FINAL REPORT

STUDY TITLE

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

STUDY NUMBER

WIL-534002

STUDY DIRECTOR

Jeannie B. Kirkpatrick, MS

STUDY INITIATION DATE

5 January 2005

STUDY COMPLETION DATE

2 September 2005

PERFORMING LABORATORY

WIL Research Laboratories, LLC
1407 George Road
Ashland, OH 44805-9281

SPONSOR

AGC Chemical
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COMPLIANCE STATEMENT

This study, designated WIL-534002, was conducted in compliance with the United States Environmental Protection Agency (EPA) Good Laboratory Practice Standards (40 CFR Part 792), September 18, 1989; the Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice [C (97) 186/Final], November 26, 1997; the standard operating procedures of WIL Research Laboratories, LLC, and the protocol as approved by the sponsor. A Certificate of Analysis for the PFHxA was provided by the sponsor and a Certificate of Analysis for the PFBS was provided by Sigma-Aldrich (presented in Appendix A); the characterization analyses were not conducted according to GLP standards.

Jeannie B. Kirkpatrick, MS
Staff Toxicologist
Study Director

2 September 2005
Date

TABLE OF CONTENTS

Page

INDEX OF TABLES

INDEX OF APPENDICES

1. SUMMARY

1.1. OBJECTIVE

The objectives of this study were to evaluate the pharmacokinetic (in blood) and excretion profiles of the test articles in cynomolgus monkeys.

1.2. STUDY DESIGN

Perfluorohexanoic acid (PFHxA) was administered as a single intravenous (bolus) injection to 3 male and 3 female Cynomolgus monkeys (Group 1) followed 7 days later by a single intravenous (bolus) dose of nonafluoro-1-butanesulfonic acid (PFBS) to the same animals (Group 2). The dosage level for both test articles was 10 mg/kg and the dose volume was 5 mL/kg.

The animals were observed twice daily for mortality and moribundity. Clinical examinations were performed twice on each day of dosing and daily on non-dosing days and detailed physical examinations were performed weekly. Individual body weights were recorded weekly. Blood samples for serum drug levels were collected from all animals/group at 0 (prior to dosing) and 1, 2, 4, 8, 24 and 48 hours following administration of each dose. Urine was collected on wet ice from all animals at 0-6, 6-12 and 12-24 hours post-dosing and then once daily until 7 days post-dosing. However, due to the limitations of the test chamber design, quantitative recovery of urine may not have been attained. Some urine may also have been adsorbed to the feces. All animals were returned to the stock colony following a 7-day monitoring period after administration of PFBS.

Serum and urine concentrations of PFHxA or PFBS were measured using a validated LC MS/MS method. The serum concentration immediately following the intravenous dose was estimated based on the first two measured values. The concentrations in serum and amounts excreted in urine were used for pharmacokinetic analysis.

1.3. RESULTS AND CONCLUSIONS

Systemic exposures to PFHxA were approximately an order of magnitude lower than exposures to PFBS at equivalent dosages. The half-life of PFHxA in serum was shorter (2.4-5.3 hours) than the half-life of PFBS in serum (8.1-15 hours). Apparent systemic clearance was approximately an order of magnitude higher for PFHxA than for PFBS and volume of distribution was two- to five-fold higher. The lower apparent volume of distribution for PFBS than for PFHxA suggests that PFBS is sequestered outside the vasculature. There did not appear to be any gender differences in the pharmacokinetics of PFHxA. For PFBS, male monkeys appeared to have higher exposure and longer half-lives than female monkeys, but the mean values were greatly influenced by one male with high exposure and a long half-life.

In conclusion, PFHxA treatment resulted in lower systemic exposure in cynomolgus monkeys than PFBS at equivalent dosages. PFHxA was cleared more rapidly and had a wider volume of distribution than PFBS.

2. INTRODUCTION

2.1. GENERAL STUDY INFORMATION

This report presents the data from "A Pharmacokinetic (in Blood) and Excretion Study in Cynomolgus Monkeys". Due to software spacing constraints, the study title appears as "A Pharmacokinetic and Excretion Study in Cynomolgus Monkeys" on the report tables.

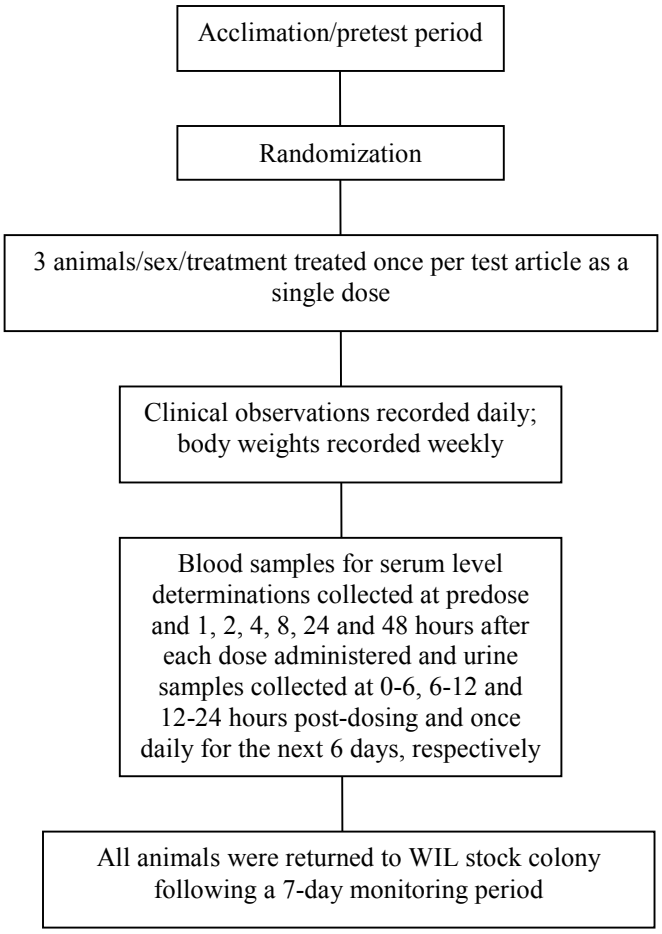
The following computer protocols were used for data collection during the study:

Computer Protocol	Type of Data Collected
WIL-534002	Main study data
WIL-534002P	Pretest data

2.2. KEY STUDY DATES

Date(s)	Event(s)
6 January 2005	Experimental starting date (animal selection from stock colony)
9 February 2005	Assignment to study groups
10 February 2005	Experimental start date (initiation of dose administration [PFHxA]; study day 0)
17 February 2005	Initiation of dose administration [PFBS]; study day 7
18 July 2005	Experimental termination (completion) date (last bioanalytical data collection)

3. STUDY DESIGN



4. EXPERIMENTAL PROCEDURES - MATERIALS AND METHODS

4.1. TEST ARTICLE AND VEHICLE

4.1.1. TEST ARTICLE IDENTIFICATION

4.1.1.1. TEST ARTICLE 1 IDENTIFICATION

Test article 1, perfluorohexanoic acid (PFHxA), was received from AGC Chemical, via Nippon Express USA, Bensenville, Illinois, on 4 January 2005, as follows:

Identification	Quantity Received	Physical Description
PFHxA Lot no. C15004301 [WIL log no. 6394A]	1 bottle Gross weight: 1403.7 g	Clear colorless liquid

A Certificate of Analysis for the PFHxA was provided by the supplier and is presented in Appendix A. Data documenting the purity and stability of the test article are on file with the sponsor. The purity of PFHxA was 98.5%, but was considered to be 100% for dose calculation purposes. PFHxA was stored at room temperature and was considered stable under this condition. A reserve sample of the PFHxA (approximately 2 g) was collected on 17 January 2005 and stored in the Archives of WIL Research Laboratories, LLC.

4.1.1.2. TEST ARTICLE 2 IDENTIFICATION

Test article 2, nonafluorobutane-1-sulfonic acid (PFBS), was received from Aldrich, Milwaukee, Wisconsin, on 30 December 2004, as follows:

Identification	Quantity Received	Physical Description
PFBS Lot no. 562629 CAS no. 375-73-5 [WIL log no. 6396A]	1 bottle Gross weight: 123.6 g	Clear colorless liquid

A Certificate of Analysis for the PFBS was provided by the supplier and is presented in Appendix A. The purity of PFBS was 99.65%, but was considered to be 100% for dose

calculation purposes. PFBS was stored refrigerated under nitrogen and was considered stable under these conditions. A reserve sample of PFBS (approximately 1 g) was collected on 14 February 2005 and stored in the Archives of WIL Research Laboratories, LLC.

4.1.2. VEHICLE IDENTIFICATION

The vehicle used in preparation of the PFHxA and PFBS formulations group was sterile water for injection, USP (lot no. C631283, exp. date: 1 October 2005, received from Hursh Drug).

4.1.3. PREPARATION

Both PFHxA and PFBS formulations were weight/volume (test article/vehicle) mixtures. The appropriate amount of the test article for each group was weighed into a tared, calibrated container. A predetermined volume of the vehicle was added to each container to bring the formulations nearly to the calibration mark. The formulations were mixed until uniform using a magnetic stirrer. A sufficient volume of the vehicle was added to each container to bring the formulations to the calibration mark. The formulation was mixed using a magnetic stirrer until uniform. All groups were sterile filtered through a 0.22-µm Durapore Bottle-top filter into sterile containers and capped with septums. The test article formulations were prepared once as single formulations for each dosage level, divided into 2 aliquots (1 for a 2-day stability test, the other for dosing) and stored refrigerated, protected from light. The test article formulations were stirred continuously throughout the preparation procedures.

4.1.4. ADMINISTRATION

PFHxA and PFBS were administered via intravenous (bolus) injection to all monkeys as a single dose (for each test article). Each animal was dosed while properly restrained. The formulations were administered by bolus push using an appropriately sized syringe. A dosage volume of 5 mL/kg was used for all animals. Individual doses were based on

the most recently recorded body weights to provide the correct mg/kg dosage. The first day of dosing was study day 0; the first week of dosing was study week 0.

The following table presents the study group assignment:

Treatment/ Group Number	Test Article	Dosage Level (mg/kg)	Dosage Volume (mL/kg)	Concentration (mg/mL)	Number of Animals Males	Females
1	PFHxA	10	5	2	3	3
2	PFBS	10	5	2	3	3

[a] = Animals were dosed once per test article. There was a 7-day non-dosing observation period between treatments. After the last dose was administered, animals were monitored for 7 days before being assigned to the WIL stock monkey colony.

The selected route of administration was intravenous (bolus) injection in order to remove the variability of absorption. The animal model, the cynomolgus monkey, is recognized as appropriate for both single and repeated-dose toxicity studies and is a widely used species for which significant historical control data are available. The number of animals used on study is the minimum number required to yield scientifically meaningful data and is consistent with agency expectations.

4.1.5. SAMPLING AND ANALYSES

Homogeneity assessments were not performed for either test article. Prior to the initiation of dose administration of PFBS (15 February 2005) duplicate samples (1 mL each) for stability determinations were collected from the middle strata of the dosing formulations and stored refrigerated and protected from light for 2 days. Stability for PFHxA was established in a previous study (Kirkpatrick, Draft) at 2-days. Samples (1 mL each) for concentration analyses were collected during the dosing period.

All analyses were conducted by the Analytical Chemistry Department, WIL Research Laboratories, LLC. The methodology and results of these analyses are presented in

Appendices B and C. The test article formulations contained the amounts of test article specified in the protocol and were stable for at least 2 days.

4.2. ANIMAL RECEIPT AND ACCLIMATION/PRETEST PERIOD

Cynomolgus monkeys (*Macaca fascicularis*) used on study were obtained from the WIL stock colony and originally were received in apparent good health from Covance Research Products Inc., Alice, Texas, on 28 June 2004. The monkeys were young adults, approximately 2 to 2.5 years old upon receipt. Each animal was weighed the following day. Animals were uniquely identified by tattoo. Animals considered suitable for the study were housed for at least a 5-week acclimation/pretest period. During this period, each animal was observed twice daily for mortality and changes in general appearance or behavior.

The monkeys tested negative for antibodies to Herpes B virus, SIV (simian immunodeficiency virus), STLV1 (simian T-lymphotropic virus) and SRV (simian retrovirus) (SRV-1 and SRV-2). All animals were vaccinated for measles and Hepatitis A by the supplier. Stool samples were collected during the acclimation period and checked for parasites; none were present. Three tuberculin (TB) tests were administered approximately 2 weeks apart, 1 by the supplier prior to shipment and 2 by WIL Research Laboratories, LLC personnel. Results of all TB tests were negative. Prior to the first dose, animals were acclimated to the handling and restraint procedures required for dosing and blood collection.

Pretest data collection began on 6 January 2005. Individual body weights were recorded and detailed physical examinations were performed periodically during the pretest period. Pretest clinical observations are presented in Appendix D.

4.3. ANIMAL HOUSING

The animals were housed individually in clean, stainless steel cages elevated above stainless steel flush pans that were cleaned daily during the acclimation period and throughout the study. All animals were maintained in accordance with the *Guide for the*

Care and Use of Laboratory Animals (National Research Council, 1996). All monkeys were provided with an enriched environment in accordance with the standard operating procedures at WIL Research Laboratories, LLC, and the Animal Welfare Act (9 CFR Part 3). The animal facilities at WIL Research Laboratories, LLC, are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

4.4. DIET, DRINKING WATER AND MAINTENANCE

Seven to 9 PMI Nutrition International, LLC, Certified Primate LabDiet® 5048 biscuits were offered twice daily. The diet utilized at WIL Research Laboratories, LLC, is a certified feed with appropriate analyses for potential contaminants performed and provided by the manufacturer. The diet was supplemented with other nutrients (such as fresh fruits) which were presented to the animals as part of the environmental enrichment program. Reverse osmosis-treated (on-site) drinking water, delivered by an automatic watering system, was provided *ad libitum* throughout the study period. Municipal water supplying the facility is sampled for contaminants according to the standard operating procedures. The results of the diet and water analyses are maintained at WIL Research Laboratories, LLC. No contaminants were present in animal feed or water at concentrations sufficient to interfere with the objectives of this study.

4.5. ENVIRONMENTAL CONDITIONS

All animals were housed throughout the acclimation period and during the study in an environmentally controlled room. The room temperature and humidity controls were set to maintain daily averages of 71 ± 5°F (22 ± 3°C) and 50 ± 20% relative humidity. Room temperature and relative humidity were monitored using the Metasys® DDC Electronic Environmental control system and were recorded approximately hourly. These data are summarized in Appendix E. Actual mean daily temperature ranged from 70.3°F to 70.6°F (21.3°C to 21.5°C) and mean daily relative humidity ranged from 36.9% to 43.4% during the study. Light timers were set to provide a 12-hour light (0600 to 1800)/12-hour dark photoperiod. Air handling units were set to provide a minimum of 10

fresh air changes per hour. The 12-hour light/12-hour dark photoperiod was interrupted as necessary to allow for the performance of protocol-specified activities.

4.6. ASSIGNMENT OF ANIMALS TO STUDY GROUPS

On 9 February 2005 (the day prior to the initiation of dose administration), all available monkeys were weighed and examined in detail for physical abnormalities. These data were collected using the WIL Toxicology Data Management System (WTDMS™) and reviewed by the study director. The animals judged suitable for assignment to the study were arbitrarily assigned to the study. The selected animals were approximately 3 years old at the initiation of dose administration; body weights ranged from 2.4 kg to 2.8 kg for the males and 2.5 kg to 2.6 kg for the females.

5. PARAMETERS EVALUATED

5.1. CLINICAL OBSERVATIONS AND SURVIVAL

The animals were observed twice daily, once in the morning and once in the afternoon, for mortality and moribundity. All animals were also observed prior to dose administration and approximately 1 to 2 hours following dose administration (designated as 1 hour post-dosing for report presentation purposes). During the non-dosing observation periods, the animals were observed once daily. All significant findings were recorded. Detailed physical examinations were conducted on all animals weekly, beginning at least 1 week prior to test article administration and prior to return to the stock colony.

5.2. BODY WEIGHTS

Individual body weights were recorded weekly, beginning approximately 1 week prior to test article administration (study week -1). Animals were weighed on the day prior to each dosing. Body weights were also recorded approximately 1 week after the last dose.

5.3. FOOD CONSUMPTION

Individual food consumption was not determined. Any decrease in appetite was recorded as a daily clinical observation.

5.4. TOXICOKINETICS

Blood samples (approximately 0.5 mL each) for test article serum level determinations were collected at predose and 1, 2, 4, 8, 24 and 48 hours after each dose administration. Blood was collected via the femoral vein into tubes containing no anticoagulant. Serum was prepared and frozen with minimal delay at approximately -20°C or -70°C. The serum samples were transferred to the WIL Analytical Chemistry Department for analysis.

After each dose administration, urine was collected on wet ice over the following intervals: 0-6, 6-12 and 12-24 hours post-dosing and then once daily for the next 6 days (until 7 days post-dosing). Urine was collected as run-off from trays under the animal

caging which did not specifically separate fecal material from urine. The equipment used did not allow quantitative recovery of all urine excreted because the animals were not prevented from urinating outside of the cages. Urine samples were frozen with minimal delay at approximately -20°C and stored at approximately -20°C until transferred to the WIL Analytical Chemistry Department for analysis.

The methods and results of these analyses are presented in Appendix F and G, and the interpretation of the toxicokinetic data is presented in Appendix H.

5.5. STATISTICAL METHODS

Because of the small group sizes, statistical inferences were not calculated.

5.6. DATA RETENTION

The sponsor has title to all documentation records, raw data, specimens or other work product generated during the performance of the study. All work product generated by WIL Research Laboratories, LLC, including raw paper data and specimens, are retained in the Archives at WIL Research Laboratories, LLC, as specified in the study protocol.

Reserve samples of the test and control articles (if appropriate), pertinent electronic storage media and the original final report are retained in the Archives at WIL Research Laboratories, LLC, in compliance with regulatory requirements.

6. RESULTS AND DISCUSSION

6.1. CLINICAL OBSERVATIONS AND SURVIVAL

Individual Data: Tables 1 through 8

All animals survived to the termination of the study. Soft feces was noted in both the PFHxA and PFBS group males and females, but was also observed for several animals before the start of treatment. Two of the three PFHxA group males had soft feces on the day after dosing. All other occurrences of soft feces were 5 to 7 days after treatment with either PFHxA or PFBS and showed no relationship to treatment. There were no other potential test article-related clinical observations. All observations were limited to single animals and/or were common findings for laboratory monkeys.

6.2. BODY WEIGHTS

Individual Data: Tables 9, 10

Body weights were unaffected by test article administration.

6.3. TOXICOKINETICS

Summary Data: Appendices F, G

Individual Data: Appendices F, G

Serum and urine concentrations of PFHxA or PFBS were measured using a validated LC-MS/MS method. The serum concentration immediately following the intravenous dose was estimated based on the first two measured values. The concentrations in serum and amounts excreted in urine were used for pharmacokinetic analysis.

The mean pharmacokinetic parameters for PFHxA and PFBS are summarized in the following table:

MEAN PHARMACOKINETIC RESULTS

10 mg/kg Intravenous Dose	SERUM C_0* (ng/mL)	$AUC_{0-\infty}$ (ng•h/mL)	Half-life (h)**	Cl (L/h/kg)	V_d (L/kg)
PFHxA					
Males	57877	84002	5.3	0.122	0.989
Females	65178	75157	2.4	0.136	0.474
PFBS					
Males	113852	1114762	15	0.0124	0.209
Females	100301	488859	8.1	0.0229	0.248

* Values were estimated.
** For the terminal elimination phase.

Systemic exposures to PFHxA were approximately an order of magnitude lower than exposures to PFBS at equivalent dosages. The half-life of PFHxA in serum was shorter (2.4-5.3 hours) than the half-life of PFBS in serum (8.1-15 hours). Apparent systemic clearance was approximately an order of magnitude higher for PFHxA than for PFBS and volume of distribution was two- to five-fold higher. The lower apparent volume of distribution for PFBS than for PFHxA suggests that PFBS is sequestered outside the vasculature. On average, only about 10-20% of the administered dose of either compound was recovered in the urine during the 7 days post-dosing. The low percentage of dose recovered in urine may be due to the incomplete capture of the urine. Therefore, the calculated half-life for urinary elimination of PFHxA and PFBS may not be meaningful. There did not appear to be any gender differences in the pharmacokinetics of PFHxA. For PFBS, male monkeys appeared to have higher exposure and longer half-lives than female monkeys, but the mean values were greatly influenced by one male with high exposure and a long half-life.

7. CONCLUSIONS

In conclusion, PFHxA treatment resulted in lower systemic exposure in cynomolgus monkeys than PFBS at equivalent dosages. PFHxA was cleared more rapidly and had a wider volume of distribution than PFBS.

8. KEY STUDY PERSONNEL AND REPORT SUBMISSION

Report Submitted By:

Jeannie B. Kirkpatrick, MS
Staff Toxicologist
Study Director

2 September 2005
Date

Report Prepared By:

A. Dan Armstrong II, BS
Study Analyst

2 Sept 05
Date

Report Reviewed By:

Christopher P. Chengelis, PhD, DABT
Director, Toxicology

2 Sep 05
Date

Jozef J.W.M. Mertens, PhD, DABT
Associate Director, General Toxicology

September 2, 2005
Date

Robert A. Wally, BS, RAC
Acting Manager, Reporting and
Regulatory Technical Services

2 September 2005
Date

Study Personnel:

Susan C. Haley, BS	Manager, Clinical Pathology
Sally A. Keets, AS	Senior Operations Manager, Vivarium
Teresa D. Morris, BS	Operations Manager, Toxicology
Theresa M. Rafeld	Group Supervisor, Formulations Laboratory

9. QUALITY ASSURANCE UNIT STATEMENT

9.1. PHASES INSPECTED

Date(s) of Inspection(s)	Phase Inspected	Date(s) Findings Reported to Study Director	Date(s) Findings Reported to Management	Auditor(s)
11-Feb-2005	Blood Measurement / Urine Collection 2/11/05	11-Feb-2005	25-Mar-2005	K.Dobbs
23-Mar-2005, 24-Mar-2005	Study Records (A-1 to A-4)	24-Mar-2005	23-Apr-2005	E.Crawford
23-Mar-2005, 24-Mar-2005	Study Records (A-1 to A-4)(Supplemental Audit)	24-Mar-2005	23-Apr-2005	E.Crawford
05-Apr-2005	Study Records (I-1)	05-Apr-2005	28-May-2005	J.House
05-Apr-2005, 14-Apr-2005	Study Records (C-1)	14-Apr-2005	28-May-2005	J.House
14-Apr-2005	Study Records (Rx-1)	14-Apr-2005	28-May-2005	J.House
22-May-2005	Reports (Draft AC Appendix-PFHxA)	23-May-2005	29-Jun-2005	E.Crawford
22-May-2005	Reports (Draft AC Appendix - PFBS)	23-May-2005	29-Jun-2005	E.Crawford
15-Jun-2005, 16-Jun-2005, 17-Jun-2005, 20-Jun-2005	Study Records (A-1, A-5 through A-11, Bioanalytical Portion Only)	20-Jun-2005	25-Jul-2005	N.Gardner, J.House
11-Jul-2005, 12-Jul-2005, 15-Jul-2005, 26-Jul-2005, 28-Jul-2005	Study Records (C-1 Supplemental, Toxicokinetic Study Data)	28-Jul-2005	25-Aug-2005	J.House, K.Shaner
11-Jul-2005, 12-Jul-2005, 15-Jul-2005, 26-Jul-2005, 28-Jul-2005	Reports (Draft Report, Toxicokinetic Appendix G)	28-Jul-2005	25-Aug-2005	J.House, K.Shaner
29-Jul-2005, 01-Aug-2005, 07-Aug-2005, 08-Aug-2005	Study Records (A-12, A-13)	08-Aug-2005	2-Sept-2005	J.House, N.Gardner

Date(s) of Inspection(s)	Phase Inspected	Date(s) Findings Reported to Study Director	Date(s) Findings Reported to Management	Auditor(s)
02-Aug-2005, 03-Aug-2005, 05-Aug-2005, 07-Aug-2005, 08-Aug-2005	Reports (Draft Report - Bioanalytical appendix - PFHxA)	08-Aug-2005	2-Sept-2005	J.House, N.Gardner
05-Aug-2005	Reports Draft Report, without Analytical, Bioanalytical, or Toxicokinetic Appendices	05-Aug-2005	2-Sept-2005	J.House
05-Aug-2005, 08-Aug-2005	Reports (Draft Report - Bioanalytical Appendix - PFBS)	25-Aug-2005	2-Sept-2005	J.House, N.Gardner

This study was inspected in accordance with the U.S. EPA Good Laboratory Practice Standards (40 CFR Part 792), the OECD Principles of Good Laboratory Practice, the standard operating procedures of WIL Research Laboratories, LLC, and the sponsor's protocol and protocol amendments with the following exceptions. The data located in Appendix A (Certificate Of Analysis) was the responsibility of the sponsor. Quality Assurance findings, derived from the inspections during the conduct of the study and from the inspections of the raw data and draft report, are documented and have been reported to the study director. A status report is submitted to management monthly.

This report accurately reflects the data generated during the study. The methods and procedures used in the study were those specified in the protocol, its amendments and the standard operating procedures of WIL Research Laboratories, LLC.

The raw data, the retention sample(s), if applicable, and the final report will be stored in the Archives at WIL Research Laboratories, LLC, or another location specified by the sponsor.

9.2. APPROVAL

This study was inspected according to the criteria discussed in Section 9.1.

Report Audited By:

Janet M. House

Janet M. House, BS, RQAP-GLP
Compliance Specialist

2 September 2005
Date

Nancy J. Gardner

Nancy J. Gardner
Associate Compliance Specialist

2 September 2005
Date

Report Released By:

for: Heather L. Osborn, BS, RQAP-GLP
Manager, Quality Assurance

2 September 2005
Date

10. REFERENCES

Kirkpatrick, J.B. Pharmacokinetic (in Blood) and Excretion Study of Perfluorohexanoic Acid and Nonafluoro-1-Butanesulfonic Acid in Rats (Study No. WIL-534004). WIL Research Laboratories, LLC, Ashland, OH, **Draft.**

National Research Council. *Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources*, Commission on Life Sciences; National Academy Press: Washington, DC, **1996**.

11. DEVIATIONS FROM THE PROTOCOL

This study was conducted in accordance with the protocol and protocol amendments, except for the following.

- **Protocol Section 5.4** states that animals will be young adults (estimated to be 2-2.5 years old) at the initiation of dosing. At the start of the study, the mean age of the animals was 3.13 years.
- **Protocol Section 7.1.2** states that each animal was inspected by a veterinarian upon receipt. The animals that were selected for study from the stock colony did not have documentation that the animals were inspected at the time of receipt.
- **Protocol Section 7.3** states that the route of administration will be intravenous (bolus) injection. On 17 February 2005 (PFBS administration), female nos. 1572 and 1576 had their doses administered to both legs. This was a result of an undetermined amount of test article being administered outside of the vein in the initial left leg administration. The remainder of the dose was administered into the right leg saphenous vein.
- **Protocol Section 7.5.2** states that for stability assessments, test article formulations will be prepared, stored refrigerated (protected from light), and sampled over a minimum of 2 days. The original assessment of the PFHxA formulations was deemed invalid and no time-zero concentration was obtained and the stability analysis was inadvertently not done. PFHxA formulations at the same concentration level were prepared in a separate study (WIL-534004). These formulations were sampled for stability over a 2-day period and were 98.4% of time-zero concentration values, demonstrating stability over 2 days.
- **Protocol Section 8.5** states that serum samples will be frozen with minimal delay at approximately -20°C and stored at approximately -20°C until transferred to the Analytical Chemistry Department for analysis. All of the samples collected on study day 0 except the 48 hour post-dose samples were stored at -70°C. Also on study day 7 all but the 48 hour post-dose samples were stored at -70°C until transfer to the Analytical Chemistry Department.
- **Protocol Section 8.5** states that serum samples will be frozen with minimal delay at approximately -20°C and stored at approximately -20°C until transferred to the Analytical Chemistry Department for analysis. The urine samples received from metabolism on 8 March 2005 were stored in a freezer at -70°C. On 14 March 2005, the samples were moved from the -70°C freezer into a -20°C freezer.

- **Protocol Section 8.6** states that after each dose administration, urine will be collected on wet ice over the following intervals: 0-6, 6-12 and 12-24 hours post-dosing and then once daily for the next 6 days. On 10 February 2005, (day of dose administration for PFHxA) animal nos. 1573 and 1576 had their 6-12 hour urine collection outside (11 and 4 minutes, respectively) protocol specified range. On 17 February 2005 (day of dose administration for PFBS) animal nos. 1563, 1572, 1576 and 1573 had their 0-6 hour urine collection earlier (2, 14, 22 and 21 minutes, respectively) than protocol specified range.

These deviations did not negatively impact the quality or integrity of the data nor the outcome of the study.

