Female	010	Platelet Count	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
84	-2	1	Female	007	Percent Neutrophils	Result	
				<i>Comment:</i> Result obtained by manual differential.			
84	-2	1	Female	008	Percent Neutrophils	Result	
				<i>Comment:</i> Result obtained by manual differential.			
84	-2	1	Female	010	Percent Neutrophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
84	30	1	Female	008	Percent Neutrophils	Result	
				<i>Comment:</i> result obtained by manual differential.			
84	72	1	Female	009	MeanPlatelet Volume	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
84	72	1	Female	009	Percent Neutrophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
84	72	1	Female	010	MeanPlatelet Volume	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
84	72	1	Female	010	Percent Neutrophils	Result	
				<i>Comment:</i> Result obtained by manual differential			
85	-2	1	Female	007	Percent Lymphocytes	Result	
				<i>Comment:</i> Result obtained by manual differential.			
85	-2	1	Female	007	Percent Monocytes	Result	
				<i>Comment:</i> Result obtained by manual differential.			
85	-2	1	Female	008	Percent Lymphocytes	Result	
				<i>Comment:</i> Result obtained by manual differential.			
85	-2	1	Female	008	Percent Monocytes	Result	
				<i>Comment:</i> Result obtained by manual differential.			
85	-2	1	Female	010	Percent Lymphocytes	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
85	-2	1	Female	010	Percent Monocytes	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
85	30	1	Female	008	Percent Lymphocytes	Result	
				<i>Comment:</i> result obtained by manual differential.			
85	72	1	Female	009	Percent Lymphocytes	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
85	72	1	Female	010	Percent Lymphocytes	Result	
				<i>Comment:</i> Result obtained by manual differential			
86	-2	1	Female	007	Percent Eosinophils	Result	
				<i>Comment:</i> Result obtained by manual differential.			
86	-2	1	Female	008	Percent Eosinophils	Result	
				<i>Comment:</i> Result obtained by manual differential.			
86	-2	1	Female	010	Percent Eosinophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
86	30	1	Female	008	Percent Monocytes	Result	
				<i>Comment:</i> result obtained by manual differential.			
86	30	1	Female	008	Percent Eosinophils	Result	
				<i>Comment:</i> result obtained by manual differential.			
86	72	1	Female	009	Percent Monocytes	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
86	72	1	Female	009	Percent Eosinophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
86	72	1	Female	010	Percent Monocytes	Result	
				<i>Comment:</i> Result obtained by manual differential			
86	72	1	Female	010	Percent Eosinophils	Result	
				<i>Comment:</i> Result obtained by manual differential			
87	-2	1	Female	007	Percent Basophils	Result	
				<i>Comment:</i> Result obtained by manual differential.			
87	-2	1	Female	007	Percent Band	Result	
				<i>Comment:</i> Result obtained by manual differential.			
87	-2	1	Female	008	Percent Basophils	Result	
				<i>Comment:</i> Result obtained by manual differential.			
87	-2	1	Female	008	Percent Band	Result	
				<i>Comment:</i> Result obtained by manual differential.			
87	-2	1	Female	010	Percent Basophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
87	30	1	Female	008	Percent Basophils	Result	
				<i>Comment:</i> result obtained by manual differential.			
87	72	1	Female	009	Percent Basophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
87	72	1	Female	010	Percent Basophils	Result	
				<i>Comment:</i> Result obtained by manual differential			
88	-2	1	Female	007	Percent Atyp Lymphocytes	Result	
				<i>Comment:</i> Result obtained by manual differential.			
88	-2	1	Female	008	Percent Atyp Lymphocytes	Result	
				<i>Comment:</i> Result obtained by manual differential.			
88	30	1	Female	008	Percent Band	Result	
				<i>Comment:</i> result obtained by manual differential.			
88	30	1	Female	008	Percent Atyp Lymphocytes	Result	
				<i>Comment:</i> result obtained by manual differential.			
88	72	1	Female	010	Percent Band	Result	
				<i>Comment:</i> Result obtained by manual differential			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
89	-2	1	Female	007	Neutrophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
89	-2	1	Female	008	Neutrophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
89	-2	1	Female	010	Neutrophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
89	30	1	Female	008	Neutrophils (Absolute)	Result	
				<i>Comment:</i> result obtained by manual differential.			
89	72	1	Female	009	Neutrophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
89	72	1	Female	010	Percent Atyp Lymphocytes	Result	
				<i>Comment:</i> Result obtained by manual differential			
89	72	1	Female	010	Neutrophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential			
90	-2	1	Female	007	Lymphocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
90	-2	1	Female	007	Monocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
90	-2	1	Female	008	Lymphocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
90	-2	1	Female	008	Monocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
90	-2	1	Female	010	Lymphocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
90	-2	1	Female	010	Monocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
90	30	1	Female	008	Lymphocytes (Absolute)	Result	
				<i>Comment:</i> result obtained by manual differential.			
90	72	1	Female	009	Lymphocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
90	72	1	Female	010	Lymphocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
91	-2	1	Female	007	Eosinophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
91	-2	1	Female	008	Eosinophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
91	-2	1	Female	010	Eosinophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
91	30	1	Female	008	Monocytes (Absolute)	Result	
				<i>Comment:</i> result obtained by manual differential.			
91	30	1	Female	008	Eosinophils (Absolute)	Result	
				<i>Comment:</i> result obtained by manual differential.			
91	72	1	Female	009	Monocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
91	72	1	Female	009	Eosinophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
91	72	1	Female	010	Monocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential			
91	72	1	Female	010	Eosinophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential			
92	-2	1	Female	007	Basophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
92	-2	1	Female	007	Band (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
92	-2	1	Female	008	Basophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
92	-2	1	Female	008	Band (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
92	-2	1	Female	010	Basophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
92	30	1	Female	008	Basophils (Absolute)	Result	
				<i>Comment:</i> result obtained by manual differential.			
92	72	1	Female	009	Basophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
92	72	1	Female	010	Basophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential			
93	-2	1	Female	007	Atyp Lymph (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
93	-2	1	Female	008	Atyp Lymph (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
93	30	1	Female	008	Band (Absolute)	Result	
				<i>Comment:</i> result obtained by manual differential.			
93	30	1	Female	008	Atyp Lymph (Absolute)	Result	
				<i>Comment:</i> result obtained by manual differential.			
93	72	1	Female	010	Band (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential			
94	-2	1	Female	007	Percent Reticulocyte	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
94	-2	1	Female	008	Percent Reticulocyte	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
94	-2	1	Female	010	Percent Reticulocyte	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
94	30	1	Female	008	Percent Reticulocyte	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
94	72	1	Female	009	Percent Reticulocyte	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
94	72	1	Female	010	Atyp Lymph (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential			
94	72	1	Female	010	Percent Reticulocyte	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
95	-2	1	Female	007	Reticulocyte (Absolute)	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
95	-2	1	Female	008	Reticulocyte (Absolute)	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
95	-2	1	Female	010	Reticulocyte (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
95	30	1	Female	008	Reticulocyte (Absolute)	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
95	72	1	Female	009	Reticulocyte (Absolute)	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
95	72	1	Female	010	Reticulocyte (Absolute)	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
96	-2	2	Female	020	White Blood Cells	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
96	-2	2	Female	020	Red Blood Cells	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
96	72	2	Female	019	White Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
96	72	2	Female	020	White Blood Cells	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
97	-2	2	Female	020	Hemoglobin	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
97	72	2	Female	019	Red Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
97	72	2	Female	020	Red Blood Cells	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
98	-2	2	Female	020	Hematocrit	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
98	72	2	Female	019	Hemoglobin	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
98	72	2	Female	019	Hematocrit	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
98	72	2	Female	020	Hemoglobin	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
98	72	2	Female	020	Hematocrit	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
99	-2	2	Female	020	MCV	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
99	-2	2	Female	020	MCH	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
99	72	2	Female	019	MCV	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
99	72	2	Female	020	MCV	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
100	-2	2	Female	020	MCHC	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
100	72	2	Female	019	MCH	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
100	72	2	Female	019	MCHC	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
100	72	2	Female	020	MCH	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
100	72	2	Female	020	MCHC	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
<span style="border: 1px solid black; padding: 0 2px;">Pr</span>	-2	2	Female	020	RDW	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
<span style="border: 1px solid black; padding: 0 2px;">Pr</span>	-2	2	Female	020	Platelet Count	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
<span style="border: 1px solid black; padding: 0 2px;">Pr</span>	72	2	Female	019	RDW	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
<span style="border: 1px solid black; padding: 0 2px;">Pr</span>	72	2	Female	020	RDW	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
102	-2	2	Female	020	MeanPlatelet Volume	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
102	72	2	Female	019	Platelet Count	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
102	72	2	Female	020	Platelet Count	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
103	-2	2	Female	020	Percent Neutrophils	Result	
				<i>Comment: Result obtained by manual differential.</i>			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
103	72	2	Female	019	MeanPlatelet Volume	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
103	72	2	Female	019	Percent Neutrophils	Result	
				<i>Comment:</i> Result obtained by manual differential			
103	72	2	Female	020	MeanPlatelet Volume	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
103	72	2	Female	020	Percent Neutrophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
104	-2	2	Female	020	Percent Lymphocytes	Result	
				<i>Comment:</i> Result obtained by manual differential.			
104	-2	2	Female	020	Percent Monocytes	Result	
				<i>Comment:</i> Result obtained by manual differential.			
104	72	2	Female	019	Percent Lymphocytes	Result	
				<i>Comment:</i> Result obtained by manual differential			
104	72	2	Female	020	Percent Lymphocytes	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
105	-2	2	Female	020	Percent Eosinophils	Result	
				<i>Comment:</i> Result obtained by manual differential.			
105	72	2	Female	019	Percent Monocytes	Result	
				<i>Comment:</i> Result obtained by manual differential			
105	72	2	Female	019	Percent Eosinophils	Result	
				<i>Comment:</i> Result obtained by manual differential			
105	72	2	Female	020	Percent Monocytes	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
105	72	2	Female	020	Percent Eosinophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
106	-2	2	Female	020	Percent Basophils	Result	
				<i>Comment:</i> Result obtained by manual differential.			
106	-2	2	Female	020	Percent Band	Result	
				<i>Comment:</i> Result obtained by manual differential.			
106	72	2	Female	019	Percent Basophils	Result	
				<i>Comment:</i> Result obtained by manual differential			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
106	72	2	Female	020	Percent Basophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
107	-2	2	Female	020	Percent Atyp Lymphocytes	Result	
				<i>Comment:</i> Result obtained by manual differential.			
107	72	2	Female	019	Percent Band	Result	
				<i>Comment:</i> Result obtained by manual differential			
108	-2	2	Female	020	Neutrophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
108	72	2	Female	019	Percent Atyp Lymphocytes	Result	
				<i>Comment:</i> Result obtained by manual differential			
108	72	2	Female	019	Neutrophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential			
108	72	2	Female	020	Neutrophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
109	-2	2	Female	020	Lymphocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
109	-2	2	Female	020	Monocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
109	72	2	Female	019	Lymphocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential			
109	72	2	Female	020	Lymphocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
110	-2	2	Female	020	Eosinophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
110	72	2	Female	019	Monocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential			
110	72	2	Female	019	Eosinophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential			
110	72	2	Female	020	Monocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
110	72	2	Female	020	Eosinophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
111	-2	2	Female	020	Basophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
111	-2	2	Female	020	Band (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
111	72	2	Female	019	Basophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential			
111	72	2	Female	020	Basophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
112	-2	2	Female	020	Atyp Lymph (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
112	72	2	Female	019	Band (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential			
113	-2	2	Female	020	Percent Reticulocyte	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
113	72	2	Female	019	Atyp Lymph (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential			
113	72	2	Female	019	Percent Reticulocyte	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
113	72	2	Female	020	Percent Reticulocyte	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
114	-2	2	Female	020	Reticulocyte (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
114	72	2	Female	019	Reticulocyte (Absolute)	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
114	72	2	Female	020	Reticulocyte (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
115	-2	3	Female	026	White Blood Cells	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
115	-2	3	Female	026	Red Blood Cells	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
115	-2	3	Female	027	White Blood Cells	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
115	-2	3	Female	027	Red Blood Cells	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
115	-2	3	Female	028	White Blood Cells	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
115	-2	3	Female	028	Red Blood Cells	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
115	17	3	Female	027	White Blood Cells	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
115	72	3	Female	029	White Blood Cells	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
116	-2	3	Female	026	Hemoglobin	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
116	-2	3	Female	027	Hemoglobin	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
116	-2	3	Female	028	Hemoglobin	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
116	17	3	Female	027	Red Blood Cells	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
116	17	3	Female	027	Hemoglobin	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
116	72	3	Female	029	Red Blood Cells	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
117	-2	3	Female	026	Hematocrit	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
117	-2	3	Female	027	Hematocrit	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
117	-2	3	Female	028	Hematocrit	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
117	17	3	Female	027	Hematocrit	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
117	72	3	Female	029	Hemoglobin	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
117	72	3	Female	029	Hematocrit	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
118	-2	3	Female	026	MCV	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
118	-2	3	Female	026	MCH	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
118	-2	3	Female	027	MCV	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
118	-2	3	Female	027	MCH	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
118	-2	3	Female	028	MCV	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
118	-2	3	Female	028	MCH	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
118	17	3	Female	027	MCV	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
118	17	3	Female	027	MCH	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
118	72	3	Female	029	MCV	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
119	-2	3	Female	026	MCHC	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
119	-2	3	Female	027	MCHC	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
119	-2	3	Female	028	MCHC	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
119	17	3	Female	027	MCHC	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
119	72	3	Female	029	MCH	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
119	72	3	Female	029	MCHC	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
120	-2	3	Female	026	RDW	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
120	-2	3	Female	026	Platelet Count	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
120	-2	3	Female	027	RDW	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
120	-2	3	Female	027	Platelet Count	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
120	-2	3	Female	028	RDW	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
120	-2	3	Female	028	Platelet Count	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
120	17	3	Female	027	RDW	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
120	72	3	Female	029	RDW	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
121	-2	3	Female	026	MeanPlatelet Volume	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
121	-2	3	Female	027	MeanPlatelet Volume	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
121	-2	3	Female	028	MeanPlatelet Volume	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
121	17	3	Female	027	Platelet Count	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
121	17	3	Female	027	MeanPlatelet Volume	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
121	72	3	Female	029	Platelet Count	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
122	-2	3	Female	026	Percent Neutrophils	Result	
				<i>Comment:</i> Result obtained by manual differential.			
122	-2	3	Female	027	Percent Neutrophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
122	-2	3	Female	028	Percent Neutrophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
122	17	3	Female	027	Percent Neutrophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
122	72	3	Female	029	MeanPlatelet Volume	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
122	72	3	Female	029	Percent Neutrophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
123	-2	3	Female	026	Percent Lymphocytes	Result	
				<i>Comment:</i> Result obtained by manual differential.			
123	-2	3	Female	026	Percent Monocytes	Result	
				<i>Comment:</i> Result obtained by manual differential.			
123	-2	3	Female	027	Percent Lymphocytes	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
123	-2	3	Female	027	Percent Monocytes	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
123	-2	3	Female	028	Percent Lymphocytes	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
123	-2	3	Female	028	Percent Monocytes	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
123	17	3	Female	027	Percent Lymphocytes	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
123	17	3	Female	027	Percent Monocytes	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
123	72	3	Female	029	Percent Lymphocytes	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
124	-2	3	Female	026	Percent Eosinophils	Result	
				<i>Comment:</i> Result obtained by manual differential.			
124	-2	3	Female	027	Percent Eosinophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
124	-2	3	Female	028	Percent Eosinophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
124	17	3	Female	027	Percent Eosinophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
124	72	3	Female	029	Percent Monocytes	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
124	72	3	Female	029	Percent Eosinophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
125	-2	3	Female	026	Percent Basophils	Result	
				<i>Comment:</i> Result obtained by manual differential.			
125	-2	3	Female	026	Percent Band	Result	
				<i>Comment:</i> Result obtained by manual differential.			
125	-2	3	Female	027	Percent Basophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
125	-2	3	Female	028	Percent Basophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
125	17	3	Female	027	Percent Basophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
125	72	3	Female	029	Percent Basophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
126	-2	3	Female	026	Percent Atyp Lymphocytes	Result	
				<i>Comment:</i> Result obtained by manual differential.			
127	-2	3	Female	026	Neutrophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
127	-2	3	Female	027	Neutrophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
127	-2	3	Female	028	Neutrophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
127	17	3	Female	027	Neutrophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
127	72	3	Female	029	Neutrophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
128	-2	3	Female	026	Lymphocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