TABLES 1 - 10

TABLE 1

PROJECT NO.:WIL-534002 A PHARMACOKINETIC AND EXCRETION STUDY IN CYNOMOLGUS MONKEYS PAGE 1

SPONSOR:AGC CHEMICAL INDIVIDUAL SURVIVAL AND DISPOSITION

ANIMAL	SEX	GROUP	TYPE OF DEATH	AGE IN WEEKS A	DATE OF DEATH	DAYS ON STUDY
1555T1	M	10 MG/KG PFHxA	RETURNED TO STOCK	161	24-FEB-05	14
1562T1	M	10 MG/KG PFHxA	RETURNED TO STOCK	161	24-FEB-05	14
1563T1	M	10 MG/KG PFHxA	RETURNED TO STOCK	161	24-FEB-05	14
1555T2	M	10 MG/KG PFBS	RETURNED TO STOCK	161	24-FEB-05	14
1562T2	M	10 MG/KG PFBS	RETURNED TO STOCK	161	24-FEB-05	14
1563T2	M	10 MG/KG PFBS	RETURNED TO STOCK	161	24-FEB-05	14

A = CALCULATED TO THE NEAREST WHOLE WEEK USING THE MEAN AGE IN WEEKS AT INITIATION OF DOSING (159)

TABLE 1

PROJECT NO.:WIL-534002 A PHARMACOKINETIC AND EXCRETION STUDY IN CYNOMOLGUS MONKEYS PAGE 2

SPONSOR:AGC CHEMICAL INDIVIDUAL SURVIVAL AND DISPOSITION

ANIMAL	SEX	GROUP	TYPE OF DEATH	AGE IN WEEKS A	DATE OF DEATH	DAYS ON STUDY
1572T1	F	10 MG/KG PFHxA	RETURNED TO STOCK	161	24-FEB-05	14
1576T1	F	10 MG/KG PFHxA	RETURNED TO STOCK	161	24-FEB-05	14
1573T1	F	10 MG/KG PFHxA	RETURNED TO STOCK	161	24-FEB-05	14
1572T2	F	10 MG/KG PFBS	RETURNED TO STOCK	161	24-FEB-05	14
1576T2	F	10 MG/KG PFBS	RETURNED TO STOCK	161	24-FEB-05	14
1573T2	F	10 MG/KG PFBS	RETURNED TO STOCK	161	24-FEB-05	14

A = CALCULATED TO THE NEAREST WHOLE WEEK USING THE MEAN AGE IN WEEKS AT INITIATION OF DOSING (159)

PDEADv4.05
07/13/2005
R:07/13/2005

TABLE RANGE: 02-10-05 TO 02-24-05

ANIMAL	SEX	GROUP	CATEGORY	DATE	TIME	GRADE	OBSERVATIONS
1555T1	M	10 MG/KG PFHxA	DISPOSITION	02-24-05	12:54	P	RETURNED TO STOCK
1562T1	M	10 MG/KG PFHxA	DISPOSITION	02-24-05	12:54	P	RETURNED TO STOCK
1563T1	M	10 MG/KG PFHxA	DISPOSITION	02-24-05	12:54	P	RETURNED TO STOCK
1555T2	M	10 MG/KG PFBS	NORMAL	02-24-05	12:46	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
1555T2	M	10 MG/KG PFBS	DISPOSITION	02-24-05	12:55	P	RETURNED TO STOCK
1555T2	M	10 MG/KG PFBS	EXCRETA	02-16-05	12:38	P	SOFT FECES
1562T2	M	10 MG/KG PFBS	NORMAL	02-16-05	12:40	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				02-24-05	12:48	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
1562T2	M	10 MG/KG PFBS	DISPOSITION	02-24-05	12:55	P	RETURNED TO STOCK
1563T2	M	10 MG/KG PFBS	NORMAL	02-16-05	12:41	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				02-24-05	12:49	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
1563T2	M	10 MG/KG PFBS	DISPOSITION	02-24-05	12:55	P	RETURNED TO STOCK
1572T1	F	10 MG/KG PFHxA	DISPOSITION	02-24-05	12:55	P	RETURNED TO STOCK
1576T1	F	10 MG/KG PFHxA	DISPOSITION	02-24-05	12:55	P	RETURNED TO STOCK
1576T1	F	10 MG/KG PFHxA	BEHAVIOR/CNS	02-12-05	7:34	P	INAPPETENCE
1573T1	F	10 MG/KG PFHxA	DISPOSITION	02-24-05	12:56	P	RETURNED TO STOCK
1572T2	F	10 MG/KG PFBS	NORMAL	02-24-05	12:49	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
1572T2	F	10 MG/KG PFBS	DISPOSITION	02-24-05	12:56	P	RETURNED TO STOCK
1572T2	F	10 MG/KG PFBS	EXCRETA	02-16-05	13:18	P	SOFT FECES
1576T2	F	10 MG/KG PFBS	NORMAL	02-24-05	12:50	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
1576T2	F	10 MG/KG PFBS	DISPOSITION	02-24-05	12:56	P	RETURNED TO STOCK
1576T2	F	10 MG/KG PFBS	BEHAVIOR/CNS	02-19-05	6:27	P	INAPPETENCE
1576T2	F	10 MG/KG PFBS	BODY/INTEGUMENT	02-16-05	13:20	P	SCABBING END OF TAIL
1573T2	F	10 MG/KG PFBS	NORMAL	02-24-05	12:51	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
1573T2	F	10 MG/KG PFBS	DISPOSITION	02-24-05	12:56	P	RETURNED TO STOCK
1573T2	F	10 MG/KG PFBS	EXCRETA	02-16-05	13:21	P	SOFT FECES

GRADE CODE: 1 - SLIGHT 2 - MODERATE 3 - SEVERE P - PRESENT

TABLE 3 (AT TIME OF DOSING - TREATMENT 1)

PROJECT NO.:WIL-534002 A PHARMACOKINETIC AND EXCRETION STUDY IN CYNOMOLGUS MONKEYS

SPONSOR:AGC CHEMICAL INDIVIDUAL CLINICAL OBSERVATIONS

TABLE RANGE: 02-10-05 TO 02-10-05

ANIMAL	SEX	GROUP	CATEGORY	DATE	TIME	GRADE	OBSERVATIONS
NO SIGNIFICANT CLINICAL OBSERVATIONS AT TIME OF DOSING							

GRADE CODE: 1 - SLIGHT 2 - MODERATE 3 - SEVERE P - PRESENT

PCRDv4.09
07/13/2005
R:07/13/2005

TABLE RANGE: 02-17-05 TO 02-17-05

ANIMAL	SEX	GROUP	CATEGORY	DATE	TIME	GRADE	OBSERVATIONS
1555T2	M	10 MG/KG PFBS	EXCRETA	02-17-05	11:51	P	SOFT FECES
1572T2	F	10 MG/KG PFBS	EXCRETA	02-17-05	11:54	P	SOFT FECES
1573T2	F	10 MG/KG PFBS	EXCRETA	02-17-05	12:17	P	SOFT FECES

GRADE CODE: 1 - SLIGHT 2 - MODERATE 3 - SEVERE P - PRESENT

TABLE 5 (1 HOUR POST-DOSING - TREATMENT 1)

PROJECT NO.:WIL-534002 A PHARMACOKINETIC AND EXCRETION STUDY IN CYNOMOLGUS MONKEYS PAGE 1

SPONSOR:AGC CHEMICAL INDIVIDUAL CLINICAL OBSERVATIONS

TABLE RANGE: 02-10-05 TO 02-10-05

ANIMAL	SEX	GROUP	CATEGORY	DATE	TIME	GRADE	OBSERVATIONS
NO SIGNIFICANT CLINICAL OBSERVATIONS 1 HOUR POST-DOSING							

GRADE CODE: 1 - SLIGHT 2 - MODERATE 3 - SEVERE P - PRESENT

PCRDv4.09
07/13/2005
R:07/13/2005

TABLE RANGE: 02-17-05 TO 02-17-05

ANIMAL	SEX	GROUP	CATEGORY	DATE	TIME	GRADE	OBSERVATIONS
1572T2	F	10 MG/KG PFBS	EXCRETA	02-17-05	13:11	P	SOFT FECES

GRADE CODE: 1 - SLIGHT 2 - MODERATE 3 - SEVERE P - PRESENT

TABLE 7 (DAILY OBSERVATIONS - RECOVERY PERIOD - TREATMENT 1)

PROJECT NO.:WIL-534002 A PHARMACOKINETIC AND EXCRETION STUDY IN CYNOMOLGUS MONKEYS

SPONSOR:AGC CHEMICAL INDIVIDUAL CLINICAL OBSERVATIONS

TABLE RANGE: 02-11-05 TO 02-16-05

ANIMAL	SEX	GROUP	CATEGORY	DATE	TIME	GRADE	OBSERVATIONS
1555T1	M	10 MG/KG PFHxA	EXCRETA	02-11-05	10:53	P	SOFT FECES
				02-15-05	9:01	P	SOFT FECES
				02-16-05	6:41	P	SOFT FECES
1563T1	M	10 MG/KG PFHxA	EXCRETA	02-11-05	10:54	P	SOFT FECES
1572T1	F	10 MG/KG PFHxA	EXCRETA	02-16-05	6:42	P	SOFT FECES

GRADE CODE: 1 - SLIGHT 2 - MODERATE 3 - SEVERE P - PRESENT

PCRDv4.09
07/13/2005

TABLE RANGE: 02-18-05 TO 02-24-05

ANIMAL	SEX	GROUP	CATEGORY	DATE	TIME	GRADE	OBSERVATIONS
1555T2	M	10 MG/KG PFBS	EXCRETA	02-23-05	11:12	P	SOFT FECES
1572T2	F	10 MG/KG PFBS	EXCRETA	02-22-05	7:56	P	SOFT FECES
1573T2	F	10 MG/KG PFBS	EXCRETA	02-22-05	7:56	P	SOFT FECES

GRADE CODE: 1 - SLIGHT 2 - MODERATE 3 - SEVERE P - PRESENT

TABLE 9

PROJECT NO.:WIL-534002 A PHARMACOKINETIC AND EXCRETION STUDY IN CYNOMOLGUS MONKEYS PAGE 1

SPONSOR:AGC CHEMICAL INDIVIDUAL BODY WEIGHTS [KG]

MALE GROUP: 10 MG/KG PFHxA

DAY	-7	-1	6	14		
ANIMAL						
1555T1	2.4	2.4	2.4	NA	RETURNED TO STOCK	14
1562T1	2.4	2.4	2.4	NA	RETURNED TO STOCK	14
1563T1	2.8	2.7	2.8	NA	RETURNED TO STOCK	14
MEAN	2.5	2.5	2.5	NA		
S.D.	0.23	0.20	0.23			
N	3	3	3			

NA = NOT APPLICABLE

TABLE 9

PROJECT NO.:WIL-534002 A PHARMACOKINETIC AND EXCRETION STUDY IN CYNOMOLGUS MONKEYS PAGE 2

SPONSOR:AGC CHEMICAL INDIVIDUAL BODY WEIGHTS [KG]

MALE GROUP: 10 MG/KG PFBS

DAY	-7	-1	6	14		
ANIMAL						
1555T2	NA	NA	2.4	2.5	RETURNED TO STOCK	14
1562T2	NA	NA	2.4	2.4	RETURNED TO STOCK	14
1563T2	NA	NA	2.8	2.8	RETURNED TO STOCK	14
MEAN	NA	NA	2.5	2.6		
S.D.			0.23	0.21		
N			3	3		

NA = NOT APPLICABLE

TABLE 9

PROJECT NO.:WIL-534002 A PHARMACOKINETIC AND EXCRETION STUDY IN CYNOMOLGUS MONKEYS PAGE 3

SPONSOR:AGC CHEMICAL INDIVIDUAL BODY WEIGHTS [KG]

FEMALE GROUP: 10 MG/KG PFHxA

DAY	-7	-1	6	14		
ANIMAL						
1572T1	2.6	2.6	2.6	NA	RETURNED TO STOCK	14
1576T1	2.4	2.5	2.5	NA	RETURNED TO STOCK	14
1573T1	2.5	2.5	2.5	NA	RETURNED TO STOCK	14
MEAN	2.5	2.5	2.5	NA		
S.D.	0.09	0.05	0.08			
N	3	3	3			

NA = NOT APPLICABLE

FEMALE GROUP: 10 MG/KG PFBS

DAY	-7	-1	6	14		
ANIMAL						
1572T2	NA	NA	2.6	2.6	RETURNED TO STOCK	14
1576T2	NA	NA	2.5	2.4	RETURNED TO STOCK	14
1573T2	NA	NA	2.5	2.5	RETURNED TO STOCK	14
MEAN	NA	NA	2.5	2.5		
S.D.			0.08	0.11		
N			3	3		

NA = NOT APPLICABLE

TABLE 10

PROJECT NO.:WIL-534002 A PHARMACOKINETIC AND EXCRETION STUDY IN CYNOMOLGUS MONKEYS

SPONSOR:AGC CHEMICAL INDIVIDUAL BODY WEIGHT CHANGES [KG]

MALE GROUP: 10 MG/KG PFHxA

DAY	-7 TO -1	-1 TO 6	6 TO 14		
ANIMAL					
1555T1	0.0	0.0	NA	RETURNED TO STOCK	14
1562T1	0.0	0.0	NA	RETURNED TO STOCK	14
1563T1	0.0	0.1	NA	RETURNED TO STOCK	14
MEAN	0.0	0.0	NA		
S.D.	0.00	0.06			
N	3	3			

NA = NOT APPLICABLE

MALE GROUP: 10 MG/KG PFBS

DAY	-7 TO -1	-1 TO 6	6 TO 14		
ANIMAL					
1555T2	NA	NA	0.1	RETURNED TO STOCK	14
1562T2	NA	NA	0.0	RETURNED TO STOCK	14
1563T2	NA	NA	0.0	RETURNED TO STOCK	14
MEAN	NA	NA	0.0		
S.D.			0.06		
N			3		

NA = NOT APPLICABLE

TABLE 10

PROJECT NO.:WIL-534002 A PHARMACOKINETIC AND EXCRETION STUDY IN CYNOMOLGUS MONKEYS PAGE 3

SPONSOR:AGC CHEMICAL INDIVIDUAL BODY WEIGHT CHANGES [KG]

FEMALE GROUP: 10 MG/KG PFHxA

DAY	-7 TO -1	-1 TO 6	6 TO 14		
ANIMAL					
1572T1	0.0	0.1	NA	RETURNED TO STOCK	14
1576T1	0.1	0.0	NA	RETURNED TO STOCK	14
1573T1	0.0	0.0	NA	RETURNED TO STOCK	14
MEAN	0.0	0.0	NA		
S.D.	0.06	0.06			
N	3	3			

NA = NOT APPLICABLE

FEMALE GROUP: 10 MG/KG PFBS

DAY	-7 TO -1	-1 TO 6	6 TO 14		
ANIMAL					
1572T2	NA	NA	0.0	RETURNED TO STOCK	14
1576T2	NA	NA	-0.1	RETURNED TO STOCK	14
1573T2	NA	NA	0.0	RETURNED TO STOCK	14
MEAN	NA	NA	0.0		
S.D.			0.06		
N			3		

NA = NOT APPLICABLE

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APPENDIX A

Certificate Of Analysis (Sponsor-Provided Data)

AGC Chemicals
ASAHI GLASS CO.,LTD.
10 Goikaigan, Ichihara-shi, Chiba
290-8566 JAPAN

CERTIFICATE OF ANALYSIS

Chemical Name: Perfluorohexanoic acid < PFHxA>
CAS No.: 307-24-4
Lot Number: C15004301
Molecular Weight: 314
Stability/Expiry date: December, 2005

Item	Unit	Analysis Results
Appearance		Clear colorless liquid
Purity <PFHxA>	Area%	98.50
Impurities		
Unknown	Area%	0.55
Unknown	Area%	0.34
Unknown	Area%	0.28
Unknown	Area%	0.21
Unknown	Area%	0.12

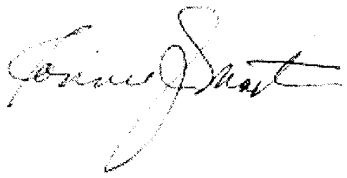
Motoki Shinohara, Safety Manager
Environment & Safety Office

SIGMA-ALDRICH

Certificate of Analysis

Product Name	Nonafluorobutane-1-sulfonic acid
Product Number	56,262-9
Product Brand	ALDRICH
CAS Number	375-73-5
Molecular Formula	$C_4HF_9O_3S$
Molecular Weight	300.10

TEST	SPECIFICATION	LOT 10410KC RESULTS
APPEARANCE		COLORLESS LIQUID
INFRARED SPECTRUM		CONFORMS TO STRUCTURE.
FLUORINE NMR		CONFORMS TO STRUCTURE.
TITRATION		99.65 % (WITH NAOH)
QUALITY CONTROL		SEPTEMBER, 2004
ACCEPTANCE DATE		



Ronnie J. Martin, Supervisor
Quality Control
Milwaukee, Wisconsin USA

APPENDIX B

Analyses Of Dosing Formulations [PFHxA] (WIL Research Laboratories, LLC)

A PHARMACOKINETIC (IN BLOOD) AND EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Analyses Of PFHxA Dosing Formulations

Analytical Chemistry Department

WIL Research Laboratories, LLC

TABLE OF CONTENTS

INDEX OF FIGURES

Page

INDEX OF TABLES

INDEX OF ATTACHMENTS

1. INTRODUCTION

This report provides a detailed description and validation of an assay for the determination of perfluorohexanoic acid (PFHxA) in aqueous formulations using high performance liquid chromatography (HPLC) tandem mass spectrometry (HPLC/MS/MS) in negative electrospray ionization (ESI-) mode. Assay specificity/selectivity, calibration reproducibility, accuracy, precision and ruggedness were assessed. Quantitation was performed using standard calibration solutions in the concentration range of 10 to 25 µg PFHxA/mL. Precision and accuracy were verified with the analysis of quality control (QC) samples at 10.0 and 90.0 mg/mL. The assay was extended to include QC samples at 2.0 mg/mL using a single validation session.

PFHxA stability in formulations stored refrigerated for a minimum of 2 days was to be assessed according to the protocol. The stability was inadvertently not conducted under this study, but was established in a separate study (Kirkpatrick, **Draft**). Formulations used for dose administration were analyzed and the resulting concentrations were within acceptance criteria (%RE within ± 15%).

2. EXPERIMENTAL

2.1. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Instrument:	Waters 2695 liquid chromatograph equipped with an autosampler, Micromass tandem quadrupole Quattro Micro™ Mass Spectrometer and MassLynx™ software, or equivalent system
Column:	2 MetaGaurd Nucleosil 5µ 300A C18 cartridges or
Column Temperature:	35°C
Mobile Phase:	50% A: deionized water 50% B: acetonitrile
Flow Rate:	0.3 mL/minute
Detector:	Mass spectrometer with conditions as described in Section 2.2.
Injection Volume:	5 µL

Retention Time:	Approximately 0.3 minutes for perfluorohexanoic acid (PFHxA)
Run Time:	1.5 minutes

Note: The retention time and run times varied depending on column performance.

2.2. MASS SPECTROMETRY

2.2.1. INSTRUMENT

A Micromass Quattro Micro™ tandem mass spectrometer equipped with an ESI- interface was used in this study. Data acquisition and analysis were performed using MassLynx™ software version 4.0.

2.2.2. SOURCE PARAMETERS

Source:	ESI-
Capillary:	3.50 kV
Cone:	40 V
Extractor:	1.0 V
RF Lens:	0 V
Source Block Temperature:	100°C
Desolvation Temperature:	300°C
Cone Gas Flow :	Approximately 100 L nitrogen/hour
Desolvation Gas Flow:	Approximately 500 L nitrogen/hour

Note: Settings varied depending on mass spectrometer performance.

2.2.3. ACQUISITION PARAMETERS

Function Type:	MRM (multiple reaction monitoring)
Precursor/Product Ion:	m/z 313/268 for PFHxA
Dwell Time:	1 second

Note: Settings varied depending on mass spectrometer performance.

2.3. DILUENT PREPARATION

The diluent was prepared by combining 500 mL of acetonitrile (ACN) and 500 mL of de-ionized (DI) water. The diluent was thoroughly mixed and vacuum degassed. The preparation was scaled as needed.

2.4. PREPARATION OF THE CALIBRATION STOCK SOLUTION

The calibration stock solution was prepared at 500 µg PFHxA/mL by accurately weighing approximately 0.02500 g of PFHxA (WIL log no. 6394A) in a tared glass weigh funnel, transferring to a 50-mL volumetric flask with rinses of ACN and diluting to volume with ACN. The solution was thoroughly mixed. The calibration stock solution was prepared fresh and scaled as needed.

2.5. PREPARATION OF THE QUALITY CONTROL STOCK SOLUTION

The QC stock solution was prepared at 5000 µg PFHxA/mL by accurately weighing approximately 0.50 g of PFHxA (WIL log no. 6394A) in a tared 100-mL volumetric flask and diluting to volume with ACN. The solution was thoroughly mixed. The QC stock solution was prepared fresh as needed.

2.6. PREPARATION OF CALIBRATION SAMPLES

Dilutions of the calibration stock solution were prepared with diluent to yield calibration samples at 10.0, 17.5 and 25.0 µg PFHxA/mL. A portion of each calibration sample was transferred to an amber autosampler vial for analysis.

2.7. PREPARATION OF QUALITY CONTROL SAMPLES

QC samples were prepared in triplicate. Aliquots of the QC stock solution were added to 50-mL polypropylene tubes containing 1.0 mL of DI water to yield low (LQC) and high (HQC) QC sample concentrations of 10.0 and 90.0 mg/mL, respectively. The assay was extended to include a QC sample at 2.0 mg/mL. Appropriate volumes of ACN were added to each tube to achieve a final volume of 40 mL.

Initial Concentration (mg/mL)	DI Water Volume (mL)	Stock Volume (mL)	ACN Volume (mL)	Total Volume (mL)	Diluted Concentration (µg/ml)
0.0	0.5	0.0	39.5	40	0.0
2.0	1.0	0.4	38.6	40	50.0
10.0	0.5	1.0	38.5	40	125
90.0	0.5	9.0	30.5	40	1125

The QC samples were thoroughly mixed and a secondary dilution was performed with diluent in new 50-mL polypropylene tubes. A portion of each QC sample was transferred to an amber autosampler vial for analysis.

Secondary Dilutions:

Initial Concentration (mg/mL)	Primary Diluent Volume (mL)	Diluent Volume (mL)	Total Volume (mL)	Final Concentration (µg/ml)
0.0	1.5	8.5	10.0	0.0
2.0	0.3	0.7	1.0	15.0
10.0	1.5	8.5	10.0	18.75
90.0	0.550	34.45	35.0	17.68

2.8. SAMPLE PROCESSING

Samples (1.0 mL) of the formulations were diluted with 39 mL of ACN in 50-mL polypropylene tubes. The samples were thoroughly mixed and a secondary dilution was performed with diluent in new polypropylene tubes. A portion of each sample was transferred to an amber autosampler vial for analysis.

Group	Dose Concentration (mg/mL)	Sample Volume (mL)	ACN Volume (mL)	Total Volume (mL)	Diluted Concentration (µg/ml)
1	2.0	1.0	39.0	40.0	50.0

Secondary Dilutions:

Group	Dose Concentration (mg/mL)	Primary Diluent Volume (mL)	Diluent Volume (mL)	Total Volume (mL)	Final Concentration (µg/mL)
1	2.0	0.300	0.700	1.0	15.0

2.9. CALIBRATION AND QUANTITATION

Single injections were made of each calibration, QC and formulation sample. A calibration curve was constructed for each set of analyses. Using MassLynx™, the PFHxA peak areas (y) and the theoretical concentrations of the calibration standards (x) were fit with least-squares regression analysis to the linear function:

$$y = ax + b$$

Concentration and percent relative error (%RE) were calculated using MassLynx™. The concentration data were transferred to an Excel spreadsheet, where appropriate summary statistics, *i.e.,* mean, standard deviation (SD), relative standard deviation (RSD) and percent relative error (%RE), were calculated and presented in tabular form. The concentrations of the dosing formulations and QC samples were calculated by applying any necessary multiplication factors.

3. RESULTS AND DISCUSSION

Under the described chromatographic conditions, the retention time of PFHxA was approximately 0.3 minutes. Figures 1, 2, 3 and 4 are typical chromatograms of a 10 µg PFHxA/mL calibration sample, a processed 2.0 mg PFHxA/mL QC sample, a processed 2 mg PFHxA/mL formulation sample and a processed vehicle sample, respectively. The total analysis time required for each run was approximately 1.5 minutes. The assay validation was established through a careful study of the assay specificity/selectivity, calibration reproducibility, precision, accuracy and ruggedness. Formulations were prepared and evaluated for concentration verification. No

homogeneity assessments were performed because the formulations were filter sterilized for IV dosing and were assumed to be solutions.

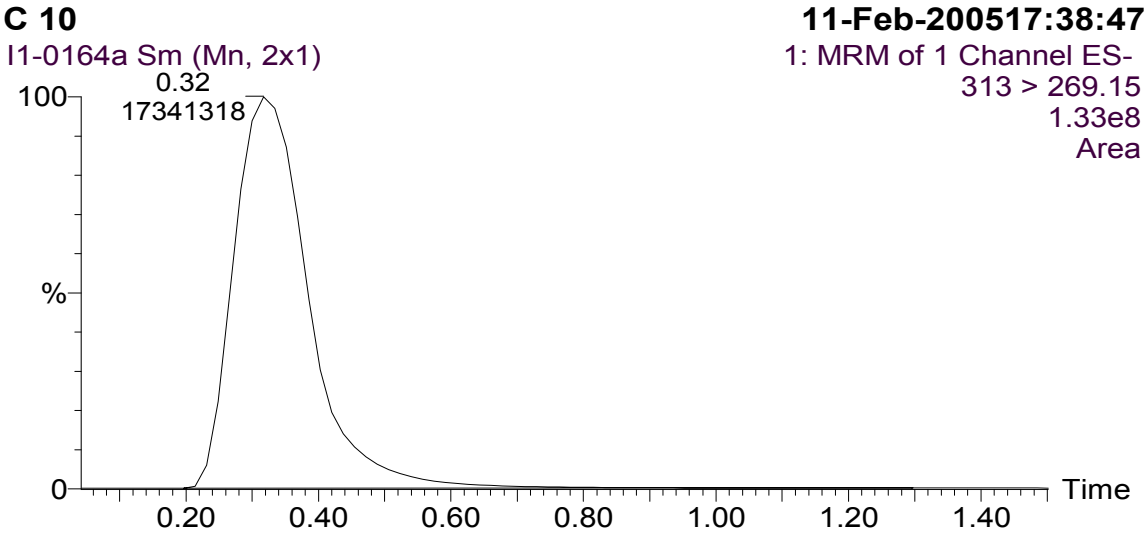


Figure 1: Representative Chromatogram Of A 10 µg/mL Calibration Sample

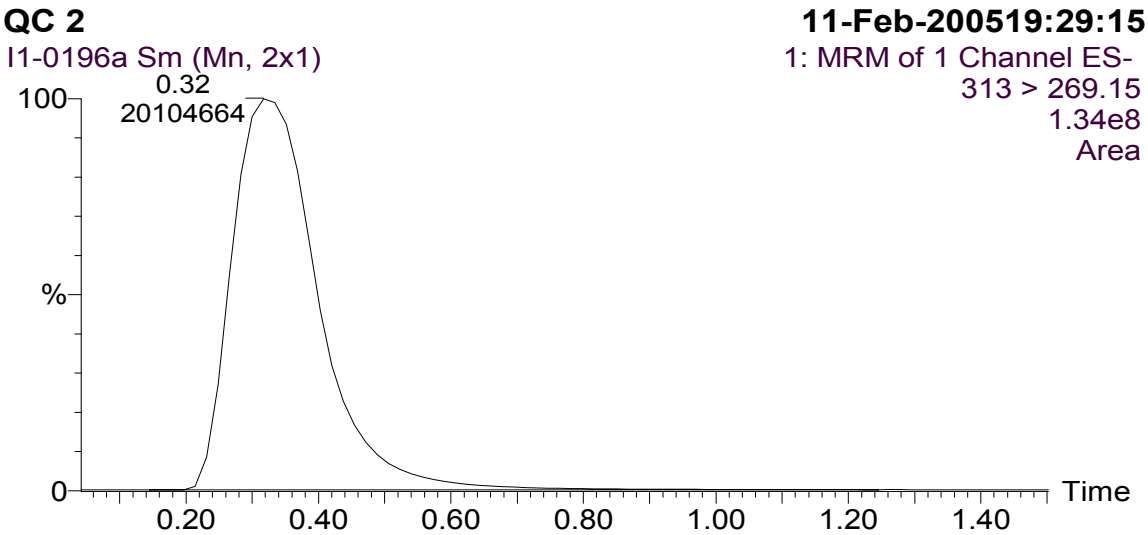


Figure 2: Representative Chromatogram Of A Processed 2.0 mg/mL QC Sample

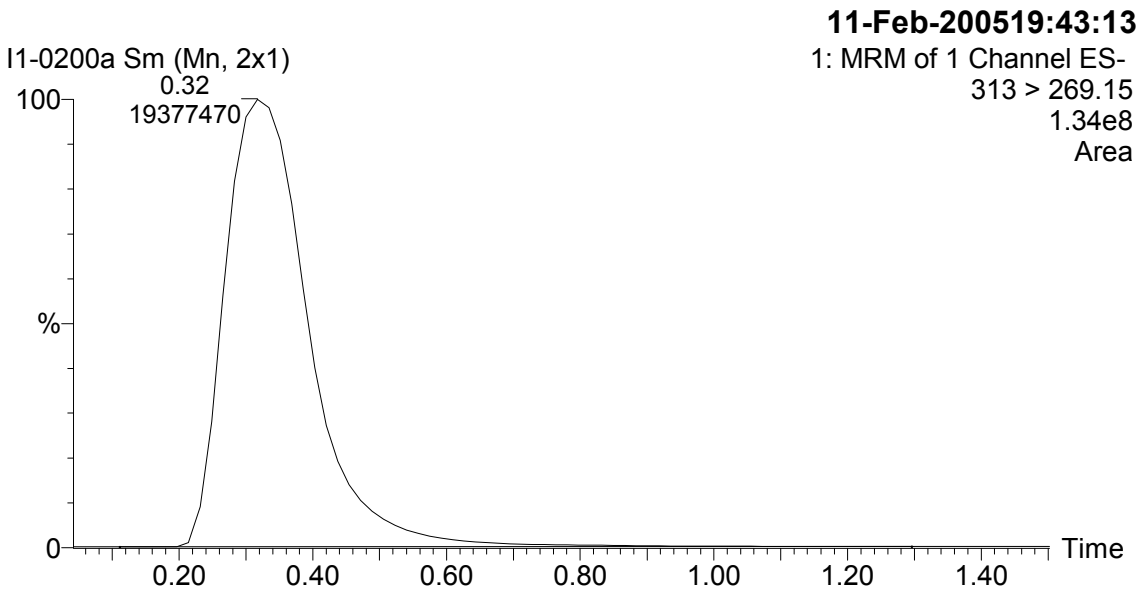


Figure 3: Representative Chromatogram Of A Processed 2 mg/mL Formulation Sample

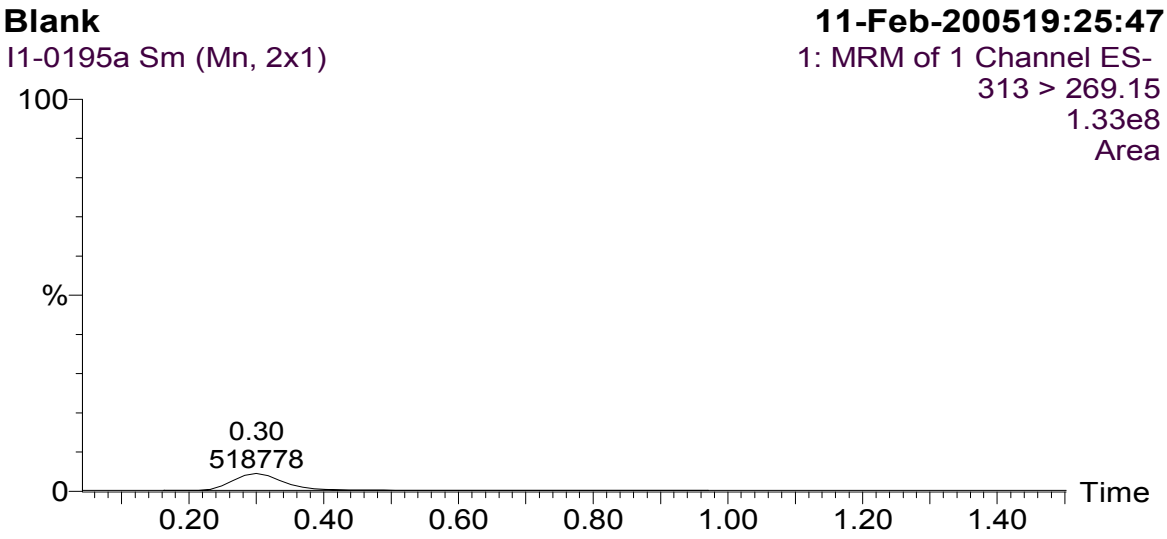


Figure 4: Representative Chromatogram Of A Processed Vehicle Sample

3.1. SPECIFICITY/SELECTIVITY

As shown in Figure 4 (and in contrast to the chromatograms shown in Figures 1 through 3), assay specificity/selectivity was confirmed when HPLC/MS/MS analysis of vehicle formulations revealed no significant peaks at or near the retention time for PFHxA (0.3 minutes).

3.2. CALIBRATION REPRODUCIBILITY

During each of three validation sessions and one extension validation session, triplicate calibration samples at three concentration levels were prepared and analyzed as described previously. Single injections were made of each processed calibration sample. The resulting peak area concentration data were fit to the linear function using least-squares regression analysis. The regression equation was used to back-calculate the corresponding concentrations from the peak area data. The reproducibility of the calibration curve data was considered valid when 1) the inter-session variability (RSD) of the back-calculated concentrations at each concentration level was ≤ 15%, except at the lowest calibration concentration where an RSD ≤ 20% was acceptable; and 2) the mean back-calculated concentrations at each concentration level were within 15% of the theoretical values (%RE within ± 15%), except at the lowest concentration level where %RE within ± 20% was acceptable.

The back-calculated concentration values and the associated intra- and inter-session statistics for the validation of the PFHxA assay calibration samples are summarized in Table 1. The inter-session variability (RSD) of the back-calculated concentrations at each level ranged from 0.43% to 1.6%. The inter-session concentration means had %RE values ranging from -0.85% to 0.97%.

The back-calculated concentration values and the associated intra-session statistics for calibration samples during the extension validation of the PFHxA assay are summarized in Table 3. The intra-session variability (RSD) of the back-calculated concentrations at each level ranged from 3.3% to 5.8%. The intra-session concentration means had %RE values ranging from -2.7% to 3.1%.

Based on the stated criteria, the reproducibility of the calibration data was acceptable.

3.3. PRECISION AND ACCURACY

During each of three validation sessions, triplicate QC samples at two concentration levels were prepared and analyzed as described previously. Single injections were made of each processed QC sample. The calibration equation was used to calculate the corresponding concentrations from these QC peak area data. The variability (RSD) of the calculated QC concentration data was used as a measure of assay precision. The precision of the method was considered acceptable when the inter-session RSD of the calculated concentrations at each QC concentration level was ≤ 15%. The difference from theoretical of the calculated QC concentration means (%RE) was used as a measure of assay accuracy. The accuracy of the method was considered acceptable when the inter-session concentration means of the calculated concentrations at each QC concentration level had %RE values within ± 15%.

The calculated concentration values and the associated intra- and inter-session statistics for the QC samples from the validation of the PFHxA assay are summarized in Table 2. The inter-session variability (RSD) of the calculated concentration means were 10% and 5.0%. The inter-session concentration means had %RE values (accuracy) of 7.2% and 4.1%.

The calculated concentration values and the associated intra-session statistics for the QC samples from the extension validation of the PFHxA assay are summarized in Table 4. The intra-session variability (RSD) of the calculated concentration mean was 4.1%. The intra-session concentration mean had a %RE value (accuracy) of 12%.

Based on the stated criteria, the precision and accuracy of the PFHxA assay were acceptable.

3.4. ASSAY RUGGEDNESS

Assay ruggedness was successfully demonstrated for this procedure since more than one analyst successfully performed at least one of the required three validation sessions.

3.5. CONCENTRATION ANALYSES OF DOSING FORMULATIONS

The results of the concentration analysis of the dosing formulation are presented in Table 5, and the mean concentration (and % of target) value are summarized in the following table.

Formulation Date	Group 1 2 mg/mL (% of Target)
8 Feb 2005	1.94 (96.9)

The analyzed formulation used for dose administration was 96.9% of target and, therefore, met the WIL SOP requirement for concentration acceptability, that is, the analyzed concentrations were within 15% of the target dose concentrations.

4. CONCLUSION

Assay specificity/selectivity, calibration reproducibility, precision, accuracy and ruggedness were assessed and validated. Formulations used for dosing were analyzed for test article concentration confirmation and the results met all acceptance criteria (within 15% of the target concentrations).

5. REFERENCES

Kirkpatrick, J.B. Pharmacokinetic (In Blood) And Excretion Study Of Perfluorohexanoic Acid And Nonafluoro-1-Butanesulfonic Acid In Rats (Study No. WIL-534004). WIL Research Laboratories, LLC, Ashland, OH, **Draft.**

6. KEY STUDY PERSONNEL AND REPORT SUBMISSION

Report Submitted By:

Jeannie B. Kirkpatrick — 2 September 2005

Jeannie B. Kirkpatrick, M.S.
Staff Toxicologist
Study Director — Date

Report Prepared By:

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Nichole R. Myers — 2 Sep. 2005

Nichole R. Myers, B.A.
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Report Reviewed By:

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Daniel W. Sved, Ph.D.
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TABLES 1 -5

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 1: Back-Calculated PFHxA Concentrations And Intra- And Inter-Session Statistics Of Calibration Samples

Theo Conc (µg/mL)	**10.0**	**17.5**	**25.0**
Set 1 - DKP	9.83	17.8	25.0
(1/18/05)	9.92	17.7	25.0
Sequence 534002j	9.94	17.6	24.7
Mean	9.90	17.7	24.9
SD	0.059	0.095	0.15
RSD	0.59	0.54	0.59
%RE	-1.0	1.2	-0.41
Set 2 - DKP	9.87	17.7	24.9
(1/19/05)	9.91	17.6	24.9
Sequence 534002k	9.98	17.7	25.0
Mean	9.92	17.7	24.9
SD	0.057	0.078	0.081
RSD	0.58	0.44	0.33
%RE	-0.83	0.95	-0.33
Set 3 - KLK	9.73	17.7	24.8
(1/19/05)	10.3	17.6	25.1
Sequence 534002l	9.78	17.6	24.9
Mean	9.93	17.6	24.9
SD	0.31	0.072	0.13
RSD	3.1	0.41	0.50
%RE	-0.67	0.78	-0.25
Inter-set Statistics			
n	9	9	9
Mean	9.92	17.7	24.9
SD	0.16	0.077	0.11
RSD	1.6	0.44	0.43
%RE	-0.85	0.97	-0.33

534002 Dosing Results.xls I
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A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 2: PFHxA Concentrations And Intra- And Inter-Session Statistics Of Quality Control Samples

Theo Conc (mg/mL)	**10.0**	**90.0**
Set 1 - DKP	10.2	91.6
(1/18/05)	10.3	87.5
Sequence 534002j	9.63	91.4
Mean	10.0	90.2
SD	0.36	2.3
RSD	3.6	2.6
%RE	0.37	0.17
Set 2 - DKP	11.0	94.6
(1/19/05)	11.1	102
Sequence 534002k	13.4	99.7
Mean	11.8	98.9
SD	1.4	4.0
RSD	12	4.0
%RE	18	9.9
Set 3 -KLK	10.3	91.7
(1/19/05)	10.2	93.0
Sequence 534002l	10.3	91.2
Mean	10.3	92.0
SD	0.039	0.92
RSD	0.37	1.0
%RE	2.9	2.2
Inter-set Statistics		
n	9	9
Mean	10.7	93.7
SD	1.1	4.6
RSD	10	5.0
%RE	7.2	4.1

534002 Dosing Results.xls II
Printed: 05/25/05 10:20 AM

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 3: Back-Calculated PFHxA Concentrations And Intra-Session Statistics Of The Extension Validation Calibration Samples

Theo Conc (µg/mL)	**10.0**	**17.5**	**25.0**
Set 1 - DKP	10.0	17.9	24.8
(1/18/05)	9.08	19.0	23.9
Sequence 534001d1	10.1	17.2	25.5
Mean	9.73	18.0	24.7
SD	0.57	0.89	0.82
RSD	5.8	5.0	3.3
%RE	-2.7	3.1	-1.1
Intra-session Statistics			
n	3	3	3
Mean	9.73	18.0	24.7
SD	0.57	0.89	0.82
RSD	5.8	5.0	3.3
%RE	-2.7	3.1	-1.1

534002 Dosing Results.xls I (2)
Printed: 05/25/05 10:20 AM

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 4: PFHxA Concentrations And Intra-Session Statistics Of The Extension Validation Quality Control Samples

Theo Conc (mg/mL)	**2.0**
Set 1 - DKP	2.14
(1/18/05)	2.25
Sequence 534001d1	2.32
Mean	2.2
SD	0.091
RSD	4.1
%RE	12
Intra-session Statistics	
n	3
Mean	2.2
SD	0.091
RSD	4.1
%RE	12

534002 Dosing Results.xls II (2)
Printed: 05/25/05 10:20 AM

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 5: Concentration Analysis Of The 08 Feb2005 PFHxA Formulations
(Analyzed 08-11 Feb2005)

Group	Dose Conc (mg/mL)	Ref # (534002 -)	Run # (534001- -)	Analyzed Conc (mg/mL)	Percent of Target (%)	Mean Conc (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)
1	2	45 - 3	I1-0200a	1.93	96.4	1.94	0.016	0.80	96.9
		45 - 4	I1-0201a	1.95	97.5				

534002 Dosing Results.xls conc.1 (2)
Printed: 05/25/05 10:20 AM

ATTACHMENT I

Supporting Data

Table A-1: Calibration and Quality Control Data of Sequence 534002J

Quantify Compound Summary Report
Printed Thu Jan 20 09:03:28 2005

Compound 1: PFHxA

Run #	Ref # (534002-)	Sample Text	RT	Area	Conc. (ug/mL)	%RE	Corr. Factor	Final Conc. (mg/ml)
I3-0289		50:50 ACN\H2O	0.78	36.39	0			
I3-0290	27-2	C 10	0.79	25440	9.83	-1.7		
I3-0291	27-3	C 10	0.79	25670	9.92	-0.78		
I3-0292	27-4	C 10	0.79	25710	9.94	-0.62		
I3-0293	27-5	C 17.5	0.79	44650	17.8	1.7		
I3-0294	27-6	C 17.5	0.79	44420	17.7	1.2		
I3-0295	27-7	C 17.5	0.79	44180	17.6	0.62		
I3-0296	27-8	C 25	0.79	61850	25.0	-0.21		
I3-0297	27-9	C 25	0.79	62000	25.0	0.035		
I3-0298	27-10	C 25	0.79	61330	24.7	-1.1		
I3-0299		50:50 ACN\H2O	0.78	43.54	0			
I3-0300	28-9	Blank	0.78	48.9	0			
I3-0301	28-10	QC 10	0.79	51930	20.8	11	0.533	11.1
I3-0302	28-11	QC 10	0.79	52200	20.9	12	0.533	11.2
I3-0303	28-12	QC 10	0.79	51380	20.6	9.9	0.533	11.0
I3-0304	28-13	QC 90	0.79	44410	17.7	0.14	5.09	90.1
I3-0305	28-14	QC 90	0.79	45100	18.0	1.8	5.09	91.6
I3-0306	28-15	QC 90	0.79	45330	18.1	2.3	5.09	92.1
I3-0307		50:50 ACN\H2O	0.78	39.95	0			
I3-0308	29-9	blank						
I3-0309	29-10	QC 10	0.79	42040	16.7	-11	0.533	8.92
I3-0310	29-11	QC 10	0.79	42020	16.7	-11	0.533	8.91
I3-0311	29-12	QC 10	0.81	39850	15.8	-16	0.533	8.43
I3-0312	29-13	QC 90	0.79	42890	17.1	-3.4	5.09	86.9
I3-0313	29-14	QC 90	0.79	42790	17.0	-3.7	5.09	86.7
I3-0314	29-15	QC 90	0.79	41500	16.5	-6.7	5.09	84.0
I3-0315		50:50 ACN\H2O	0.78	33.82	0			
I3-0316	30z-9	blank	0.79	26.98	0	-100		
I3-0317	30z-10	QC 10	0.79	47730	19.1	1.8	0.533	10.2
I3-0318	30z-11	QC 10	0.79	48330	19.3	3.1	0.533	10.3
I3-0319	30z-12	QC 10	0.79	45250	18.1	2.1	0.533	9.63
I3-0320	30z-13	QC 90	0.79	45110	18.0	1.8	5.09	91.6
I3-0321	30z-14	QC 90	0.79	43150	17.2	-2.8	5.09	87.5
I3-0322	30z-15	QC 90	0.79	45000	18.0	1.5	5.09	91.4
I3-0323		50:50 ACN\H2O	0.78	29.37	0			

Table A-2: Calibration and Quality Control Data of Sequence 534002K

Quantify Compound Summary Report
Printed Thu Jan 20 08:04:27 2005

Compound 1: PFHxA

Run #	Ref # (534002-)	Sample Text	RT	Response	Conc. (ug/mL)	%RE	Corr. Factor	Final Conc. (mg/mL)
I3-0324		sys suit	0.79	24610	10.0			
I3-0325		sys suit	0.79	24370	9.91			
I3-0326		sys suit	0.79	24290	9.88			
I3-0327		50:50 ACN\H2O	0.78	25.88	0			
I3-0328	30b1-2	C 10	0.79	24260	9.87	-1.4		
I3-0329	30b1-3	C 10	0.79	24370	9.91	-0.92		
I3-0330	30b1-4	C 10	0.79	24530	10.0	-0.22		
I3-0331	30b1-5	C 17.5	0.79	43220	17.7	1.3		
I3-0332	30b1-6	C 17.5	0.79	42850	17.6	0.43		
I3-0333	30b1-7	C 17.5	0.79	43130	17.7	1.1		
I3-0334	30b1-8	C 25	0.79	60410	24.9	-0.56		
I3-0335	30b1-9	C 25	0.79	60460	24.9	-0.47		
I3-0336	30b1-10	C 25	0.79	60770	25.0	0.043		
I3-0337		50:50 ACN\H2O	0.78	26.67	0			
I3-0338	31d-9	Blank	0.78	9.943	0			
I3-0339	31d-10	QC 10	0.79	50390	20.7	10	0.533	11.0
I3-0340	31d-11	QC 10	0.79	50430	20.7	11	0.533	11.1
I3-0341	31d-12	QC 10	0.79	61220	25.2	34	0.533	13.4
I3-0342	31d-13	QC 90	0.79	45270	18.6	5.1	5.09	94.6
I3-0343	31d-14	QC 90	0.79	49020	20.1	14	5.09	102
I3-0344	31d-15	QC 90	0.79	47670	19.6	11	5.09	99.7
I3-0345		50:50 ACN\H2O	0.79	26.84	0	-100		

Table A-3: Calibration, Quality Control, and Homogeneity Data of Sequence 534002L

Quantify Compound Summary Report
Printed Thu Jan 20 08:04:08 2005

Compound 1: PFHxA

Run #	Ref # (534002-)	Sample Text	RT	Response	Conc. (ug/mL)	%RE	Corr. Factor	Final Conc. (mg/mL)
I3-0346		50:50 ACN\H2O	0.79	24.61	0			
I3-0347	32aaa-2	C 10	0.79	24670	9.73	-2.7		
I3-0348	32aaa-3	C 10	0.79	25980	10.3	2.9		
I3-0349	32aaa-4	C 10	0.79	24770	9.78	-2.3		
I3-0350	32aaa-5	C 17.5	0.79	43580	17.7	1.3		
I3-0351	32aaa-6	C 17.5	0.79	43270	17.6	0.52		
I3-0352	32aaa-7	C 17.5	0.79	43280	17.6	0.55		
I3-0353	32aaa-8	C 25	0.79	60350	24.8	-0.78		
I3-0354	32aaa-9	C 25	0.79	60940	25.1	0.22		
I3-0355	32aaa-10	C 25	0.79	60660	24.9	-0.26		
I3-0356		50:50 ACN\H2O	0.79	33.26	0			
I3-0357	33aaa-9	Blank	0.78	9.409	0			
I3-0358	33aaa-10	QC 10	0.79	47410	19.3	3.1	0.533	10.3
I3-0359	33aaa-11	QC 10	0.79	47110	19.2	2.5	0.533	10.2
I3-0360	33aaa-12	QC 10	0.79	47390	19.3	3.1	0.533	10.3
I3-0361	33aaa-13	QC 90	0.79	44290	18.0	1.9	5.09	91.7
I3-0362	33aaa-14	QC 90	0.79	44880	18.3	3.3	5.09	93.0
I3-0363	33aaa-15	QC 90	0.79	44050	17.9	1.4	5.09	91.2
I3-0364		50:50 ACN\H2O	0.79	26.41	0	-100		
I3-0365	534001-31-20	Grp 1 mid	0.76	6.515	0			0
I3-0366	534001-31-21	Grp 2 top	0.79	51700	21.2		0.533	11.3
I3-0367	534001-31-22	Grp 2 top	0.79	52360	21.4		0.533	11.4
I3-0368	534001-31-23	Grp 2 Mid	0.79	51890	21.2		0.533	11.3
I3-0369	534001-31-24	Grp 2 Mid	0.79	51030	20.9		0.533	11.1
I3-0370	534001-31-25	Grp 2 Btm	0.79	50840	20.8		0.533	11.1
I3-0371	534001-31-26	Grp 2 Btm	0.79	51660	21.1		0.533	11.3
I3-0372	534001-31-27	Grp 3 top	0.79	47730	19.5		1.6	31.2
I3-0373	534001-31-28	Grp 3 top	0.79	48070	19.6		1.6	31.4
I3-0374	534001-31-29	Grp 3 mid	0.79	47060	19.2		1.6	30.7
I3-0375	534001-31-30	Grp 3 mid	0.79	47520	19.4		1.6	31.0
I3-0376	534001-31-31	Grp 3 Btm	0.79	48240	19.7		1.6	31.5
I3-0377	534001-31-32	Grp 3 Btm	0.79	48020	19.6		1.6	31.3
I3-0378	534001-31-33	Grp 4 top	0.79	46340	18.9		5.33	101
I3-0379	534001-31-34	Grp 4 top	0.79	47810	19.5		5.33	104
I3-0380	534001-31-35	Grp 4 Mid	0.79	47630	19.4		5.33	104
I3-0381	534001-31-36	Grp 4 Mid	0.79	46540	19.0		5.33	101
I3-0382	534001-31-37	Grp 4 Btm	0.79	47250	19.3		5.33	103
I3-0383	534001-31-38	Grp 4 Btm	0.79	45600	18.6		5.33	99.1
I3-0384		50:50 ACN\H2O	0.79	32.01	0			

Table A-4: Calibration, Quality Control, and Concentration Data of Sequence 534002d1

Quantify Compound Summary Report

Sample List:C:\MASSLYNX\534001.PRO\SampleDB\534001(PFHxA)d1
Last modified:Fri Feb 11 17:18:25 2005
Method:C:\MASSLYNX\534001.PRO\MethDB\534001b
Last modified:Fri Feb 11 16:31:06 2005
Job Code:

Printed:Sat Feb 12 11:13:45 2005

Compound 1: PFHxA

Run #	Ref # (534002-)	Sample Text	RT	Response	Conc. (ug/mL)	%RE
I1-0159a	136-1	system suit	0.32	16314253	7.75	
I1-0160a	136-1	system suit	0.32	17058910	9.39	
I1-0161a	136-1	system suit	0.32	16830688	8.89	
I1-0162a	136-1	system suit	0.32	17166236	9.63	
I1-0163a		50:50 ACN\H2O	0.3	97966	0	
I1-0164a	136-1	C 10	0.32	17341318	10.0	0.10
I1-0165a	136-2	C 10	0.32	16918104	9.08	-9.2
I1-0166a	136-3	C 10	0.32	17382420	10.1	1.0
I1-0167a	136-4	C 17.5	0.32	20933584	17.9	2.4
I1-0168a	136-5	C 17.5	0.32	21421894	19.0	8.5
I1-0169a	136-6	C 17.5	0.32	20615640	17.2	-1.6
I1-0170a	136-7	C 25	0.33	24047074	24.8	-0.93
I1-0171a	136-8	C 25	0.33	23649236	23.9	-4.4
I1-0172a	136-9	C 25	0.33	24394162	25.5	2.1
I1-0173a		50:50 ACN\H2O	0.3	65161	0	
I1-0174a	137-8	Blank	0.3	172388	0	
I1-0175a	137-9	QC 6	0.32	21428650	6.08	1.4
I1-0176a	137-10	QC 6	0.33	21856984	6.38	6.4
I1-0177a	137-11	QC 6	0.32	21394798	6.06	0.96
I1-0178a	137-12	QC 42	0.32	19141774	37.2	-12
I1-0179a	137-13	QC 42	0.32	20236844	43.6	3.8
I1-0180a	137-14	QC 42	0.32	20324502	44.1	5.0
I1-0181a		50:50 ACN\H2O	0.3	83666	0	
I1-0182a	135-13	Grp 2 top	0.33	23418920	6.22	24
I1-0183a	135-14	Grp 2 top	0.33	23273246	6.14	23
I1-0184a	135-15	Grp 2 Btm	0.33	23286850	6.14	23
I1-0185a	135-16	Grp 2 Btm	0.33	23310398	6.16	23
I1-0186a	135-17	Grp 3 top	0.32	21439844	15.2	1.2
I1-0187a	135-18	Grp 3 top	0.32	21905612	16.0	6.7
I1-0188a	135-19	Grp 3 Btm	0.32	21587220	15.4	3.0

I1-0189a	135-20	Grp 3 Btm	0.32	21982176	16.1	7.6
I1-0190a	135-21	Grp 4 top	0.33	22935824	59.4	32
Run #	Ref # (534002-)	Sample Text	RT	Response	Conc. (ug/mL)	%RE
I1-0191a	135-22	Grp 4 top	0.32	18323248	32.4	-28
I1-0192a	135-23	Grp 4 Btm	0.32	17774260	29.2	-35
I1-0193a	135-24	Grp 4 Btm	0.32	18439754	33.1	-27
I1-0194a		50:50 ACN\H2O	0.3	87461	0	
I1-0195a	534002-46-5	Blank	0.3	518778	0	
I1-0196a	534002-46-6	QC 2	0.32	20104664	2.14	7.0
I1-0197a	534002-46-7	QC 2	0.32	20473366	2.25	12
I1-0198a	534002-46-8	QC 2	0.32	20720050	2.32	16
I1-0199a		50:50 ACN\H2O	0.3	86369	0	
I1-0200a	534002-45-3	Grp 1	0.32	19377470	1.93	-3.6
I1-0201a	534002-45-4	Grp 1	0.32	19450874	1.95	-2.6
I1-0202a		50:50 ACN\H2O	0.3	124593	0	

APPENDIX C

Analyses Of Dosing Formulations [PFBS] (WIL Research Laboratories, LLC)

A PHARMACOKINETIC (IN BLOOD) AND EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Analyses Of PFBS Dosing Formulations

Analytical Chemistry Department

WIL Research Laboratories, LLC

TABLE OF CONTENTS

INDEX OF FIGURES

Page

INDEX OF TABLES

INDEX OF ATTACHMENTS

1. INTRODUCTION

This report provides a detailed description and validation of an assay for the determination of nonafluoro-1-butanesulfonic acid (PFBS) in aqueous formulations using a high performance liquid chromatography (HPLC) mass spectrometry (HPLC/MS) in negative electrospray ionization (ESI-) mode. Assay specificity/selectivity, calibration reproducibility, accuracy, precision and ruggedness were assessed. Quantitation was performed using standard calibration solutions in the concentration range of 200 to 800 ng PFBS/mL. Precision and accuracy were verified with the analysis of quality control (QC) samples at 1.0 mg/mL.

PFBS stability in formulations stored refrigerated for 2 days was assessed and the results met WIL SOP acceptance criteria. Formulations used for dose administration were analyzed and the resulting concentrations were within the acceptance criteria (%RE within ± 15%).

2. EXPERIMENTAL

2.1. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Instrument:	Waters 2695 liquid chromatograph equipped with an autosampler, Micromass tandem quadrupole Quattro Micro™ Mass Spectrometer and MassLynx™ software, or equivalent system
Column:	2 MetaGaurd Nucleosil 5µ 300A C18 cartridges or equivalent
Column Temperature:	35°C
Mobile Phase:	50% A: de-ionized water 50% B: acetonitrile
Flow Rate:	1.0 mL/minute split post-column to 0.3 mL/minute
Detector:	Mass spectrometer with conditions as described in Section 2.2.
Injection Volume:	5 µL

Retention Time:	Approximately 0.3 minutes for nonafluorobutane-1-sulfonic acid (PFBS)
Run Time:	1.5 minutes

Note: The retention time and run varied depending on column performance.

2.2. MASS SPECTROMETRY

2.2.1. INSTRUMENT

A Micromass Quattro Micro™ tandem mass spectrometer equipped with an ESI- interface was used in this study. Data acquisition and analysis were performed using MassLynx™ software version 4.0.

2.2.2. SOURCE PARAMETERS

Source:	ESI-
Capillary:	3.0 kV
Cone:	45 V
Extractor:	1.0 V
RF Lens:	0 V
Source Block Temperature:	100°C
Desolvation Temperature:	250°C
Cone Gas Flow :	Approximately 100 L nitrogen/hour
Desolvation Gas Flow:	Approximately 500 L nitrogen/hour

Note: Settings varied depending on mass spectrometer performance.

2.2.3. ACQUISITION PARAMETERS

Function Type:	SIR (selected ion recording)
Precursor/Product Ion:	m/z 299 for PFBS
Dwell Time:	0.1 second

Note: Settings varied depending on mass spectrometer performance.

2.3. DILUENT PREPARATION

The diluent was prepared by combining 500 mL of acetonitrile (ACN) and 500 mL of de-ionized (DI) water. The diluent was thoroughly mixed and vacuum degassed. The preparation was scaled as needed.

2.4. PREPARATION OF THE CALIBRATION STOCK SOLUTION

The calibration stock solution was prepared at 500 µg PFBS/mL by accurately weighing approximately 0.05 g PFBS (WIL log no. 6396A) in a tared glass weigh funnel, transferring to a 100-mL volumetric flask with rinses of ACN and diluting to volume with ACN. The solution was thoroughly mixed. The calibration stock solution was prepared fresh and scaled as needed.

2.5. PREPARATION OF THE QUALITY CONTROL STOCK SOLUTION

The QC stock solution was prepared at 5000 µg PFBS/mL by accurately weighing approximately 0.50 g of PFBS (WIL log no. 6396A) in a tared 100-mL volumetric flask and diluting to volume with ACN. The solution was thoroughly mixed. The QC stock solution was prepared fresh as needed.

2.6. PREPARATION OF CALIBRATION SAMPLES

Dilutions of the calibration stock solution were prepared with diluent to yield calibration samples at 200, 400 and 800 ng PFBS/mL. A portion of each calibration sample was transferred to an amber autosampler vial for analysis.

2.7. PREPARATION OF QUALITY CONTROL SAMPLES

QC samples were prepared in triplicate. Aliquots of the QC stock solution were added to 50-mL polypropylene tubes containing 1.0 mL of sterile water to yield QC sample concentrations of 1.0 mg/mL. Appropriate volumes of ACN were added to each tube to achieve a final volume of 40.5 mL.

Level	Initial Concentration (mg/mL)	Sterile Water Volume (mL)	Stock Volume (mL)	ACN Volume (mL)	Total Volume (mL)	Diluted Concentration (µg/ml)
Blank	0.0	1.0	0.0	39.5	40.5	0.0
QC	1.0	1.0	0.200	39.3	40.5	24.7

The QC samples were thoroughly mixed and a secondary dilution with diluent was performed in 15.0-mL polypropylene tubes. A portion of each QC sample was transferred to an amber autosampler vial for analysis.