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128	-2	3	Female	026	Monocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
128	-2	3	Female	027	Lymphocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
128	-2	3	Female	027	Monocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
128	-2	3	Female	028	Lymphocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
128	-2	3	Female	028	Monocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
128	17	3	Female	027	Lymphocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
128	17	3	Female	027	Monocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
128	72	3	Female	029	Lymphocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
129	-2	3	Female	026	Eosinophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
129	-2	3	Female	027	Eosinophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
129	-2	3	Female	028	Eosinophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
129	17	3	Female	027	Eosinophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
129	72	3	Female	029	Monocytes (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
129	72	3	Female	029	Eosinophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
130	-2	3	Female	026	Basophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
130	-2	3	Female	026	Band (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
130	-2	3	Female	027	Basophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
130	-2	3	Female	028	Basophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
130	17	3	Female	027	Basophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
130	72	3	Female	029	Basophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
131	-2	3	Female	026	Atyp Lymph (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential.			
132	-2	3	Female	026	Percent Reticulocyte	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
132	-2	3	Female	027	Percent Reticulocyte	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
132	-2	3	Female	028	Percent Reticulocyte	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
132	17	3	Female	027	Percent Reticulocyte	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
132	72	3	Female	029	Percent Reticulocyte	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
133	-2	3	Female	026	Reticulocyte (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
133	-2	3	Female	027	Reticulocyte (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
133	-2	3	Female	028	Reticulocyte (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
133	17	3	Female	027	Reticulocyte (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
133	72	3	Female	029	Reticulocyte (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
134	-2	4	Female	040	White Blood Cells	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
134	-2	4	Female	040	Red Blood Cells	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
134	17	4	Female	039	White Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
134	72	4	Female	038	White Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
134	72	4	Female	040	White Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
135	-2	4	Female	040	Hemoglobin	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
135	17	4	Female	039	Red Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
135	17	4	Female	039	Hemoglobin	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
135	72	4	Female	038	Red Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
135	72	4	Female	040	Red Blood Cells	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
136	-2	4	Female	040	Hematocrit	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
136	17	4	Female	039	Hematocrit	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
136	72	4	Female	038	Hemoglobin	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
136	72	4	Female	038	Hematocrit	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
136	72	4	Female	040	Hemoglobin	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
136	72	4	Female	040	Hematocrit	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
137	-2	4	Female	040	MCV	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
137	-2	4	Female	040	MCH	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
137	17	4	Female	039	MCV	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
137	17	4	Female	039	MCH	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
137	72	4	Female	038	MCV	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
137	72	4	Female	040	MCV	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
138	-2	4	Female	040	MCHC	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
138	17	4	Female	039	MCHC	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
138	72	4	Female	038	MCH	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
138	72	4	Female	038	MCHC	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
138	72	4	Female	040	MCH	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
138	72	4	Female	040	MCHC	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
139	-2	4	Female	040	RDW	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
139	-2	4	Female	040	Platelet Count	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
139	17	4	Female	039	RDW	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
139	72	4	Female	038	RDW	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
139	72	4	Female	040	RDW	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
140	-2	4	Female	040	MeanPlatelet Volume	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
140	17	4	Female	039	Platelet Count	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
140	17	4	Female	039	MeanPlatelet Volume	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
140	72	4	Female	038	Platelet Count	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
140	72	4	Female	040	Platelet Count	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
141	-2	4	Female	040	Percent Neutrophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
141	17	4	Female	039	Percent Neutrophils	Result	
				<i>Comment:</i> results obtained by manual differential			
141	72	4	Female	038	MeanPlatelet Volume	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
141	72	4	Female	038	Percent Neutrophils	Result	
				<i>Comment:</i> Result obtained by manual differential			
141	72	4	Female	040	MeanPlatelet Volume	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
141	72	4	Female	040	Percent Neutrophils	Result	
				<i>Comment:</i> Result obtained by manual differential			
142	-2	4	Female	040	Percent Lymphocytes	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
142	-2	4	Female	040	Percent Monocytes	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
142	17	4	Female	039	Percent Lymphocytes	Result	
				<i>Comment:</i> results obtained by manual differential			
142	17	4	Female	039	Percent Monocytes	Result	
				<i>Comment:</i> results obtained by manual differential			
142	72	4	Female	038	Percent Lymphocytes	Result	
				<i>Comment:</i> Result obtained by manual differential			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
142	72	4	Female	040	Percent Lymphocytes	Result	
				<i>Comment:</i> Result obtained by manual differential			
143	-2	4	Female	040	Percent Eosinophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
143	17	4	Female	039	Percent Eosinophils	Result	
				<i>Comment:</i> results obtained by manual differential			
143	72	4	Female	038	Percent Monocytes	Result	
				<i>Comment:</i> Result obtained by manual differential			
143	72	4	Female	038	Percent Eosinophils	Result	
				<i>Comment:</i> Result obtained by manual differential			
143	72	4	Female	040	Percent Monocytes	Result	
				<i>Comment:</i> Result obtained by manual differential			
143	72	4	Female	040	Percent Eosinophils	Result	
				<i>Comment:</i> Result obtained by manual differential			
144	-2	4	Female	040	Percent Basophils	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
144	17	4	Female	039	Percent Basophils	Result	
				<i>Comment:</i> results obtained by manual differential			
144	72	4	Female	038	Percent Basophils	Result	
				<i>Comment:</i> Result obtained by manual differential			
144	72	4	Female	040	Percent Basophils	Result	
				<i>Comment:</i> Result obtained by manual differential			
145	17	4	Female	039	Percent Band	Result	
				<i>Comment:</i> results obtained by manual differential			
145	17	4	Female	039	Percent Atyp Lymphocytes	Result	
				<i>Comment:</i> results obtained by manual differential			
145	72	4	Female	038	Percent Band	Result	
				<i>Comment:</i> Result obtained by manual differential			
145	72	4	Female	040	Percent Band	Result	
				<i>Comment:</i> Result obtained by manual differential			
146	-2	4	Female	040	Neutrophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
146	17	4	Female	039	Neutrophils (Absolute)	Result	
				<i>Comment: results obtained by manual differential</i>			
146	72	4	Female	038	Percent Atyp Lymphocytes	Result	
				<i>Comment: Result obtained by manual differential</i>			
146	72	4	Female	038	Neutrophils (Absolute)	Result	
				<i>Comment: Result obtained by manual differential</i>			
146	72	4	Female	040	Percent Atyp Lymphocytes	Result	
				<i>Comment: Result obtained by manual differential</i>			
146	72	4	Female	040	Neutrophils (Absolute)	Result	
				<i>Comment: Result obtained by manual differential</i>			
147	-2	4	Female	040	Lymphocytes (Absolute)	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
147	-2	4	Female	040	Monocytes (Absolute)	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
147	17	4	Female	039	Lymphocytes (Absolute)	Result	
				<i>Comment: results obtained by manual differential</i>			
147	17	4	Female	039	Monocytes (Absolute)	Result	
				<i>Comment: results obtained by manual differential</i>			
147	72	4	Female	038	Lymphocytes (Absolute)	Result	
				<i>Comment: Result obtained by manual differential</i>			
147	72	4	Female	040	Lymphocytes (Absolute)	Result	
				<i>Comment: Result obtained by manual differential</i>			
148	-2	4	Female	040	Eosinophils (Absolute)	Result	
				<i>Comment: Result obtained by repeat analysis.</i>			
148	17	4	Female	039	Eosinophils (Absolute)	Result	
				<i>Comment: results obtained by manual differential</i>			
148	72	4	Female	038	Monocytes (Absolute)	Result	
				<i>Comment: Result obtained by manual differential</i>			
148	72	4	Female	038	Eosinophils (Absolute)	Result	
				<i>Comment: Result obtained by manual differential</i>			
148	72	4	Female	040	Monocytes (Absolute)	Result	
				<i>Comment: Result obtained by manual differential</i>			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
148	72	4	Female	040	Eosinophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential			
149	-2	4	Female	040	Basophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
149	17	4	Female	039	Basophils (Absolute)	Result	
				<i>Comment:</i> results obtained by manual differential			
149	72	4	Female	038	Basophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential			
149	72	4	Female	040	Basophils (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential			
150	17	4	Female	039	Band (Absolute)	Result	
				<i>Comment:</i> results obtained by manual differential			
150	17	4	Female	039	Atyp Lymph (Absolute)	Result	
				<i>Comment:</i> results obtained by manual differential			
150	72	4	Female	038	Band (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential			
150	72	4	Female	040	Band (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential			
151	-2	4	Female	040	Percent Reticulocyte	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			
151	17	4	Female	039	Percent Reticulocyte	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
151	72	4	Female	038	Atyp Lymph (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential			
151	72	4	Female	038	Percent Reticulocyte	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
151	72	4	Female	040	Atyp Lymph (Absolute)	Result	
				<i>Comment:</i> Result obtained by manual differential			
151	72	4	Female	040	Percent Reticulocyte	Result	
				<i>Comment:</i> Confirmed by Repeat Analysis			
152	-2	4	Female	040	Reticulocyte (Absolute)	Result	
				<i>Comment:</i> Result obtained by repeat analysis.			

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with [Proprietary] [Pr] in Male and Female Beagle Dogs

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Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
152	17	4	Female	039	Reticulocyte (Absolute)	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
152	72	4	Female	038	Reticulocyte (Absolute)	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
152	72	4	Female	040	Reticulocyte (Absolute)	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			

Key Page**General Footnotes**

Provantis version 10.1.0.1

"." indicates Not Applicable

Statistical significance indicated on a group with an N < 3 is not valid

**Quality Flags**

<u>Symbol</u>	<u>IES Status</u>	<u>Description</u>
I	Included	Include

**Measurement Descriptions**

<u>Headings Used</u>	<u>Description</u>
White Blood Cells	White Blood Cells
Red Blood Cells	Red Blood Cells
Hemoglobin	Hemoglobin
Hematocrit	Hematocrit
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
RDW	Red Blood Cell Distribution Width
Platelet Count	Platelet Count
MeanPlatelet Volume	Mean Platelet Volume
Percent Neutrophils	Percent Neutrophils
Percent Lymphocytes	Percent Lymphocytes
Percent Monocytes	Percent Monocytes
Percent Eosinophils	Percent Eosinophils
Percent Basophils	Percent Basophils
Percent Band	Percent BAND
Percent Atyp Lymphocytes	Percent Atypical Lymphocytes
Neutrophils (Absolute)	Absolute Neutrophils
Lymphocytes (Absolute)	Absolute Lymphocytes

Key Page**Measurement Descriptions (Continued)**

<u>Headings Used</u>	<u>Description</u>
Monocytes (Absolute)	Absolute Monocytes
Eosinophils (Absolute)	Absolute Eosinophils
Basophils (Absolute)	Absolute Basophils
Band (Absolute)	Absolute BAND
Atyp Lymph (Absolute)	Atypical Lymphocytes (Absolute)
Percent Reticulocyte	Percent Reticulocytes
Reticulocyte (Absolute)	Absolute Reticulocytes

**Unit Descriptions**

<u>Headings Used</u>	<u>Description</u>
%	%
10^9/L	10^9/L
fL	fL
g/dL	g/dL
pg	pg
x10^3/uL	x10^3/uL
x10^6/uL	x10^6/uL

**Measurement/Statistics**

<u>Measurement</u>	<u>Descriptive</u>
White Blood Cells	Mean Standard Deviation Count (N)
Red Blood Cells	Mean Standard Deviation Count (N)

Key Page**Measurement/Statistics (Continued)**

<u>Measurement</u>	<u>Descriptive</u>
Hemoglobin	Mean Standard Deviation Count (N)
Hematocrit	Mean Standard Deviation Count (N)
MCV	Mean Standard Deviation Count (N)
MCH	Mean Standard Deviation Count (N)
MCHC	Mean Standard Deviation Count (N)
RDW	Mean Standard Deviation Count (N)
Platelet Count	Mean Standard Deviation Count (N)
MeanPlatelet Volume	Mean Standard Deviation Count (N)
Percent Neutrophils	Mean Standard Deviation Count (N)



Key Page**Measurement/Statistics (Continued)**

<u>Measurement</u>	<u>Descriptive</u>
Percent Lymphocytes	Mean Standard Deviation Count (N)
Percent Monocytes	Mean Standard Deviation Count (N)
Percent Eosinophils	Mean Standard Deviation Count (N)
Percent Basophils	Mean Standard Deviation Count (N)
Percent Band	Mean Standard Deviation Count (N)
Percent Atyp Lymphocytes	Mean Standard Deviation Count (N)
Neutrophils (Absolute)	Mean Standard Deviation Count (N)
Lymphocytes (Absolute)	Mean Standard Deviation Count (N)
Monocytes (Absolute)	Mean Standard Deviation Count (N)

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Key Page

**Measurement/Statistics (Continued)**

<u>Measurement</u>	<u>Descriptive</u>
Eosinophils (Absolute)	Mean Standard Deviation Count (N)
Basophils (Absolute)	Mean Standard Deviation Count (N)
Band (Absolute)	Mean Standard Deviation Count (N)
Atyp Lymph (Absolute)	Mean Standard Deviation Count (N)
Percent Reticulocyte	Mean Standard Deviation Count (N)
Reticulocyte (Absolute)	Mean Standard Deviation Count (N)

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings 1-4</u>		
1	Excipient	Control	Group 1	0	mg/kg SC
2	<span style="border: 1px solid black;">Propriet</span> <span style="border: 1px solid black;">Pr</span> (0.4)	Dose	Group 2	0.4	mg/kg SC
3	<span style="border: 1px solid black;">Propriet</span> <span style="border: 1px solid black;">Pr</span> (3.64)	Dose	Group 3	3.64	mg/kg SC
4	<span style="border: 1px solid black;">Propriet</span> <span style="border: 1px solid black;">Pr</span> (14.55)	Dose	Group 4	14.55	mg/kg SC

**Comment Abbreviations**

RC = Result Comment

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with [Propriet] [Pr] in Male and Female Beagle Dogs

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End of Print

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**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix I-2**