Secondary Dilutions:

Level	Initial Concentration (mg/mL)	Primary Diluent Volume (mL)	Diluent Volume (mL)	Total Volume (mL)	Final Concentration (ng/ml)
Blank	0.0	0.100	4.90	5.0	0.0
QC	1.0	0.100	4.90	5.0	494

2.8. SAMPLE PROCESSING

Formulation samples (1.0 mL) were extracted with 39.0 mL of ACN in 50-mL polypropylene tubes. The samples were thoroughly mixed and a secondary dilution was performed with diluent in new polypropylene tubes. A portion of each sample was transferred to an amber autosampler vial for analysis.

Group	Dose Concentration (mg/mL)	Sample Volume (mL)	ACN Volume (mL)	Total Volume (mL)	Diluted Concentration (µg/ml)
1	2.0	1.0	39.0	40.0	50.0

Secondary Dilutions:

Group	Dose Concentration (mg/mL)	Primary Diluent Volume (mL)	Diluent Volume (mL)	Total Volume (mL)	Final Concentration (ng/ml)
1	2.0	0.100	9.90	10.0	500

2.9. CALIBRATION AND QUANTITATION

Single injections were made of each calibration, QC and formulation sample. A calibration curve was constructed for each set of analyses. Using MassLynx™, the PFBS peak areas (y) and the theoretical concentrations of the calibration standards (x) were fit with least-squares regression analysis to the linear function:

$$y = ax + b$$

Concentration and percent relative error (%RE) were calculated using MassLynx™. The concentration data were transferred to an Excel spreadsheet, where appropriate summary statistics, *i.e.*, mean, standard deviation (SD), relative standard deviation (RSD) and percent relative error (%RE), were calculated and presented in tabular form. The concentrations of the dosing formulations and QC samples were calculated by applying any necessary multiplication factors.

3. RESULTS AND DISCUSSION

Under the described chromatographic conditions, the retention time for PFBS was approximately 0.3 minutes. Figures 1, 2, 3 and 4 are typical chromatograms of a 200 ng PFBS/mL calibration sample, a processed 1.0 mg PFBS/mL QC sample, a processed 2 mg PFBS/mL formulation sample, and a processed vehicle sample, respectively. The total analysis time required for each run was approximately 1.5 minutes. The assay validation was established through a careful study of the assay specificity/selectivity, calibration reproducibility, precision, accuracy, and ruggedness. Formulations were prepared and evaluated for concentration verification. No

homogeneity assessments were performed as instructed by the study director notification dated 07 Feb 2005. Test article stability in formulations stored refrigerated for 2 days was assessed. These formulations were sampled using a 1-cc syringe, and sampled again using the IV apparatus used for dosing the animals. Comparison of the sample concentrations was used to determine whether or not the test article adsorbed to the dosing apparatus.

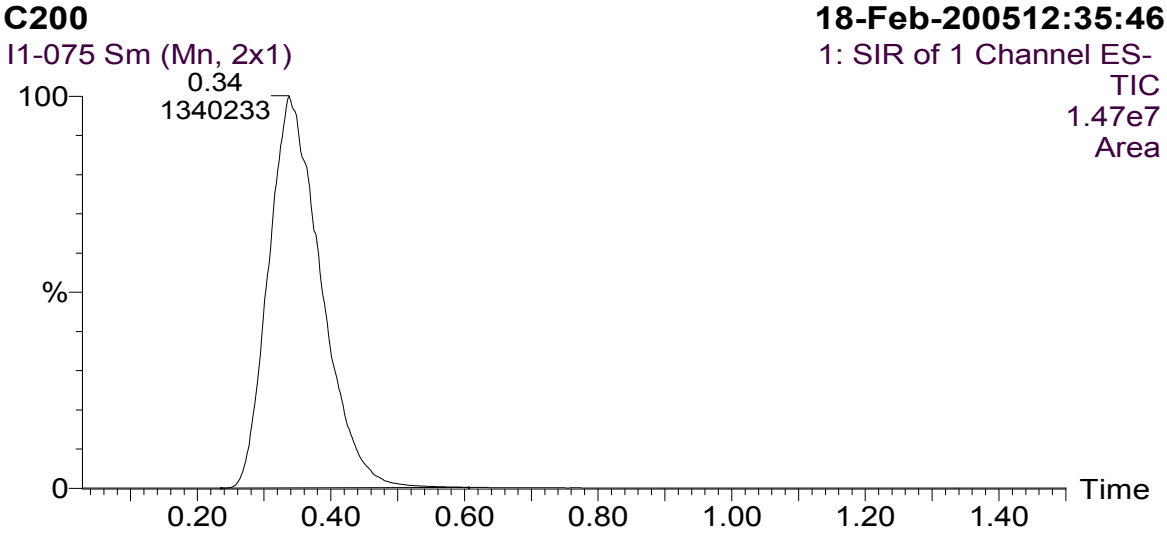

Figure 1: Representative Chromatogram Of A 200 ng PFBS/mL Calibration Sample

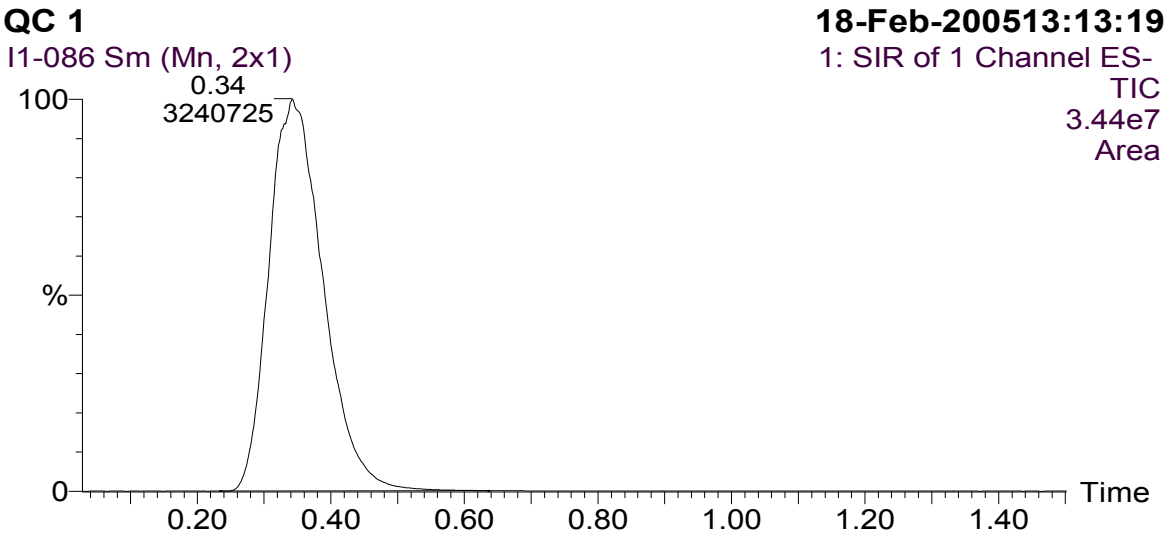

Figure 2: Representative Chromatogram Of A Processed 1.0 mg PFBS/mL QC Sample

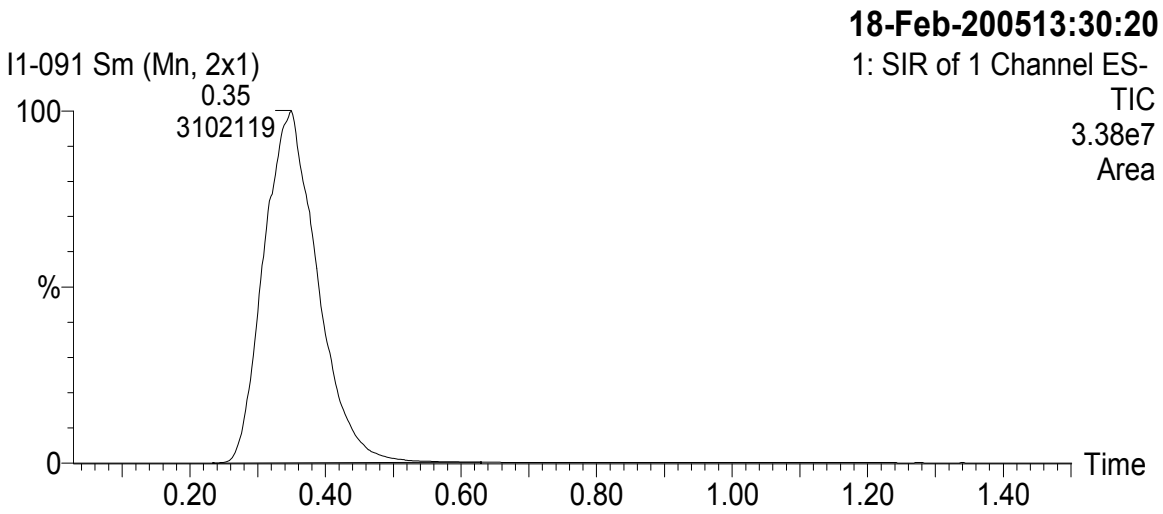

Figure 3: Representative Chromatogram Of A Processed 2 mg PFBS/mL Formulation Sample

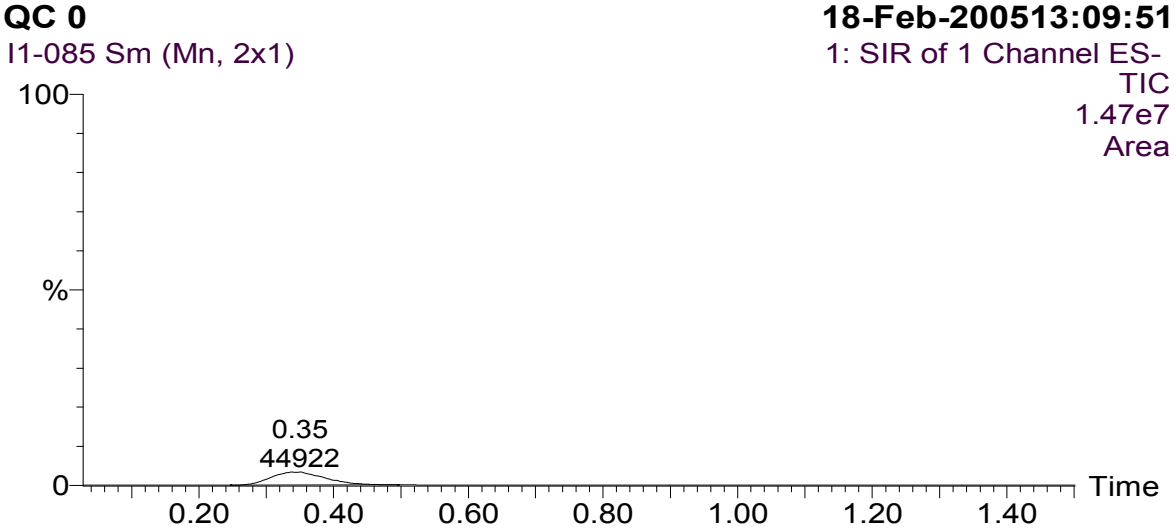

Figure 4: Representative Chromatogram Of A Processed Vehicle Sample

3.1. SPECIFICITY/SELECTIVITY

As shown in Figure 4 (and in contrast to the chromatograms shown in Figures 1 through 3), assay specificity/selectivity was confirmed when HPLC/MS analysis of vehicle formulations revealed that there were no significant peaks at or near the retention time for PFBS (0.3 minutes).

3.2. CALIBRATION REPRODUCIBILITY

During each of three validation sessions, triplicate calibration samples at three concentration levels were prepared and analyzed as described previously. Single injections were made of each processed calibration sample. The resulting peak area concentration data were fit to the linear function using least-squares regression analysis. The regression equation was used to back-calculate the corresponding concentrations from the peak area data. The reproducibility of the calibration curve data was considered valid when 1) the inter-session variability (RSD) of the back-calculated concentrations at each concentration level was ≤ 15%, except at the lowest calibration concentration where an RSD ≤ 20% was acceptable; and 2) the mean back-calculated concentrations at each concentration level were within 15% of the theoretical values (%RE within ± 15%), except at the lowest concentration level where %RE within ± 20% was acceptable.

The back-calculated concentration values and the associated intra- and inter-session statistics for the PFBS assay calibration samples are summarized in Table 1. The inter-session variability (RSD) of the back-calculated concentrations at each level ranged from 4.3% to 8.7%. The inter-session concentration means had %RE values ranging from -3.4% to 2.3%.

Based on the stated criteria, the reproducibility of the calibration data was acceptable.

3.3. PRECISION AND ACCURACY

During each of three validation sessions, triplicate QC samples at one concentration level were prepared and analyzed as described previously. Single injections were made of each processed QC sample. The calibration equation was used to calculate the corresponding concentrations from these QC peak area data. The variability (RSD) of these calculated QC concentration data was used as a measure of assay precision. The precision of the method was considered acceptable when the inter-session RSD of the calculated concentrations at each QC concentration level was ≤ 15%. The difference from theoretical of the calculated QC concentration means (%RE) was used as a measure

of assay accuracy. The accuracy of the method was considered acceptable when the inter-session concentration means of the calculated concentrations at each QC concentration level had %RE values within ± 15%.

The calculated concentration values and the associated intra- and inter-session statistics for the PFBS assay QC samples are summarized in Table 2. The inter-session variability (RSD) of the calculated concentrations was 4.0%. The inter-session concentration mean had a %RE value (accuracy) -0.029%.

Based on the stated criteria, the precision and accuracy of the PFBS assay were acceptable.

3.4. ASSAY RUGGEDNESS

Assay ruggedness was successfully demonstrated for this procedure since more than one analyst successfully performed at least one of the required three validation sessions.

3.5. CONCENTRATION ANALYSES OF DOSING FORMULATIONS

The results of the concentration analysis of the dosing formulation are presented in Table 3, and the mean concentration (and % of target) value is summarized in the following table.

Formulation Date	Group 1 2 mg/mL (% of Target)
15 Feb 2005	2.06 (103)

The analyzed formulation used for dose administration was 103% of target and, therefore, met the WIL SOP requirement for concentration acceptability, that is, the analyzed concentrations were within 15% of the target dose concentrations.

3.6. STABILITY

The dosing formulation, analyzed on the day the preparation was completed (15-16 Feb 2005), was stored refrigerated for 2 days, at which time samples were collected and analyzed to assess test article stability. Samples were collected from the formulation on 16 Feb 2005 and then again after refrigerated storage on 18 Feb 2005. Samples were collected with 1-cc syringes and the IV dosing apparatus to determine whether or not the test article adsorbed to the IV apparatus. The results of the stability assessment are presented in Table 4 with the mean concentrations and (percent of time-zero) values summarized as follows:

Sample Technique	% of Time Zero
1-cc syringe	1.90 (92.5%)
IV dosing apparatus	1.89 (91.7%)

The test article in the formulation tested was stable during 2 days of refrigerated storage since the post-storage concentrations met WIL SOP criteria, *i.e.*, the post-storage values were greater than or equal to 90% of the corresponding time-zero values. The mean concentration of the IV dosing apparatus samples was equivalent to the mean concentration of the 1-cc syringe samples which indicated that there was no adsorption of the test article to the dosing apparatus.

4. CONCLUSION

Assay specificity/selectivity, calibration reproducibility, precision, accuracy and ruggedness were assessed and validated. Formulations used for dosing were analyzed for test article concentration confirmation and the results met all acceptance criteria (within 15% of the target concentrations). Two-day refrigerated stability was assessed and the results met WIL SOP acceptance criteria. There was no adsorption of the test article to the dosing apparatus when the formulation was sampled using the IV dosing apparatus versus a 1-cc syringe.

5. KEY STUDY PERSONNEL AND REPORT SUBMISSION

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TABLES 1 - 4

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 1: Back-Calculated PFBS Concentrations And Intra- And Inter-Session Statistics Of Calibration Samples

Theo Conc (ng/mL)	**200**	**400**	**800**
Set 1 - DKP	201	397	811
(2/14/05)	196	416	771
Sequence 534002a	198	396	815
Mean	198	403	799
SD	2.7	11	25
RSD	1.3	2.8	3.1
%RE	-1.0	0.72	-0.12
Set 2 - KLK	154	439	766
(2/15/05)	188	405	823
Sequence 534002b	210	427	787
Mean	184	424	792
SD	28	17	29
RSD	15	4.1	3.6
%RE	-7.9	5.9	-0.99
Set 3 - DKP	0*	395	833
(2/16/05)	198	445	735
Sequence 534002c1	200	363	831
Mean	199	401	800
SD	1.6	42	56
RSD	0.78	10	7.0
%RE	-0.42	0.21	-0.035
Interset Statistics			
n	8	9	9
Mean	193	409	797
SD	17	26	34
RSD	8.7	6.3	4.3
%RE	-3.4	2.3	-0.38

* Sample Prep error. Not included in calculations. 534002 Val sulfonic acid.xls I
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A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 2: PFBS Concentrations And Intra- And Inter-Session Statistics Of Quality Control Samples

Theo Conc (mg/mL)	**1.0**
Set 1 - DKP	0.965
(2/14/05)	1.03
Sequence 534002a	0.944
Mean	0.980
SD	0.046
RSD	4.7
%RE	-2.0
Set 2 - KLK	1.03
(2/15/05)	0.948
Sequence 534002b	1.04
Mean	1.00
SD	0.049
RSD	4.9
%RE	0.42
Set 3 - DKP	0.979
(2/16/05)	1.02
Sequence 534002c1	1.05
Mean	1.01
SD	0.033
RSD	3.3
%RE	1.5
Interset Statistics	
n	9
Mean	1.00
SD	0.040
RSD	4.0
%RE	-0.029

534002 Val sulfonic acid.xls II
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A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 3: Concentration Analysis Of The 15 Feb2005 Formulations
(Analyzed 15-16 Feb2005)

Group (PFBS)	Dose Conc (mg/mL)	Ref # (534002 -)	Run #	Analyzed Conc (mg/mL)	Percent of Target (%)	Mean Conc (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)
1	2	57 - 3	I1-063a	2.16	108	2.06	0.15	7.1	103
		57 - 4	I1-064a	1.95	97.7				

534002 Val sulfonic acid.xls conc.1
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A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 4: 2-Day Refrigerated Stability Analysis Of The 15 Feb2005 PFBS Formulations
(Analyzed 18 Feb2005)

Group	Strata	Dose Conc (mg/mL)	Ref # (534002 -)	Run #	Analyzed Conc (mg/mL)	Percent of Target (%)	Mean Conc (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)	Percent of Time Zero (%)
1	NA	2	66 - 5	I1-090	2.00	99.9	1.90	0.14	7.2	95.1	92.5
			66 - 6	I1-091	1.81	90.3					
*1	NA	2	66 - 7	I1-092	2.00	100	1.89	0.17	8.9	94.3	91.7
			66 - 8	I1-093	1.77	88.4					

* These samples collected as per study director notification with the syringe apparatus used in dosing of the animals.

	Group	(mg/mL)
Time Zero Concentration:	2	2.06

534002 Val sulfonic acid.xls 2D R Stab
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PFBS

ATTACHMENT I

Supporting Data

Table A-1: Calibration and Quality Control Data of Sequence 534002a

Quantify Compound Summary Report

Sample List:C:\MASSLYNX\534002.PRO\SampleDB\534002a
Last modified:Mon Feb 14 17:13:23 2005
Method:C:\MASSLYNX\534002.PRO\MethDB\534002
Last modified:Tue Feb 15 08:22:29 2005
Job Code:

Printed:Tue Feb 15 08:30:52 2005

Compound 1: PFBS

Run #	Ref. # (534002-)	Sample Text	RT	Area	Flags	Conc. (ng/mL)	% RE	Corr. Factor	Final Conc. (mg/mL)
I1-001	48-2	system suit	0.28	715060	bd	206			
I1-002	48-2	system suit	0.29	716585	bb	206			
I1-003	48-2	system suit	0.28	720787	bb	208			
I1-004									
I1-005	48-2	C200	0.29	699976	bb	201	0.49		
I1-006	48-3	C200	0.29	683292	bd	196	-2.1		
I1-007	48-4	C200	0.29	689102	bb	198	-1.2		
I1-008	48-5	C400	0.29	1321295	bb	397	-0.86		
I1-009	48-6	C400	0.29	1382147	bb	416	3.9		
I1-010	48-7	C400	0.29	1320525	bb	396	-0.92		
I1-011	48-8	C800	0.29	2638805	bb	811	1.4		
I1-012	48-9	C800	0.29	2509279	bb	771	-3.7		
I1-013	48-10	C800	0.29	2650903	bd	815	1.9		
I1-014									
I1-015	49-6								
I1-016	49-7	QC 500	0.29	1578937	bb	478		0.00202	0.965
I1-017	49-8	QC 500	0.28	1683646	bb	511		0.00202	1.03
I1-018	49-9	QC 500	0.28	1545565	bb	467		0.00202	0.944
I1-019									
I1-020	51-5								
I1-021	51-6	QC 500	0.28	1629238	bb	494	-1.3		
I1-022	51-7	QC 500	0.29	1498174	bb	452	-9.5		
I1-023	51-8	QC 500	0.28	1602891	bb	485	-3.0		
I1-024									

Table A-2: Calibration and Quality Control Data of Sequence 534002b

Quantify Compound Summary Report

Sample List:C:\MASSLYNX\534002.PRO\SampleDB\534002b
Last modified:Tue Feb 15 16:19:42 2005
Method:C:\MASSLYNX\534002.PRO\MethDB\534002
Last modified:Tue Feb 15 08:22:29 2005
Job Code:

Printed:Wed Feb 16 11:22:52 2005

Compound 1: PFBS

Run #	Ref. #	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-025		system suit	0.28	540512	bb	0	156	
I1-026		system suit	0.28	586264	bb	0	173	
I1-027		system suit	0.28	521992	bb	0	149	
I1-028						0		
I1-029	52-2	C200	0.28	535498	MM	0	154	-23
I1-030	52-3	C200	0.29	627223	bb	0	188	-5.8
I1-031	52-4	C200	0.29	685006	bb	0	210	4.9
I1-032	52-5	C400	0.28	1302060	bb	0	439	9.7
I1-033	52-6	C400	0.28	1210163	bb	0	405	1.2
I1-034	52-7	C400	0.28	1271039	bb	0	427	6.8
I1-035	52-8	C800	0.28	2184190	bb	0	766	-4.2
I1-036	52-9	C800	0.28	2336838	bb	0	823	2.9
I1-037	52-10	C800	0.28	2239647	bb	0	787	-1.6
I1-038						0		
I1-039	53-6					0		
I1-040	53-7	QC 1	0.29	1490057	bb	2.02	1028	2.8
I1-041	53-8	QC 1	0.29	1384239	bb	2.02	948	-5.2
I1-042	53-9	QC 1	0.28	1502545	bb	2.02	1037	3.7
I1-043						0		

Table A-3: Calibration, Quality Control and Concentration Data of Sequence 534002c1

Quantify Compound Summary Report

Sample List:C:\MASSLYNX\534002.PRO\SampleDB\534002b
Quantify Compound Summary Report

Sample List:C:\MASSLYNX\534002.PRO\SampleDB\534002c1
Last modified:Wed Feb 16 11:41:56 2005
Method:C:\MASSLYNX\534002.PRO\MethDB\534002
Last modified:Tue Feb 15 08:22:29 2005
Job Code:

Printed:Wed Feb 16 12:06:30 2005

Compound 1: PFBS

Run #	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc, (ng/mL)	% RE
I1-044a		system suit	0.27	272216	bb	0	0	
I1-045a		system suit	0.27	301122	bb	0	0	
I1-046a		system suit	0.27	270011	bb	0	0	
I1-047a						0		
I1-048a	54-2	C200	0.27	286018	bbX	0	0	-100
I1-049a	54-3	C200	0.27	1541738	bb	0	198	-0.97
I1-050a	54-4	C200	0.27	1554039	bb	0	200	0.13
I1-051a	54-5	C400	0.27	2641196	bb	0	395	-1.3
I1-052a	54-6	C400	0.27	2920591	bb	0	445	11
I1-053a	54-7	C400	0.27	2460610	bb	0	363	-9.3
I1-054a	54-8	C800	0.27	5085377	bb	0	833	4.1
I1-055a	54-9	C800	0.27	4540488	bb	0	735	-8.1
I1-056a	54-10	C800	0.27	5078479	bb	0	831	3.9
I1-057a						0		
I1-058a	55-6	QC 0	0.27	1603	bb	0	0	
I1-059a	55-7	QC 1	0.28	3143402	bb	2.02	979	-2.1
I1-060a	55-8	QC 1	0.28	3253684	bb	2.02	1019	1.9
I1-061a	55-9	QC 1	0.27	3326685	bb	2.02	1046	4.6
I1-062a						0		
I1-063a	57-3	Grp 1	0.28	3451435	bb	4	2160	8.0
I1-064a	57-4	Grp 1	0.27	3164368	bb	4	1955	-2.3
I1-065a						0		
I1-066a	56-6	QC 0	0.28	6226	bb	0	0	
I1-067a	56-7	QC 1	0.27	3261408	bb	2.02	1022	2.2
I1-068a	56-8	QC 1	0.27	3319041	bb	2.02	1043	4.3
I1-069a	56-9	QC 1	0.28	3374058	bb	2.02	1063	6.3
I1-070a		50;50 acn/water	0.26	1219	bb	0	0	

Table A-4: Calibration, Quality Control and Stability Data of Sequence 534002d

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534002.PRO\SampleDB\534002d
Last modified: Fri Feb 18 12:18:51 2005
Method: C:\MASSLYNX\534002.PRO\MethDB\534002
Last modified: Tue Feb 15 08:22:29 2005
Job Code:

Printed: Fri Feb 18 13:52:39 2005

Compound 1: PFBS

Run #	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc, (ng/mL)	% RE
I1-071		system suit	0.34	1357228	bb	0	154	
I1-072		system suit	0.34	1296103	bb	0	144	
I1-073		system suit	0.33	1336772	bb	0	150	
I1-074						0		
I1-075	64-2	C200	0.34	1340233	bb	0	151	-24
I1-076	64-3	C200	0.35	1587337	bb	0	193	-3.4
I1-077	64-4	C200	0.34	1381325	bb	0	158	-21
I1-078	64-5	C400	0.35	3559164	bb	0	529	32
I1-079	64-6	C400	0.34	2558334	bb	0	359	-10
I1-080	64-7	C400	0.35	3145328	bb	0	459	15
I1-081	64-8	C800	0.34	4800900	bb	0	741	-7.4
I1-082	64-9	C800	0.35	5712563	bb	0	896	12
I1-083	64-10	C800	0.35	4642687	bb	0	714	-11
Run #	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc, (µg/mL)	% RE
I1-084						0		
I1-085	65-6	QC 0	0.35	44922	bb	0	0	
I1-086	65-7	QC 1	0.34	3240725	bb	2.02	959	-4.1
I1-087	65-8	QC 1	0.34	2793539	bb	2.02	805	-19
I1-088	65-9	QC 1	0.34	3087466	bb	2.02	907	-9.3
I1-089		50;50 acn/water	0.34	2862	bb	0	0	
I1-090	66-5	Grp 1	0.34	3385869	bb	4	1999	-0.057
I1-091	66-6	Grp 1	0.35	3102119	bb	4	1805	-9.7
I1-092	66-7	Grp 1	0.33	3393377	bb	4	2004	0.20
I1-093	66-8	Grp 1	0.34	3046565	bb	4	1768	-12
I1-094		50;50 acn/water	0.35	10530	bb	0	0	

APPENDIX D

Pretest Clinical Observations

PRETEST CLINICAL OBSERVATIONS

PROJECT NO.:WIL-534002P A PHARMACOKINETIC AND EXCRETION STUDY IN CYNOMOLGUS MONKEYS PAGE 1

SPONSOR:AGC CHEMICAL INDIVIDUAL CLINICAL OBSERVATIONS

TABLE RANGE: 01-06-05 TO 02-09-05

ANIMAL	SEX	GROUP	CATEGORY	DATE	TIME	GRADE	OBSERVATIONS
1555	M	PRETEST	NORMAL	01-17-05	9:28	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				01-31-05	7:56	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				02-03-05	10:35	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
1555	M	PRETEST	EXCRETA	01-06-05	9:43	P	SOFT FECES
				01-24-05	8:51	P	SOFT FECES
1562	M	PRETEST	NORMAL	01-06-05	9:43	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				01-17-05	9:30	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				01-24-05	8:52	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				01-31-05	7:57	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
1562	M	PRETEST	EXCRETA	02-03-05	10:38	P	SOFT FECES
1563	M	PRETEST	NORMAL	01-06-05	9:44	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				01-17-05	9:31	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				01-24-05	8:53	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				01-31-05	7:58	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				02-03-05	10:38	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
1563	M	PRETEST	BODY/INTEGUMENT	01-26-05	7:48	P	SWOLLEN UROGENITAL AREA
1572	F	PRETEST	NORMAL	01-17-05	9:32	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				01-24-05	8:54	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				01-31-05	7:59	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				02-03-05	10:39	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
1572	F	PRETEST	BEHAVIOR/CNS	02-08-05	8:48	P	INAPPETENCE
1572	F	PRETEST	EXCRETA	01-06-05	9:45	P	SOFT FECES
1576	F	PRETEST	NORMAL	01-06-05	9:46	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				01-17-05	9:35	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				01-24-05	8:55	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				01-31-05	8:00	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				02-03-05	10:39	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
1573	F	PRETEST	NORMAL	01-17-05	9:35	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				01-24-05	8:55	P	NO SIGNIFICANT CLINICAL OBSERVATIONS

GRADE CODE: 1 - SLIGHT 2 - MODERATE 3 - SEVERE P - PRESENT

TABLE RANGE: 01-06-05 TO 02-09-05

ANIMAL	SEX	GROUP	CATEGORY	DATE	TIME	GRADE	OBSERVATIONS
1573	F	PRETEST	NORMAL	01-31-05	8:01	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
				02-03-05	10:39	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
1573	F	PRETEST	EXCRETA	01-06-05	9:47	P	SOFT FECES