**INDIVIDUAL CLINICAL CHEMISTRY**

Page 1355 of 3822 to Page 1458 of 3822

Withheld pursuant to exemption

Proprietary Info

of the Freedom of Information and Privacy Act



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
1	-4	1	Male	014	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
1	-4	1	Male	014	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
1	-4	1	Male	005	Blood Urea Nitrogen	Sample	
				<i>Comment: 1+ Hemolysis</i>			
1	-4	1	Male	005	Creatinine	Sample	
				<i>Comment: 1+ Hemolysis</i>			
1	30	1	Male	014	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
1	72	1	Male	004	Blood Urea Nitrogen	Sample	
				<i>Comment: 1+ Hemolysis</i>			
1	72	1	Male	005	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
2	-4	1	Male	014	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
2	-4	1	Male	005	Glucose	Sample	
				<i>Comment: 1+ Hemolysis</i>			
2	30	1	Male	014	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
2	30	1	Male	014	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
2	72	1	Male	004	Creatinine	Sample	
				<i>Comment: 1+ Hemolysis</i>			
2	72	1	Male	005	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
3	-4	1	Male	014	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
3	-4	1	Male	014	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
3	-4	1	Male	005	AST	Sample	
				<i>Comment: 1+ Hemolysis</i>			
3	-4	1	Male	005	ALT	Sample	
				<i>Comment: 1+ Hemolysis</i>			
3	30	1	Male	014	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
3	72	1	Male	004	Glucose	Sample	
				<i>Comment: 1+ Hemolysis</i>			
3	72	1	Male	004	AST	Sample	
				<i>Comment: 1+ Hemolysis</i>			
3	72	1	Male	005	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
3	72	1	Male	005	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
4	-4	1	Male	014	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
4	-4	1	Male	005	Alkaline Phosphatase	Sample	
				<i>Comment: 1+ Hemolysis</i>			
4	30	1	Male	014	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
4	72	1	Male	004	ALT	Sample	
				<i>Comment: 1+ Hemolysis</i>			
4	72	1	Male	005	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
5	-4	1	Male	002	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
5	-4	1	Male	003	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
5	-4	1	Male	014	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
5	-4	1	Male	014	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
5	-4	1	Male	004	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
5	-4	1	Male	005	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
5	-4	1	Male	005	Total Bilirubin	Sample	
				<i>Comment: 1+ Hemolysis</i>			
5	30	1	Male	002	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
5	30	1	Male	003	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
5	30	1	Male	014	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
5	30	1	Male	014	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
5	30	1	Male	014	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
5	72	1	Male	004	Alkaline Phosphatase	Sample	
				<i>Comment: 1+ Hemolysis</i>			
5	72	1	Male	004	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
5	72	1	Male	004	Total Bilirubin	Sample	
				<i>Comment: 1+ Hemolysis</i>			
5	72	1	Male	005	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
5	72	1	Male	005	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
5	72	1	Male	005	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
6	-4	1	Male	014	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
6	-4	1	Male	014	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
6	-4	1	Male	005	Sodium	Sample	
				<i>Comment: 1+ Hemolysis</i>			
6	-4	1	Male	005	Potassium	Sample	
				<i>Comment: 1+ Hemolysis</i>			
6	30	1	Male	014	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
6	72	1	Male	004	Sodium	Sample	
				<i>Comment: 1+ Hemolysis</i>			
6	72	1	Male	005	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
7	-4	1	Male	014	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
7	-4	1	Male	005	Chloride	Sample	
				<i>Comment: 1+ Hemolysis</i>			
7	30	1	Male	014	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
7	30	1	Male	014	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
7	72	1	Male	004	Potassium	Sample	
				<i>Comment: 1+ Hemolysis</i>			
7	72	1	Male	005	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
8	-4	1	Male	014	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
8	-4	1	Male	014	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
8	-4	1	Male	005	Calcium	Sample	
				<i>Comment: 1+ Hemolysis</i>			
8	-4	1	Male	005	Phosphorus	Sample	
				<i>Comment: 1+ Hemolysis</i>			
8	30	1	Male	014	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
8	72	1	Male	004	Chloride	Sample	
				<i>Comment: 1+ Hemolysis</i>			
8	72	1	Male	004	Calcium	Sample	
				<i>Comment: 1+ Hemolysis</i>			
8	72	1	Male	005	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
8	72	1	Male	005	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
9	-4	1	Male	014	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
9	-4	1	Male	005	Total Protein	Sample	
				<i>Comment: 1+ Hemolysis</i>			
9	30	1	Male	014	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
9	72	1	Male	004	Phosphorus	Sample	
				<i>Comment: 1+ Hemolysis</i>			
9	72	1	Male	005	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
10	-4	1	Male	014	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
10	-4	1	Male	005	Albumin	Sample	
				<i>Comment: 1+ Hemolysis</i>			
10	30	1	Male	014	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
10	30	1	Male	014	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
10	72	1	Male	004	Total Protein	Sample	
				<i>Comment: 1+ Hemolysis</i>			
10	72	1	Male	004	Albumin	Sample	
				<i>Comment: 1+ Hemolysis</i>			
10	72	1	Male	005	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
10	72	1	Male	005	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
11	-4	1	Male	014	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
11	-4	1	Male	014	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
11	-4	1	Male	005	Globulin	Sample	
				<i>Comment: 1+ Hemolysis</i>			
11	-4	1	Male	005	Alb/Glo Ratio	Sample	
				<i>Comment: 1+ Hemolysis</i>			
11	30	1	Male	014	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
11	72	1	Male	004	Globulin	Sample	
				<i>Comment: 1+ Hemolysis</i>			
11	72	1	Male	005	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
12	-4	1	Male	014	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
12	-4	1	Male	005	Cholesterol	Sample	
				<i>Comment: 1+ Hemolysis</i>			
12	30	1	Male	014	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
12	30	1	Male	014	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
12	72	1	Male	004	Alb/Glo Ratio	Sample	
				<i>Comment: 1+ Hemolysis</i>			
12	72	1	Male	005	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
13	-4	1	Male	014	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
13	-4	1	Male	005	Triglyceride	Sample	
				<i>Comment: 1+ Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
13	30	1	Male	014	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
13	72	1	Male	004	Cholesterol	Sample	
				<i>Comment: 1+ Hemolysis</i>			
13	72	1	Male	004	Triglyceride	Sample	
				<i>Comment: 1+ Hemolysis</i>			
13	72	1	Male	005	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
13	72	1	Male	005	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
14	-4	2	Male	011	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
14	-4	2	Male	011	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
14	-4	2	Male	012	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
14	-4	2	Male	012	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
14	-4	2	Male	013	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
14	-4	2	Male	013	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
14	-4	2	Male	015	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
14	-4	2	Male	015	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
15	-4	2	Male	011	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
15	-4	2	Male	012	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
15	-4	2	Male	013	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
15	-4	2	Male	015	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
16	-4	2	Male	011	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
16	-4	2	Male	011	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
16	-4	2	Male	012	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
16	-4	2	Male	012	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
16	-4	2	Male	013	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
16	-4	2	Male	013	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
16	-4	2	Male	015	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
16	-4	2	Male	015	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
17	-4	2	Male	011	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
17	-4	2	Male	012	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
17	-4	2	Male	013	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
17	-4	2	Male	015	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
18	-4	2	Male	011	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
18	-4	2	Male	011	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
18	-4	2	Male	012	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
18	-4	2	Male	012	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
18	-4	2	Male	013	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
18	-4	2	Male	013	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
18	-4	2	Male	001	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
18	-4	2	Male	015	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
18	-4	2	Male	015	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
18	30	2	Male	011	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
18	30	2	Male	012	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
18	30	2	Male	013	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
18	72	2	Male	001	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
18	72	2	Male	015	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
19	-4	2	Male	011	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
19	-4	2	Male	011	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
19	-4	2	Male	012	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
19	-4	2	Male	012	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
19	-4	2	Male	013	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
19	-4	2	Male	013	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
19	-4	2	Male	015	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
19	-4	2	Male	015	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
20	-4	2	Male	011	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
20	-4	2	Male	012	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
20	-4	2	Male	013	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
20	-4	2	Male	015	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
21	-4	2	Male	011	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
21	-4	2	Male	011	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
21	-4	2	Male	012	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
21	-4	2	Male	012	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
21	-4	2	Male	013	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
21	-4	2	Male	013	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
21	-4	2	Male	015	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
21	-4	2	Male	015	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
22	-4	2	Male	011	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
22	-4	2	Male	012	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
22	-4	2	Male	013	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
22	-4	2	Male	015	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
23	-4	2	Male	011	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
23	-4	2	Male	012	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
23	-4	2	Male	013	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
23	-4	2	Male	015	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
24	-4	2	Male	011	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
24	-4	2	Male	011	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
24	-4	2	Male	012	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
24	-4	2	Male	012	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
24	-4	2	Male	013	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
24	-4	2	Male	013	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
24	-4	2	Male	015	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
24	-4	2	Male	015	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
25	-4	2	Male	011	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
25	-4	2	Male	012	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
25	-4	2	Male	013	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
25	-4	2	Male	015	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
26	-4	2	Male	011	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
26	-4	2	Male	012	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
26	-4	2	Male	013	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
26	-4	2	Male	015	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
27	-4	3	Male	021	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
27	-4	3	Male	021	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
27	-4	3	Male	022	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
27	-4	3	Male	022	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
27	-4	3	Male	023	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
27	-4	3	Male	023	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
27	17	3	Male	021	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
27	17	3	Male	022	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
27	17	3	Male	023	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
27	72	3	Male	024	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
28	-4	3	Male	021	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
28	-4	3	Male	022	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
28	-4	3	Male	023	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
28	17	3	Male	021	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
28	17	3	Male	021	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
28	17	3	Male	022	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
28	17	3	Male	022	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
28	17	3	Male	023	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
28	17	3	Male	023	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
28	72	3	Male	024	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
29	-4	3	Male	021	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
29	-4	3	Male	021	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
29	-4	3	Male	022	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
29	-4	3	Male	022	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
29	-4	3	Male	023	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
29	-4	3	Male	023	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
29	17	3	Male	021	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
29	17	3	Male	022	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
29	17	3	Male	023	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
29	72	3	Male	024	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
29	72	3	Male	024	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
30	-4	3	Male	021	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
30	-4	3	Male	022	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
30	-4	3	Male	023	Alkaline Phosphatase	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
30	-4	3	Male	023	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
30	17	3	Male	021	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
30	17	3	Male	021	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
30	17	3	Male	022	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
30	17	3	Male	022	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
30	17	3	Male	023	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
30	17	3	Male	023	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
30	72	3	Male	024	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
31	-4	3	Male	021	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
31	-4	3	Male	021	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
31	-4	3	Male	022	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
31	-4	3	Male	022	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
31	-4	3	Male	023	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
31	-4	3	Male	023	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
31	-4	3	Male	024	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
31	-4	3	Male	025	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
31	17	3	Male	021	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
31	17	3	Male	021	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
31	17	3	Male	022	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
31	17	3	Male	022	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
31	17	3	Male	023	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
31	17	3	Male	023	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
31	72	3	Male	024	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
31	72	3	Male	024	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
31	72	3	Male	024	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
31	72	3	Male	025	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
32	-4	3	Male	021	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
32	-4	3	Male	021	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
32	-4	3	Male	022	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
32	-4	3	Male	022	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
32	-4	3	Male	023	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
32	-4	3	Male	023	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
32	17	3	Male	021	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
32	17	3	Male	022	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
32	17	3	Male	023	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
32	72	3	Male	024	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
33	-4	3	Male	021	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
33	-4	3	Male	022	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
33	-4	3	Male	023	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
33	17	3	Male	021	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
33	17	3	Male	021	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
33	17	3	Male	022	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
33	17	3	Male	022	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
33	17	3	Male	023	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
33	17	3	Male	023	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
33	72	3	Male	024	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
34	-4	3	Male	021	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
34	-4	3	Male	021	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
34	-4	3	Male	022	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
34	-4	3	Male	022	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
34	-4	3	Male	023	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
34	-4	3	Male	023	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
34	17	3	Male	021	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
34	17	3	Male	022	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
34	17	3	Male	023	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
34	72	3	Male	024	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
34	72	3	Male	024	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
35	-4	3	Male	021	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
35	-4	3	Male	022	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
35	-4	3	Male	023	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
35	17	3	Male	021	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
35	17	3	Male	021	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
35	17	3	Male	022	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
35	17	3	Male	022	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
35	17	3	Male	023	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
35	17	3	Male	023	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
35	72	3	Male	024	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
36	-4	3	Male	021	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
36	-4	3	Male	022	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
36	-4	3	Male	023	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
36	17	3	Male	021	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
36	17	3	Male	022	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
36	17	3	Male	023	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
36	72	3	Male	024	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
36	72	3	Male	024	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
37	-4	3	Male	021	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
37	-4	3	Male	021	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
37	-4	3	Male	022	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
37	-4	3	Male	022	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
37	-4	3	Male	023	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
37	-4	3	Male	023	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
37	17	3	Male	021	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
37	17	3	Male	022	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
37	17	3	Male	023	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
37	72	3	Male	024	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
38	-4	3	Male	021	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
38	-4	3	Male	022	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
38	-4	3	Male	023	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
38	17	3	Male	021	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
38	17	3	Male	021	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
38	17	3	Male	022	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
38	17	3	Male	022	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
38	17	3	Male	023	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
38	17	3	Male	023	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
38	72	3	Male	024	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
39	-4	3	Male	021	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
39	-4	3	Male	022	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
39	-4	3	Male	023	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
39	17	3	Male	021	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
39	17	3	Male	022	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
39	17	3	Male	023	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
39	72	3	Male	024	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
39	72	3	Male	024	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
40	-4	4	Male	031	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
40	-4	4	Male	031	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
40	-4	4	Male	034	Blood Urea Nitrogen	Sample	
				<i>Comment: 1+ Hemolysis</i>			
40	-4	4	Male	034	Creatinine	Sample	
				<i>Comment: 1+ Hemolysis</i>			
40	17	4	Male	031	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
40	17	4	Male	033	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
40	72	4	Male	034	Blood Urea Nitrogen	Sample	
				<i>Comment: 2+ Hemolysis</i>			
41	-4	4	Male	031	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
41	-4	4	Male	034	Glucose	Sample	
				<i>Comment: 1+ Hemolysis</i>			
41	17	4	Male	031	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
41	17	4	Male	031	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
41	17	4	Male	033	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
41	17	4	Male	033	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
41	72	4	Male	034	Creatinine	Sample	
				<i>Comment: 2+ Hemolysis</i>			
42	-4	4	Male	031	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
42	-4	4	Male	031	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
42	-4	4	Male	034	AST	Sample	
				<i>Comment: 1+ Hemolysis</i>			
42	-4	4	Male	034	ALT	Sample	
				<i>Comment: 1+ Hemolysis</i>			
42	17	4	Male	031	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
42	17	4	Male	033	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
42	72	4	Male	034	Glucose	Sample	
				<i>Comment: 2+ Hemolysis</i>			
42	72	4	Male	034	AST	Sample	
				<i>Comment: 2+ Hemolysis</i>			
43	-4	4	Male	031	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
43	-4	4	Male	034	Alkaline Phosphatase	Sample	
				<i>Comment: 1+ Hemolysis</i>			
43	17	4	Male	031	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
43	17	4	Male	031	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
43	17	4	Male	033	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
43	17	4	Male	033	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
43	72	4	Male	034	ALT	Sample	
				<i>Comment: 2+ Hemolysis</i>			
44	-4	4	Male	031	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
44	-4	4	Male	031	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
44	-4	4	Male	032	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
44	-4	4	Male	033	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
44	-4	4	Male	034	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
44	-4	4	Male	034	Total Bilirubin	Sample	
				<i>Comment: 1+ Hemolysis</i>			
44	-4	4	Male	035	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
44	17	4	Male	031	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
44	17	4	Male	031	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
44	17	4	Male	032	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
44	17	4	Male	033	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
44	17	4	Male	033	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
44	72	4	Male	034	Alkaline Phosphatase	Sample	
				<i>Comment: 2+ Hemolysis</i>			
44	72	4	Male	034	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
44	72	4	Male	034	Total Bilirubin	Sample	
				<i>Comment: 2+ Hemolysis</i>			
44	72	4	Male	035	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
45	-4	4	Male	031	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
45	-4	4	Male	031	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
45	-4	4	Male	034	Sodium	Sample	
				<i>Comment: 1+ Hemolysis</i>			
45	-4	4	Male	034	Potassium	Sample	
				<i>Comment: 1+ Hemolysis</i>			
45	17	4	Male	031	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
45	17	4	Male	033	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
45	72	4	Male	034	Sodium	Sample	
				<i>Comment: 2+ Hemolysis</i>			
46	-4	4	Male	031	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
46	-4	4	Male	034	Chloride	Sample	
				<i>Comment: 1+ Hemolysis</i>			
46	17	4	Male	031	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
46	17	4	Male	031	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
46	17	4	Male	033	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
46	17	4	Male	033	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
46	72	4	Male	034	Potassium	Sample	
				<i>Comment: 2+ Hemolysis</i>			
47	-4	4	Male	031	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
47	-4	4	Male	031	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
47	-4	4	Male	034	Calcium	Sample	
				<i>Comment: 1+ Hemolysis</i>			
47	-4	4	Male	034	Phosphorus	Sample	
				<i>Comment: 1+ Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
47	17	4	Male	031	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
47	17	4	Male	033	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
47	72	4	Male	034	Chloride	Sample	
				<i>Comment: 2+ Hemolysis</i>			
47	72	4	Male	034	Calcium	Sample	
				<i>Comment: 2+ Hemolysis</i>			
48	-4	4	Male	031	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
48	-4	4	Male	034	Total Protein	Sample	
				<i>Comment: 1+ Hemolysis</i>			
48	17	4	Male	031	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
48	17	4	Male	031	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
48	17	4	Male	033	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
48	17	4	Male	033	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
48	72	4	Male	034	Phosphorus	Sample	
				<i>Comment: 2+ Hemolysis</i>			
49	-4	4	Male	031	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
49	-4	4	Male	034	Albumin	Sample	
				<i>Comment: 1+ Hemolysis</i>			
49	17	4	Male	031	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
49	17	4	Male	033	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
49	72	4	Male	034	Total Protein	Sample	
				<i>Comment: 2+ Hemolysis</i>			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
49	72	4	Male	034	Albumin	Sample	
				<i>Comment: 2+ Hemolysis</i>			
50	-4	4	Male	031	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
50	-4	4	Male	031	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
50	-4	4	Male	034	Globulin	Sample	
				<i>Comment: 1+ Hemolysis</i>			
50	-4	4	Male	034	Alb/Glo Ratio	Sample	
				<i>Comment: 1+ Hemolysis</i>			
50	17	4	Male	031	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
50	17	4	Male	033	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
50	72	4	Male	034	Globulin	Sample	
				<i>Comment: 2+ Hemolysis</i>			
51	-4	4	Male	031	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
51	-4	4	Male	034	Cholesterol	Sample	
				<i>Comment: 1+ Hemolysis</i>			
51	17	4	Male	031	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
51	17	4	Male	031	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
51	17	4	Male	033	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
51	17	4	Male	033	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
51	72	4	Male	034	Alb/Glo Ratio	Sample	
				<i>Comment: 2+ Hemolysis</i>			
52	-4	4	Male	031	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
52	-4	4	Male	034	Triglyceride	Sample	
				<i>Comment: 1+ Hemolysis</i>			
52	17	4	Male	031	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
52	17	4	Male	033	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
52	72	4	Male	034	Cholesterol	Sample	
				<i>Comment: 2+ Hemolysis</i>			
52	72	4	Male	034	Triglyceride	Sample	
				<i>Comment: 2+ Hemolysis</i>			
53	-2	1	Female	006	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
53	-2	1	Female	006	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
53	-2	1	Female	007	Blood Urea Nitrogen	Sample	
				<i>Comment: 1+ Hemolysis</i>			
53	-2	1	Female	007	Creatinine	Sample	
				<i>Comment: 1+ Hemolysis</i>			
53	-2	1	Female	008	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
53	-2	1	Female	008	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
53	-2	1	Female	009	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
53	-2	1	Female	009	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
53	-2	1	Female	010	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
53	-2	1	Female	010	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
53	72	1	Female	009	Blood Urea Nitrogen	Sample	
				<i>Comment: 1+ Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
53	72	1	Female	010	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
54	-2	1	Female	006	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
54	-2	1	Female	007	Glucose	Sample	
				<i>Comment: 1+ Hemolysis</i>			
54	-2	1	Female	008	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
54	-2	1	Female	009	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
54	-2	1	Female	010	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
54	72	1	Female	009	Creatinine	Sample	
				<i>Comment: 1+ Hemolysis</i>			
54	72	1	Female	010	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
55	-2	1	Female	006	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
55	-2	1	Female	007	AST	Sample	
				<i>Comment: 1+ Hemolysis</i>			
55	-2	1	Female	008	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
55	-2	1	Female	009	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
55	-2	1	Female	010	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
55	72	1	Female	009	Glucose	Sample	
				<i>Comment: 1+ Hemolysis</i>			
55	72	1	Female	009	AST	Sample	
				<i>Comment: 1+ Hemolysis</i>			
55	72	1	Female	010	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
55	72	1	Female	010	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
56	-2	1	Female	006	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
56	-2	1	Female	006	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
56	-2	1	Female	007	ALT	Sample	
				<i>Comment: 1+ Hemolysis</i>			
56	-2	1	Female	007	Alkaline Phosphatase	Sample	
				<i>Comment: 1+ Hemolysis</i>			
56	-2	1	Female	008	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
56	-2	1	Female	008	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
56	-2	1	Female	009	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
56	-2	1	Female	009	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
56	-2	1	Female	010	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
56	-2	1	Female	010	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
56	72	1	Female	009	ALT	Sample	
				<i>Comment: 1+ Hemolysis</i>			
56	72	1	Female	010	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
57	-2	1	Female	006	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
57	-2	1	Female	006	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
57	-2	1	Female	007	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
57	-2	1	Female	007	Total Bilirubin	Sample	
				<i>Comment: 1+ Hemolysis</i>			
57	-2	1	Female	008	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
57	-2	1	Female	008	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
57	-2	1	Female	009	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
57	-2	1	Female	009	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
57	-2	1	Female	010	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
57	-2	1	Female	010	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
57	30	1	Female	006	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
57	30	1	Female	007	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
57	30	1	Female	008	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
57	72	1	Female	009	Alkaline Phosphatase	Sample	
				<i>Comment: 1+ Hemolysis</i>			
57	72	1	Female	009	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
57	72	1	Female	009	Total Bilirubin	Sample	
				<i>Comment: 1+ Hemolysis</i>			
57	72	1	Female	010	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
57	72	1	Female	010	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
57	72	1	Female	010	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
58	-2	1	Female	006	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
58	-2	1	Female	006	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
58	-2	1	Female	007	Sodium	Sample	
				<i>Comment: 1+ Hemolysis</i>			
58	-2	1	Female	007	Potassium	Sample	
				<i>Comment: 1+ Hemolysis</i>			
58	-2	1	Female	008	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
58	-2	1	Female	008	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
58	-2	1	Female	009	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
58	-2	1	Female	009	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
58	-2	1	Female	010	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
58	-2	1	Female	010	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
58	72	1	Female	009	Sodium	Sample	
				<i>Comment: 1+ Hemolysis</i>			
58	72	1	Female	010	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
59	-2	1	Female	006	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
59	-2	1	Female	007	Chloride	Sample	
				<i>Comment: 1+ Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
59	-2	1	Female	008	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
59	-2	1	Female	009	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
59	-2	1	Female	010	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
59	72	1	Female	009	Potassium	Sample	
				<i>Comment: 1+ Hemolysis</i>			
59	72	1	Female	010	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
60	-2	1	Female	006	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
60	-2	1	Female	007	Calcium	Sample	
				<i>Comment: 1+ Hemolysis</i>			
60	-2	1	Female	008	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
60	-2	1	Female	009	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
60	-2	1	Female	010	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
60	72	1	Female	009	Chloride	Sample	
				<i>Comment: 1+ Hemolysis</i>			
60	72	1	Female	009	Calcium	Sample	
				<i>Comment: 1+ Hemolysis</i>			
60	72	1	Female	010	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
60	72	1	Female	010	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
61	-2	1	Female	006	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
61	-2	1	Female	006	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
61	-2	1	Female	007	Phosphorus	Sample	
				<i>Comment: 1+ Hemolysis</i>			
61	-2	1	Female	007	Total Protein	Sample	
				<i>Comment: 1+ Hemolysis</i>			
61	-2	1	Female	008	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
61	-2	1	Female	008	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
61	-2	1	Female	009	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
61	-2	1	Female	009	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
61	-2	1	Female	010	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
61	-2	1	Female	010	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
61	72	1	Female	009	Phosphorus	Sample	
				<i>Comment: 1+ Hemolysis</i>			
61	72	1	Female	010	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
62	-2	1	Female	006	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
62	-2	1	Female	007	Albumin	Sample	
				<i>Comment: 1+ Hemolysis</i>			
62	-2	1	Female	008	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
62	-2	1	Female	009	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
62	-2	1	Female	010	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
62	72	1	Female	009	Total Protein	Sample	
				<i>Comment: 1+ Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
62	72	1	Female	009	Albumin	Sample	
				<i>Comment: 1+ Hemolysis</i>			
62	72	1	Female	010	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
62	72	1	Female	010	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
63	-2	1	Female	006	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
63	-2	1	Female	006	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
63	-2	1	Female	007	Globulin	Sample	
				<i>Comment: 1+ Hemolysis</i>			
63	-2	1	Female	007	Alb/Glo Ratio	Sample	
				<i>Comment: 1+ Hemolysis</i>			
63	-2	1	Female	008	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
63	-2	1	Female	008	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
63	-2	1	Female	009	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
63	-2	1	Female	009	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
63	-2	1	Female	010	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
63	-2	1	Female	010	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
63	72	1	Female	009	Globulin	Sample	
				<i>Comment: 1+ Hemolysis</i>			
63	72	1	Female	010	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
64	-2	1	Female	006	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
64	-2	1	Female	007	Cholesterol	Sample	
				<i>Comment: 1+ Hemolysis</i>			
64	-2	1	Female	008	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
64	-2	1	Female	009	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
64	-2	1	Female	010	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
64	72	1	Female	009	Alb/Glo Ratio	Sample	
				<i>Comment: 1+ Hemolysis</i>			
64	72	1	Female	010	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
65	-2	1	Female	006	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
65	-2	1	Female	007	Triglyceride	Sample	
				<i>Comment: 1+ Hemolysis</i>			
65	-2	1	Female	008	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
65	-2	1	Female	009	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
65	-2	1	Female	010	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
65	72	1	Female	009	Cholesterol	Sample	
				<i>Comment: 1+ Hemolysis</i>			
65	72	1	Female	009	Triglyceride	Sample	
				<i>Comment: 1+ Hemolysis</i>			
65	72	1	Female	010	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
65	72	1	Female	010	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
66	-2	2	Female	016	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
66	-2	2	Female	016	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
66	-2	2	Female	017	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
66	-2	2	Female	017	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
66	-2	2	Female	018	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
66	-2	2	Female	018	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
66	-2	2	Female	019	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
66	-2	2	Female	019	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
66	-2	2	Female	020	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
66	-2	2	Female	020	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
66	30	2	Female	018	Blood Urea Nitrogen	Sample	
				<i>Comment: 1+ Hemolysis</i>			
66	72	2	Female	019	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
66	72	2	Female	020	Blood Urea Nitrogen	Sample	
				<i>Comment: 1+ Hemolysis</i>			
67	-2	2	Female	016	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
67	-2	2	Female	017	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
67	-2	2	Female	018	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
67	-2	2	Female	019	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
67	-2	2	Female	020	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
67	30	2	Female	018	Creatinine	Sample	
				<i>Comment: 1+ Hemolysis</i>			
67	30	2	Female	018	Glucose	Sample	
				<i>Comment: 1+ Hemolysis</i>			
67	72	2	Female	019	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
67	72	2	Female	020	Creatinine	Sample	
				<i>Comment: 1+ Hemolysis</i>			
68	-2	2	Female	016	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
68	-2	2	Female	017	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
68	-2	2	Female	018	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
68	-2	2	Female	019	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
68	-2	2	Female	020	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
68	30	2	Female	018	AST	Sample	
				<i>Comment: 1+ Hemolysis</i>			
68	72	2	Female	019	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
68	72	2	Female	019	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
68	72	2	Female	020	Glucose	Sample	
				<i>Comment: 1+ Hemolysis</i>			
68	72	2	Female	020	AST	Sample	
				<i>Comment: 1+ Hemolysis</i>			
69	-2	2	Female	016	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
69	-2	2	Female	016	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
69	-2	2	Female	017	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
69	-2	2	Female	017	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
69	-2	2	Female	018	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
69	-2	2	Female	018	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
69	-2	2	Female	019	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
69	-2	2	Female	019	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
69	-2	2	Female	020	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
69	-2	2	Female	020	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
69	30	2	Female	018	ALT	Sample	
				<i>Comment: 1+ Hemolysis</i>			
69	72	2	Female	019	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
69	72	2	Female	020	ALT	Sample	
				<i>Comment: 1+ Hemolysis</i>			
70	-2	2	Female	016	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
70	-2	2	Female	016	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
70	-2	2	Female	017	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
70	-2	2	Female	017	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
70	-2	2	Female	018	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
70	-2	2	Female	018	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
70	-2	2	Female	019	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
70	-2	2	Female	019	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
70	-2	2	Female	020	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
70	-2	2	Female	020	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
70	30	2	Female	016	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
70	30	2	Female	017	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
70	30	2	Female	018	Alkaline Phosphatase	Sample	
				<i>Comment: 1+ Hemolysis</i>			
70	30	2	Female	018	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
70	30	2	Female	018	Total Bilirubin	Sample	
				<i>Comment: 1+ Hemolysis</i>			
70	72	2	Female	019	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
70	72	2	Female	019	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
70	72	2	Female	019	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
70	72	2	Female	020	Alkaline Phosphatase	Sample	
				<i>Comment: 1+ Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
70	72	2	Female	020	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
70	72	2	Female	020	Total Bilirubin	Sample	
				<i>Comment: 1+ Hemolysis</i>			
71	-2	2	Female	016	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
71	-2	2	Female	016	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
71	-2	2	Female	017	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
71	-2	2	Female	017	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
71	-2	2	Female	018	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
71	-2	2	Female	018	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
71	-2	2	Female	019	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
71	-2	2	Female	019	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
71	-2	2	Female	020	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
71	-2	2	Female	020	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
71	30	2	Female	018	Sodium	Sample	
				<i>Comment: 1+ Hemolysis</i>			
71	72	2	Female	019	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
71	72	2	Female	020	Sodium	Sample	
				<i>Comment: 1+ Hemolysis</i>			
72	-2	2	Female	016	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
72	-2	2	Female	017	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
72	-2	2	Female	018	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
72	-2	2	Female	019	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
72	-2	2	Female	020	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
72	30	2	Female	018	Potassium	Sample	
				<i>Comment: 1+ Hemolysis</i>			
72	30	2	Female	018	Chloride	Sample	
				<i>Comment: 1+ Hemolysis</i>			
72	72	2	Female	019	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
72	72	2	Female	020	Potassium	Sample	
				<i>Comment: 1+ Hemolysis</i>			
73	-2	2	Female	016	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
73	-2	2	Female	017	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
73	-2	2	Female	018	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
73	-2	2	Female	019	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
73	-2	2	Female	020	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
73	30	2	Female	018	Calcium	Sample	
				<i>Comment: 1+ Hemolysis</i>			
73	72	2	Female	019	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
73	72	2	Female	019	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
73	72	2	Female	020	Chloride	Sample	
				<i>Comment: 1+ Hemolysis</i>			
73	72	2	Female	020	Calcium	Sample	
				<i>Comment: 1+ Hemolysis</i>			
74	-2	2	Female	016	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
74	-2	2	Female	016	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
74	-2	2	Female	017	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
74	-2	2	Female	017	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
74	-2	2	Female	018	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
74	-2	2	Female	018	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
74	-2	2	Female	019	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
74	-2	2	Female	019	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
74	-2	2	Female	020	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
74	-2	2	Female	020	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
74	30	2	Female	018	Phosphorus	Sample	
				<i>Comment: 1+ Hemolysis</i>			
74	72	2	Female	019	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
74	72	2	Female	020	Phosphorus	Sample	
				<i>Comment: 1+ Hemolysis</i>			
75	-2	2	Female	016	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
75	-2	2	Female	017	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
75	-2	2	Female	018	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
75	-2	2	Female	019	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
75	-2	2	Female	020	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
75	30	2	Female	018	Total Protein	Sample	
				<i>Comment: 1+ Hemolysis</i>			
75	30	2	Female	018	Albumin	Sample	
				<i>Comment: 1+ Hemolysis</i>			
75	72	2	Female	019	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
75	72	2	Female	019	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
75	72	2	Female	020	Total Protein	Sample	
				<i>Comment: 1+ Hemolysis</i>			
75	72	2	Female	020	Albumin	Sample	
				<i>Comment: 1+ Hemolysis</i>			
76	-2	2	Female	016	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
76	-2	2	Female	016	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
76	-2	2	Female	017	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
76	-2	2	Female	017	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
76	-2	2	Female	018	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
76	-2	2	Female	018	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
76	-2	2	Female	019	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
76	-2	2	Female	019	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
76	-2	2	Female	020	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
76	-2	2	Female	020	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
76	30	2	Female	018	Globulin	Sample	
				<i>Comment: 1+ Hemolysis</i>			
76	72	2	Female	019	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
76	72	2	Female	020	Globulin	Sample	
				<i>Comment: 1+ Hemolysis</i>			
77	-2	2	Female	016	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
77	-2	2	Female	017	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
77	-2	2	Female	018	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
77	-2	2	Female	019	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
77	-2	2	Female	020	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
77	30	2	Female	018	Alb/Glo Ratio	Sample	
				<i>Comment: 1+ Hemolysis</i>			
77	30	2	Female	018	Cholesterol	Sample	
				<i>Comment: 1+ Hemolysis</i>			
77	72	2	Female	019	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
77	72	2	Female	020	Alb/Glo Ratio	Sample	
				<i>Comment: 1+ Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
78	-2	2	Female	016	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
78	-2	2	Female	017	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
78	-2	2	Female	018	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
78	-2	2	Female	019	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
78	-2	2	Female	020	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
78	30	2	Female	018	Triglyceride	Sample	
				<i>Comment: 1+ Hemolysis</i>			
78	72	2	Female	019	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
78	72	2	Female	019	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
78	72	2	Female	020	Cholesterol	Sample	
				<i>Comment: 1+ Hemolysis</i>			
78	72	2	Female	020	Triglyceride	Sample	
				<i>Comment: 1+ Hemolysis</i>			
79	-2	3	Female	026	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
79	-2	3	Female	026	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
79	-2	3	Female	027	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
79	-2	3	Female	027	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
79	-2	3	Female	028	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
79	-2	3	Female	028	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
79	-2	3	Female	029	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
79	-2	3	Female	029	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
79	-2	3	Female	030	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
79	-2	3	Female	030	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
79	17	3	Female	026	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
79	17	3	Female	027	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
79	17	3	Female	028	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
79	72	3	Female	030	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
80	-2	3	Female	026	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
80	-2	3	Female	027	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
80	-2	3	Female	028	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
80	-2	3	Female	029	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
80	-2	3	Female	030	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
80	17	3	Female	026	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
80	17	3	Female	026	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
80	17	3	Female	027	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
80	17	3	Female	027	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
80	17	3	Female	028	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
80	17	3	Female	028	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
80	72	3	Female	030	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
81	-2	3	Female	026	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
81	-2	3	Female	027	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
81	-2	3	Female	028	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
81	-2	3	Female	029	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
81	-2	3	Female	030	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
81	17	3	Female	026	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
81	17	3	Female	027	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
81	17	3	Female	028	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
81	72	3	Female	030	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
81	72	3	Female	030	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
82	-2	3	Female	026	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
82	-2	3	Female	026	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
82	-2	3	Female	027	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
82	-2	3	Female	027	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
82	-2	3	Female	028	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
82	-2	3	Female	028	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
82	-2	3	Female	029	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
82	-2	3	Female	029	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
82	-2	3	Female	030	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
82	-2	3	Female	030	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
82	17	3	Female	026	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
82	17	3	Female	026	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
82	17	3	Female	027	ALT	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
82	17	3	Female	027	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
82	17	3	Female	027	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
82	17	3	Female	028	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
82	17	3	Female	028	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
82	72	3	Female	030	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
83	-2	3	Female	026	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
83	-2	3	Female	026	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
83	-2	3	Female	027	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
83	-2	3	Female	027	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
83	-2	3	Female	028	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
83	-2	3	Female	028	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
83	-2	3	Female	029	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
83	-2	3	Female	029	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
83	-2	3	Female	030	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
83	-2	3	Female	030	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
83	17	3	Female	026	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
83	17	3	Female	026	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
83	17	3	Female	027	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
83	17	3	Female	027	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
83	17	3	Female	028	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
83	17	3	Female	028	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
83	72	3	Female	029	Total Bilirubin	Replacement	<0.15
				<i>Comment:</i> Result below technical limit.			
83	72	3	Female	030	Alkaline Phosphatase	Sample	
				<i>Comment:</i> Trace Hemolysis			
83	72	3	Female	030	Total Bilirubin	Replacement	<0.15
				<i>Comment:</i> Result below technical limit.			
83	72	3	Female	030	Total Bilirubin	Sample	
				<i>Comment:</i> Trace Hemolysis			
84	-2	3	Female	026	Sodium	Sample	
				<i>Comment:</i> Trace Hemolysis			
84	-2	3	Female	026	Potassium	Sample	
				<i>Comment:</i> Trace Hemolysis			
84	-2	3	Female	027	Sodium	Sample	
				<i>Comment:</i> Trace Hemolysis			
84	-2	3	Female	027	Potassium	Sample	
				<i>Comment:</i> Trace Hemolysis			
84	-2	3	Female	028	Sodium	Sample	
				<i>Comment:</i> Trace Hemolysis			
84	-2	3	Female	028	Potassium	Sample	
				<i>Comment:</i> Trace Hemolysis			
84	-2	3	Female	029	Sodium	Sample	
				<i>Comment:</i> Trace Hemolysis			
84	-2	3	Female	029	Potassium	Sample	
				<i>Comment:</i> Trace Hemolysis			
84	-2	3	Female	030	Sodium	Sample	
				<i>Comment:</i> Trace Hemolysis			
84	-2	3	Female	030	Potassium	Sample	
				<i>Comment:</i> Trace Hemolysis			
84	17	3	Female	026	Sodium	Sample	
				<i>Comment:</i> Trace Hemolysis			
84	17	3	Female	027	Sodium	Sample	
				<i>Comment:</i> Trace Hemolysis			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
84	17	3	Female	028	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
84	72	3	Female	030	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
85	-2	3	Female	026	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
85	-2	3	Female	027	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
85	-2	3	Female	028	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
85	-2	3	Female	029	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
85	-2	3	Female	030	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
85	17	3	Female	026	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
85	17	3	Female	026	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
85	17	3	Female	027	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
85	17	3	Female	027	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
85	17	3	Female	028	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
85	17	3	Female	028	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
85	72	3	Female	030	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
86	-2	3	Female	026	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
86	-2	3	Female	027	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
86	-2	3	Female	028	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
86	-2	3	Female	029	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
86	-2	3	Female	030	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
86	17	3	Female	026	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
86	17	3	Female	027	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
86	17	3	Female	028	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
86	72	3	Female	030	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
86	72	3	Female	030	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
87	-2	3	Female	026	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
87	-2	3	Female	026	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
87	-2	3	Female	027	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
87	-2	3	Female	027	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
87	-2	3	Female	028	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
87	-2	3	Female	028	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
87	-2	3	Female	029	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
87	-2	3	Female	029	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
87	-2	3	Female	030	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
87	-2	3	Female	030	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
87	17	3	Female	026	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
87	17	3	Female	026	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
87	17	3	Female	027	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
87	17	3	Female	027	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
87	17	3	Female	028	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
87	17	3	Female	028	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
87	72	3	Female	030	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
88	-2	3	Female	026	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
88	-2	3	Female	027	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
88	-2	3	Female	028	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
88	-2	3	Female	029	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
88	-2	3	Female	030	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
88	17	3	Female	026	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
88	17	3	Female	027	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
88	17	3	Female	028	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
88	72	3	Female	030	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
88	72	3	Female	030	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
89	-2	3	Female	026	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
89	-2	3	Female	026	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
89	-2	3	Female	027	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
89	-2	3	Female	027	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
89	-2	3	Female	028	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
89	-2	3	Female	028	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
89	-2	3	Female	029	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
89	-2	3	Female	029	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
89	-2	3	Female	030	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
89	-2	3	Female	030	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
89	17	3	Female	026	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
89	17	3	Female	027	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
89	17	3	Female	028	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
89	72	3	Female	030	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
90	-2	3	Female	026	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
90	-2	3	Female	027	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
90	-2	3	Female	028	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
90	-2	3	Female	029	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
90	-2	3	Female	030	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
90	17	3	Female	026	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
90	17	3	Female	026	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
90	17	3	Female	027	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
90	17	3	Female	027	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
90	17	3	Female	028	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
90	17	3	Female	028	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
90	72	3	Female	030	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
91	-2	3	Female	026	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
91	-2	3	Female	027	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
91	-2	3	Female	028	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
91	-2	3	Female	029	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
91	-2	3	Female	030	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
91	17	3	Female	026	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
91	17	3	Female	027	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
91	17	3	Female	028	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
91	72	3	Female	030	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
91	72	3	Female	030	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
92	-2	4	Female	036	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
92	-2	4	Female	036	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
92	-2	4	Female	037	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
92	-2	4	Female	037	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
92	-2	4	Female	038	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
92	-2	4	Female	038	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
92	-2	4	Female	039	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
92	-2	4	Female	039	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
92	-2	4	Female	040	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
92	-2	4	Female	040	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
92	17	4	Female	036	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
92	17	4	Female	037	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
92	72	4	Female	040	Blood Urea Nitrogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
93	-2	4	Female	036	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
93	-2	4	Female	037	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
93	-2	4	Female	038	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
93	-2	4	Female	039	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
93	-2	4	Female	040	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
93	17	4	Female	036	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
93	17	4	Female	036	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
93	17	4	Female	037	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
93	17	4	Female	037	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
93	72	4	Female	040	Creatinine	Sample	
				<i>Comment: Trace Hemolysis</i>			
94	-2	4	Female	036	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
94	-2	4	Female	037	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
94	-2	4	Female	038	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
94	-2	4	Female	039	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
94	-2	4	Female	040	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
94	17	4	Female	036	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
94	17	4	Female	037	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
94	17	4	Female	039	AST	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
94	72	4	Female	040	Glucose	Sample	
				<i>Comment: Trace Hemolysis</i>			
94	72	4	Female	040	AST	Sample	
				<i>Comment: Trace Hemolysis</i>			
95	-2	4	Female	036	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
95	-2	4	Female	036	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
95	-2	4	Female	037	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
95	-2	4	Female	037	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
95	-2	4	Female	038	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
95	-2	4	Female	038	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
95	-2	4	Female	039	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
95	-2	4	Female	039	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
95	-2	4	Female	040	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
95	-2	4	Female	040	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
95	17	4	Female	036	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
95	17	4	Female	036	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
95	17	4	Female	037	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
95	17	4	Female	037	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
95	17	4	Female	039	ALT	Result	
				<i>Comment: Confirmed by Repeat Analysis</i>			
95	72	4	Female	040	ALT	Sample	
				<i>Comment: Trace Hemolysis</i>			
96	-2	4	Female	036	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
96	-2	4	Female	036	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
96	-2	4	Female	037	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
96	-2	4	Female	037	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
96	-2	4	Female	038	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
96	-2	4	Female	038	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
96	-2	4	Female	039	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
96	-2	4	Female	039	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
96	-2	4	Female	040	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
96	-2	4	Female	040	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
96	17	4	Female	036	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
96	17	4	Female	036	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
96	17	4	Female	037	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
96	17	4	Female	037	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
96	17	4	Female	039	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
96	72	4	Female	038	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
96	72	4	Female	040	Alkaline Phosphatase	Sample	
				<i>Comment: Trace Hemolysis</i>			
96	72	4	Female	040	Total Bilirubin	Replacement	<0.15
				<i>Comment: Result below technical limit.</i>			
96	72	4	Female	040	Total Bilirubin	Sample	
				<i>Comment: Trace Hemolysis</i>			
97	-2	4	Female	036	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
97	-2	4	Female	036	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
97	-2	4	Female	037	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
97	-2	4	Female	037	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
97	-2	4	Female	038	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
97	-2	4	Female	038	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
97	-2	4	Female	039	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
97	-2	4	Female	039	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
97	-2	4	Female	040	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
97	-2	4	Female	040	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
97	17	4	Female	036	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
97	17	4	Female	037	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
97	72	4	Female	040	Sodium	Sample	
				<i>Comment: Trace Hemolysis</i>			
98	-2	4	Female	036	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
98	-2	4	Female	037	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
98	-2	4	Female	038	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
98	-2	4	Female	039	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
98	-2	4	Female	040	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
98	17	4	Female	036	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
98	17	4	Female	036	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
98	17	4	Female	037	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
98	17	4	Female	037	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
98	72	4	Female	040	Potassium	Sample	
				<i>Comment: Trace Hemolysis</i>			
99	-2	4	Female	036	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
99	-2	4	Female	037	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
99	-2	4	Female	038	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
99	-2	4	Female	039	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
99	-2	4	Female	040	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
99	17	4	Female	036	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
99	17	4	Female	037	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
99	72	4	Female	040	Chloride	Sample	
				<i>Comment: Trace Hemolysis</i>			
99	72	4	Female	040	Calcium	Sample	
				<i>Comment: Trace Hemolysis</i>			
100	-2	4	Female	036	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
100	-2	4	Female	036	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
100	-2	4	Female	037	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
100	-2	4	Female	037	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
100	-2	4	Female	038	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
100	-2	4	Female	038	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
100	-2	4	Female	039	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
100	-2	4	Female	039	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
100	-2	4	Female	040	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
100	-2	4	Female	040	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
100	17	4	Female	036	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
100	17	4	Female	036	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
100	17	4	Female	037	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
100	17	4	Female	037	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
100	72	4	Female	040	Phosphorus	Sample	
				<i>Comment: Trace Hemolysis</i>			
<span style="border: 1px solid black; padding: 0 2px;">Pr</span>	-2	4	Female	036	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
<span style="border: 1px solid black; padding: 0 2px;">Pr</span>	-2	4	Female	037	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
<span style="border: 1px solid black; padding: 0 2px;">Pr</span>	-2	4	Female	038	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
<span style="border: 1px solid black; padding: 0 2px;">Pr</span>	-2	4	Female	039	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
<span style="border: 1px solid black; padding: 0 2px;">Pr</span>	-2	4	Female	040	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
<span style="border: 1px solid black; padding: 0 2px;">Pr</span>	17	4	Female	036	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
<u>Pr</u>	17	4	Female	037	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
<u>Pr</u>	72	4	Female	040	Total Protein	Sample	
				<i>Comment: Trace Hemolysis</i>			
<u>Pr</u>	72	4	Female	040	Albumin	Sample	
				<i>Comment: Trace Hemolysis</i>			
102	-2	4	Female	036	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
102	-2	4	Female	036	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
102	-2	4	Female	037	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
102	-2	4	Female	037	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
102	-2	4	Female	038	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
102	-2	4	Female	038	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
102	-2	4	Female	039	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
102	-2	4	Female	039	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
102	-2	4	Female	040	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
102	-2	4	Female	040	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
102	17	4	Female	036	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
102	17	4	Female	037	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			
102	72	4	Female	040	Globulin	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
103	-2	4	Female	036	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
103	-2	4	Female	037	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
103	-2	4	Female	038	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
103	-2	4	Female	039	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
103	-2	4	Female	040	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
103	17	4	Female	036	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
103	17	4	Female	036	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
103	17	4	Female	037	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
103	17	4	Female	037	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
103	72	4	Female	040	Alb/Glo Ratio	Sample	
				<i>Comment: Trace Hemolysis</i>			
104	-2	4	Female	036	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
104	-2	4	Female	037	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
104	-2	4	Female	038	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
104	-2	4	Female	039	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
104	-2	4	Female	040	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
104	17	4	Female	036	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			