GRADE CODE: 1 - SLIGHT 2 - MODERATE 3 - SEVERE P - PRESENT

APPENDIX E

Animal Room Environmental Conditions

PROJECT NO.:WIL- 534002 TEMPERATURE/HUMIDITY - DAILY SUMMARY REPORT BY STUDY
SPONSOR: AGC CHEMICAL

STUDY SPECIFICATIONS: 534002 DATE IN: 01/06/05 TIME IN: 7:00
DATE OUT: 02/24/05 TIME OUT: 16:00

ROOM SPECIFICATIONS: B ROOM 82 LOW TEMPERATURE °F: 66.0 HIGH TEMPERATURE °F: 76.0 LOW HUMIDITY: 30.0
SPECIES: MONKEY LOW TEMPERATURE °C: 18.9 HIGH TEMPERATURE °C: 24.4 HIGH HUMIDITY: 70.0

	TEMPERATURE		HUMIDITY
DATE	MEAN (°F)	MEAN (°C)	MEAN (%RH)
06-Jan-05	70.3	21.3	41.5
07-Jan-05	70.5	21.4	40.9
08-Jan-05	70.4	21.4	41.0
09-Jan-05	70.5	21.4	40.6
10-Jan-05	70.4	21.4	40.1
11-Jan-05	70.5	21.4	41.3
12-Jan-05	70.4	21.3	43.4
13-Jan-05	70.5	21.4	42.0
14-Jan-05	70.4	21.3	41.1
15-Jan-05	70.4	21.3	39.2
16-Jan-05	70.4	21.3	38.1
17-Jan-05	70.4	21.4	36.9
18-Jan-05	70.4	21.3	37.4
19-Jan-05	70.5	21.4	39.3
20-Jan-05	70.3	21.3	39.2
21-Jan-05	70.5	21.4	38.2
22-Jan-05	70.5	21.4	37.3
23-Jan-05	70.4	21.3	37.2
24-Jan-05	70.5	21.4	38.9
25-Jan-05	70.6	21.5	40.9
26-Jan-05	70.4	21.3	40.2
27-Jan-05	70.4	21.4	38.2
28-Jan-05	70.4	21.3	38.9

NOTE: + = VALUE WAS GREATER THAN HIGH RANGE
- = VALUE WAS LESS THAN LOW RANGE
NOTE: MEANS REPRESENT THE MEAN OF THE DAILY VALUES

REPORT 4
VERSION 1.09
06/29/2005 13:35

STUDY SPECIFICATIONS: 534002 DATE IN: 01/06/05 TIME IN: 7:00
DATE OUT: 02/24/05 TIME OUT: 16:00

ROOM SPECIFICATIONS: B ROOM 82 LOW TEMPERATURE °F: 66.0 HIGH TEMPERATURE °F: 76.0 LOW HUMIDITY: 30.0
SPECIES: MONKEY LOW TEMPERATURE °C: 18.9 HIGH TEMPERATURE °C: 24.4 HIGH HUMIDITY: 70.0

	TEMPERATURE		HUMIDITY
DATE	MEAN (°F)	MEAN (°C)	MEAN (%RH)
29-Jan-05	70.4	21.3	39.7
30-Jan-05	70.5	21.4	40.7
31-Jan-05	70.4	21.3	39.6
01-Feb-05	70.5	21.4	39.1
02-Feb-05	70.4	21.3	40.2
03-Feb-05	70.5	21.4	41.4
04-Feb-05	70.5	21.4	39.3
05-Feb-05	70.5	21.4	39.9
06-Feb-05	70.5	21.4	40.2
07-Feb-05	70.5	21.4	39.6
08-Feb-05	70.6	21.4	40.3
09-Feb-05	70.5	21.4	41.5
10-Feb-05	70.4	21.3	41.5
11-Feb-05	70.6	21.4	41.2
12-Feb-05	70.6	21.5	41.2
13-Feb-05	70.5	21.4	40.9
14-Feb-05	70.5	21.4	40.3
15-Feb-05	70.4	21.4	40.5
16-Feb-05	70.5	21.4	41.2
17-Feb-05	70.4	21.3	41.0
18-Feb-05	70.4	21.3	40.0
19-Feb-05	70.5	21.4	40.8
20-Feb-05	70.5	21.4	41.6

NOTE: + = VALUE WAS GREATER THAN HIGH RANGE
- = VALUE WAS LESS THAN LOW RANGE
NOTE: MEANS REPRESENT THE MEAN OF THE DAILY VALUES

STUDY SPECIFICATIONS: 534002 DATE IN: 01/06/05 TIME IN: 7:00
DATE OUT: 02/24/05 TIME OUT: 16:00

ROOM SPECIFICATIONS: B ROOM 82 LOW TEMPERATURE °F: 66.0 HIGH TEMPERATURE °F: 76.0 LOW HUMIDITY: 30.0
SPECIES: MONKEY LOW TEMPERATURE °C: 18.9 HIGH TEMPERATURE °C: 24.4 HIGH HUMIDITY: 70.0

	TEMPERATURE		HUMIDITY
DATE	MEAN (°F)	MEAN (°C)	MEAN (%RH)
21-Feb-05	70.6	21.4	41.4
22-Feb-05	70.4	21.3	41.6
23-Feb-05	70.4	21.3	41.7
24-Feb-05	70.4	21.3	40.8

GRAND STATS	MEAN	MIN	MAX
TEMPERATURE °F	70.5	70.3	70.6
TEMPERATURE °C	21.4	21.3	21.5
HUMIDITY (%RH)	40.2	36.9	43.4
N DAYS	50		

NOTE: + = VALUE WAS GREATER THAN HIGH RANGE
- = VALUE WAS LESS THAN LOW RANGE
NOTE: MEANS REPRESENT THE MEAN OF THE DAILY VALUES

ROOM SPECIFICATIONS:	B ROOM 82		
SPECIES:	MONKEY		
LOW TEMPERATURE:	66.0	DATE IN:	01/06/05
HIGH TEMPERATURE:	76.0	TIME IN:	7:00
LOW HUMIDITY:	30.0	DATE OUT:	02/24/05
HIGH HUMIDITY:	70.0	TIME OUT:	16:00

ROOM B ROOM 82 SUMMARY

	TEMPERATURE	HUMIDITY
MEAN	70.5	40.2
MIN	69.2	23.7
MAX	71.3	58.5
SD	0.24	3.06
N SAMPLES	1184	1184
FIRST DAY	01/06/05	
LAST DAY	02/24/05	
N DAYS	50	

NOTE: TEMPERATURE UNITS = DEGREES FAHRENHEIT
HUMIDITY UNITS = % RELATIVE HUMIDITY
NOTE: MEANS REPRESENT THE MEAN OF ALL VALUES

STUDY 534002 SUMMARY

MEAN	70.5	40.2
MIN	69.2	23.7
MAX	71.3	58.5
SD	0.24	3.06
N SAMPLES	1184	1184
FIRST DAY	01/06/05	
LAST DAY	02/24/05	
N DAYS	50	

NOTE: TEMPERATURE UNITS = DEGREES FAHRENHEIT
HUMIDITY UNITS = % RELATIVE HUMIDITY
NOTE: MEANS REPRESENT THE MEAN OF ALL VALUES

APPENDIX F

Bioanalytical Report (WIL Research Laboratories, LLC) [PFHxA]

A PHARMACOKINETIC (IN BLOOD) AND EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Analyses Of PFHxA Serum and Urine Samples

Analytical Chemistry Department

WIL Research Laboratories, LLC

TABLE OF CONTENTS

Page

INDEX OF TABLES

Page

INDEX OF FIGURES

Page

Page

Page

Page

INDEX OF ATTACHMENTS

1. INTRODUCTION

This report provides a detailed description and validation of a high performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS) method in the negative electrospray ionization (ESI-) mode for the determination of perfluorohexanoic acid (PFHxA) in monkey serum. The method was cross-validated to determine PFHxA in monkey urine. Method sensitivity, specificity/selectivity, calibration reproducibility, accuracy and precision were assessed and validated. Stability of PFHxA in processed samples, in samples during frozen storage (-20°C) and after short-term (4 hour) room temperature storage and in samples after the freeze-thaw process was evaluated.

This report details the analytical results from the determination of PFHxA in monkey serum and urine samples. Analysis of serum samples resulted in levels ranging from not detected (ND) to 25,993 ng PFHxA/mL. Analysis of urine samples resulted in levels ranging from less than the lower limit of quantitation (LLOQ) to 538,105 ng PFHxA/mL.

2. BLANK MATRIX IDENTIFICATION

Blank monkey serum and urine were obtained from Bioreclamation, Inc., East Meadow, New York.

3. EXPERIMENTAL

3.1. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Instrument:	Hewlett Packard 1100 liquid chromatograph equipped with a diode array detector, autosampler, Micromass tandem quadrupole Quattro Ultima™ Mass Spectrometer and MassLynx™ software, or equivalent system
Column:	ACE C8 50 x 2.1 mm with a C8 guard cartridge, or equivalent
Column Temperature:	35°C
Mobile Phase:	A 1 mM ammonium acetate B Acetonitrile (ACN)

Gradient	Time (minutes)	Solvent A (%)	Solvent B (%)	Flow (mL/minute)
	0.00	80.0	20.0	0.3
	0.50	80.0	20.0	0.3
	1.50	10.0	90.0	0.3
	3.50	10.0	90.0	0.3
	3.60	80.0	20.0	0.4
	9.90	80.0	20.0	0.4
	10.0	80.0	20.0	0.3

Flow Rate: 0.3 mL/minute

Detector: Mass spectrometer with conditions as described in Section 3.2.

Injection Volume: 10 µL

Retention Time: Approximately 3.8 minutes for PFHxA

Run Time: 10 minutes

Note: The retention times and run times varied depending on column performance.

3.2. MASS SPECTROMETRY

3.2.1. INSTRUMENT

A Micromass Quattro Ultima™ (or equivalent system) tandem mass spectrometer equipped with an ESI- interface was used in this study. Data acquisition and analysis were performed using MassLynx™ software version 3.4.

3.2.2. SOURCE PARAMETERS

Source:	ESI-
Capillary:	3.0 kV
Cone:	45 V
Hexapole 1:	0 V
Aperture 1:	0 V

Hexapole 2:	0 V
Source Block Temperature:	100°C
Desolvation Temperature:	300°C
Cone Gas Flow :	Approximately 100 L nitrogen/hour
Desolvation Gas Flow:	Approximately 500 L nitrogen/hour

Note: Settings varied depending on mass spectrometer performance.

3.2.3. ACQUISITION PARAMETERS

Function Type:	MRM (multiple reaction monitoring)
Precursor/Product Ion:	m/z 313/269 for PFHxA m/z 444/381 for 9H-hexadecafluorononanoic acid
Dwell Time:	0.5 second

Note: Settings varied depending on mass spectrometer performance.

3.3. PREPARATION OF 1 MM AMMONIUM ACETATE

This solution was prepared by dissolving approximately 77 mg of ammonium acetate in 1 L of deionized (DI) water. The solution was thoroughly mixed to achieve complete dissolution and vacuum degassed. The preparation was scaled as needed, *i.e.*, if the volume of the preparation was doubled, then the stated amounts of any constituents were doubled.

3.4. PREPARATION OF INTERNAL STANDARD FORTIFICATION SOLUTION

A stock solution of 9H-hexadecafluorononanoic acid (WIL log no. ARS-0354A) was prepared at a concentration of 1000 µg/mL in acetonitrile (ACN). The solution was stirred to achieve complete dissolution. An aliquot of the stock solution was diluted with ACN to yield a solution of 1 µg/mL.

3.5. PREPARATION OF PRIMARY STOCK SOLUTION

A stock solution of PFHxA (WIL log no. 6394A) was prepared at a concentration of 1000 µg/mL in ACN. The solution was stirred to achieve complete dissolution.

3.6. PREPARATION OF CALIBRATION SAMPLES

An aliquot of the primary stock solution was diluted with ACN to yield a secondary stock solution at 25 µg PFHxA/mL. Aliquots of this secondary stock solution were diluted with ACN to yield fortification solutions from 0.15 to 25 µg PFHxA/mL.

Calibration samples containing 30 to 5000 ng/mL PFHxA were prepared by addition of 20 µL of the appropriate fortification solution to 0.1 mL of blank (control) matrix in 1.5-mL conical tubes. The calibration samples were processed as described in Sections 3.8. and 3.9. (Sample Processing).

3.7. PREPARATION OF QUALITY CONTROL STOCK SOLUTIONS AND QUALITY CONTROL SAMPLES

An aliquot of the primary stock solution was diluted with ACN to yield a secondary stock solution at 25 µg PFHxA/mL. Aliquots of this secondary stock solution were diluted with ACN to yield fortification solutions ranging from 0.15 to 25 µg PFHxA/mL.

Quality control (QC) samples at concentrations of 30, 300 and 5000 ng PFHxA/mL were prepared by adding 20 µL of the appropriate fortification solution to 0.1 mL of blank (control) matrix in 1.5-mL conical tubes. Dilutional QC samples were prepared at a concentration of 10,000 ng PFHxA/mL by adding 10 µL of the primary stock solution to 990 µL of blank matrix. A 10 µL aliquot of the dilutional QC was diluted to 1 mL with blank matrix. The QC samples were processed as described in Sections 3.8. and 3.9. (Serum and Urine Sample Processing).

3.8. SAMPLE PROCESSING (VALIDATION)

Aliquots (0.1 mL) of the calibration and QC samples were transferred into 1.5-mL conical tubes. An aliquot (50 µL) of the internal standard (IS) solution was added to each tube except the solvent blank and matrix blank samples. The 1.5-mL tubes containing the calibration and QC samples were capped and mixed with vortex action for approximately 10 seconds. ACN (300 µL) was added to each tube. The tubes were capped, mixed with vortex action for approximately 10 seconds and centrifuged at a minimum of 5000 rpm

for approximately 10 minutes at approximately 4°C. A portion of each supernatant fraction was transferred to an autosampler vial for analysis.

3.9. SAMPLE PROCESSING (CROSS VALIDATION AND ROUTINE ANALYSIS)

Aliquots (0.1 mL) of the experimental samples were transferred into 1.5-mL conical tubes. ACN (20 µL) was added to the experimental samples to simulate the analyte fortification step of the standards and QC samples. The 1.5-mL tubes containing the calibration, QC and experimental samples were capped and mixed with vortex action for approximately 10 seconds. ACN (350 µL) was added to each tube. The tubes were capped, mixed with vortex action for approximately 10 seconds and centrifuged at a minimum of 3650 rpm for approximately 10 minutes at approximately 4°C. A portion of each supernatant fraction was transferred to an autosampler vial for analysis.

3.10. CONCENTRATION QUANTITATION

An external standard method of quantitation was used for determination of PFHxA in serum and urine. A calibration curve was constructed for each set of analyses. Using the Quantify program in MassLynx™, the peak area of PFHxA (y) and the theoretical concentrations of the calibration standards (x) were fit with least-squares regression analysis to the ln-quadratic function (excluding zero):

$$\ln (y) = a \times [\ln (x)]^2 + b \times \ln (x) + c$$

Concentrations were back-calculated from the results of the regression analysis using the Quantify program in the MassLynx™ software.

4. RESULTS AND DISCUSSION

4.1. METHOD VALIDATION AND CROSS VALIDATIONS

A method was developed and validated for the determination of PFHxA in monkey serum where five validation sessions were conducted with the addition of the IS to the samples. Method sensitivity, specificity/selectivity, calibration reproducibility, accuracy and precision were assessed and validated. Though the IS was added to each sample, the

resulting data revealed the IS response was too variable and the data were quantitated using the external standard method. The validated monkey serum assay was cross-validated for the determination of PFHxA in monkey serum without the addition of the IS to the samples in a single validation session. The monkey serum assay was then cross-validated for the determination of PFHxA in monkey urine in a single validation session. The results of the monkey serum assay validation and the subsequent cross-validation are summarized in Tables 1 through 3 (calibration samples) and 4 through 6 (quality control samples). Stability of PFHxA in processed samples during frozen storage (-20°C) and after short-term (4 hour) room temperature storage as well as in samples after the freeze-thaw process was evaluated.

Under the described chromatographic conditions, the retention time of PFHxA was approximately 3.8 minutes. The total run time for each analysis was approximately 10 minutes.

Figures 1 through 26 illustrate typical chromatograms for a processed solvent blank (Figure 1), a processed serum blank (Figure 2), processed serum calibration samples (Figures 3 through 8), processed serum QC samples (Figures 9 through 12) and experimental serum samples (Figures 13 through 26).

Figures 27 through 56 illustrate typical chromatograms for a processed solvent blank (Figure 27), a processed urine blank (Figure 28), processed urine calibration samples (Figures 29 through 34), processed urine QC samples (Figures 35 through 38) and experimental urine samples (Figures 39 through 56).

4.2. SENSITIVITY

According to WIL SOP, the lower limit of quantitation (LLOQ) can be defined as the lowest calibration concentration that meets the validation acceptance criteria, *i.e.*, percent relative standard deviation (%RSD) ≤ 20% and percent relative error (%RE) within ± 20%. As shown in Tables 1 through 3, the LLOQ was 30 ng/mL for PFHxA in monkey

serum and urine. The monkey serum and urine validation sessions resulted in mean %RSD and %RE values at the LLOQ for each analyte as indicated in the following table.

	%RE	%RSD
Serum with IS	3.8	4.1
Serum without IS	5.2	10
Urine	2.9	14

4.3. SPECIFICITY/SELECTIVITY

Assay specificity/selectivity refers to the ability of the assay chromatography to specifically detect and quantitate the analyte(s) of interest from potentially interfering compounds. Assay specificity/selectivity was confirmed when assessment of the assay accuracy and precision met the acceptance criteria.

4.4. CALIBRATION ACCEPTABILITY

During each validation session, triplicate calibration samples at each concentration level were prepared and analyzed as described previously. Single injections were made for each processed calibration sample. The resulting peak area versus concentration data were fit to the ln quadratic function using least squares regression analysis, excluding zero. The regression equation was used to back calculate the corresponding concentrations from the peak areas. The reproducibility of the calibration curve data was considered valid when 1) the inter-session variability (RSD) of the back calculated concentrations at each concentration level was ≤ 15%, except at the lowest concentration level where ≤ 20% was acceptable; and 2) the mean back calculated concentration at each concentration level was within ± 15% of the theoretical values (%RE within ± 15%), except at the lowest concentration level where %RE within ± 20% was acceptable.

The back-calculated concentration values and the associated intra- and inter-session statistics for PFHxA calibration samples used during the five validation sessions for monkey serum are shown in Table 1. The intra-session variability of back-calculated

concentrations at each level used for the cross-validation without IS are shown in Table 2. The intra-session variability of back-calculated concentrations at each level used for the cross-validation to urine are shown in Table 3. The intra- and inter-session statistics for the calibration samples are summarized in the following tables.

	%RE Range	%RSD Range
Serum with IS	-6.7 to 6.5	4.1 to 9.4
Serum without IS	-7.4 to 9.0	1.9 to 10
Urine	-3.0 to 5.6	4.1 to 14

Based on the results in Tables 1 through 3, the reproducibility of the calibration data was acceptable for the determination of PFHxA concentration in monkey serum and urine.

4.5. ACCURACY AND PRECISION

During each validation session, triplicate QC samples at each concentration level were prepared and analyzed as described previously. Single injections were made of each processed QC sample. The regression equation was used to calculate the corresponding concentrations from the QC peak area data. The variability (RSD) of calculated QC concentration data was used as a measure of assay precision. For the serum and urine assays, the precision of the method was considered acceptable when the inter-session RSD of the calculated concentrations at each QC concentration level was ≤ 15%, except at the lowest concentration level where ≤ 20% was acceptable. The accuracy of the method was considered acceptable when inter-session concentration means of the calculated concentrations at each QC concentration level had %RE values within ± 15%, except at the lowest concentration level where ≤ 20% was acceptable.

The back calculated concentration values and the associated intra- and inter-session statistics for the PFHxA QC samples used during the five validation sessions for monkey serum are shown in Table 4. The intra-session variability of back-calculated concentrations at each level used for the cross-validation without IS are shown in

Table 5. The intra-session variability of back-calculated concentrations at each level used for the cross-validation to urine are shown in Table 6. The intra- and inter-session statistics for the QC samples are summarized in the following tables.

	%RE Range	%RSD Range
Serum with IS	-6.6 to 2.7	7.5 to 14
Serum without IS	-11 to -3.7	3.1 to 5.3
Urine	-8.2 to 12	4.4 to 10

Based on the results in Tables 4 through 6, the accuracy and precision of the QC sample data were acceptable for the determination of PFHxA concentration in monkey serum and urine.

4.6. STABILITY

Stability of PFHxA in processed samples, in samples during frozen storage (-20°C) and after short-term (4 hour) room temperature storage, and in samples after the freeze-thaw process was evaluated. According to WIL SOP, stability is indicated if the mean measured post-storage (or treatment) analyte(s) concentration is not less than 90% of the corresponding time-zero concentration.

4.6.1. STABILITY OF PFHXA IN PROCESSED SERUM AND URINE SAMPLES

Stability of PFHxA in processed monkey serum and urine samples was evaluated. The samples were re-injected after refrigerated storage to evaluate the test article stability in processed samples. The mean concentrations of the reanalyzed samples were compared to the corresponding time zero PFHxA concentrations.

The mean measured concentrations in serum samples after 5 days of refrigerated storage ranged from 85.4% to 137% of the corresponding time-zero concentrations (Table 7). Since post-storage concentrations of PFHxA met the specified criteria (with the exception of the 100 ng/mL level), PFHxA in monkey serum samples was considered to be stable through 5 days of refrigerated storage.

The mean measured concentrations in urine samples after 1 day of refrigerated storage were 96.1% and 120% of the corresponding time-zero concentrations (Table 8). Since post-storage concentrations of PFHxA met the specified criteria, PFHxA in monkey urine samples was considered to be stable through 1 day of refrigerated storage.

4.6.2. LONG TERM FROZEN STABILITY OF PFHXA IN SERUM AND URINE SAMPLES

Frozen (-20°C) storage stability of PFHxA in monkey serum and urine was assessed. Chemical degradation usually occurs at a rate described by a monoexponential decay function: $C_t = C_0 \times e(kt)$. The natural logarithm transformation of this function is: $\ln(C_t) = \ln(C_0) + kt$. Here, C_0 is the analyte concentration at t = 0; and C_t is the analyte concentration after storage time = t days. Tables 9 and 10 summarize and graphically represent the ln (concentration) vs storage time in days for monkey serum and urine. The data were fit using least-squares regression analysis to the monoexponential function yielding degradation constants (k), summarized in the following table.

	Serum	Urine
300 ng/mL	$-1.53e^{-03}$	$9.23e^{-05}$
5000 ng/mL	$1.34e^{-03}$	$1.24e^{-03}$

When fit to the exponential function shown previously, the 5000 ng/mL serum samples and both levels of urine samples resulted in positive degradation constants (k), indicating no detectable loss during the storage of the samples. The largest serum degradation constant (-0.00153) would result in a 10% loss in 69 days, 50% in 453 days, etc.

4.6.3. STABILITY OF PFHXA IN SERUM AND URINE SAMPLES AT ROOM TEMPERATURE

Serum and urine samples were fortified with PFHxA. Triplicate samples were used to evaluate the stability of the analyte after 4-hours of room temperature storage. The mean

test article concentrations of the stored samples were compared to the corresponding time-zero PFHxA concentrations.

The mean measured concentrations in serum samples after 4-hours of room temperature storage were 94.2% and 85.3% of the corresponding time-zero concentrations (Table 11). The 300 ng/mL post-storage concentrations of PFHxA met the specified criteria, but the 5000 ng/mL post-storage concentrations did not. Therefore, in future studies samples should be processed as soon as thawed.

The mean measured concentrations in urine samples after 4-hours of room temperature storage were 84.6% and 82.6% of the corresponding time-zero concentrations (Table 12). Post-storage concentrations of PFHxA did not meet the specified criteria; therefore, in future studies, samples should be processed as soon as thawed.

4.6.4. FREEZE/THAW STABILITY OF PFHXA IN SERUM AND URINE SAMPLES

The freeze-thaw stability of PFHxA in monkey serum and urine was evaluated. Serum and urine samples were fortified at 300 and 5000 ng PFHxA/mL. Triplicate samples from each concentration level were used to evaluate the stability of the analytes after each of 3 freeze-thaw cycles. The samples were frozen and thawed (1 cycle), and the process repeated 2 more times (cycles 2 and 3) for the analysis of freeze-thaw stability.

Serum samples for the second freeze-thaw cycle for the 300 ng/mL level were not analyzed individually. The mean measured concentrations in serum samples for up to 3 freeze-thaw cycles ranged from 83.7% to 110% of the corresponding time-zero concentrations (Table 13). The post-storage concentrations of the 5000 ng PFHxA/mL samples met the specified criteria, however the 300 ng PFHxA/mL sample did not. Therefore, in future studies, precautions should be taken to limit the number of freeze-thaw cycles to which the low level samples are subjected.

The mean measured concentrations in urine samples for up to 3 freeze-thaw cycles ranged from 97.2% to 106% of the corresponding time-zero concentrations (Table 14). Since post-storage concentrations of PFHxA met the specified criteria, PFHxA in monkey urine samples was considered to be stable through 3 freeze-thaw cycles.

4.7. ANALYSIS OF EXPERIMENTAL SAMPLES

Monkey serum and urine samples were analyzed for PFHxA and the results are summarized in Tables 15 and 16, respectively. In addition to the experimental (unknown) samples, each set of analyses consisted of at least duplicate calibration samples, one solvent blank, one control (blank) matrix sample and at least triplicate QC samples at each concentration level. For an analytical run for serum or urine samples to be considered valid, at least two-thirds of the QC samples, with at least 1 at each concentration level, could not deviate more than ± 15% from the QC target concentration values, except at the lowest concentration level where ≤ 20% was acceptable. Based on the criteria mentioned above, all the PFHxA analyses were acceptable.

5. CONCLUSION

An HPLC/MS/MS ESI- method for the determination of PFHxA in monkey serum was developed and validated. The validated monkey serum assay was cross-validated for the determination of PFHxA in monkey urine. Stability of PFHxA in processed samples, in samples during frozen storage (-20°C) and after short-term (4 hour) room temperature storage, and in samples after the freeze-thaw process was evaluated. Analysis of serum samples resulted in levels ranging from ND to 25,993 ng PFHxA/mL. Analysis of urine samples resulted in levels ranging from LLOQ to 538,105 ng PFHxA/mL.

6. KEY STUDY PERSONNEL AND REPORT SUBMISSION

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2 September 2005
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2 Sep. 2005
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TABLES 1 - 16

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 1: PFHxA Back-Calculated Concentrations And Intra-Session And Inter-Session Statistics Of Calibration Samples In Monkey Serum With IS

Theo. Conc. (ng/mL)	30	100	300	1000	3000	5000
Set 1 (3/30/05), 534002(PFHxA)CMS, analyst KLK						
Samp 1	32.3	92.1	310	1048	2899	4701
Samp 2	29.5	96.0	308	1033	2975	5656
Samp 3	30.2	96.7	305	1000	2859	4845
Mean	30.7	94.9	308	1027	2911	5067
SD	1.5	2.5	2.9	25	59	515
RSD	4.8	2.6	0.95	2.4	2.0	10
%RE	2.2	-5.1	2.6	2.7	-3.0	1.3
Set 2 (3/30/05), 534002(PFHxA)DMS1, analyst KLK						
Samp 1	32.1	87.3	318	1060	3011	4971
Samp 2	29.7	96.1	304	1042	3125	4829
Samp 3	32.6	87.0	305	1066	2915	4750
Mean	31.5	90.1	309	1056	3017	4850
SD	1.6	5.2	8.1	13	105	112
RSD	5.0	5.7	2.6	1.2	3.5	2.3
%RE	4.9	-9.9	3.0	5.6	0.57	-3.0
Set 3 (3/31/05), 534002(PFHxA)EMS, analyst LEB2, DKP						
Samp 1	32.8	92.5	304	1074	3025	4764
Samp 2	30.9	91.0	305	1163	3067	4674
Samp 3	31.3	86.8	281	1091	3050	4759
Mean	31.6	90.1	297	1110	3047	4732
SD	1.0	2.9	14	47	21	50
RSD	3.2	3.3	4.7	4.3	0.69	1.1
%RE	5.5	-9.9	-1.1	11	1.6	-5.4

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 1: PFHxA Back-Calculated Concentrations And Intra-Session And Inter-Session Statistics Of Calibration Samples In Monkey Serum With IS

Theo. Conc. (ng/mL)	**30**	**100**	**300**	**1000**	**3000**	**5000**
Set 4 (3/31/05), 534002(PFHxA)FMS, analyst LEB2, DKP						
Samp 1	28.3	98.1	321	754	3430	5187
Samp 2	31.4	99.3	249	1164	2834	4354
Samp 3	31.0	102	323	1135	3191	*
Mean	30	100	298	1017	3152	4771
SD	1.7	1.7	42	229	300	589
RSD	5.6	1.7	14	22	9.5	12
%RE	0.71	-0.32	-0.71	1.7	5.1	-4.6
Set 5 (4/5/05), 534002(PFHxA)GFMS, analyst KLK						
Samp 1	31.8	98.9	285	1176	3074	4726
Samp 2	31.2	90.6	294	1082	2985	4751
Samp 3	32.2	85.9	281	1086	3120	4777
Mean	31.7	91.8	287	1115	3060	4751
SD	0.52	6.6	6.3	54	69	25
RSD	1.6	7.2	2.2	4.8	2.3	0.53
%RE	5.8	-8.2	-4.4	11	2.0	-5.0
Interset Statistics						
n	15	15	15	15	15	14
Mean	31.1	93.3	300	1065	3037	4839
SD	1.3	5.2	19	100	149	295
RSD	4.1	5.5	6.4	9.4	4.9	6.1
%RE	3.8	-6.7	-0.13	6.5	1.2	-3.2

* Data point excluded due to suspected sample prep error.