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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

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Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
104	17	4	Female	037	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			
104	72	4	Female	040	Cholesterol	Sample	
				<i>Comment: Trace Hemolysis</i>			
104	72	4	Female	040	Triglyceride	Sample	
				<i>Comment: Trace Hemolysis</i>			

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

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Key Page**General Footnotes**

Provantis version 10.1.0.1

"." indicates Not Applicable

Statistical significance indicated on a group with an N &lt; 3 is not valid

**Replacement Values**

<u>Value</u>	<u>Description</u>
<0.15	<0.15

**Measurement Descriptions**

<u>Headings Used</u>	<u>Description</u>
Blood Urea Nitrogen	Blood Urea Nitrogen
Creatinine	Creatinine
Glucose	Glucose
AST	Aspartate Aminotransferase
ALT	Alanine Aminotransferase
Alkaline Phosphatase	Alkaline Phosphatase
Total Bilirubin	Total Bilirubin
Sodium	Sodium
Potassium	Potassium
Chloride	Chloride
Calcium	Calcium
Phosphorus	Phosphorus
Total Protein	Total Protein
Albumin	Albumin
Globulin	Globulin
Alb/Glo Ratio	Albumin/Globulin Ratio
Cholesterol	Cholesterol
Triglyceride	Triglyceride

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

---

Key Page**Unit Descriptions**

<u>Headings Used</u>	<u>Description</u>
g/dL	g/dL
mg/dL	mg/dL
mmol/L	mmol/L
U/L	U/L

**Measurement/Statistics**

<u>Measurement</u>	<u>Descriptive</u>
Blood Urea Nitrogen	Mean Standard Deviation Count (N)
Creatinine	Mean Standard Deviation Count (N)
Glucose	Mean Standard Deviation Count (N)
AST	Mean Standard Deviation Count (N)
ALT	Mean Standard Deviation Count (N)
Alkaline Phosphatase	Mean Standard Deviation Count (N)
Total Bilirubin	Count (N)

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

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Key Page**Measurement/Statistics (Continued)**