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 2: PFHxA Back-Calculated Concentrations And Intra-Session Statistics Of Calibration Samples In Monkey Serum With No IS

Theo. Conc. (ng/mL)	**30**	**100**	**300**	**1000**	**3000**	**5000**
Set 1 (4/5/05), 534002(PFHxA)HMS, analyst KLK						
Samp 1	32.7	102	294	1110	3022	4882
Samp 2	34.0	88.7	303	1105	2901	4935
Samp 3	28.0	87.5	281	1055	3073	4754
Intra-set Statistics						
n	3	3	3	3	3	3
Mean	31.6	92.6	293	1090	2999	4857
SD	3.2	7.8	11	31	88	93
RSD	10	8.4	3.7	2.8	2.9	1.9
%RE	5.2	-7.4	-2.4	9.0	-0.035	-2.9

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 3: PFHxA Back-Calculated Concentrations And Intra-Session Statistics Of Calibration Samples In Monkey Urine

Theo. Conc. (ng/mL)	**30**	**100**	**300**	**1000**	**3000**	**5000**
Set 1 (4/18/05), 534002(PFHxA)KMU1, KMU2, analyst KLK						
Samp 1	35.0	97.1	265	1020	2897	4910
Samp 2	26.3	108	292	1063	3258	4851
Samp 1, re-injection	28.4	90.1	304	928	2915	4696
Samp 2, re-injection	33.8	95.9	303	1213	2989	5181
Intra-set Statistics						
n	4	4	4	4	4	4
Mean	30.9	97.8	291	1056	3015	4910
SD	4.2	7.6	18	119	167	202
RSD	14	7.8	6.2	11	5.5	4.1
%RE	2.9	-2.2	-3.0	5.6	0.49	-1.8

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 4: PFHxA Concentrations And Intra-Session And Inter-Session Statistics Of Quality Control Samples In Monkey Serum With IS

Theo. Conc. (ng/mL)	**30**	**300**	**5000**
Set 1 (3/30/05), 534002(PFHxA)CMS, analyst KLK			
Samp 1	35.8	282	4334
Samp 2	31.0	290	4218
Samp 3	32.4	290	4605
Mean	33.1	287	4386
SD	2.4	5.0	198
RSD	7.4	1.7	4.5
%RE	10	-4.2	-12
Set 2 (3/30/05), 534002(PFHxA)DMS1, analyst KLK			
Samp 1	35.2	261	3862
Samp 2	34.7	264	4381
Samp 3	33.9	248	4401
Mean	34.6	258	4215
SD	0.69	8.4	305
RSD	2.0	3.2	7.2
%RE	15	-14	-16
Set 3 (3/31/05), 534002(PFHxA)EMS, analyst LEB2, DKP			
Samp 1	29.5	279	4798
Samp 2	29.4	260	4935
Samp 3	29.5	269	4997
Mean	29.5	269	4910
SD	0.060	9.3	102
RSD	0.20	3.5	2.1
%RE	-1.7	-10	-1.8

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 4: PFHxA Concentrations And Intra-Session And Inter-Session Statistics Of Quality Control Samples In Monkey Serum With IS

Theo. Conc. (ng/mL)	**30**	**300**	**5000**
Set 4 (3/31/05), 534002(PFHxA)FMS, analyst LEB2, DKP			
Samp 1	20.5	258	3858
Samp 2	22.5	289	4358
Samp 3	28.9	311	5733
Mean	24.0	286	4650
SD	4.4	26	971
RSD	18	9.2	21
%RE	-20	-4.7	-7.0
Set 5 (4/5/05), 534002(PFHxA)GFMS, analyst KLK			
Samp 1	32.4	301	4851
Samp 2	34.7	279	5588
Samp 3	31.8	324	5274
Mean	33.0	301	5237
SD	1.5	22	370
RSD	4.6	7.5	7.1
%RE	9.9	0.42	4.7
Interset Statistics			
n	15	15	15
Mean	30.8	280	4680
SD	4.4	21	564
RSD	14	7.5	12
%RE	2.7	-6.6	-6.4

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 5: PFHxA Concentrations And Intra-Session Statistics Of Quality Control Samples In Monkey Serum With No IS

Theo. Conc. (ng/mL)	**30**	**300**	**5000**
Set 1 (3/30/05), 534002(PFHxA)HMS, analyst KLK			
Samp 1	27.4	297	4466
Samp 2	25.8	273	4857
Samp 3	26.5	297	4932
Intra-set Statistics			
n	3	3	3
Mean	27	289	4752
SD	0.84	14	250
RSD	3.1	4.9	5.3
%RE	-11	-3.7	-5.0

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 6: PFHxA Concentrations And Intra-Session Statistics Of Quality Control Samples In Monkey Urine

Theo. Conc. (ng/mL)	**30**	**300**	**5000**	**10000**
Set 1 (4/18/05), 534002(PFHxA)KMU1, KMU2, analyst KLK				
Samp 1	*67.9	309	4245	11091
Samp 2	33.2	258	4276	10450
Samp 2, re-injection	32.1	275	4593	12306
Samp 3	35.0	304	5239	11042
Intra-set Statistics				
n	4	4	4	4
Mean	33.4	287	4588	11222
SD	1.5	24	461	779
RSD	4.4	8.4	10	6.9
%RE	11	-4.5	-8.2	12

* Data point not included in summary of statistics based on test for outliers.

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 7: 5-Day Refrigerated Stability Analysis Of Processed Monkey Serum PFHxA Samples

Time Point	Date Analyzed	Theo. Conc (ng/mL)	Ref # (534002 -)	Run #	Area	Percent of Time Zero (%)
Calibration Standards						
T = 0	3/31/2005	30.0	109 - 7	I1-485	67789	
5-Day	4/5/2005		109 - 7	I1-559	65286	96.3
T = 0	3/31/2005	100	109 - 10	I1-488	174045	
5-Day	4/5/2005		109 - 10	I1-560	148588	85.4
T = 0	3/31/2005	300	109 - 13	I1-491	435922	
5-Day	4/5/2005		109 - 13	I1-561	421700	96.7
T = 0	3/31/2005	1000	109 - 16	I1-494	853168	
5-Day	4/5/2005		109 - 16	I1-562	902427	106
T = 0	3/31/2005	3000	109 - 19	I1-497	2883927	
5-Day	4/5/2005		109 - 19	I1-563	2963086	103
T = 0	3/31/2005	5000	109 - 22	I1-500	4042899	
5-Day	4/5/2005		109 - 22	I1-564	4226770	105

Time Point	Date Analyzed	Theo. Conc (ng/mL)	Ref # (534002 -)	Run #	Area	Percent of Time Zero (%)
Quality Control Samples						
T = 0	3/31/2005	30.0	110 - 1	I1-505	53339	
5-Day	4/5/2005		110 - 1	I1-565	73254	137
T = 0	3/31/2005	300	110 - 4	I1-508	367394	
5-Day	4/5/2005		110 - 4	I1-566	400553	109
T = 0	3/31/2005	5000	110 - 7	I1-511	3173879	
5-Day	4/5/2005		110 - 7	I1-567	4009309	126

534002 PFHxA bio tables.xls 5-day pss
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A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 8: 1-Day Refrigerated Stability Analysis Of Processed Rat Urine PFHxA Samples

Time Point	Date Analyzed	Theo. Conc (ng/mL)	Ref # (534002 -)	Run #	Area	Mean Area	Percent of Time Zero (%)
Quality Control Samples							
T = 0	7/7/2005	300	242 - 1	I1-1765	1095795	1080275	---
			242 - 2	I1-1766	1101517		
			242 - 3	I1-1767	1043513		
1-Day	7/8/2005		242 - 1	I1-1765a	1086927	1038631	96.1
			242 - 2	I1-1766a	1018952		
			242 - 3	I1-1767a	1010015		
T = 0	7/7/2005	5000	242 - 4	I1-1768	7124059	7223925	---
			242 - 5	I1-1769	6736695		
			242 - 6	I1-1770	7811020		
1-Day	7/8/2005		242 - 4	I1-1768a	8638501	8666203	120
			242 - 5	I1-1769a	8993384		
			242 - 6	I1-1770a	8366723		

534002 PFHxA bio tables.xls PSS(PFBS) MU
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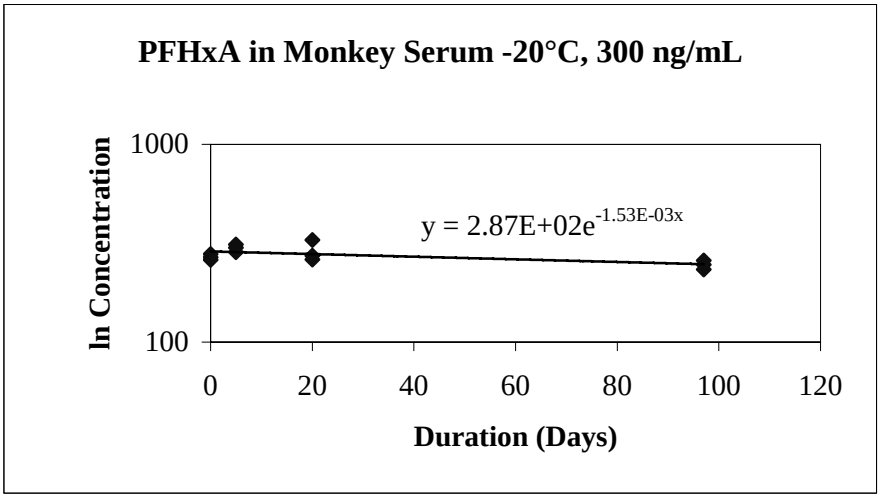
A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 9: Stability of PFHxA in Monkey Serum - Long-Term Frozen Storage at -20°C

Theo. Conc. (ng/mL)	Storage Duration (Days)	Run# (534002-)	Assay Conc. (ng/mL)	Mean Conc. (ng/mL)	k
300	0	I1-467	279	269	-1.53E-03
	0	I1-468	260		
	0	I1-469	269		
	5	I1-613	312	299	
	5	I1-614	285		
	5	I1-615	300		
	20	I1-1037	262	288	
	20	I1-1038	274		
	20	I1-1039	328		
	97	I1-1720a	259	246	
	97	I1-1721a	233		
	97	I1-1722a	246		



534002 PFHxA bio tables.xls LT (PFHxA) MS
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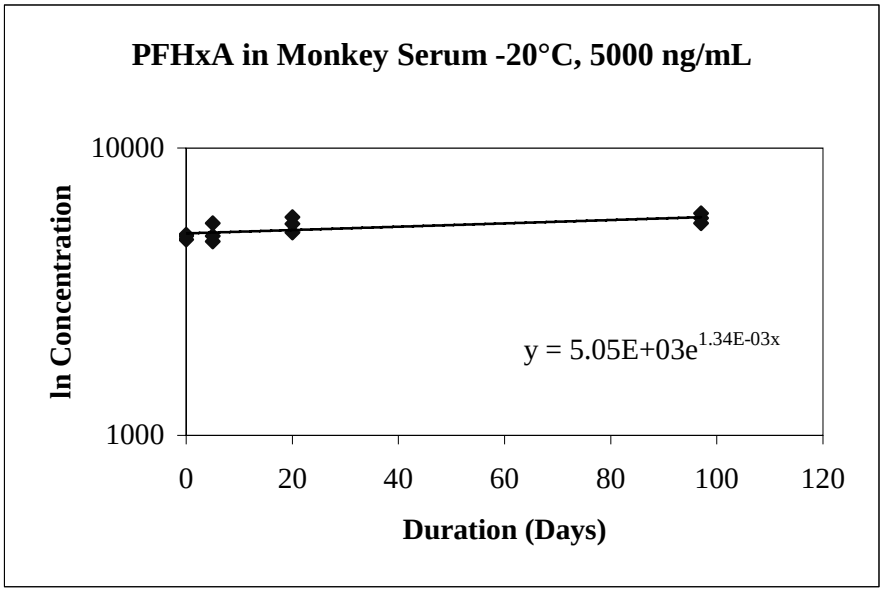
A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 9: Stability of PFHxA in Monkey Serum - Long-Term Frozen Storage at -20°C

Theo. Conc. (ng/mL)	Storage Duration (Days)	Run# (534002-)	Assay Conc. (ng/mL)	Mean Conc. (ng/mL)	k
5000	0	I1-470	4798	4910	1.34E-03
	0	I1-471	4935		
	0	I1-472	4997		
	5	I1-617	5468	5047	
	5	I1-618	4939		
	5	I1-619a	4733		
	20	I1-1028	5080	5425	
	20	I1-1029	5746		
	20	I1-1030	5449		
	97	I1-1728a	5468	5697	
	97	I1-1729a	5699		
	97	I1-1730a	5924		



534002 PFHxA bio tables.xls LT (PFHxA) MS
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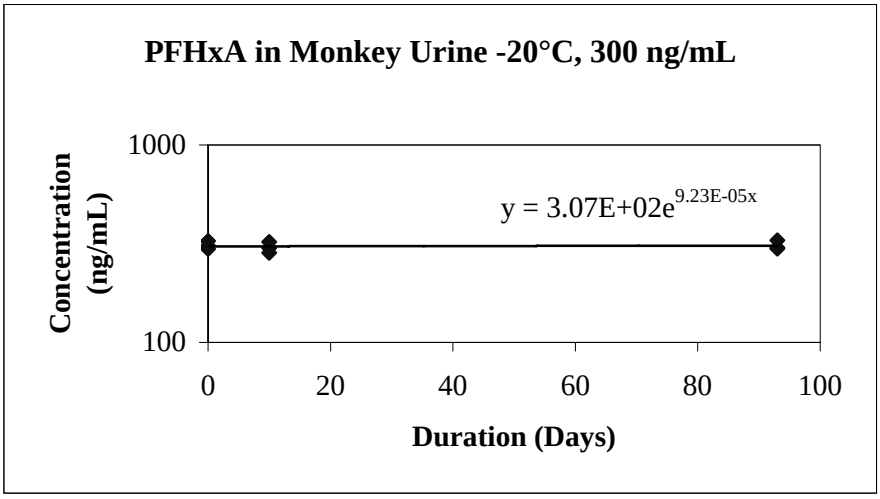
A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 10: Stability of PFHxA in Monkey Urine - Long-Term Frozen Storage at -20°C

Theo. Conc. (ng/mL)	Storage Duration (Days)	Run# (534002-)	Assay Conc. (ng/mL)	Mean Conc. (ng/mL)	k
300	0	I1-653	309	311	9.23E-05
	0	I1-654	327		
	0	I1-655	297		
	10	I1-955	301	303	
	10	I1-956	323		
	10	I1-957	284		
	93	I1-1765a	329	310	
	93	I1-1766a	302		
	93	I1-1767a	299		



534002 PFHxA bio tables.xls LT (PFHxA) MU
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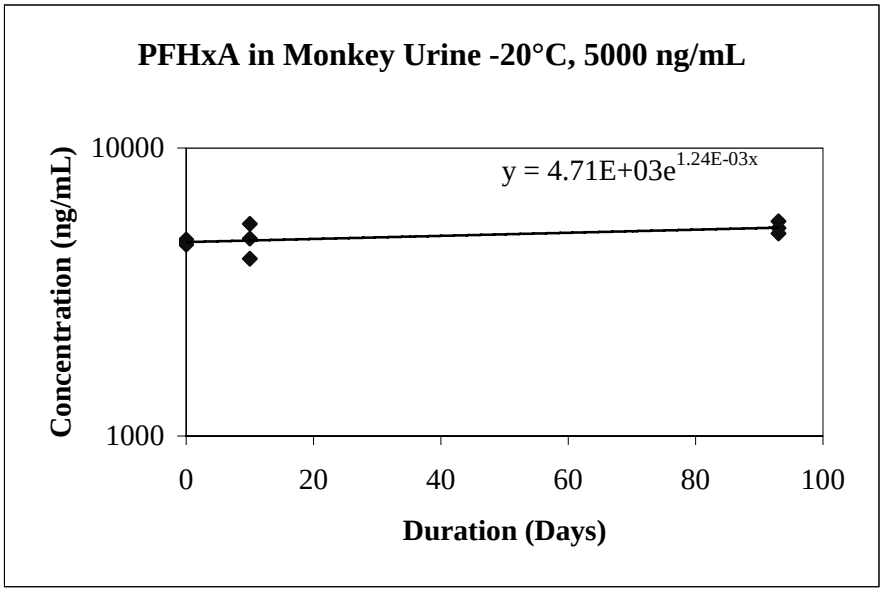
A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 10: Stability of PFHxA in Monkey Urine - Long-Term Frozen Storage at -20°C

Theo. Conc. (ng/mL)	Storage Duration (Days)	Run# (534002-)	Assay Conc. (ng/mL)	Mean Conc. (ng/mL)	k
5000	0	I1-645	4696	4710	1.24E-03
	0	I1-646	4618		
	0	I1-647	4816		
	10	I1-964	5454	4810	
	10	I1-965	4129		
	10	I1-966	4847		
	93	I1-1768a	5271	5295	
	93	I1-1769a	5566		
	93	I1-1770a	5047		



534002 PFHxA bio tables.xls LT (PFHxA) MU
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A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 11: 4-Hour Room Temperature Stability Of PFHxA In Monkey Serum

Theo. Conc. (ng/mL)	Time hr (hr)	Run # (534002-)	Analyzed Conc (ng/mL)	% Target (%)	Mean Conc (ng/mL)	RSD (%)	Mean % Target (%)	% of Time Zero (%)
300	0	I1-1720a	259	86.2	246	5.2	82.0	***
		I1-1721a	233	77.7				
		I1-1722a	246	82.0				
	4	I1-1731a	225	74.8	232	2.9	77.3	94.2
		I1-1732a	233	77.6				
		I1-1733a	238	79.3				
5000	0	I1-1728a	5468	109	5697	4.0	114	***
		I1-1729a	5699	114				
		I1-1730a	5924	118				
	4	I1-1734a	4755	95.1	4861	2.1	97.2	85.3
		I1-1735a	4956	99.1				
		I1-1736a	4871	97.4				

534002 PFHxA bio tables.xls 4hr RTS (PFHxA) MS
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A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 12: 4-Hour Room Temperature Stability Of PFHxA In Monkey Urine

Theo. Conc. (ng/mL)	Time hr (hr)	Run # (534002-)	Analyzed Conc (ng/mL)	% Target (%)	Mean Conc (ng/mL)	RSD (%)	Mean % Target (%)	% of Time Zero (%)
300	0	I1-1765a	329	110	310	5.4	103	***
		I1-1766a	302	101				
		I1-1767a	299	99.6				
	4	I1-1776a	262	87.3	262	5.9	87.5	84.6
		I1-1777a	247	82.4				
		I1-1778a	278	92.6				
5000	0	I1-1768a	5271	105	5295	4.9	106	***
		I1-1769a	5566	111				
		I1-1770a	5047	101				
	4	I1-1779a	4351	87.0	4375	0.5	87.5	82.6
		I1-1780a	4389	87.8				
		I1-1781a	4384	87.7				

534002 PFHxA bio tables.xls 4hr RTS (PFHxA) MU
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A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 13: Freeze-Thaw Stability of PFHxA in Monkey Serum

Theo. Conc. (ng/mL)	# of Cycles	Run # (534002-)	Analyzed Conc (ng/mL)	% Target (%)	Mean Conc (ng/mL)	RSD (%)	Mean % Target (%)	% of Time Zero (%)
Monkey Serum								
300	0	I1-467	279	93.0	269	3.5	89.8	na
		I1-468	260	86.8				
		I1-469	269	89.6				
	1	I1-1711a	246	82.1	249	6.3	83.0	92.5
		I1-1712a	235	78.4				
		I1-1713a	266	88.6				
	3	I1-1714a	216	72.1	225	10.2	75.1	83.7
		I1-1715a	241	80.2				
		I1-1716a	216	72.1				
		I1-1717a	224	74.6				
		I1-1718a	194	64.7				
		I1-1719a	261	86.8				
5000	0	I1-470	4798	96.0	4910	2.1	98.2	na
		I1-471	4935	98.7				
		I1-472	4997	100				
	1	I1-1028	5080	102	5425	6.2	109	110
		I1-1029	5746	115				
		I1-1030	5449	109				
	2	I1-1031	4853	97	4982	9.6	100	101
		I1-1032	4579	91.6				
		I1-1033	5514	110				
	3	I1-1034	4588	91.8	4807	4.3	96.1	97.9
		I1-1035	4836	96.7				
		I1-1036	4998	100				

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 14: Freeze-Thaw Stability of PFHxA in Monkey Urine

Theo. Conc. (ng/mL)	# of Cycles	Run # (534002-)	Analyzed Conc (ng/mL)	% Target (%)	Mean Conc (ng/mL)	RSD (%)	Mean % Target (%)	% of Time Zero (%)
Monkey Urine								
300	0	I1-653	309	103	311	4.7	104	na
		I1-654	327	109				
		I1-655	297	99.1				
	1	I1-955	300	100	302	6.3	101	97.2
		I1-956	322	107				
		I1-957	284	94.7				
	2	I1-958	324	108	314	3.4	105	101
		I1-959	303	101				
		I1-960	315	105				
	3	I1-961	341	114	328	4.0	109	106
		I1-962	329	110				
		I1-963	315	105				
5000	0	I1-645	4696	93.9	4710	2.1	94.2	na
		I1-646	4618	92.4				
		I1-647	4816	96.3				
	1	I1-964	5454	109	4810	14	96.2	102
		I1-965	4129	82.6				
		I1-966	4847	96.9				
	2	I1-967	4891	97.8	4958	1.2	99.2	105
		I1-968	5002	100				
		I1-969	4980	99.6				
	3	I1-970	5000	100	4893	2.2	97.9	104
		I1-971	4788	95.8				
		I1-972	4892	97.8				

534002 PFHxA bio tables.xls FT (PFHxA) MU
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A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 15: Monkey Serum Experimental Sample PFHxA Concentrations

Run #	Ref #	Animal #	Sex	Timept. (hrs)	PFHxA ng/mL
I1-711a	129-1	1555T1	M	0	ND
I1-1045	147-1	1555T1	M	1	18004
I1-723	129-13	1555T1	M	2	5106
I1-729	129-19	1555T1	M	4	1414
I1-1058	147-14	1555T1	M	8	972
I1-1064	147-20	1555T1	M	24	108
I1-1070	147-26	1555T1	M	48	< LLOQ
I1-712a	129-2	1562T1	M	0	ND
I1-1046	147-2	1562T1	M	1	25993
I1-1051	147-7	1562T1	M	2	11419
I1-730	129-20	1562T1	M	4	4235
I1-1059	147-15	1562T1	M	8	1902
I1-1065	147-21	1562T1	M	24	32.8
I1-1071	147-27	1562T1	M	48	< LLOQ
I1-713a	129-3	1563T1	M	0	ND
I1-1047	147-3	1563T1	M	1	21259
I1-1052	147-8	1563T1	M	2	8866
I1-731	129-21	1563T1	M	4	2079
I1-1060	147-16	1563T1	M	8	567
I1-1066	147-22	1563T1	M	24	30.3
I1-1072	147-28	1563T1	M	48	35.5
I1-714a	129-4	1572T1	F	0	ND
I1-1048	147-4	1572T1	F	1	25140
I1-1053	147-9	1572T1	F	2	7447
I1-1055	147-11	1572T1	F	4	2332
I1-1061	147-17	1572T1	F	8	341
I1-1067	147-23	1572T1	F	24	< LLOQ
I1-1073	147-29	1572T1	F	48	< LLOQ
I1-716a	129-6	1573T1	F	0	ND
I1-1050	147-6	1573T1	F	1	17469
I1-728	129-18	1573T1	F	2	5086
I1-1057	147-13	1573T1	F	4	2006
I1-1063	147-19	1573T1	F	8	272
I1-1069	147-25	1573T1	F	24	< LLOQ
I1-1075	148-1	1573T1	F	48	< LLOQ
I1-715a	129-5	1576T1	F	0	ND
I1-1049	147-5	1576T1	F	1	20004
I1-1054	147-10	1576T1	F	2	7898
I1-1056	147-12	1576T1	F	4	3078
I1-1062	147-18	1576T1	F	8	438
I1-1068	147-24	1576T1	F	24	84.3
I1-1074	147-30	1576T1	F	48	< LLOQ

< LLOQ = less than the lower limit of quantitation (30 ng/mL).

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 16: Monkey Urine Experimental Sample PFHxA Concentrations

Run #	Ref #	Animal #	Sex	Timept.	Day	PFHxA	Urine Vol	PFHxA
	(534002-)			(hrs)		ng/mL	(mL)	(µg)
I1-955a	142-1	1555T1	M	0-6	0	538105	8	4305
I1-960a	142-4	1555T1	M	6-12	0	22638	10	226
I1-964a	142-7	1555T1	M	12-24	0	6987	39	273
I1-968a	136-14	1555T1	M	---	2	2113	65	137
I1-971a	136-17	1555T1	M	---	3	497	73	36.3
I1-976a	136-22	1555T1	M	---	4	751	36	27.1
I1-982a	136-28	1555T1	M	---	5	759	11	8.35
I1-974	137-2	1555T1	M	---	6	324	35	11.3
I1-980	137-8	1555T1	M	---	7	117	30	3.51
I1-956a	142-2	1562T1	M	0-6	0	514407	5	2572
		1562T1	M	6-12	0	---	---	ISV
		1562T1	M	12-24	0	---	---	ISV
		1562T1	M	---	2	---	---	ISV
		1562T1	M	---	3	---	---	ISV
I1-977a	136-23	1562T1	M	---	4	734	13	9.5
		1562T1	M	---	5	---	---	ISV
I1-975	137-3	1562T1	M	---	6	521	1	0.521
		1562T1	M	---	7	---	---	ISV
I1-957a	136-3	1563T1	M	0-6	0	148	8	1.19
I1-961a	142-5	1563T1	M	6-12	0	10098	15	151
I1-965a	136-11	1563T1	M	12-24	0	3497	95	332
I1-969a	136-15	1563T1	M	---	2	2324	106	246
I1-972a	136-18	1563T1	M	---	3	444	180	79.9
I1-978a	136-24	1563T1	M	---	4	1521	47	71.5
I1-983a	136-29	1563T1	M	---	5	698	50	34.9
I1-976	137-4	1563T1	M	---	6	231	82	19.0
I1-981	137-9	1563T1	M	---	7	74.7	57	4.26

ISV = insufficient sample volume to analyze.

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 16: Monkey Urine Experimental Sample PFHxA Concentrations

Run #	Ref #	Animal #	Sex	Timept.	Day	PFHxA	Urine Vol	PFHxA
	(534002-)			(hrs)		ng/mL	(mL)	(µg)
I1-958a	142-3	1572T1	F	0-6	0	248101	38	9428
I1-962a	142-6	1572T1	F	6-12	0	10423	6	62.5
I1-966a	136-12	1572T1	F	12-24	0	2297	91	209
I1-970a	136-16	1572T1	F	---	2	564	80	45.1
I1-973a	136-19	1572T1	F	---	3	468	75	35.1
I1-979a	136-25	1572T1	F	---	4	416	51	21.2
I1-984a	136-30	1572T1	F	---	5	259	29	7.51
I1-977	137-5	1572T1	F	---	6	33.2	22	0.730
I1-982	137-10	1572T1	F	---	7	85.7	50	4.28
I1-959a	136-5	1573T1	F	0-6	0	1717	3	5.15
I1-963a	136-9	1573T1	F	6-12	0	1337	5	6.68
I1-967a	142-8	1573T1	F	12-24	0	13526	4	54.1
		1573T1	F	---	2	---	---	ISV
I1-975a	136-21	1573T1	F	---	3	145	24	3.49
I1-981a	136-27	1573T1	F	---	4	587	2	1.17
I1-973	137-1	1573T1	F	---	5	95.8	20	1.92
I1-979	137-7	1573T1	F	---	6	159	32	5.08
I1-984	137-12	1573T1	F	---	7	62.7	32	2.01
		1576T1	F	0-6	0	---	---	ISV
		1576T1	F	6-12	0	---	---	ISV
		1576T1	F	12-24	0	---	---	ISV
		1576T1	F	---	2	---	---	ISV
I1-974a	136-20	1576T1	F	---	3	1591	31	49.3
I1-980a	136-26	1576T1	F	---	4	1437	1	1.44
		1576T1	F	---	5	---	---	ISV
I1-978	137-6	1576T1	F	---	6	190	6	1.14
I1-983	137-11	1576T1	F	---	7	<LLOQ	75	< LLOQ

ISV = insufficient sample volume to analyze.
< LLOQ = less than the lower limit of quantitation (30 ng/mL).