<u>Measurement</u>	<u>Descriptive</u>
Sodium	Mean
	Standard Deviation
	Count (N)
Potassium	Mean
	Standard Deviation
	Count (N)
Chloride	Mean
	Standard Deviation
	Count (N)
Calcium	Mean
	Standard Deviation
	Count (N)
Phosphorus	Mean
	Standard Deviation
	Count (N)
Total Protein	Mean
	Standard Deviation
	Count (N)
Albumin	Mean
	Standard Deviation
	Count (N)
Globulin	Mean
	Standard Deviation
	Count (N)
Alb/Glo Ratio	Mean
	Standard Deviation
	Count (N)

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Key Page

**Measurement/Statistics (Continued)**

<u>Measurement</u>	<u>Descriptive</u>
Cholesterol	Mean Standard Deviation Count (N)
Triglyceride	Mean Standard Deviation Count (N)

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings 1-4</u>		
1	Excipient	Control	Group 1	0	mg/kg SC
2	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (0.4)	Dose	Group 2	0.4	mg/kg SC
3	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (3.64)	Dose	Group 3	3.64	mg/kg SC
4	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (14.55)	Dose	Group 4	14.55	mg/kg SC

**Comment Abbreviations**

RC = Result Comment, SC = Sample Comment



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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with [Propriet] [Pr] in Male and Female Beagle Dogs

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End of Print

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**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix I-3**

**INDIVIDUAL COAGULATION**

Page 1531 of 3822 to Page 1554 of 3822

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

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Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
1	-4	1	Male	004	Prothrombin Time	Sample	
				<i>Comment: Trace Hemolysis</i>			
1	-4	1	Male	004	Activated PTT	Sample	
				<i>Comment: Trace Hemolysis</i>			
1	-4	1	Male	005	Prothrombin Time	Sample	
				<i>Comment: 1+ Hemolysis</i>			
1	-4	1	Male	005	Activated PTT	Sample	
				<i>Comment: 1+ Hemolysis</i>			
1	30	1	Male	002	Prothrombin Time	Sample	
				<i>Comment: Trace Hemolysis</i>			
1	30	1	Male	003	Prothrombin Time	Sample	
				<i>Comment: Trace Hemolysis</i>			
1	30	1	Male	014	Prothrombin Time	Sample	
				<i>Comment: Trace Hemolysis</i>			
2	-4	1	Male	003	Fibrinogen	Result	
				<i>Comment: Result: dilution changed</i>			
2	-4	1	Male	004	Fibrinogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
2	-4	1	Male	005	Fibrinogen	Sample	
				<i>Comment: 1+ Hemolysis</i>			
2	30	1	Male	002	Activated PTT	Sample	
				<i>Comment: Trace Hemolysis</i>			
2	30	1	Male	002	Fibrinogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
2	30	1	Male	003	Activated PTT	Sample	
				<i>Comment: Trace Hemolysis</i>			
2	30	1	Male	003	Fibrinogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
2	30	1	Male	014	Activated PTT	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
2	30	1	Male	014	Fibrinogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
3	72	1	Male	004	Fibrinogen	Result	
				<i>Comment: Result: dilution changed</i>			
3	72	1	Male	005	Fibrinogen	Result	
				<i>Comment: Result: dilution changed</i>			
4	-4	2	Male	015	Prothrombin Time	Sample	
				<i>Comment: Trace Hemolysis</i>			
4	-4	2	Male	015	Activated PTT	Sample	
				<i>Comment: Trace Hemolysis</i>			
4	30	2	Male	011	Prothrombin Time	Sample	
				<i>Comment: Trace Hemolysis</i>			
4	30	2	Male	012	Prothrombin Time	Sample	
				<i>Comment: Trace Hemolysis</i>			
4	72	2	Male	015	Prothrombin Time	Sample	
				<i>Comment: Trace Hemolysis</i>			
5	-4	2	Male	013	Fibrinogen	Result	
				<i>Comment: Result: dilution changed</i>			
5	-4	2	Male	015	Fibrinogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
5	30	2	Male	011	Activated PTT	Sample	
				<i>Comment: Trace Hemolysis</i>			
5	30	2	Male	011	Fibrinogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
5	30	2	Male	012	Activated PTT	Sample	
				<i>Comment: Trace Hemolysis</i>			
5	30	2	Male	012	Fibrinogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
5	72	2	Male	015	Activated PTT	Sample	
				<i>Comment: Trace Hemolysis</i>			
6	72	2	Male	015	Fibrinogen	Sample	
				<i>Comment: Trace Hemolysis</i>			



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
7	-4	3	Male	021	Prothrombin Time	Sample	
				<i>Comment: Trace Hemolysis</i>			
7	-4	3	Male	021	Activated PTT	Sample	
				<i>Comment: Trace Hemolysis</i>			
8	-4	3	Male	021	Fibrinogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
8	-4	3	Male	022	Fibrinogen	Result	
				<i>Comment: Result: dilution changed</i>			
8	-4	3	Male	025	Fibrinogen	Result	
				<i>Comment: Result: dilution changed</i>			
9	72	3	Male	025	Fibrinogen	Result	
				<i>Comment: Result: dilution changed</i>			
10	72	4	Male	034	Prothrombin Time	Sample	
				<i>Comment: Trace Hemolysis</i>			
11	72	4	Male	034	Activated PTT	Sample	
				<i>Comment: Trace Hemolysis</i>			
12	72	4	Male	034	Fibrinogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
12	72	4	Male	035	Fibrinogen	Result	
				<i>Comment: Result: dilution changed</i>			
14	-2	1	Female	007	Fibrinogen	Result	
				<i>Comment: Result: dilution changed</i>			
14	-2	1	Female	008	Fibrinogen	Result	
				<i>Comment: Result: dilution changed</i>			
14	-2	1	Female	010	Fibrinogen	Result	
				<i>Comment: Result: dilution changed</i>			
15	72	1	Female	010	Fibrinogen	Result	
				<i>Comment: Result: dilution changed</i>			
16	-2	2	Female	020	Prothrombin Time	Sample	
				<i>Comment: Trace Hemolysis</i>			
16	-2	2	Female	020	Activated PTT	Sample	
				<i>Comment: Trace Hemolysis</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
16	72	2	Female	019	Prothrombin Time	Sample	
				<i>Comment: Trace Hemolysis</i>			
16	72	2	Female	020	Prothrombin Time	Sample	
				<i>Comment: Trace Hemolysis</i>			
17	-2	2	Female	016	Fibrinogen	Result	
				<i>Comment: Result: dilution changed</i>			
17	-2	2	Female	017	Fibrinogen	Result	
				<i>Comment: Result: dilution changed</i>			
17	-2	2	Female	018	Fibrinogen	Result	
				<i>Comment: Result: dilution changed</i>			
17	-2	2	Female	019	Fibrinogen	Result	
				<i>Comment: Result: dilution changed</i>			
17	-2	2	Female	020	Fibrinogen	Result	
				<i>Comment: Result: dilution changed</i>			
17	-2	2	Female	020	Fibrinogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
17	72	2	Female	019	Activated PTT	Sample	
				<i>Comment: Trace Hemolysis</i>			
17	72	2	Female	020	Activated PTT	Sample	
				<i>Comment: Trace Hemolysis</i>			
18	72	2	Female	019	Fibrinogen	Result	
				<i>Comment: Result: dilution changed</i>			
18	72	2	Female	019	Fibrinogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
18	72	2	Female	020	Fibrinogen	Result	
				<i>Comment: Result: dilution changed</i>			
18	72	2	Female	020	Fibrinogen	Sample	
				<i>Comment: Trace Hemolysis</i>			
20	-2	3	Female	029	Fibrinogen	Result	
				<i>Comment: Result: dilution changed</i>			
20	-2	3	Female	030	Fibrinogen	Result	
				<i>Comment: Result: dilution changed</i>			

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
21	72	3	Female	029	Fibrinogen	Result	
				<i>Comment:</i> Result: dilution changed			
22	-2	4	Female	038	Prothrombin Time	Sample	
				<i>Comment:</i> Trace Hemolysis			
22	-2	4	Female	038	Activated PTT	Sample	
				<i>Comment:</i> Trace Hemolysis			
22	72	4	Female	040	Prothrombin Time	Sample	
				<i>Comment:</i> Trace Hemolysis			
23	-2	4	Female	036	Fibrinogen	Result	
				<i>Comment:</i> Result: dilution changed			
23	-2	4	Female	037	Fibrinogen	Result	
				<i>Comment:</i> Result: dilution changed			
23	-2	4	Female	038	Fibrinogen	Result	
				<i>Comment:</i> Result: dilution changed			
23	-2	4	Female	038	Fibrinogen	Sample	
				<i>Comment:</i> Trace Hemolysis			
23	72	4	Female	040	Activated PTT	Sample	
				<i>Comment:</i> Trace Hemolysis			
24	72	4	Female	038	Fibrinogen	Result	
				<i>Comment:</i> Result: dilution changed			
24	72	4	Female	040	Fibrinogen	Result	
				<i>Comment:</i> Result: dilution changed			
24	72	4	Female	040	Fibrinogen	Sample	
				<i>Comment:</i> Trace Hemolysis			

Key Page**General Footnotes**

Provantis version 10.1.0.1

"." indicates Not Applicable

Statistical significance indicated on a group with an N &lt; 3 is not valid

**Measurement Descriptions**

<u>Headings Used</u>	<u>Description</u>
Prothrombin Time	PT (Prothrombin Time)
Activated PTT	Activated partial thromboplastin time
Fibrinogen	Fibrinogen

**Unit Descriptions**

<u>Headings Used</u>	<u>Description</u>
mg/dL	mg/dL
Seconds	Seconds

**Measurement/Statistics**

<u>Measurement</u>	<u>Descriptive</u>
Prothrombin Time	Mean Standard Deviation Count (N)
Activated PTT	Mean Standard Deviation Count (N)
Fibrinogen	Mean Standard Deviation Count (N)

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with [Propriet] [Pr] in Male and Female Beagle Dogs

---

Key Page**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings 1-4</u>		
1	Excipient	Control	Group 1	0	mg/kg SC
2	[Propriet] [Pr] (0.4)	Dose	Group 2	0.4	mg/kg SC
3	[Propriet] [Pr] (3.64)	Dose	Group 3	3.64	mg/kg SC
4	[Propriet] [Pr] (14.55)	Dose	Group 4	14.55	mg/kg SC

**Comment Abbreviations**

RC = Result Comment, SC = Sample Comment



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with [Propriet] [Pr] in Male and Female Beagle Dogs

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End of Print

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**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix J**

**INDIVIDUAL ANIMAL URINALYSIS**

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix J-1**

**INDIVIDUAL URINALYSIS**

Page 1565 of 3822 to Page 1636 of 3822

Withheld pursuant to exemption

Proprietary Info

of the Freedom of Information and Privacy Act

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

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Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
3	72	1	Male	004	Urinary Blood	Result	
				<i>Comment:</i> TRACE-I = TRACE-INTACT			
39	72	1	Female	010	Urinary Blood	Result	
				<i>Comment:</i> TRACE-I = TRACE-INTACT			
56	17	3	Female	026	Urinary Blood	Result	
				<i>Comment:</i> TRACE-I = TRACE-INTACT			
66	72	4	Female	040	UA pH	Replacement	>=9.0



Key Page**General Footnotes**

Provantis version 10.1.0.1

"." indicates Not Applicable

Statistical significance indicated on a group with an N &lt; 3 is not valid

**Replacement Values**

<u>Value</u>	<u>Description</u>
>=9.0	>=9.0

**Measurement Descriptions**

<u>Headings Used</u>	<u>Description</u>
Specific Gravity	Specific Gravity
Urinary Glucose	Urinary Glucose
Urinary Bilirubin	Urinary Bilirubin
Urinary Ketones	Urinary Ketones
Urinary Blood	Urinary Blood
UA pH	UA pH
Urinary Protein	Urinary Protein
UA Urobilinogen	UA Urobilinogen
Urinary Nitrite	Urinary Nitrite
Urinary Leukocytes	Urinary Leukocytes
Urinary WBC	Urinary WBC
Urinary RBC	Urinary RBC
Urinary Epit helial Cells	Urinary Epithelial Cells
Urinary Amorphus Cry	Urinary Amorphus Crystals
Urinary Bacteria	Urinary Bacteria
Urinary Triple Phosp	Urinary Triple Phosphate
Urinary Mucus Thread	Urinary Mucus Thread
Urinary Sperm	Urinary Sperm
Urinary Budding Yeas	Urinary Budding Yeast

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

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Key Page**Measurement Descriptions (Continued)**

<u>Headings Used</u>	<u>Description</u>
Fine Granula	Fine Granular Cast

**Unit Descriptions**

<u>Headings Used</u>	<u>Description</u>
EU/dL	EU/dL

**Measurement/Statistics**

<u>Measurement</u>	<u>Descriptive</u>
Specific Gravity	Mean Standard Deviation Count (N)
Urinary Glucose	Count (N)
Urinary Bilirubin	Count (N)
Urinary Ketones	Count (N)
Urinary Blood	Count (N)
UA pH	Mean Standard Deviation Count (N)
Urinary Protein	Count (N)
UA Urobilinogen	Mean Standard Deviation Count (N)
Urinary Nitrite	Count (N)
Urinary Leukocytes	Count (N)
Urinary WBC	Count (N)
Urinary RBC	Count (N)
Urinary Epithelial Cells	Count (N)

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

---

Key Page**Measurement/Statistics (Continued)**

<u>Measurement</u>	<u>Descriptive</u>
Urinary Amorphus Cry	Count (N)
Urinary Bacteria	Count (N)
Urinary Triple Phosp	Count (N)
Urinary Mucus Thread	Count (N)
Urinary Sperm	Count (N)
Urinary Budding Yeas	Count (N)
Fine Granula	Count (N)

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings 1-4</u>		
1	Excipient	Control	Group 1	0	mg/kg SC
2	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (0.4)	Dose	Group 2	0.4	mg/kg SC
3	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (3.64)	Dose	Group 3	3.64	mg/kg SC
4	<span style="border: 1px solid black; padding: 0 2px;">Propriet</span> <span style="border: 1px solid black; padding: 0 2px;">Pr</span> (14.55)	Dose	Group 4	14.55	mg/kg SC

**Comment Abbreviations**

RC = Result Comment

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study with [Propriet] [Pr] in Male and Female Beagle Dogs

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End of Print

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix J-2**

**INDIVIDUAL URINE COLOR AND CLARITY**



Page 1643 of 3822 to Page 1650 of 3822

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with [Propriet] [Pr] in Male and Female Beagle Dogs

---

Key Page

**General Footnotes**

Provantis version 10.1.0.1

"-" indicates Not Applicable

**Measurement Descriptions**

Headings Used

Urine Color

Urine Clarity

Description

Urine Color

Urine Clarity

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings 1-4</u>			
1	Excipient	Control	Group 1	0		mg/kg SC
2	[Propriet] [Pr] (0.4)	Dose	Group 2	0.4		mg/kg SC
3	[Propriet] [Pr] (3.64)	Dose	Group 3	3.64		mg/kg SC
4	[Propriet] [Pr] (14.55)	Dose	Group 4	14.55		mg/kg SC

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix K**

**INDIVIDUAL ORGAN WEIGHTS**

Page 1653 of 3822 to Page 1732 of 3822

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

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Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
1	72	1	Male	004	Adrenal Glands Wt	Out of Range	<
3	30	1	Male	002	Testes Weight	Out of Range	>
3	72	1	Male	004	Liver Weight	Out of Range	<
4	30	1	Male	002	Thymus Weight	Out of Range	<
4	30	1	Male	003	Thymus Weight	Out of Range	<
4	72	1	Male	004	Thymus Weight	Out of Range	<
12	72	2	Male	001	Heart Weight	Out of Range	>
12	72	2	Male	015	Heart Weight	Out of Range	>
14	30	2	Male	011	Thymus Weight	Out of Range	<
14	72	2	Male	001	Thymus Weight	Out of Range	<
14	72	2	Male	015	Thymus Weight	Out of Range	<
21	17	3	Male	021	Adrenal Glands Wt	Out of Range	>
21	17	3	Male	021	Heart Weight	Out of Range	>
24	17	3	Male	021	Thymus Weight	Out of Range	<
24	17	3	Male	022	Thymus Weight	Out of Range	<
24	17	3	Male	023	Thymus Weight	Out of Range	<
24	72	3	Male	025	Thymus Weight	Out of Range	<
32	72	4	Male	035	Heart Weight	Out of Range	>
34	17	4	Male	031	Thymus Weight	Out of Range	<
34	17	4	Male	032	Thymus Weight	Out of Range	<
34	17	4	Male	033	Thymus Weight	Out of Range	<
34	72	4	Male	034	Thymus Weight	Out of Range	<
41	30	1	Female	006	Brain Weight	Out of Range	<
42	30	1	Female	007	Liver Weight	Out of Range	<
43	30	1	Female	007	Ovaries Weight	Out of Range	>
43	30	1	Female	008	Ovaries Weight	Out of Range	>
43	72	1	Female	009	Liver Weight	Out of Range	<
43	72	1	Female	010	Liver Weight	Out of Range	<
43	72	1	Female	010	Ovaries Weight	Out of Range	>
44	72	1	Female	009	Thymus Weight	Out of Range	<



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
44	72	1	Female	010	Thymus Weight	Out of Range	<
52	30	2	Female	016	Liver Weight	Out of Range	<
52	30	2	Female	018	Liver Weight	Out of Range	<
53	72	2	Female	019	Liver Weight	Out of Range	<
53	72	2	Female	019	Ovaries Weight	Out of Range	>
53	72	2	Female	020	Liver Weight	Out of Range	<
54	30	2	Female	016	Thymus Weight	Out of Range	<
54	30	2	Female	017	Thymus Weight	Out of Range	<
54	30	2	Female	018	Thymus Weight	Out of Range	<
54	72	2	Female	019	Thymus Weight	Out of Range	<
54	72	2	Female	020	Thymus Weight	Out of Range	<
61	17	3	Female	028	Adrenal Glands Wt	Out of Range	<
61	72	3	Female	029	Brain Weight	Out of Range	<
62	17	3	Female	026	Liver Weight	Out of Range	<
63	72	3	Female	030	Liver Weight	Out of Range	<
64	17	3	Female	026	Thymus Weight	Out of Range	<
64	17	3	Female	028	Thymus Weight	Out of Range	<
64	72	3	Female	029	Thymus Weight	Out of Range	<
64	72	3	Female	030	Thymus Weight	Out of Range	<
71	17	4	Female	037	Brain Weight	Out of Range	<
71	17	4	Female	039	Brain Weight	Out of Range	<
71	72	4	Female	038	Adrenal Glands Wt	Out of Range	>
71	72	4	Female	040	Brain Weight	Out of Range	<
73	72	4	Female	038	Liver Weight	Out of Range	<
73	72	4	Female	040	Liver Weight	Out of Range	<
73	72	4	Female	040	Ovaries Weight	Out of Range	>
74	17	4	Female	036	Thymus Weight	Out of Range	<
74	17	4	Female	037	Thymus Weight	Out of Range	<
74	17	4	Female	039	Thymus Weight	Out of Range	<
74	72	4	Female	038	Thymus Weight	Out of Range	<

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

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Key Page

**General Footnotes**

Provantis version 10.1.0.1

"-" indicates Not Applicable

< or >: Data falls outside of the normal range defined in the software

**Measurement Descriptions**

<u>Headings Used</u>	<u>Description</u>
Adrenal Glands Wt	Adrenal Glands Weight
Brain Weight	Brain Weight
Heart Weight	Heart Weight
Kidneys Weight	Kidneys Weight
Liver Weight	Liver Weight
Ovaries Weight	Ovaries Weight
Testes Weight	Testes Weight
Thymus Weight	Thymus Weight
Adrenal/BW Ratio	Adrenals/Terminal BW Ratio (kg)
Brain/BW Ratio	Brain/Terminal BW Ratio (kg)
Heart/BW Ratio	Heart/Terminal BW Ratio (kg)
Kidney/BW Ratio	Kidney/Terminal BW Ratio (kg)
Liver/BW Ratio	Liver/Terminal BW Ratio (kg)
Ovaries/BW Ratio	Ovaries/Terminal BW Ratio (kg)
Testes/BW Ratio	Testes/Terminal BW Ratio (kg)
Thymus/BW Ratio	Thymus/Terminal BW Ratio (kg)
Adrenal/ Brain	Adrenals/Brain Ratio
Heart/ Brain	Heart/Brain Ratio
Kidneys/ Brain	Kidneys/Brain Ratio
Liver/ Brain	Liver/Brain Ratio
Ovaries/ Brain	Ovaries/Brain Ratio
Testes/ Brain	Testes/Brain Ratio
Thymus/ Brain	Thymus/Brain Ratio