FIGURES 1 - 56

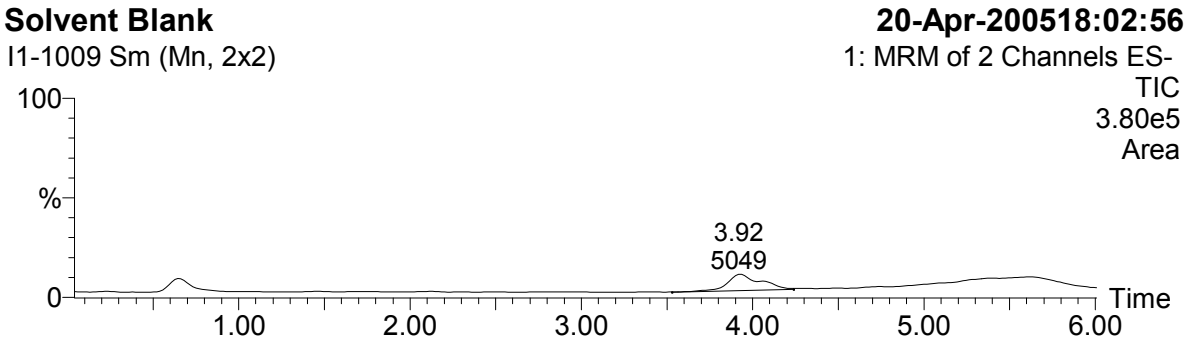

Figure 1: Representative Chromatogram Of A Processed Solvent Blank (Serum Assay)

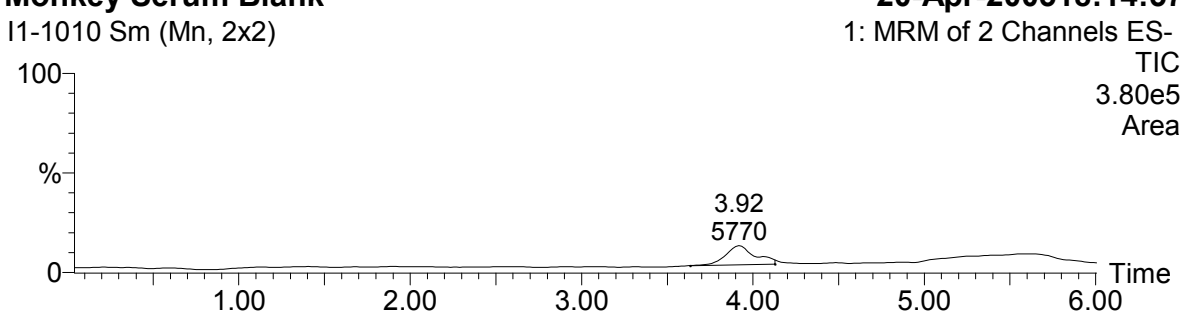

Figure 2: Representative Chromatogram Of A Processed Serum Blank

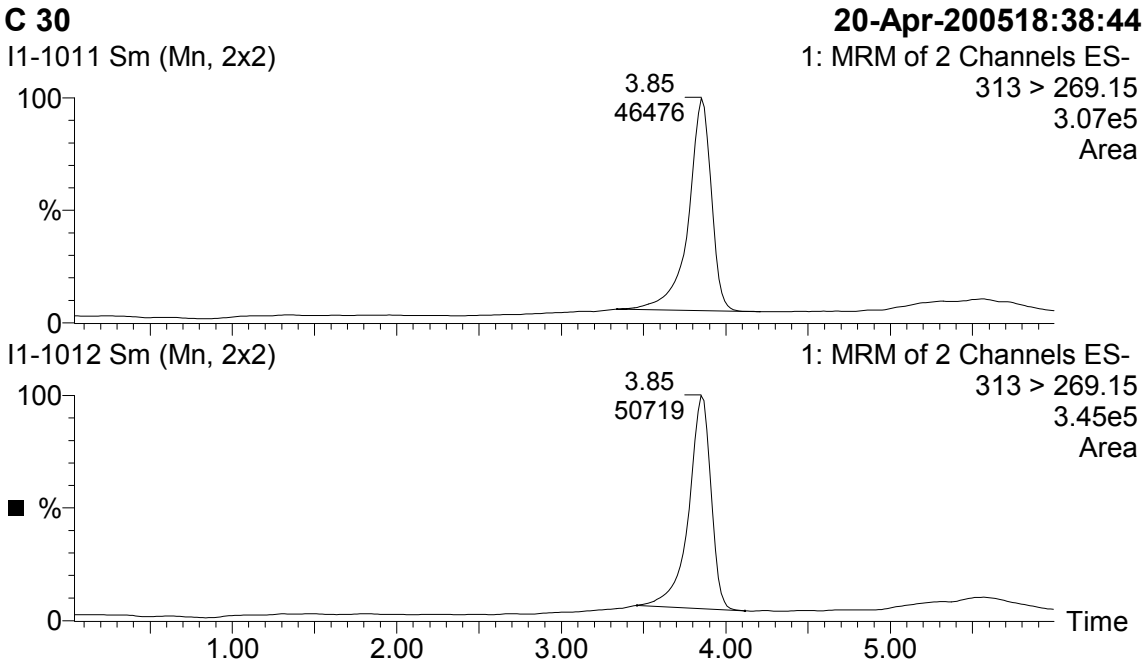

Figure 3: Representative Chromatogram Of 30 ng/mL Serum Calibration Samples

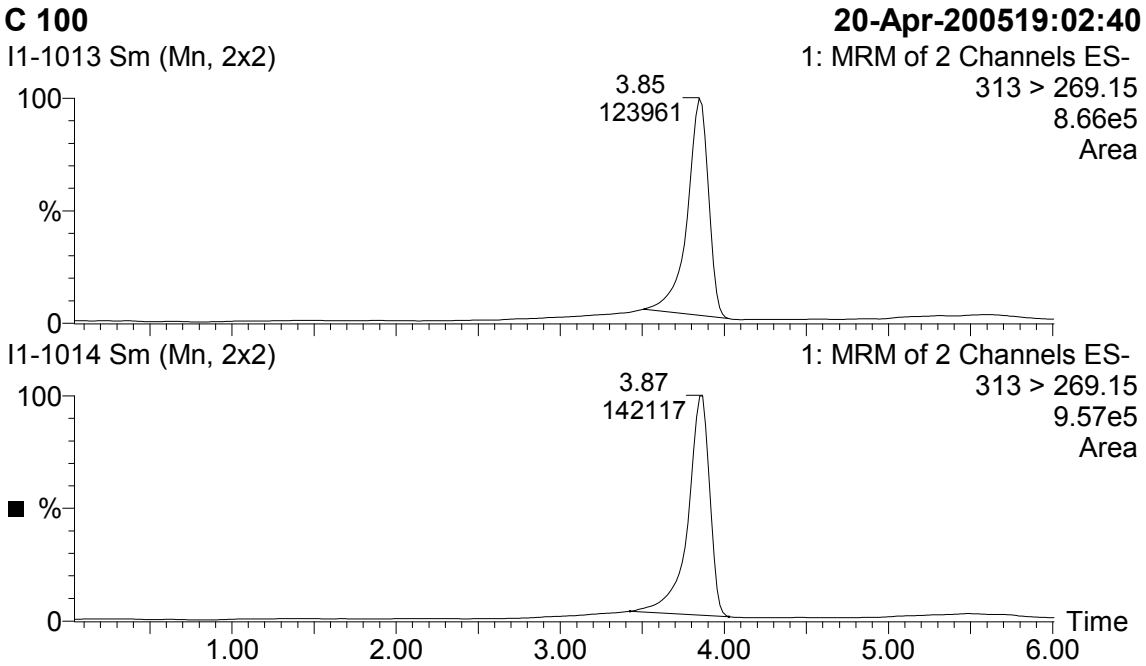

Figure 4: Representative Chromatogram Of 100 ng/mL Serum Calibration Samples

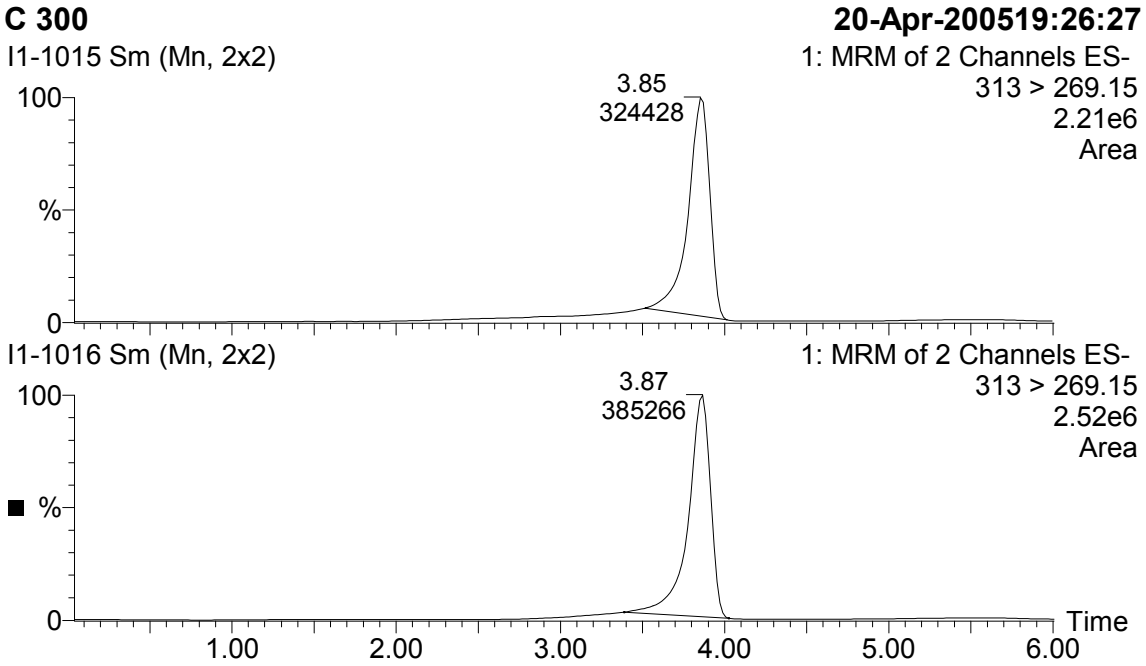

Figure 5: Representative Chromatogram Of 300 ng/mL Serum Calibration Samples

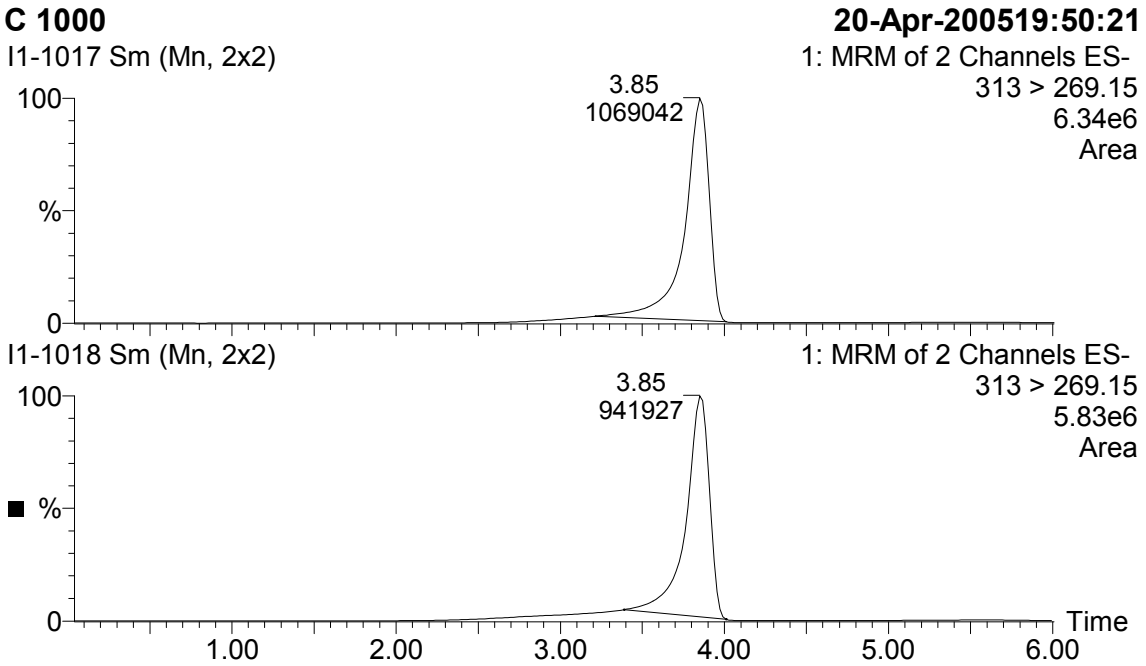

Figure 6: Representative Chromatogram Of 1000 ng/mL Serum Calibration Samples

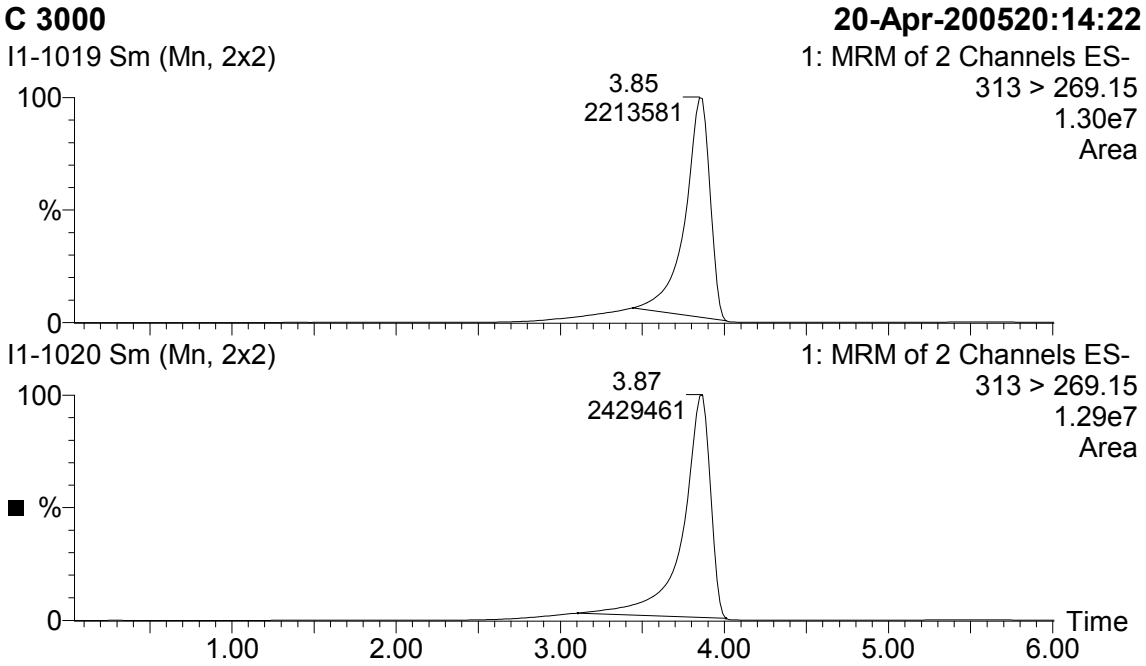

Figure 7: Representative Chromatogram Of 3000 ng/mL Serum Calibration Samples

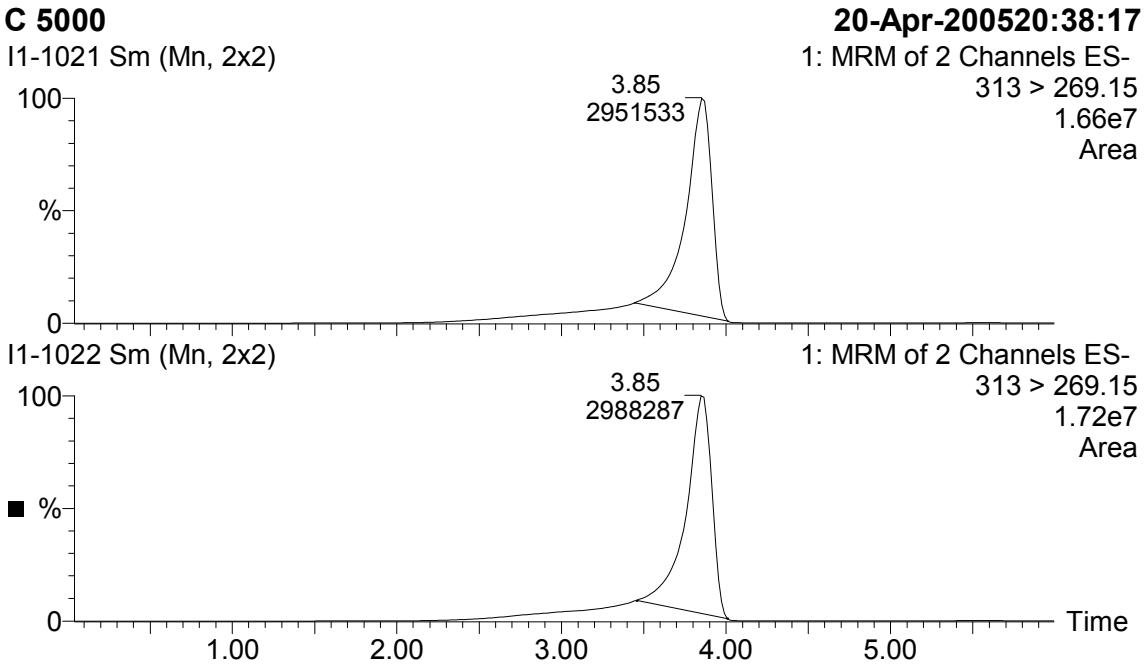

Figure 8: Representative Chromatogram Of 5000 ng/mL Serum Calibration Samples

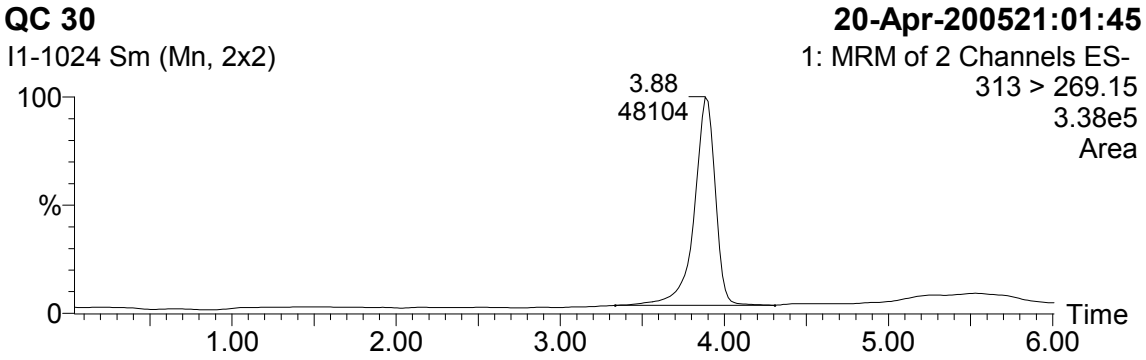

Figure 9: Representative Chromatogram Of A 30 ng/mL Serum Quality Control Sample

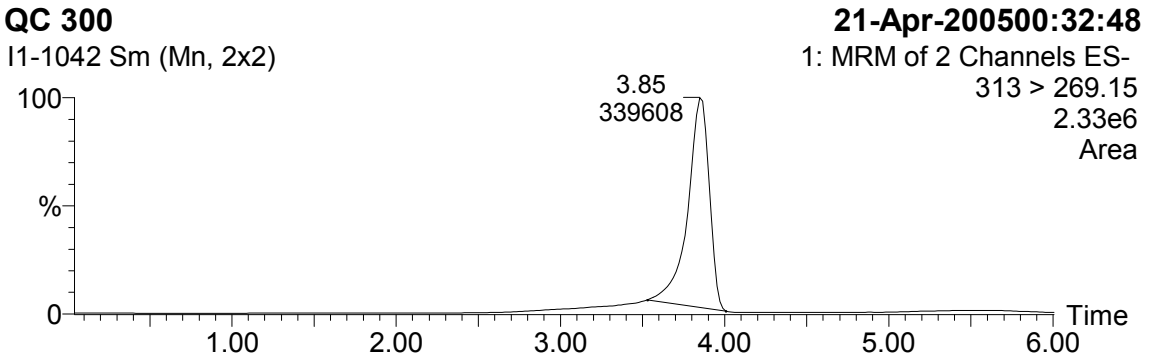

Figure 10: Representative Chromatogram Of A 300 ng/mL Serum Quality Control Sample

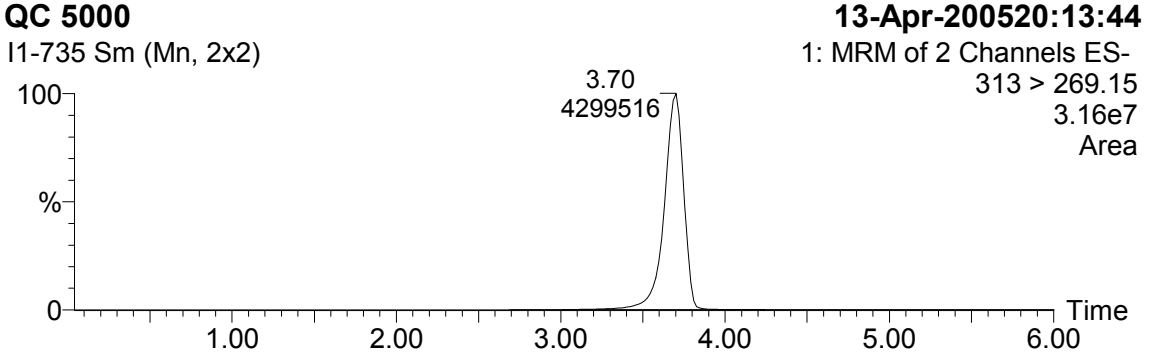

Figure 11: Representative Chromatogram Of A 5000 ng/mL Serum Quality Control Sample

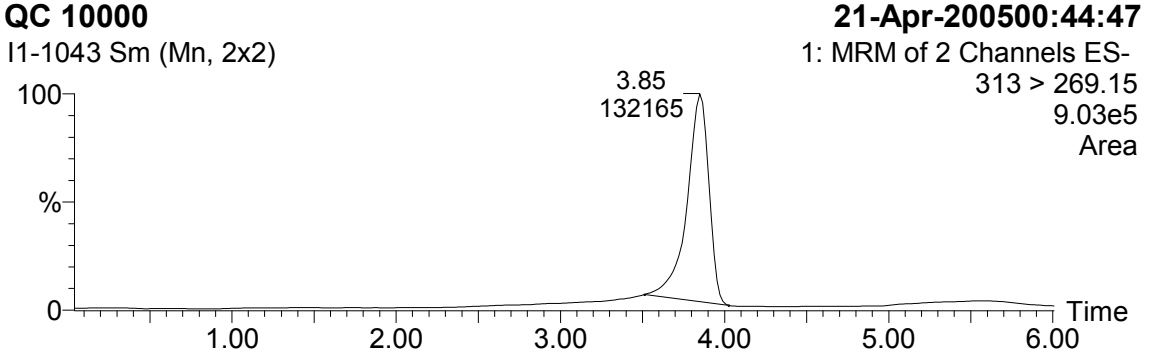

Figure 12: Representative Chromatogram Of A 10,000 ng/mL Serum Quality Control Sample

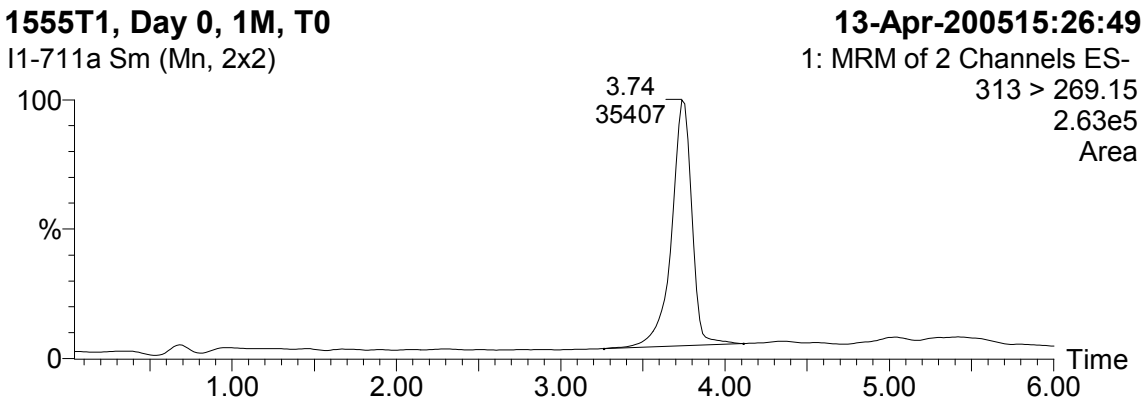

Figure 13: Chromatogram Of Animal No. 1555, Group T1, Male, Pre-Dose Serum Sample

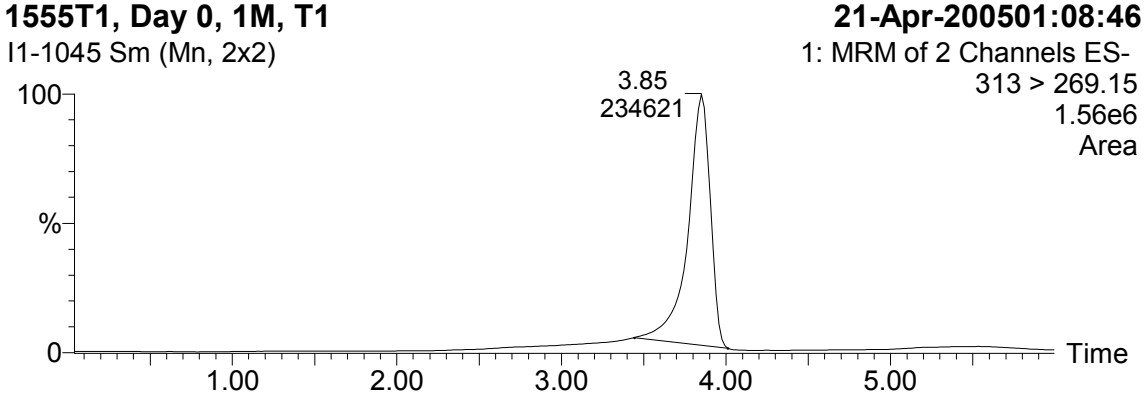

Figure 14: Chromatogram Of Animal No. 1555, Group T1, Male, 1 Hour Post-Dose Serum Sample

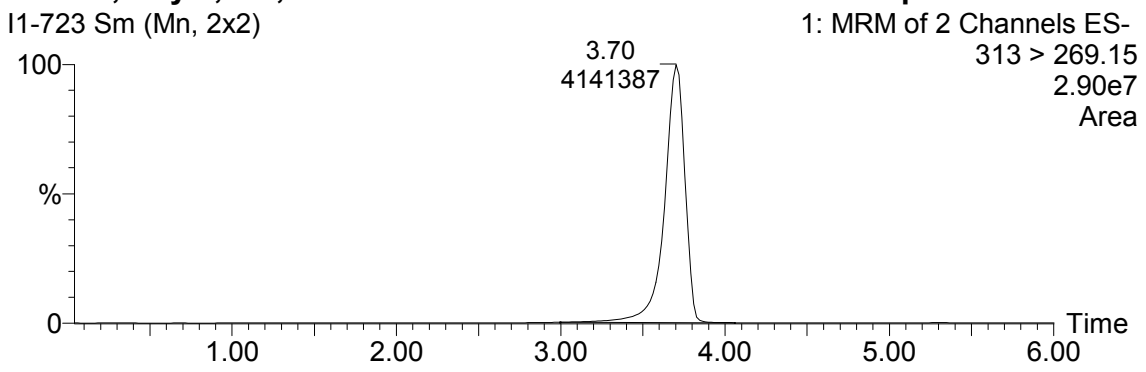

Figure 15: Chromatogram Of Animal No. 1555, Group T1, Male, 2 Hours Post-Dose Serum Sample

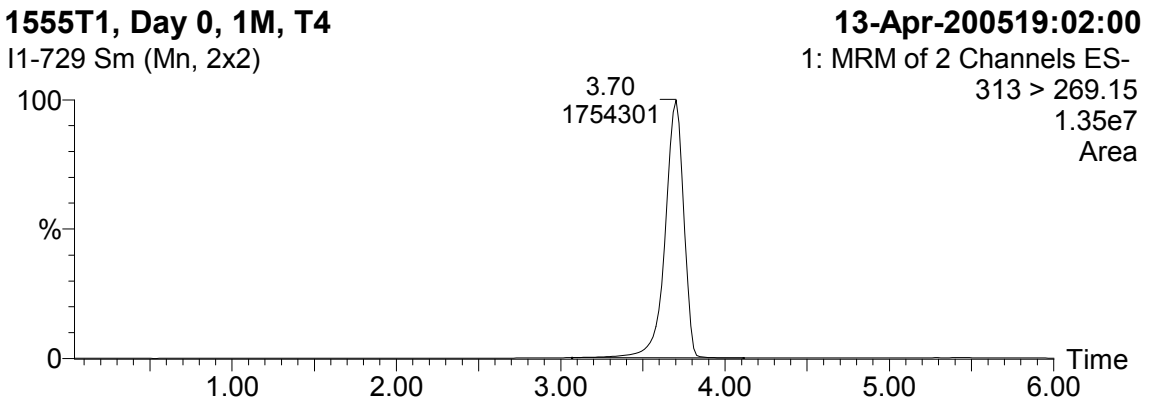

Figure 16: Chromatogram Of Animal No. 1555, Group T1, Male, 4 Hours Post-Dose Serum Sample

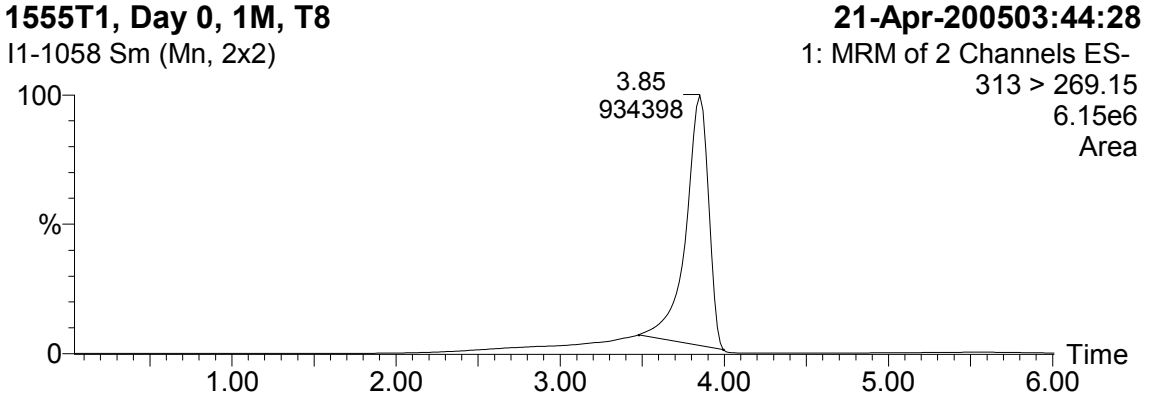

Figure 17: Chromatogram Of Animal No. 1555, Group T1, Male, 8 Hours Post-Dose Serum Sample