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

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Key Page

**Unit Descriptions**

Headings Used

%

g

Description

%

g

**Measurement/Statistics**

Measurement

Adrenal Glands Wt

Brain Weight

Heart Weight

Kidneys Weight

Liver Weight

Ovaries Weight

Testes Weight

Descriptive

Mean

Standard Deviation

Count (N)

Mean

Standard Deviation

Count (N)

Mean

Standard Deviation

Count (N)

Mean

Standard Deviation

Count (N)

Mean

Standard Deviation

Count (N)

Mean

Standard Deviation

Count (N)

Mean

Standard Deviation

Count (N)

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

---

Key Page

**Measurement/Statistics (Continued)**

<u>Measurement</u>	<u>Descriptive</u>
Thymus Weight	Mean Standard Deviation Count (N)
Adrenal/BW Ratio	Mean Standard Deviation Count (N)
Brain/BW Ratio	Mean Standard Deviation Count (N)
Heart/BW Ratio	Mean Standard Deviation Count (N)
Kidney/BW Ratio	Mean Standard Deviation Count (N)
Liver/BW Ratio	Mean Standard Deviation Count (N)
Ovaries/BW Ratio	Mean Standard Deviation Count (N)
Testes/BW Ratio	Mean Standard Deviation Count (N)
Thymus/BW Ratio	Mean Standard Deviation Count (N)

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

Key Page

**Measurement/Statistics (Continued)**

<u>Measurement</u>	<u>Descriptive</u>
Adrenal/ Brain	Mean
	Standard Deviation
	Count (N)
Heart/ Brain	Mean
	Standard Deviation
	Count (N)
Kidneys/ Brain	Mean
	Standard Deviation
	Count (N)
Liver/ Brain	Mean
	Standard Deviation
	Count (N)
Ovaries/ Brain	Mean
	Standard Deviation
	Count (N)
Testes/ Brain	Mean
	Standard Deviation
	Count (N)
Thymus/ Brain	Mean
	Standard Deviation
	Count (N)

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings 1-4</u>		
1	Excipient	Control	Group 1	0	mg/kg SC
2	<u>Propriet</u> <u>Pr</u> (0.4)	Dose	Group 2	0.4	mg/kg SC
3	<u>Propriet</u> <u>Pr</u> (3.64)	Dose	Group 3	3.64	mg/kg SC



M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

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Key Page**Group Information (Continued)**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings 1-4</u>		
4	<span>Propriet</span> <span>Pr</span> (14.55)	Dose	Group 4	14.55	mg/kg SC

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix L**

**NECROPSY OBSERVATIONS AND PATHOLOGY**

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix L-1**

**PATHOLOGY NARRATIVE**

**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Pathology Narrative**

**Methods:**

Protocol-listed retained tissues, with the exception of eyes, optic nerves, and testes for Groups 1 and 4, all gross lesions, unscheduled deaths, and identified target tissues for all groups were retained in 10% neutral-buffered formalin, processed routinely, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E), and examined microscopically by a veterinary pathologist. Eyes, optic nerves, and testes were placed in Davidson's solution and processed similarly.

Microscopic data were recorded in Provantis pathology ver. 10.1.0.1; histopathology data are presented in summary and individual animal tables generated using Provantis software. A four-step grading system (minimal, mild, moderate, and marked) was used to define gradable changes. Terminology for data capture was consistent with International Harmonization of Nomenclature and Diagnostic Criteria (INHAND), as promulgated by the Society of Toxicologic Pathology. Records of necropsy findings and changes found at tissue processing in the histology laboratory were available when evaluating the formalin-fixed, paraffin-embedded, hematoxylin and eosin-stained tissue sections.

The following tissue sections were missing and not evaluated: Mammary gland in females (006, 008, 036, 037, and 039), sciatic nerve (007), and skeletal muscle (007). Mammary gland, however, was only required if within the plane of section in males; females were required examination, however, in animals with immature/non-cycling ovaries (006, 008, 036, 037, and 039), mammary gland will not be developed and will, therefore, not be present in tissue sections. The absence of these tissues was not considered to have compromised the ability to interpret the microscopic data.

Proprietary Info

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Page 1745 of 3822

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**GLP-Multiple (5 Weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with Proprietary Pro in Male and Female Beagle Dogs  
SRI Study No. M397-18**

Redacted by agreement

Redacted by agreement

05/09/2019

Date

SMW/cb

**References:**

Greaves, P. (2012) Hemopoietic and Lymphatic Systems - Thymus. *In: Histopathology of Preclinical Toxicity Studies*. Elsevier, London. 4:127.

McInnes E. (2012) Dog. *In: Background Lesions in Laboratory Animals. A Color Atlas*. Saunders Elsevier. 3:37-44.

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix L-2**

**HISTOPATHOLOGY SUMMARY**

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

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Key Page

**General Footnotes**

"." indicates Not Applicable

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**Report Request Items**

Animals Excluded: None  
Groups: All  
Observation Type: Histo - Neoplastic and Non-Neoplastic  
Tissues: All  
Removal Reasons: All

**Measurement/Statistics**

<u>Measurement</u>	<u>Descriptive</u>	<u>Comparative</u>	<u>Arithmetic</u> <u>/Adjusted</u>	<u>Transformation</u>
Pathology Observation	Count Positives			

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings</u>		
1	Excipient	Control	Group 1	0	mg/kg SC
2	<u>Propriet</u> <u>Pr</u> (0.4)	Dose	Group 2	0.4	mg/kg SC
3	<u>Propriet</u> <u>Pr</u> (3.64)	Dose	Group 3	3.64	mg/kg SC
4	<u>Propriet</u> <u>Pr</u> (14.55)	Dose	Group 4	14.55	mg/kg SC

**Removal Reason Grouping**

<u>Grouping Name</u>	<u>Abbreviation</u>	<u>Removal Reasons</u>
Interim Sacrifice	Int	Interim Sacrifice
Main Sacrifice	Main	Main Sacrifice
Recovery Sacrifice	Rec	Recovery Sacrifice
Moribund Sacrifice	Mori	Moribund Sacrifice
Found Dead	FD	Found Dead
Unscheduled Sacrifice	UnS	Unscheduled Sacrifice
Terminal Sacrifice	Term	Terminal Sacrifice
Mechanically Killed	Mech	Mechanically Killed
Other, See Text Comments	Oth	Other, See Text Comments
Removed from Study	R	Removed from Study



**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix L-3**

**INDIVIDUAL HISTOPATHOLOGY**

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

---

Key Page

**Codes**

(TGL) = Trackable Gross Lesion, (MPF) = Major Pathological Finding, (?) = Questionable, (E) = Excluded,  
(C) = Clinical Observation, (M) = Mass, (G) = Gross Pathology, (H) = Histopathology

**General Footnotes**

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**Report Request Items**

Animals Included: All  
Groups: All  
Observation Type: Histo  
Tissues: All  
Removal Reasons: All

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>
1	Excipient	Control
2	<u>Propriet</u> <u>Pr</u> (0.4)	Dose
3	<u>Propriet</u> <u>Pr</u> (3.64)	Dose
4	<u>Propriet</u> <u>Pr</u> (14.55)	Dose

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix L-4**

**NECROPSY OBSERVATIONS SUMMARY**

Page 1822 of 3822 to Page 1836 of 3822  
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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

---

Key Page

**General Footnotes**

Provantis version 10.1.0.1

"." indicates Not Applicable

**Report Request Items**

Animals Excluded: None  
Groups: All  
Observation Type: Gross  
Tissues: All  
Removal Reasons: All

**Measurement/Statistics**

<u>Measurement</u>	<u>Descriptive</u>	<u>Comparative</u>	<u>Arithmetic</u> <u>/Adjusted</u>	<u>Transformation</u>
Pathology Observation	Count Positives			

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings</u>		
1	Excipient	Control	Group 1	0	mg/kg SC
2	<u>Propriet</u> <u>Pr</u> (0.4)	Dose	Group 2	0.4	mg/kg SC
3	<u>Propriet</u> <u>Pr</u> (3.64)	Dose	Group 3	3.64	mg/kg SC
4	<u>Propriet</u> <u>Pr</u> (14.55)	Dose	Group 4	14.55	mg/kg SC

**Removal Reason Grouping**

<u>Grouping Name</u>	<u>Abbreviation</u>	<u>Removal Reasons</u>
Interim Sacrifice	Int	Interim Sacrifice
Main Sacrifice	Main	Main Sacrifice
Recovery Sacrifice	Rec	Recovery Sacrifice
Moribund Sacrifice	Mori	Moribund Sacrifice
Found Dead	FD	Found Dead
Unscheduled Sacrifice	UnS	Unscheduled Sacrifice
Terminal Sacrifice	Term	Terminal Sacrifice
Mechanically Killed	Mech	Mechanically Killed
Other, See Text Comments	Oth	Other, See Text Comments
Removed from Study	R	Removed from Study

**GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and Toxicokinetics Study  
with [Proprietary Info] [Pro] in Male and Female Beagle Dogs  
SRI Study No. M397-18**

**Appendix L-5**

**INDIVIDUAL NECROPSY OBSERVATIONS**

Page 1839 of 3822 to Page 1846 of 3822

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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with ~~Proprietary~~ Pr in Male and Female Beagle Dogs

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End Of Print  
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Page 1848 of 3822 to Page 1887 of 3822  
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M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

---

Markers

<u>Animal</u>	<u>Measurement</u>	<u>Marker and Description</u>
002	Testes (Fixed With Modified Davidsons)	>
002	Thymus	<
003	Thymus	<
004	Adrenal Glands	<
004	Liver	<
004	Thymus	<
006	Brain (Fore-, Mid-, Hindbrain)	<
007	Liver	<
007	Ovaries	>
008	Ovaries	>
009	Liver	<
009	Thymus	<
010	Liver	<
010	Ovaries	>
010	Thymus	<
011	Thymus	<
001	Heart	>
001	Thymus	<
015	Heart	>
015	Thymus	<
016	Liver	<
016	Thymus	<
017	Thymus	<
018	Liver	<
018	Thymus	<
019	Liver	<
019	Ovaries	>
019	Thymus	<
020	Liver	<
020	Thymus	<
021	Adrenal Glands	>
021	Heart	>
021	Thymus	<
022	Thymus	<
023	Thymus	<
025	Thymus	<
026	Liver	<
026	Thymus	<
028	Adrenal Glands	<
028	Thymus	<
029	Brain (Fore-, Mid-, Hindbrain)	<
029	Thymus	<
030	Liver	<
030	Thymus	<
031	Thymus	<

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with Proprietary Pr in Male and Female Beagle Dogs

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Markers (Continued)

<u>Animal</u>	<u>Measurement</u>	<u>Marker and Description</u>
032	Thymus	<
033	Thymus	<
034	Thymus	<
035	Heart	>
036	Thymus	<
037	Brain (Fore-, Mid-, Hindbrain)	<
037	Thymus	<
038	Adrenal Glands	>
038	Liver	<
038	Thymus	<
039	Brain (Fore-, Mid-, Hindbrain)	<
039	Thymus	<
040	Brain (Fore-, Mid-, Hindbrain)	<
040	Liver	<
040	Ovaries	>

M397-18 - GLP-Multiple (5-weekly) Repeat Subcutaneous Dose Toxicity and  
Toxicokinetics Study with Propriet Pr in Male and Female Beagle Dogs

---

Key Page

**Codes**

(TGL) = Trackable Gross Lesion, (MPF) = Major Pathological Finding, (?) = Questionable, (E) = Excluded,  
(C) = Clinical Observation, (M) = Mass, (G) = Gross Pathology, (H) = Histopathology

**General Footnotes**

Provantis version 10.1.0.1

**Report Request Items**

Animals Included: All  
Groups: All  
Observation Type: Gross  
Tissues: All  
Removal Reasons: All

**Unit Descriptions**

<u>Headings Used</u>	<u>Description</u>
g	g

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>
1	Excipient	Control
2	<u>Propriet</u> <u>Pr</u> (0.4)	Dose
3	<u>Propriet</u> <u>Pr</u> (3.64)	Dose
4	<u>Propriet</u> <u>Pr</u> (14.55)	Dose

Final Report • November 19, 2019

# GLP-MULTIPLE (5-WEEKLY) REPEAT SUBCUTANEOUS DOSE TOXICITY STUDY WITH [Proprietary Info] [Proprietary Info] IN SPRAGUE DAWLEY RATS

**Author:**

[Redacted by agreement]

**Testing Facility:**

SRI International  
Biosciences Division  
333 Ravenswood Avenue  
Menlo Park, CA 94025

**SRI Study Number:**

M398-18

**SRI Project Number:**

P25035.412

**Study Initiation:**

December 6, 2018

**Experimental Work Performed:****Start:**

December 12, 2018

**Finish:**

July 26, 2019

**Study Completion:**

November 19, 2019

**Sponsor:**

National Institute of Allergy and Infectious Diseases  
Division of AIDS  
5601 Fishers Lane [Redacted by agreement]  
Bethesda, MD 20892-9830

**Sponsor's Representative:**

[Redacted by agreement]

**NIAID Contract Number:**

HHSN272201400006I/TO- HHSN27200008

**GLP-Multiple (5-Weekly) Repeat Subcutaneous Dose Toxicity Study**  
**with Proprietary Pro in Sprague Dawley Rats**  
**SRI Study No. M398-18**

**APPROVAL SIGNATURES**

Written By:

Redacted by agreement

11/19/19  
Date

Approved By:

11-7-19  
Date

SRI International  
Biosciences Division  
333 Ravenswood Avenue  
Menlo Park, CA 94025



**GLP-Multiple (5-Weekly) Repeat Subcutaneous Dose Toxicity Study**  
**with Proprietary Info Pro in Sprague Dawley Rats**  
**SRI Study No. M398-18**

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**GLP-Multiple (5-Weekly) Repeat Subcutaneous Dose Toxicity Study**  
**with [Proprietary Info] [Prop] in Sprague Dawley Rats**  
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**SUMMARY**

The objective of this study was to determine potential toxicity of [Proprietary] [Prop] a new formulation of [Proprietary] [Proprietary] 12 mg/ml), [Proprietary] [Proprietary] 6.9 mg/ml) and [Proprietary] [Proprietary] 3.3 mg/ml), in adult male and female Sprague Dawley rats following a 5-weekly repeat subcutaneous (s.c.) administration.

Male and female Sprague Dawley rats (15/sex) were given a weekly subcutaneous (s.c.) administration for 5 weeks of [Proprietary] [Prop] at 1.5 (1.5 mg/kg [Proprietary] 0.8625 mg/kg [Proprietary] 0.4125 mg/kg [Proprietary] 15 (15 mg/kg [Proprietary] 8.625 mg/kg [Proprietary] 4.125 mg/kg [Proprietary] and 30 mg/kg (30 mg/kg [Proprietary] 17.25 mg/kg [Proprietary] 8.25 mg/kg [Proprietary] A control group, (15/sex) was given a weekly s.c. administration for 5 weeks of Excipient Control at an equivalent volume. The Excipient Control group received the amount of excipients that was equivalent to the high dose group (30 mg/kg). Animals were sacrificed on Days 30 and 72 (10/sex and 5/sex at each Main and Recovery necropsy, respectively). The following parameters were evaluated: mortality/morbidity, clinical observations, body weights, food consumption, ophthalmology, clinical pathology, urinalysis and gross necropsy observations and microscopic histopathologic evaluations. Doses expressed throughout the report are based on the doses of [Proprietary] administered (1.5, 15 and 30 mg/kg).

Proprietary Info

**GLP-Multiple (5-Weekly) Repeat Subcutaneous Dose Toxicity Study**  
**with [Proprietary Info] [Prop] in Sprague Dawley Rats**  
**SRI Study No. M398-18**

Proprietary Info



**GLP-Multiple (5-Weekly) Repeat Subcutaneous Dose Toxicity Study**  
**with [Proprietary] [Pro] in Sprague Dawley Rats**  
**SRI Study No. M398-18**

**QUALITY ASSURANCE UNIT**

**Final Report and  
Conflict of Interest Statement**

SRI's Quality Assurance Unit assures that the study *GLP-Multiple (5-Weekly) Repeat Subcutaneous Dose Toxicity Study* with [Proprietary] [Pro] in Sprague Dawley Rats, SRI Study Number M398-18, has been reviewed for adherence to U.S. Food and Drug Administration Good Laboratory Practice Regulations (21 CFR Part 58).

The following inspections were conducted during this study:

<u>Phase Inspected</u>	<u>Date of Inspection</u>	<u>Date Findings Reported to Management/Study Director</u>
Protocol	10-02-18	10-02-18
Protocol Amendment No. 1	12-10-18	12-10-18
Protocol Amendment No. 2	12-14-18	12-14-18
Protocol Amendment No. 3	01-10-19	01-10-19
Protocol Amendment No. 4	02-13-19	02-13-19
Protocol Amendment No. 5	11-11-19	11-11-19
Dosing, Body Weights, Bleeds	12-12-18	12-12-18
Dose Preparation	12-12-18	12-12-18
Clinical Observations	12-18-18	12-18-18
Necropsy	01-10-19	01-10-19
Bleeds, Necropsy	02-21-19	02-21-19
COAs	05-09-19	05-09-19
Plasma Analysis	07-24-19	07-25-19
Raw Data	05-16-19	05-16-19
	07-26-19	07-26-19
	08-08-19	08-08-19
	10-03-19	10-03-19
	11-18-19	11-18-19
Draft Final Report	05-16-19	05-16-19
	08-08-19	08-08-19
	10-03-19	10-03-19
	10-10-19	10-10-19
	10-14-19	10-14-19
Final Report Verification	11-19-19	11-19-19

This statement certifies that the personnel listed below participated in the inspections and audit of this study. These personnel have not been involved in the generation or evaluation of the data. Participation by the individuals listed below poses no conflict of interest.

Redacted by agreement

Redacted by agreement

19 Nov 19  
Date

**GLP-Multiple (5-Weekly) Repeat Subcutaneous Dose Toxicity Study  
with [Proprietary Info] [Prop] in Sprague Dawley Rats  
SRI Study No. M398-18**

**[Proprietary Info] QUALITY ASSURANCE STATEMENT**

[Proprietary Info]

**QUALITY ASSURANCE FINAL CERTIFICATION**

Study Title: GLP-Multiple (5-Weekly) Repeat Subcutaneous Dose Toxicity Study with  
[Proprietary Info] [Pr] in Sprague Dawley Rats

Client Study: M398-18

[Redacted by agreement]

[Proprietary Info] Project Number: 748-120

The following aspects of this study were inspected by the Quality Assurance Unit of Experimental Pathology Laboratories, Inc. Dates inspections were performed and findings reported to the EPL Principal Investigator and Management are indicated below.

Area Inspected	Dates	
	Inspection	Reporting
[Proprietary Info] Project Sheets	12/27/18; 3/15/19	12/27/18; 3/15/19
Project Setup	12/27/18; 1/17/19; 2/28/19; 3/15/19	12/27/18; 1/17/19; 2/28/19; 3/15/19
Data Review	2/12/19; 3/4,5,18,21,28/19	2/12/19; 2/13/19; 3/4,5,18,21,28/19

Date reported to Study Director/Management 9/16/19

Date of last annual facility inspection 8/19

[Redacted by agreement]

Date

16 September 2019

**GLP-Multiple (5-Weekly) Repeat Subcutaneous Dose Toxicity Study**  
**with [Proprietary Info] [Prop] in Sprague Dawley Rats**  
**SRI Study No. M398-18**

**KEY PERSONNEL**

<b>Name</b>	<b>Functional Role</b>
Redacted by agreement	

**GLP-Multiple (5-Weekly) Repeat Subcutaneous Dose Toxicity Study**  
**with [Proprietary Info] [Prop] in Sprague Dawley Rats**  
**SRI Study No. M398-18**

**I. PURPOSE OF STUDY**

The purpose of this study was to provide data of suitable quality and integrity to support applications to the U.S. Food and Drug Administration (FDA) and other regulatory agencies. Therefore, this study was performed in accordance with the U.S. FDA “Good Laboratory Practice for Nonclinical Laboratory Studies” (GLP) as described in 21 CFR Part 58.

**II. OBJECTIVE OF STUDY**

The objective of this study was to determine potential toxicity of [Proprietary] [Prop] a new formulation of [Proprietary] [Proprietary] 12 mg/ml), [Proprietary] [Proprietary] 6.9 mg/ml) and [Proprietary] [Proprietary] 3.3 mg/ml), in adult male and female Sprague Dawley rats following a 5-weekly repeat subcutaneous (s.c.) administration.

The protocol and amendments are presented in Appendix A.

**III. EXPERIMENTAL DESIGN**

Group	Treatment	Dose Level (mg/kg) <sup>a</sup>	Dose Conc. (mg/ml)	Volume (ml/kg)	Total No. of Animals	No. of Animals at Necropsy		TK Satellite Group <sup>b</sup>
						Day 30 (Main)	Day 72 (Recovery)	
1	Excipient	Equivalent to 30 mg/kg [Prop]	0	5	15M/15F	10M/10F	5M/5F	-
2	[Proprietary] [Pro]	1.5	0.3	5	24M/24F	10M/10F	5M/5F	9M/9F
3	[Proprietary] [Pro]	15	3	5	24M/24F	10M/10F	5M/5F	9M/9F
4	[Proprietary] [Pro]	30	6	5	24M/24F	10M/10F	5M/5F	9M/9F
<b>Total No. of Rats</b>					<b>87M/87F</b>	<b>40M/40F</b>	<b>20M/20F</b>	<b>27M/27F</b>

<sup>a</sup> Based on [Prop] for [Proprietary] [Pro] fixed dose [Proprietary Info] combination. The doses in mg/kg for each component were as follows: Group 2: 1.5 mg/kg [Propri] 0.8625 mg/kg [Propri] 0.4125 mg/kg [Propri] Group 3: 15 mg/kg [Propri] 8.625 mg/kg [Propri] 4.125 mg/kg [Propri] Group 4: 30 mg/kg [Propri] 17.25 mg/kg [Propri] 8.25 mg/kg [Propri]

<sup>b</sup> Satellite group was used to collect toxicokinetic samples only. Body weights were recorded for dose administration calculations and were not included in body weight analyses. No other in-life evaluations were conducted on this subset of animals. Animals were euthanized by an overdose intraperitoneal injection of sodium pentobarbital after their last blood collection. Necropsy was not performed on any animal in the TK satellite groups and no other terminal samples were collected from these animals.

**Species and Strain**

Sprague Dawley rat

**GLP-Multiple (5-Weekly) Repeat Subcutaneous Dose Toxicity Study**  
**with [Proprietary Info] [Prop] in Sprague Dawley Rats**  
**SRI Study No. M398-18**

**Route of Administration**

Subcutaneous (s.c.)

**Frequency**

Once a week for 5 weeks

**Dosing Volume**

Dose volumes were calculated based on each animal's most recent body weight to achieve the target dose levels based on 12 mg/ml [Proprietary] anchored [Proprietary] [Proprietary] concentration of dose solution. Maximum injection volume was in accordance with IACUC guidelines.

**Duration of In-Life Phase**

72 days

**IV. MATERIALS AND METHODS**

**A. Test and Control Articles**

**1. Test Article**

[Proprietary] [Prop] a new formulation of [Proprietary] [Proprietary] 12 mg/ml), [Proprietary] [Proprietary] 6.9 mg/ml) and [Proprietary] [Proprietary] 3.3 mg/ml)

**Supplier**

[Proprietary Info]

**Manufacturer**

[Proprietary Info]

**Lot Numbers**

[Proprietary Info]

**Physical Description**

White, turbid suspension

**Storage Conditions**

2°-8°C



**GLP-Multiple (5-Weekly) Repeat Subcutaneous Dose Toxicity Study**  
**with [Proprietary Info] [Prop] in Sprague Dawley Rats**  
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**Characterization of Test Article**

The Sponsor was responsible for characterization and stability of the test article and provided Certificates of Analysis (CofA) to SRI for inclusion in the final report (Appendix B). The raw data generated by the Sponsor in support of these CofA were not verified or maintained by SRI.

**2. Vehicle Control**

Excipient Control

**Supplier**

[Proprietary Info]

**Manufacturer**

[Proprietary Info]

**Lot Number**

[Proprietary Info]

**Physical Description**

White, turbid suspension

**Storage Conditions**

2°-8°C

**Characterization of Vehicle Control**

The Sponsor was responsible for characterization and stability of the excipient control under the specified storage conditions and provided the CofA to SRI for inclusion in the final report. Information on the identity, purity, and stability of the excipient control article was obtained by recording all of the pertinent information provided on the CofA provided by the supplier.

**3. Preparation of Dose Formulations**

Dose formulations were provided by the Sponsor as ready-to-dose formulations at the concentrations specified in the table in Section III.

**Storage of Dose Formulations**

Dose formulations were stored refrigerated, at 2-8°C, until the day of use. Formulations were brought to 37° ± 1°C temperature prior to administration to the animals.

**GLP-Multiple (5-Weekly) Repeat Subcutaneous Dose Toxicity Study**  
**with [Proprietary Info] [Prop] in Sprague Dawley Rats**  
**SRI Study No. M398-18**

**4. Characterization of Dose Formulations**

The Sponsor was responsible for stability, homogeneity, and concentration analyses of the test articles in the vehicle. The raw analytical data generated in support of this study were not verified or maintained by SRI. SRI relied on the formal CofA provided with the formulation for confirmation of concentration, quality and stability.

**5. Test Article Handling**

At a minimum, personnel handling the test, and control article formulations wore eye protection, gloves, and a protective smock or laboratory coat.

**6. Disposition**

At the end of the study, any remaining partially used and unused containers of excipient control or test article (kept refrigerated) will be shipped to the Sponsor unless the Sponsor issues other directions.

Residual dose formulations will be discarded when the final report is submitted.

Empty control and test article containers will be destroyed by SRI on submission of the final report to the Sponsor.

See Section V.B, "Regulatory Compliance," for information about retention of records and study samples.

**7. Method for Assuring Correct Dosing**

The administration of each dose formulation was properly documented, and the amount administered to each animal was recorded.

**B. Test System**

**1. Species**

Rat

**Strain**

Sprague Dawley

**Supplier**

[Proprietary Info]

**Number of Animals**

174 assigned to test

**Sex**

87 males and 87 females

**GLP-Multiple (5-Weekly) Repeat Subcutaneous Dose Toxicity Study**  
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**Age at First Dose**

8 weeks (males) and 10 weeks (females)

**Weight Range at First Dose**

238-334 g (males); 191-241 g (females)

Multiple male body weights exceeded the protocol-specified maximum of 260 g on Day 1. This deviation had no impact on the study as the animals' weights were age-appropriate and the animals were dosed according to their actual individual body weights.

**2. Animal Care**

General procedures for animal care and housing were in accordance with the current Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) recommendations, current requirements stated in the Guide for the Care and Use of Laboratory Animals (National Research Council), and current requirements as stated by the U.S. Department of Agriculture through the Animal Welfare Act and Animal Welfare Regulations (November 2013).

**Quarantine**

3 days

**Housing**

1-3 per cage

**Cages**

Microisolator cages with hardwood chip bedding

**Light Cycle**

12 hr light/12 hr dark

**Temperature**

67°–74°F; on Day 31 (females) and Day 32 (males), the temperature in the housing room was recorded to be lower than the protocol-specified minimum of 68°F for a total of 5 hr. This deviation had no impact on the study as it was later determined that the decreased reading was due to a hygrothermograph malfunction and the actual room temperature had not fallen out of range. Further observations indicated the animals appeared healthy and unaffected.

**Humidity**

30–56%

**GLP-Multiple (5-Weekly) Repeat Subcutaneous Dose Toxicity Study**  
**with [Proprietary Info] [Prop] in Sprague Dawley Rats**  
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**Ventilation**

At least 10 room volumes per hour, with no recirculation of air

**Food**

Envigo Teklad Certified Global 18% rodent diet, #2018C, *ad libitum*. Feed was analyzed periodically to ensure that contaminants known to be capable of interfering with the study and reasonably expected to be present in such feed were not present at levels that would affect the study. Documentation of feed analyses is maintained at SRI for reference. A copy of the lot specific reports provided by the supplier is maintained in the study records.

**Water**

Water (purified, reverse osmosis) was provided *ad libitum*. Based on previous reports, no contaminants that could interfere with and affect the results of the study were expected to be present in the water. Copies of annual analysis reports are maintained at SRI for reference.

**3. Assignment of Animals to Study**

**Day**

5 days (males) and 6 days (females) before initiation of treatment

**Randomization**

Animals were randomly assigned to treatment groups via a computerized body weight stratification procedure (Provantis version 10.1.0.1). One animal was excluded from randomization based on health observation.

**Identification**

Animals were individually identified by a unique ear punch.

**4. Welfare of the Animals**

Every effort was made to minimize, if not eliminate, pain and suffering in all animals in this study. [Proprietary Info]

[Proprietary Info]

The

Study Director made every effort to protect the scientific validity of the study.

**C. Experimental Procedure (In-Life Evaluations)**

**1. Preparation of Animals**

Animals were not fasted before dose administration.



**GLP-Multiple (5-Weekly) Repeat Subcutaneous Dose Toxicity Study**  
**with [Proprietary Info] [Prop] in Sprague Dawley Rats**  
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**2. Dose Administration**

Weekly s.c. injections for 5 weeks were made to a single site on the dorsal region. The amount of dose volume administration was calculated based on the most recent body weight. The injection site was cleaned, fur shaved, and skin wiped clean with an alcohol pad and allowed to dry prior to administration of the test article. Injection sites began on the dorsal region at either side of the spine (right or left) and, on subsequent weeks, alternated to the opposite side (left or right) of the spine, progressing 0.5 cm posterior to the prior site. The injection sites progressed weekly in a zig-zag pattern to avoid dosing to the same area. Additionally, each injection site was marked adjacent to the injection location. This marking was refreshed, as needed, to maintain a visual identification of the injection site. Subcutaneous administration is proposed for clinical use of the test article in humans.

On Day 1, the water bath containing all vials of test article for Groups 1-4 reached a temperature of 39°C, exceeding the protocol-specified maximum of 37°C ± 1°C. This deviation had minimal impact on the study as the temperature exceeded the maximum for less than 2 min and the animal that received the dose formulation during the time of the deviation had a similar reaction to the dose administration as the other animals in the same dose group.

**3. Mortality/Morbidity**

Animals were checked at least once daily.

**4. Clinical Observations**

Recorded once daily and approximately 2–4 hr postdose on Days 1, 8, 15, 22 and 29, then weekly during the Recovery phase, or more often as clinical signs warranted, and on necropsy days. Animals were examined for any altered clinical signs, including gross motor and behavioral activity, and observable changes in appearance.

On Day 8, Animal #026 in Group 1 did not receive a 2-4 hr postdose clinical observation. This deviation had minimal impact on the study as immediate- and 7 hr-postdose observations, were made. [Proprietary Info]

[Proprietary Info] which had also been reported during the immediate postdose observation.

**5. Body Weights**

Body weights were recorded on Days 1, 8, 15, 22 and 29 (predose on each dosing day) for the purpose of dose calculation, weekly thereafter and at each necropsy.

[Proprietary Info]

**6. Food Consumption**

Quantitatively measured for approximately a 24-hr period once weekly for each cage throughout the study. The total cage consumption per interval was divided by the



**GLP-Multiple (5-Weekly) Repeat Subcutaneous Dose Toxicity Study**  
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number of animals in the cage to determine the average daily food consumption per animal.

**7. Ophthalmologic Examination**

All animals (including extras) had a pretest ophthalmic examination performed by [Redacted by agreement] a board-certified veterinary ophthalmologist, and all surviving animals were re-examined by [Redacted by] within the week before their scheduled necropsy (Main necropsy Day 30). Two rats in the Main subset of animals had findings that were not considered to be toxicologically-related ophthalmologic effects; all other animals were considered normal. Therefore, ophthalmologic examinations were not performed on the Recovery animals.

**8. Plasma Drug Levels**

**Method of Collection**

Blood was collected from the retro-orbital sinus of TK Satellite rats under 60:40% CO<sub>2</sub>:O<sub>2</sub> anesthesia into tubes containing K<sub>2</sub>EDTA, processed to plasma, and then stored frozen at ≤-60°C.

**Volume**

~ 300 µl whole blood (~150 µl of plasma) per sample

**Frequency**

TK Satellite Groups 2-4 rats were sampled 3 rats/sex/time point as follows:

1 hr predose and 1 hr postdose on dosing days (Days 1, 8, 15, 22 and 29).

Additional samples were collected on Days 36, 43, 50, 57, 64 and 71.

**Method of Analysis**

Drug levels of [Proprietary] [Proprietary] and [Proprietary] were measured in plasma samples using a bioanalytical method provided by the Sponsor and validated by SRI. Details of the bioanalytical method and validation results will be included in a separate validation report (SRI Study No. B181-18).

**9. Toxicokinetics Analysis**

TK data analysis was performed on the measurable plasma concentrations of each test compound from Appendix G (Bioanalytical Chemistry). The plasma concentration data of each test compound were analyzed using Phoenix<sup>®</sup> WinNonlin<sup>®</sup> (version 6.3) software to perform non-compartmental and sparse sampling analyses. Individual animal plasma concentrations at each actual time of blood collection were used in TK data analysis. The dose administered in each dose group was entered into the program as mg/kg, and as a result no additional corrections for individual body weights of the animals were necessary. Plasma concentration data were not available after Day 15 for male Animal #163. [Proprietary Info]

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**with [Proprietary Info] [Prop] in Sprague Dawley Rats**  
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Plasma concentrations that were less than the lower limit of quantitation (LLOQ) of 5.00 ng/ml for [Propri] and [Propri] and 25.00 ng/ml for [Proprie] of the bioanalytical assay were not included in TK data analysis. Mean plasma concentrations that were less than the LLOQ of each compound were assigned a value of 2.00 ng/ml only to illustrate apparent troughs in the concentration vs. time profiles. The following TK parameters were determined for each test compound in [Proprietary] [Pro] using the administration of the first dose on Day 1 as time zero: overall apparent maximal plasma concentration ( $C_{max}$ ) and area under the plasma concentration time curve up to the last blood collection time ( $AUC_{last}$ ).

**Disposition**

Residual bioanalytical samples will be discarded on submission of the final report.

**10. Clinical Pathology Evaluations**

**Preparation of Animals**

Animals were not fasted before blood collection.

**Method of Collection**

Blood was collected from the retro-orbital sinus of rats under 60% CO<sub>2</sub>/40% O<sub>2</sub> anesthesia. Hematology samples were collected using K<sub>3</sub>EDTA as the anticoagulant. No anticoagulant was used for clinical chemistry samples.

**Frequency**

Day 30 (Main Group) and Day 72 (Recovery Group).

Parameters that were evaluated are listed below. In some cases, automated analyzers reported additional parameters not specified in the protocol. Results for the additional parameters were included in the data package, but were not summarized, analyzed, or reported, and their collection was not considered a deviation from the protocol.

Manual WBC differential counts were conducted and some parameters that were not specified in the protocol were evaluated and reported. This was not considered a deviation from the protocol.

**Hematology Parameters**

- Hematocrit (HCT)
- Hemoglobin (HGB)
- Red blood cell count (RBC)
- Red blood cell distribution width (RDW)
- White blood cell count (WBC)
- WBC differential and absolute counts
  - Absolute neutrophil (ANE)
  - Percent neutrophil (PNE)

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- Absolute lymphocyte (ALY)
- Percent lymphocyte (PLY)
- Absolute monocyte (AMO)
- Percent monocyte (PMO)
- Absolute eosinophil (AEO)
- Percent eosinophil (PEO)
- Absolute basophil (ABA)
- Percent basophil (PBA)
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular volume (MCV)
- Mean corpuscular hemoglobin concentration (MCHC)
- Platelet count (PLT)
- Mean platelet volume (MPV)
- Absolute Reticulocyte (ARET)
- Percent Reticulocyte (PRET)

**Clinical Chemistry Parameters**

- Total Bilirubin (TBI)
- Creatinine (CRE)
- Sodium (SOD)
- Potassium (POT)
- Chloride (CHL)
- Cholesterol (CHO)
- Triglyceride (TRI)
- Glucose (GLU)
- Blood urea nitrogen (BUN)
- Aspartate aminotransferase (AST)
- Alanine aminotransferase (ALT)
- Alkaline phosphatase (ALP)
- Calcium (CAL)
- Phosphorus (PHO)
- Total protein (TPR)
- Albumin (ALB)
- Albumin/globulin ratio (AGR)
- Globulin (GLO)

**11. Urinalysis**

**Method of Collection**

Urine was collected by placing animals in metabolism cages overnight. No preservative was used for the collection. Total urine volume was recorded.



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**Frequency**

Urine was collected on Day 28 (prior to Day 30 Main necropsy) and on Day 72 (prior to Day 72 Recovery necropsy).

**Urinalysis Parameters**

- Color (noted at time of collection)
- Total Volume (total urine output measured at time of collection)
- Clarity (noted at time of collection)
- Specific gravity
- Microscopic examination of urine sediment
- Bilirubin
- Glucose
- Ketones
- Leukocytes
- Nitrite
- Occult blood
- pH
- Protein
- Urobilinogen

**D. Necropsy**

**Interval**

Day 30 (Main Group) and Day 72 (Recovery Group).

[Proprietary Info]

[Proprietary Info]

**Euthanasia**

CO<sub>2</sub> compressed gas inhalation in conjunction with an approved secondary method (bilateral thoracotomy) was used for Main and Recovery animals.

**Observations**

External examination of all body orifices and an examination of all cranial, thoracic, and abdominal organs was performed, and all gross findings were recorded.

**Tissues Retained**

The following tissues were collected from all animals in the Main Group and the Recovery Group, including those found dead. Tissues were retained in 10% neutral buffered formalin, except where noted:

- All gross lesions (including tissue masses and abnormal regional lymph nodes)
- Adrenal glands

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- Aorta
- Bone (femur with femoro-tibial joint)
- Bone, sternum (marrow histology)
- Bone marrow smear, sternum (for cytology, except for found dead animals)
- Brain (fore-, mid-, and hindbrain)
- Cecum
- Cervix
- Colon
- Duodenum
- Epididymides
- Esophagus
- Eyes, with optic nerve (fixed with modified Davidson's solution)
- Heart
- Identification; (retained in formalin; not processed for histology)
- Ileum
- Injection site(s) tissue. Only representative sections of the injection site(s) were collected
- Jejunum
- Kidneys
- Liver
- Lungs with bronchi
- Lymph nodes, mandibular and mesenteric
- Mammary gland (females, males when present)
- Ovaries
- Pancreas
- Pituitary gland
- Prostate
- Rectum
- Salivary gland, mandibular
- Sciatic nerve
- Seminal vesicles
- Skeletal muscle
- Skin, ventral abdomen, taken with mammary gland
- Spinal cord retained within spinal column (thoracolumbar only)
- Spleen
- Stomach (including nonglandular stomach)
- Testes (fixed with modified Davidson's solution)
- Thymus
- Thyroid/parathyroid glands
- Trachea
- Urinary bladder
- Uterus



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- Vagina

**Final Body/Organ Weights**

Body weights were recorded on the day of necropsy for body-to-organ weight ratios. The following organs were weighed. Paired organs were weighed together.

- Adrenal glands
- Brain
- Heart
- Kidneys
- Liver
- Spleen
- Ovaries
- Testes, without epididymides
- Thymus

Organ weights were recorded for the one animal found dead, but these data were not included in statistical evaluations.

**E. Histopathologic Examination**

**Tissues**

Tissues listed under “Retained Tissues” were processed and evaluated as follows:

- All Main animals in the control and high dose groups (Groups 1 and 4)
- Animal with an unscheduled death
- Injection site, identified as a target organ by the pathologist, was examined in all dose groups
- All gross lesions were processed for all animals
- Analysis of only one parathyroid gland per animal was considered sufficient
- Neurological clinical signs were not present; therefore, only a representative sample of the thoracolumbar (or thoracic) section of the spinal cord was collected, processed and evaluated.

**Tissue Sections**

Sections of the tissues were embedded in paraffin, cut approximately 5 µm thick, and stained with hematoxylin and eosin [Proprietary Info]  
[Proprietary Info] a histology laboratory qualified by SRI.

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**Evaluated By**

Histopathology specimens were evaluated by [Redacted by agreement]  
[Redacted by agreement], a board-certified veterinary pathologist.

**Method**

Each lesion was listed and coded by the most specific topographic and morphologic diagnoses, severity, and distribution, using International Harmonization of Nomenclature and Diagnostic Criteria for Lesions (INHAND) as a guide. A four-step grading system (minimal, mild, moderate, and marked) was used to define gradable lesions for comparison between treated and control groups. Data were recorded and summarized using Provantis® version 10.1.0.1. Records of gross findings for a specimen from postmortem observations were available to the pathologist when examining that specimen microscopically.

**F. Evaluation of Data Parameters**

Mean and standard deviation were calculated for body weight, clinical pathology, food consumption, urinalysis pH, urobilinogen and specific gravity and organ weight data at each evaluation interval. Calculations were performed using Provantis® version 10.1.0.1, and MS Excel 2010 and 2016.

**Statistical Tests**

Body weight, food consumption, clinical pathology, urinalysis pH, urobilinogen and specific gravity and organ weight data were evaluated by one-way analysis of variance (ANOVA), followed by Dunnett's test (if the ANOVA was significant). All other numeric parameters were evaluated by Student's t-test, unless specified otherwise. For clinical pathology data, values for parameters that were not within the detection threshold were not included in the statistical evaluation.

**Criteria for Null Hypothesis Rejection**

The criteria for rejection of the null hypothesis was  $p \leq 0.05$

**G. Control of Bias**

While evaluating the responses of the animals and conducting the analyses, the technical staff were aware of the treatment history of each animal and sample. Based on the relatively objective endpoints to be examined, bias did not influence the results of the study.

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**V. REGULATORY COMPLIANCE**

**A. Good Laboratory Practice Compliance**

This study is intended to be submitted to and reviewed by the U.S. FDA or an equivalent regulatory agency, and this study therefore was performed in accordance with the U.S. FDA “Good Laboratory Practice for Nonclinical Laboratory Studies,” as described in 21 CFR Part 58, with the following exceptions:

- Receipt of animals was performed prior to the approval of the protocol, but because it was conducted before the protocol was signed, it may not be considered by the FDA to have been conducted in compliance with GLP requirements. This activity was conducted according to testing facility SOPs and is not considered to have impacted the study.
- Animal water, bedding, and food analysis were not performed under GLP compliance by the vendors. Based on previous reports, no contaminants that could interfere with and affect the results of the study were expected to be present in the water. Bedding and feed were analyzed periodically to ensure that contaminants known to be capable of interfering with the study and reasonably expected to be present in such feed were not present at levels that would affect the study; therefore, water, bedding, and food analyses not performed under GLP compliance by the vendors had no impact on the study.

**B. Retention of Records and Study Samples**

A copy of the original protocol, amendments, final report, including all appendices, raw data, histopathology slides and other pathology materials (blocks and wet tissue specimens) specific to this study will be retained and stored by SRI International for a period of one year. An archival sample of the test and control articles will be maintained by SRI for at least 5 years or as long as samples afford evaluation (21 CFR 58.105[d]). At the end of the retention period, the Sponsor will be contacted for instructions regarding disposition of these materials.

**VI. RESULTS**

**A. Mortality/Morbidity and Clinical Observations**

Clinical observations are summarized in Table 1. Mortality/morbidity and individual animal clinical observations are presented in Appendix C.

[Proprietary Info]



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**B. Body Weights**

Body weight and body weight changes are summarized in Tables 2 and 3, respectively. Individual animal body weights and body weight changes are presented in Appendices D-1 and D-2, respectively.

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**C. Food Consumption**

Food consumption is summarized in Table 4. Individual animal food consumption is presented in Appendix E.

Slight statistically significant changes in food consumption in

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**D. Ophthalmology**

Ophthalmology reports are included in Appendix F.

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**with [Proprietary Info] [Prop] in Sprague Dawley Rats**  
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[Proprietary Info]

**E. Plasma Drug Levels**

The time-course of plasma concentrations of [Propri] [Propri] and [Propri] is shown in Figures 1 to 3, respectively. [Proprietary Info]

[Proprietary Info]

[Proprietary Info]

Individual animal plasma concentration data are presented in Appendix G.

**F. Toxicokinetics Analysis**

The toxicokinetic parameters for [Propri] [Propri] and [Propri] are presented in Table 5.

[Proprietary Info]

**G. Clinical Pathology Evaluations**

Hematology and clinical chemistry results are summarized in Tables 6 and 7, respectively. Individual animal clinical pathology data are presented in Appendix I.



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## **H. Urinalysis**

Urinalysis results are summarized in Table 8. Individual animal urinalysis data are presented in Appendix J.

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## **I. Organ Weights**

Organ weights are summarized in Table 9. Individual animal organ weights are presented in Appendix K.

Statistically significant decreases in heart-to-body weight (↓11%) were observed

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## **J. Necropsy Observations and Histopathology**

### Necropsy Observations

Necropsy observations are presented in Appendices L-4 to L-5.

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Histopathology

The histopathology report is presented in Appendix L-1 to L-3.

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with [Proprietary Info] [Prop] in Sprague Dawley Rats  
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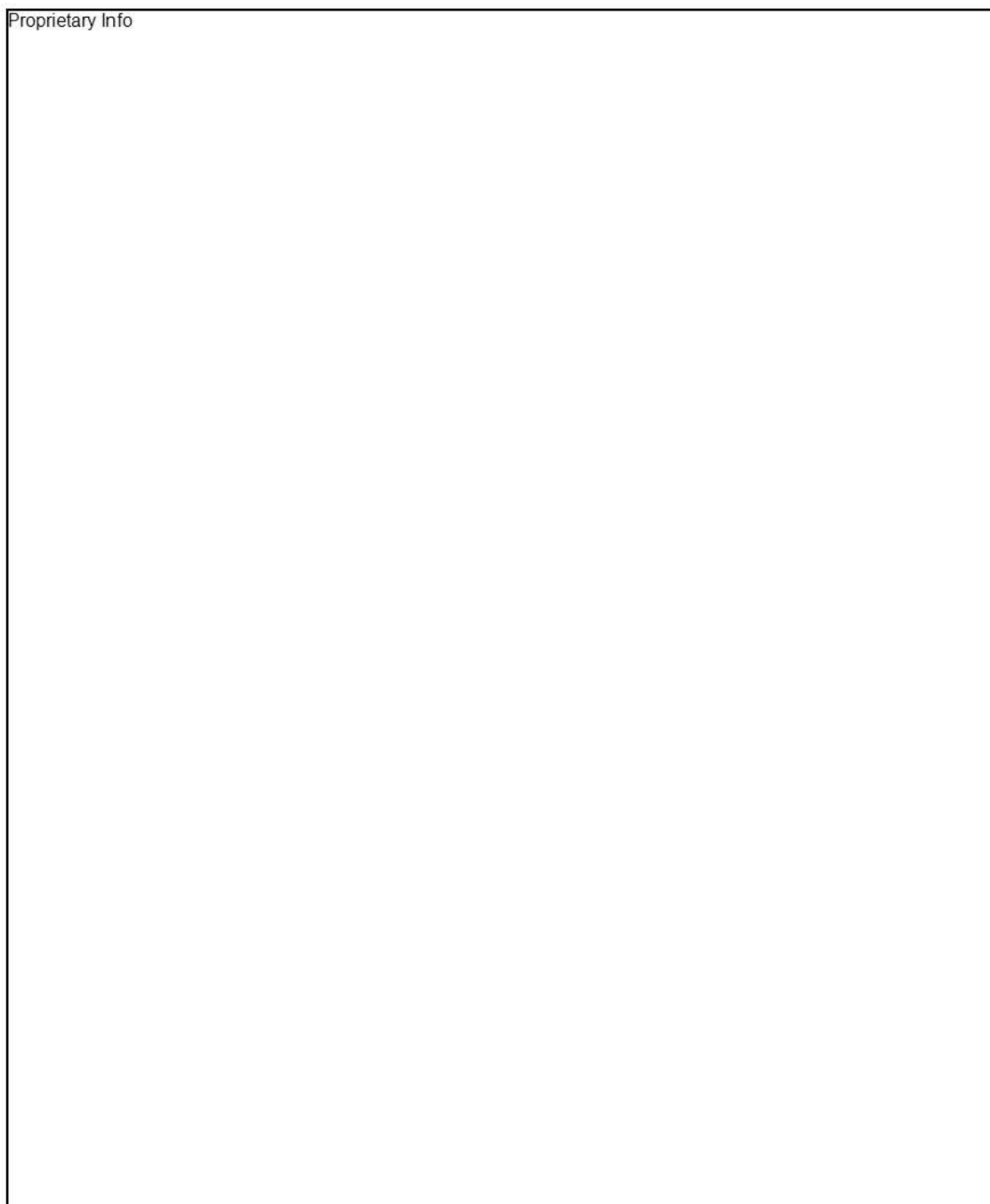
Proprietary Info

**VIII. REFERENCES**

McInnes E. (2012). Wistar and Sprague-Dawley Rats. *In: Background Lesions in Laboratory Animals. A Color Atlas.* Saunders Elsevier. **2**:17-36.

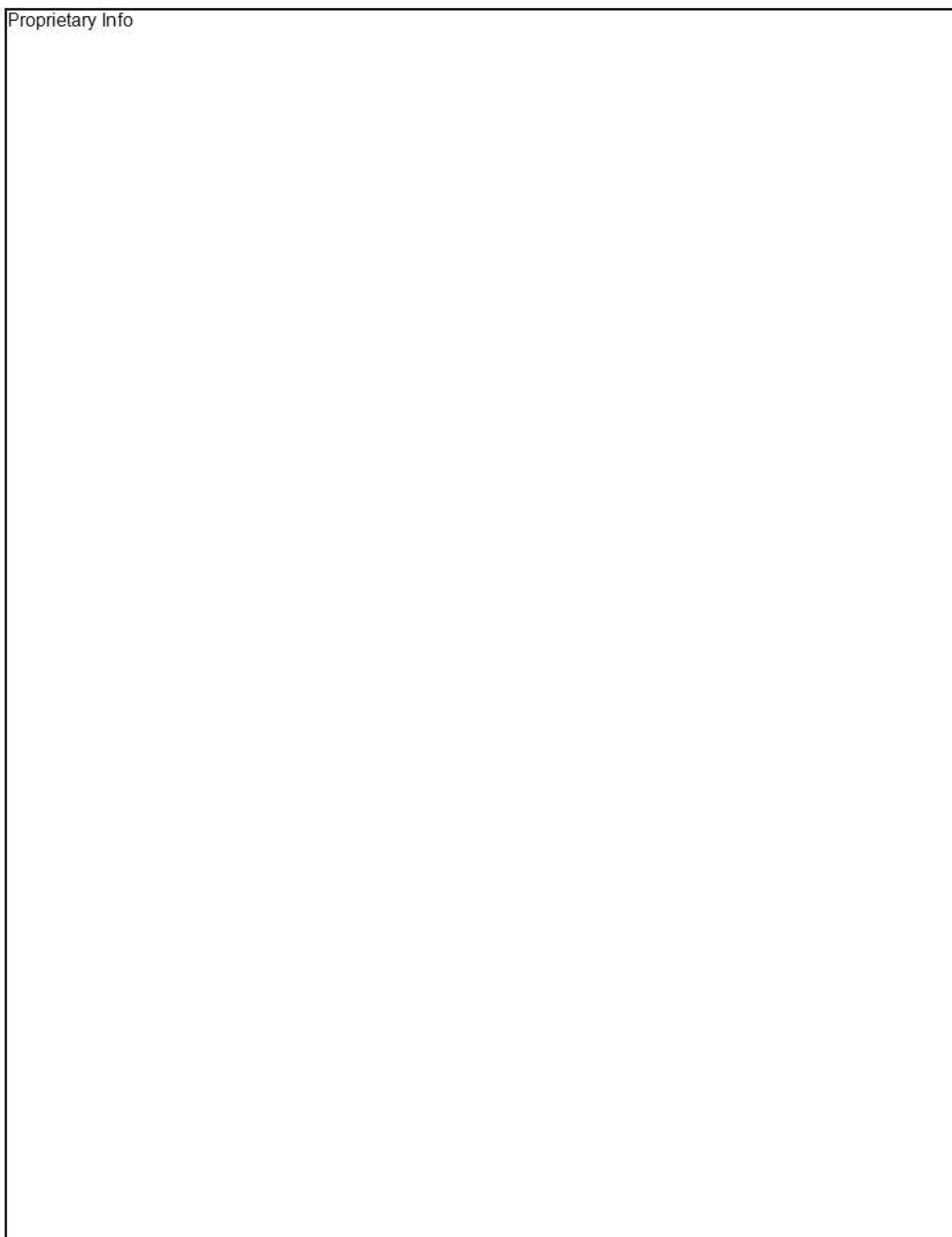


**GLP-Multiple (5-Weekly) Repeat Subcutaneous Dose Toxicity Study**  
**with [Proprietary Info] [Pro] in Sprague Dawley Rats**  
**SRI Study No. M398-18**



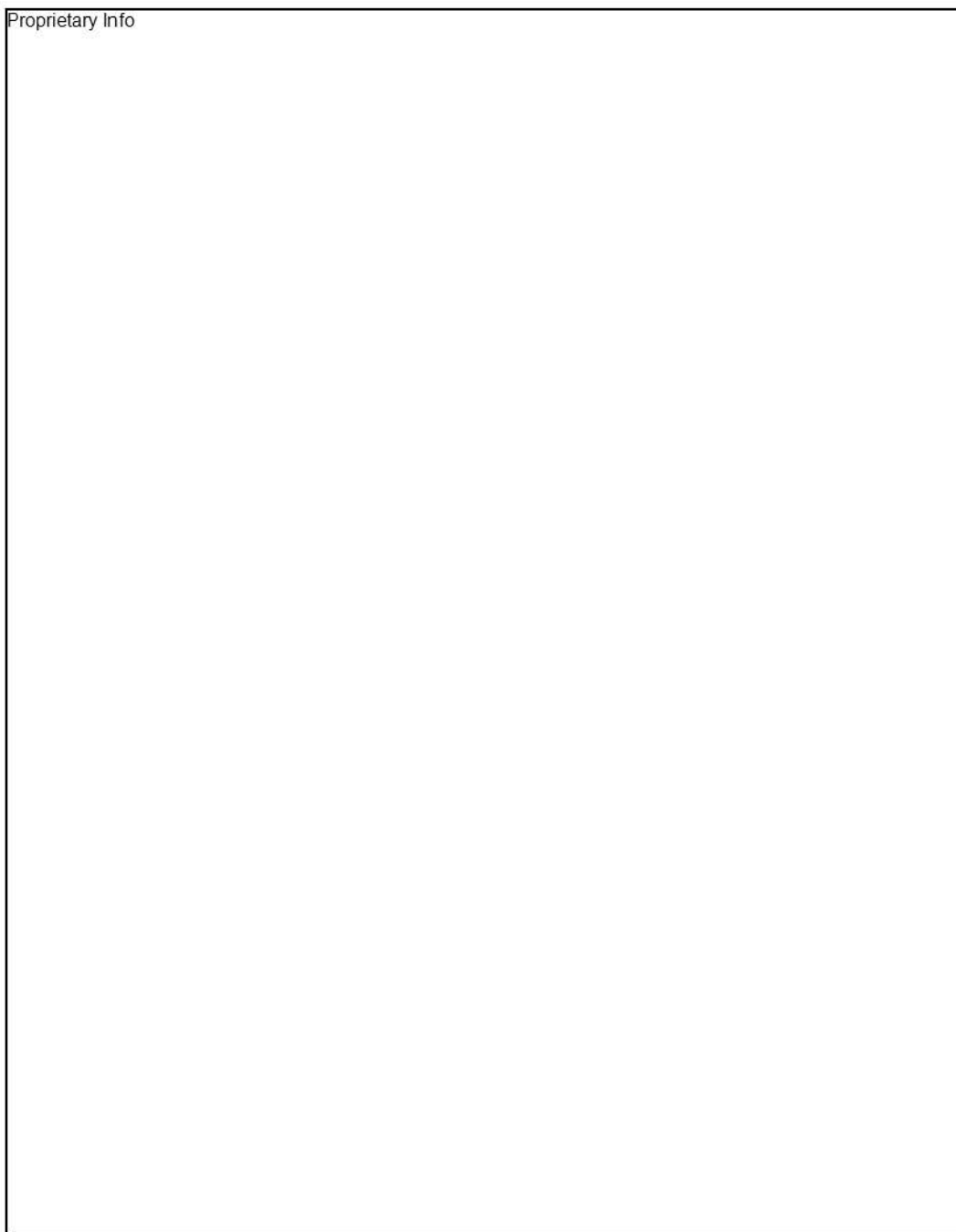
**Figure 1.** Time-course of plasma concentrations of [Proprietary] in male (top) and female (bottom) rats after sc administration of [Proprietary] [Prop] a formulation of [Proprietary] [Proprietary] and [Proprietary] [Proprietary] [Pro] was administered weekly for 5 weeks. [Proprietary] doses were 1.5 mg/kg (Group 2), 15 mg/kg (Group 3), and 30 mg/kg (Group 4). Nominal times are plotted using dose administration on Day 1 as time zero. Plasma [Proprietary] concentrations that were less than LLOQ (5.00 ng/ml) were assigned a value of 2 ng/ml for graphical illustration purposes only.

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**Figure 2.** Time-course of plasma concentrations of [Proprietary] in male (top) and female (bottom) rats after sc administration of [Proprietary] [Prop] a formulation of [Proprietary] [Proprietary] and [Proprietary] [Proprietary] [Pro] was administered weekly for 5 weeks. [Proprietary] doses were 0.8625 mg/kg (Group 2), 8.625 mg/kg (Group 3), and 17.25 mg/kg (Group 4). Nominal times are plotted using dose administration on Day 1 as time zero. Plasma [Proprietary] concentrations that were less than LLOQ (25.00 ng/ml) were assigned a value of 2 ng/ml for graphical illustration purposes only.

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**with [Proprietary Info] [Pro] in Sprague Dawley Rats**  
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**Figure 3.** Time-course of plasma concentrations of [Proprietary] in male (top) and female (bottom) rats after sc administration of [Proprietary] [Prop] a formulation of [Proprietary] [Proprietary] and [Proprietary] [Proprietary] [Pro] was administered weekly for 5 weeks. [Proprietary] doses were 0.4125 mg/kg (Group 2), 4.125 mg/kg (Group 3), and 8.25 mg/kg (Group 4). Nominal times are plotted using dose administration on Day 1 as time zero. Plasma [Proprietary] concentrations that were less than LLOQ (5.00 ng/ml) were assigned a value of 2 ng/ml for graphical illustration purposes only.

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**General Footnotes**

"-" indicates Not Applicable  
Provantis version 10.1.0.1

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings</u>		
1	Excipient (0)	Control	Group 1	0 mg/kg	SC
2	Propriet Pr (1.5)	Dose	Group 2	1.5 mg/kg	SC
3	Propriet Pr (15)	Dose	Group 3	15 mg/kg	SC
4	Propriet Pr (30)	Dose	Group 4	30 mg/kg	SC



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**General Footnotes**

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**Measurement Descriptions**

<u>Headings Used</u>	<u>Description</u>
Body Weight	Body Weight

**Measurement/Statistics**

<u>Measurement</u>	<u>Descriptive</u>	<u>Comparative</u>	<u>Arithmetic /Adjusted</u>	<u>Transformation</u>
Body Weight	Mean Standard Deviation Count (N)	Anova & Dunnett's 2 Sided	Arithmetic	Automatic

**Automatic Transformations**

<u>Measurement</u>	<u>Transformation Order</u>
Body Weight	Identity (No Transformation), Log, Rank

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Type</u>	<u>Report Headings 1-4</u>		
1	Excipient (0)	Control	Group 1	0 mg/kg	SC
2	Propriet [Pr] (1.5)	Dose	Group 2	1.5 mg/kg	SC
3	Propriet [Pr] (15)	Dose	Group 3	15 mg/kg	SC
4	Propriet [Proprie]	Dose	Group 4	30 mg/kg	SC

**Pairwise Comparisons**

<u>Group</u>	<u>Vs</u>	<u>Group</u>
1		2
1		3
1		4