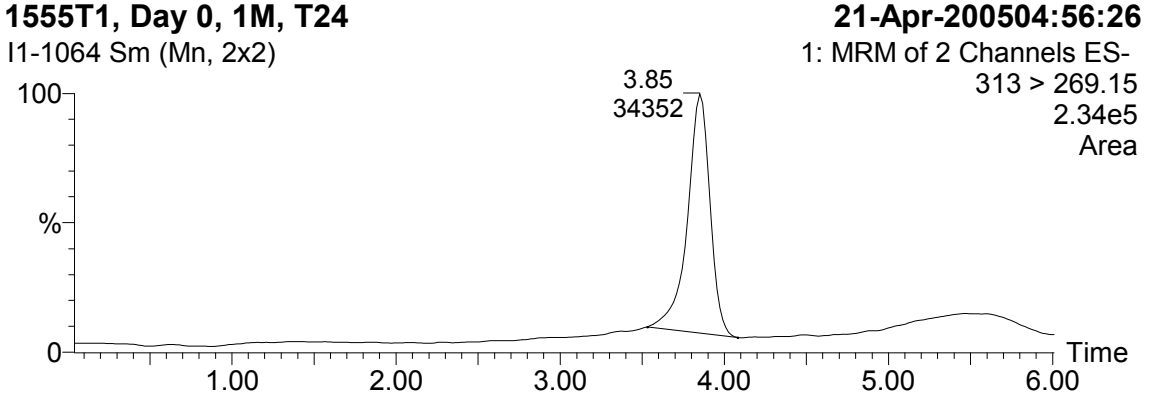

Figure 18: Chromatogram Of Animal No. 1555, Group T1, Male, 24 Hours Post-Dose Serum Sample

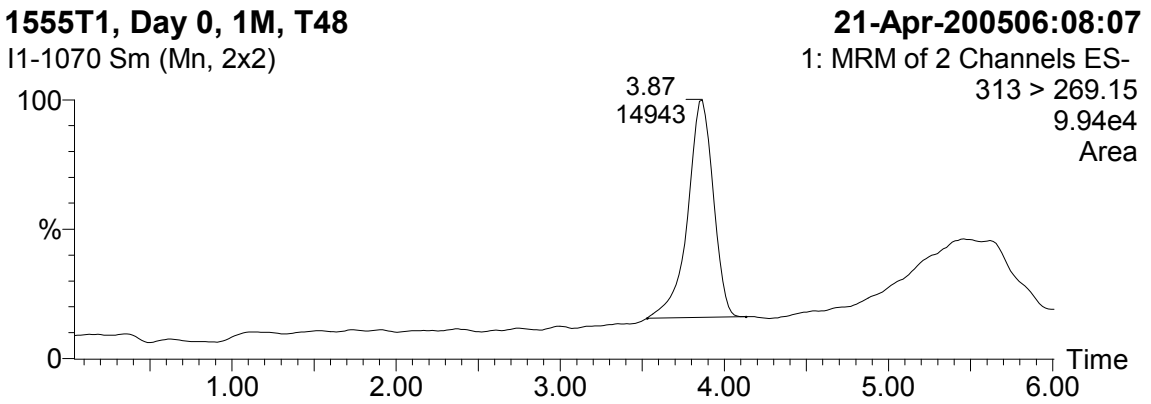

Figure 19: Chromatogram Of Animal No. 1555, Group T1, Male, 48 Hours Post-Dose Serum Sample

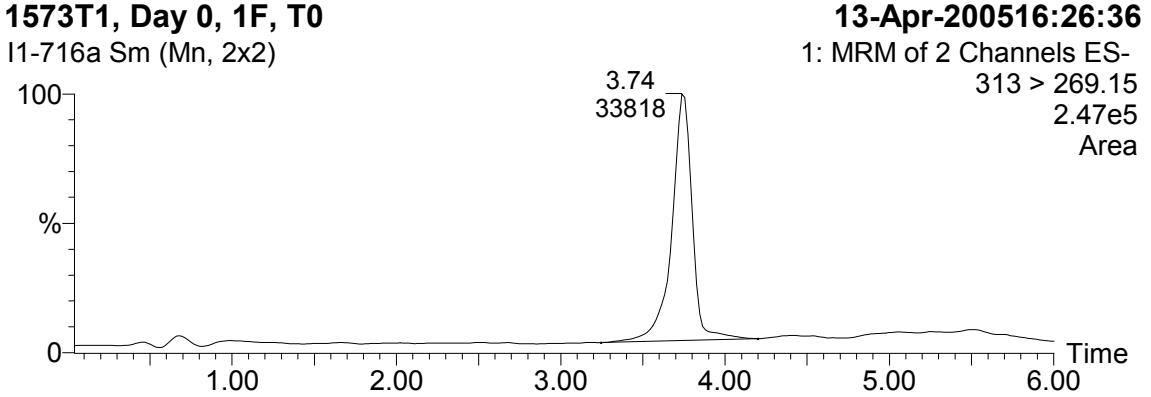

Figure 20: Chromatogram Of Animal No. 1573, Group T1, Female, Pre-Dose Serum Sample

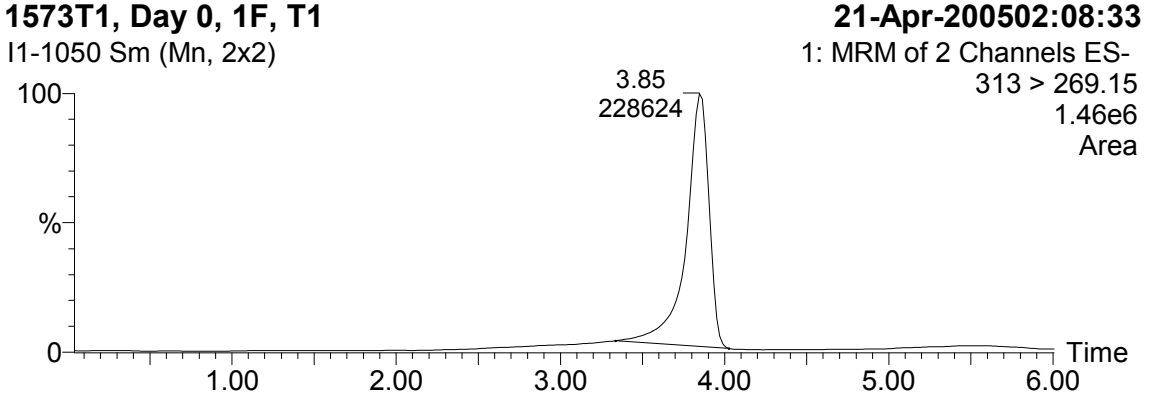

Figure 21: Chromatogram Of Animal No. 1573, Group T1, Female, 1 Hour Post-Dose Serum Sample

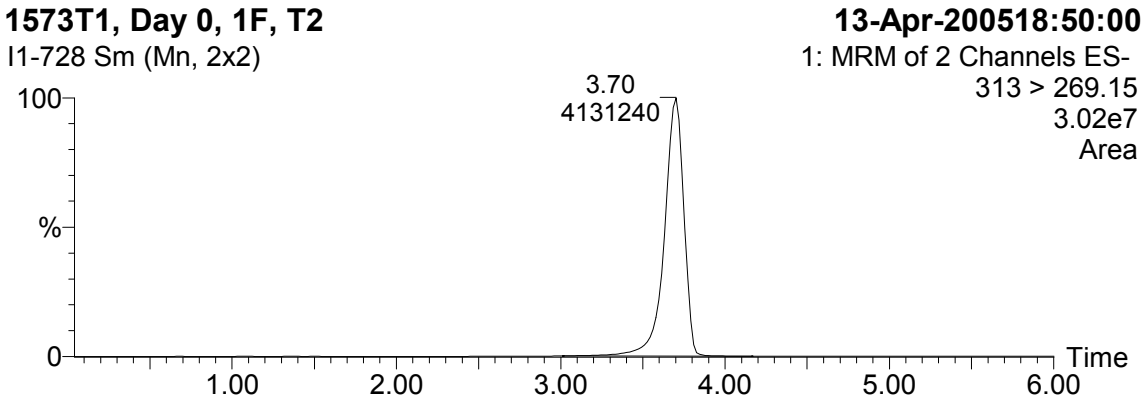

Figure 22: Chromatogram Of Animal No. 1573, Group T1, Female, 2 Hours Post-Dose Serum Sample

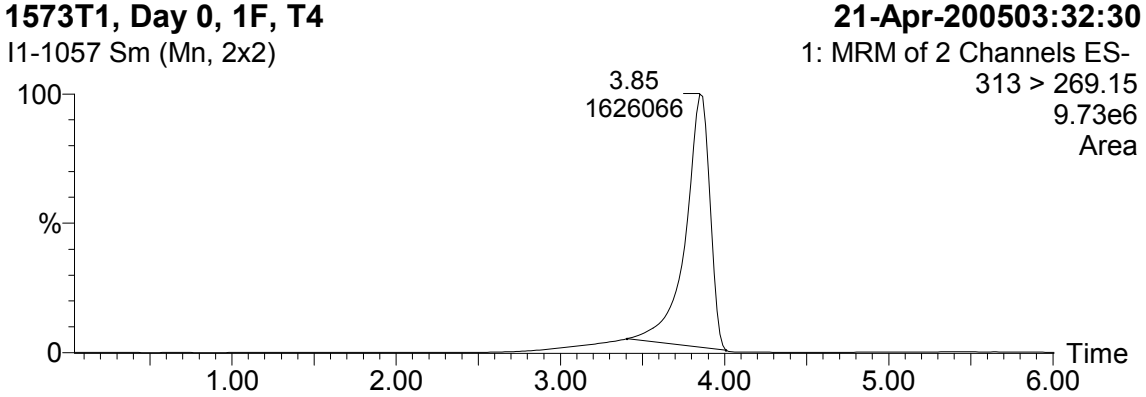

Figure 23: Chromatogram Of Animal No. 1573, Group T1, Female, 4 Hours Post-Dose Serum Sample

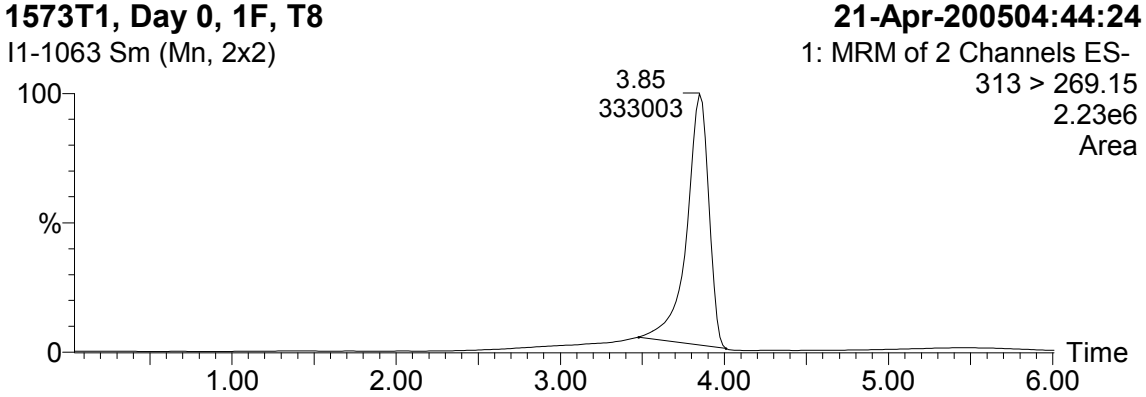

Figure 24: Chromatogram Of Animal No. 1573, Group T1, Female, 8 Hours Post-Dose Serum Sample

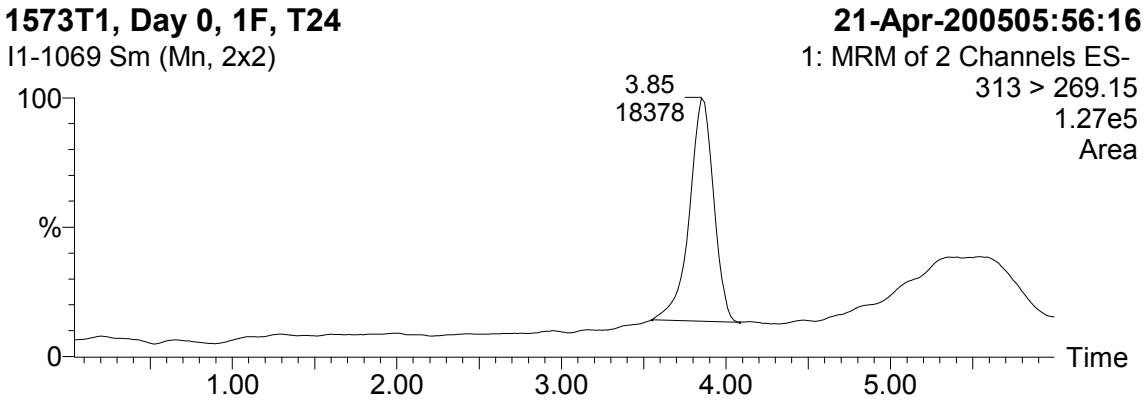

Figure 25: Chromatogram Of Animal No. 1573, Group T1, Female, 24 Hours Post-Dose Serum Sample

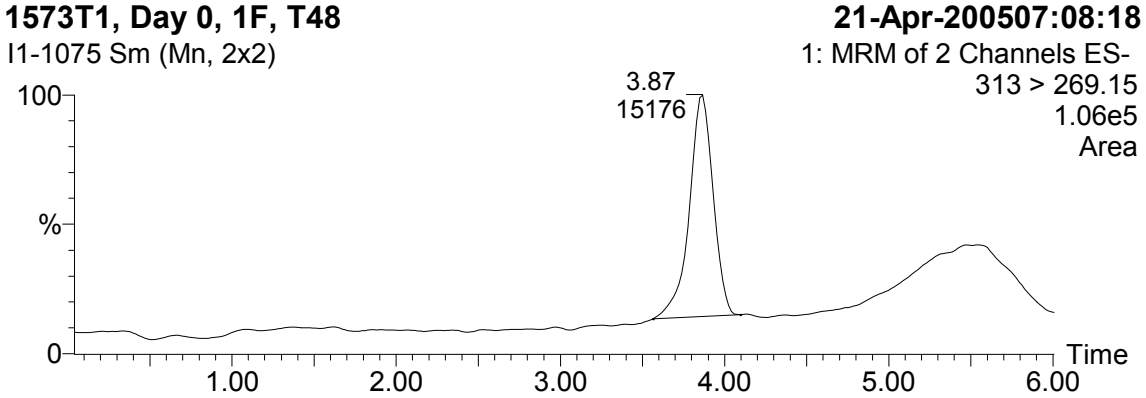

Figure 26: Chromatogram Of Animal No. 1573, Group T1, Female, 48 Hours Post-Dose Serum Sample

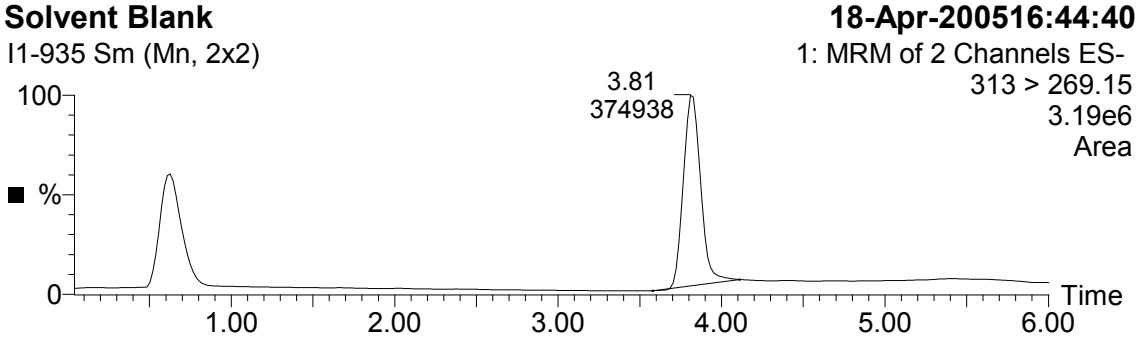

Figure 27: Representative Chromatogram Of A Processed Solvent Blank (Urine Assay)

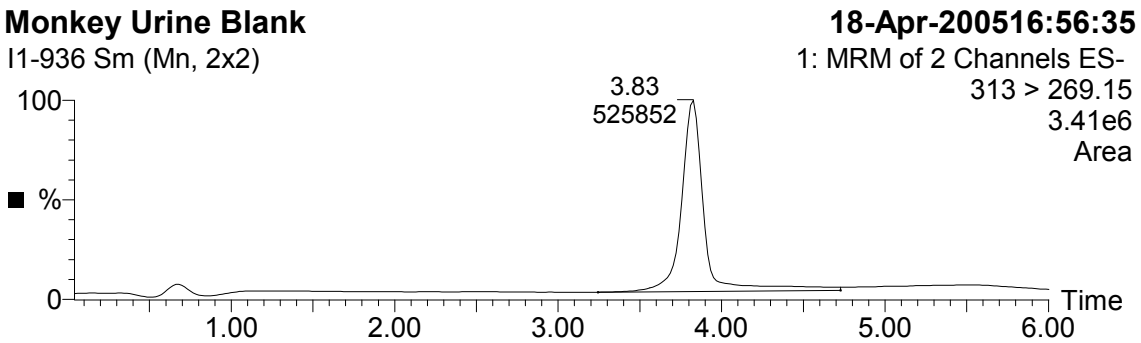

Figure 28: Representative Chromatogram Of A Processed Urine Blank

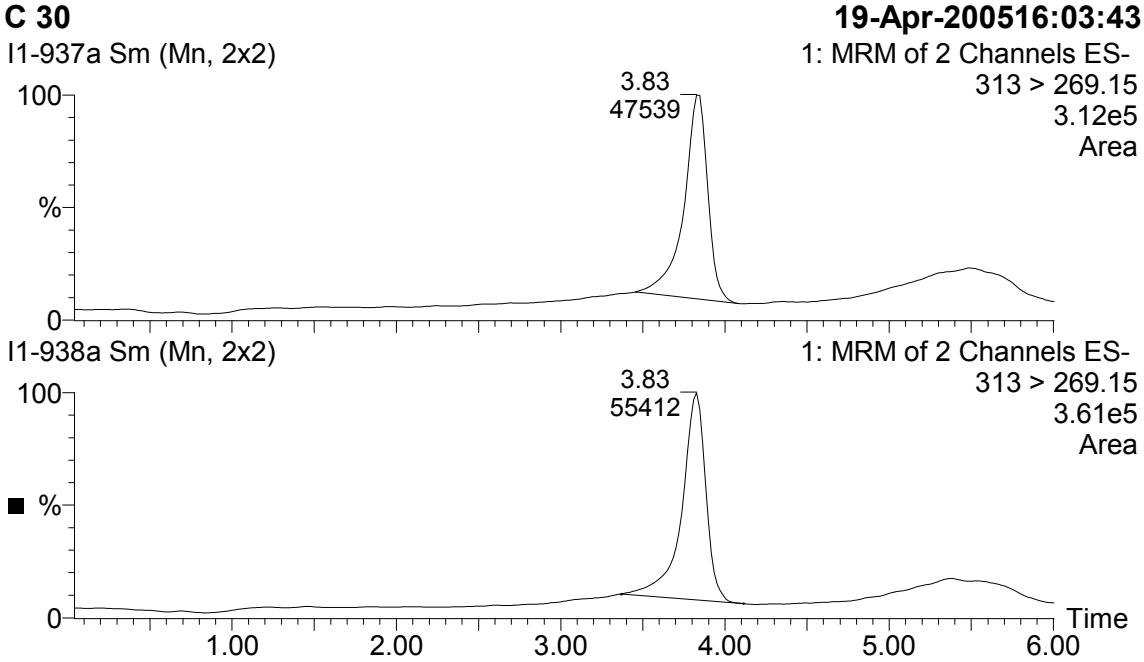

Figure 29: Representative Chromatogram Of 30 ng/mL Urine Calibration Samples

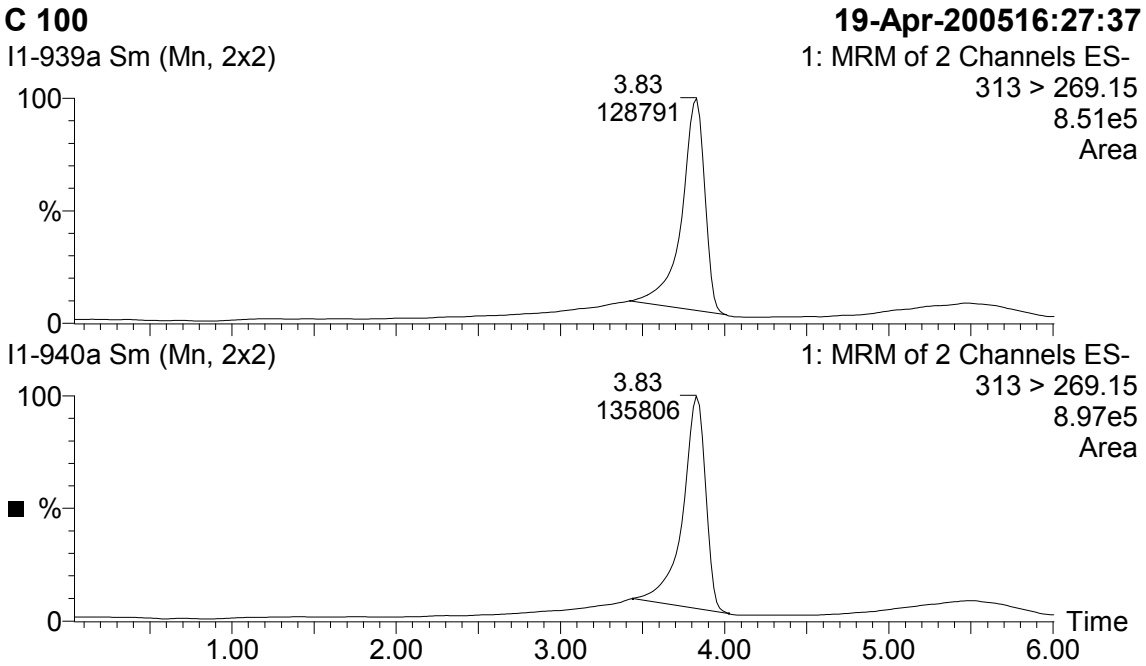

Figure 30: Representative Chromatogram Of 100 ng/mL Urine Calibration Samples

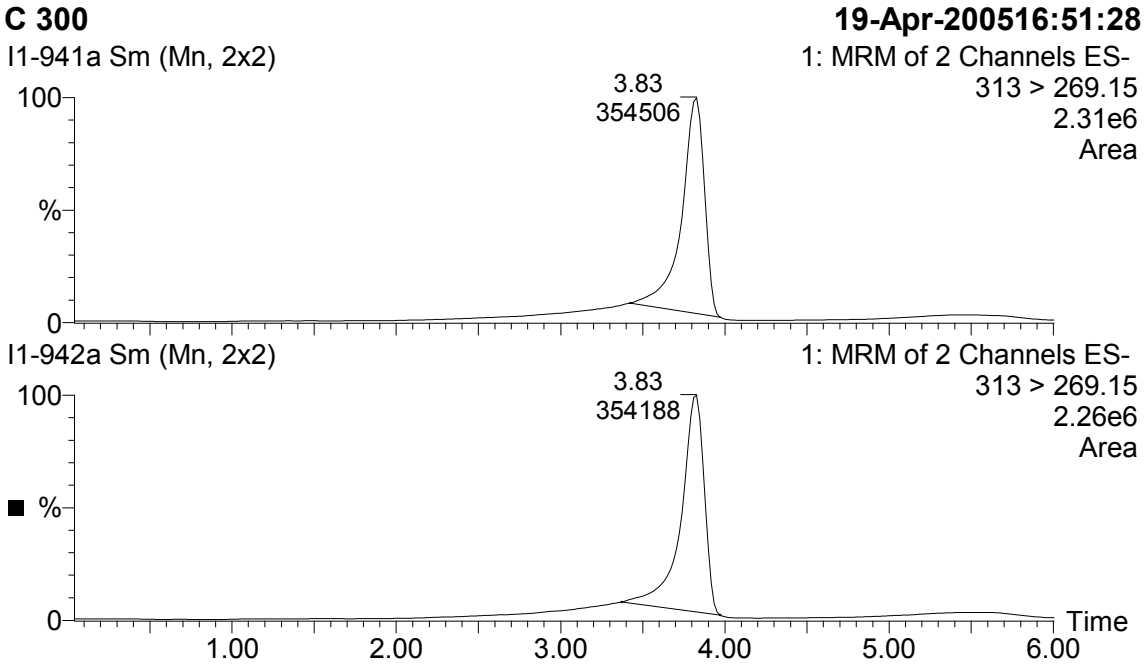

Figure 31: Representative Chromatogram Of 300 ng/mL Urine Calibration Samples

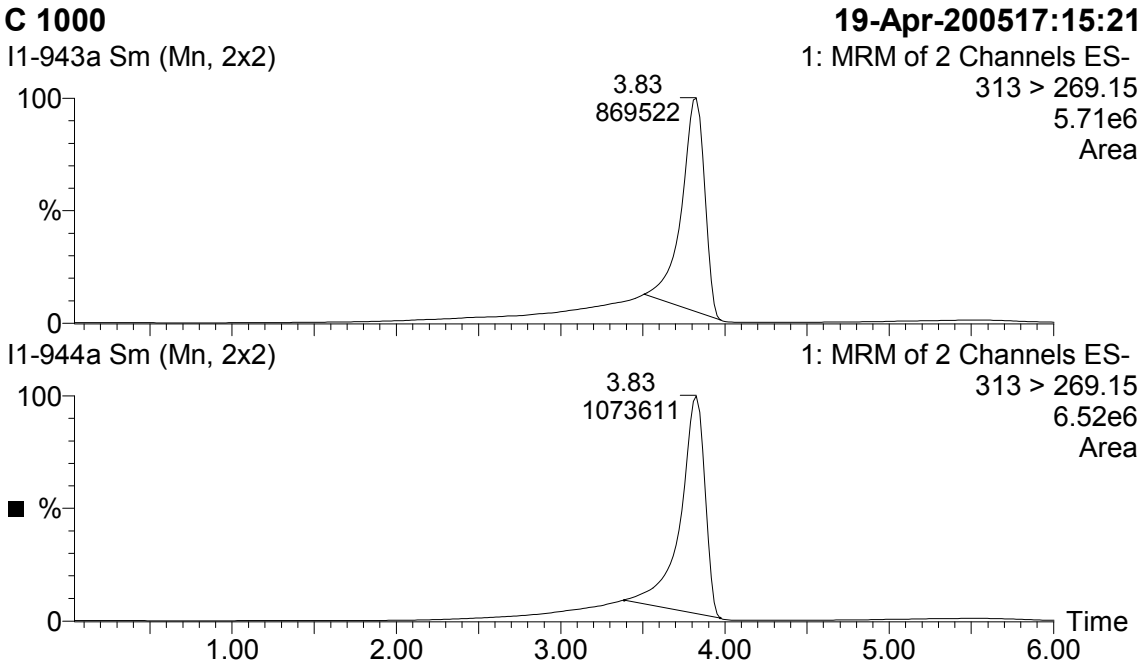

Figure 32: Representative Chromatogram Of 1000 ng/mL Urine Calibration Samples

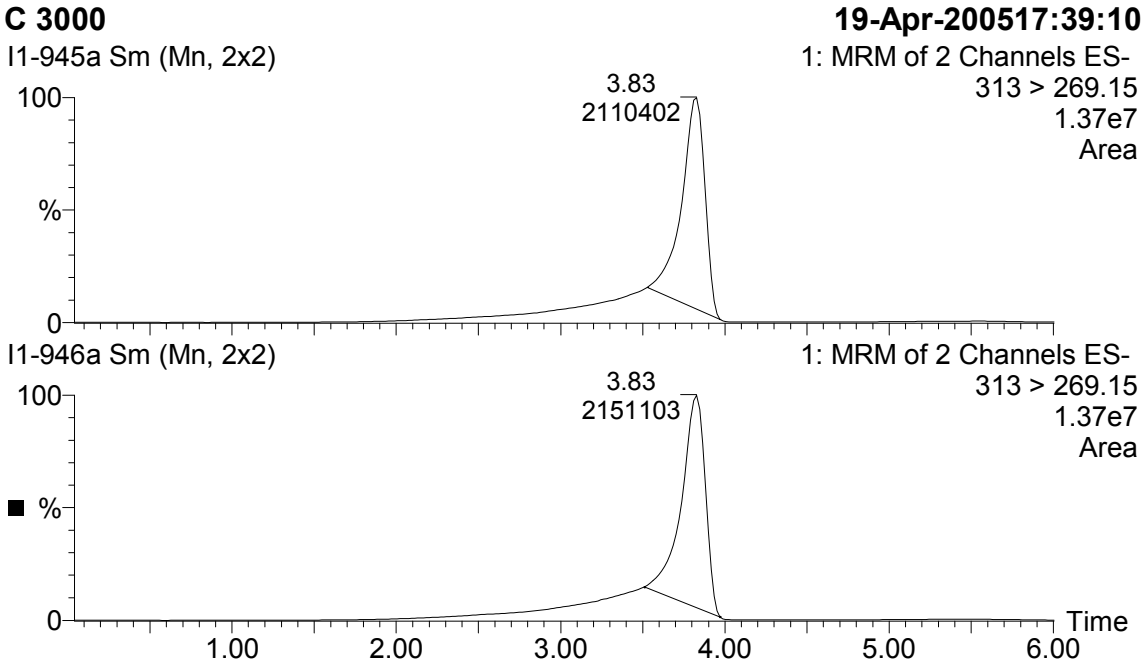

Figure 33: Representative Chromatogram Of 3000 ng/mL Urine Calibration Samples

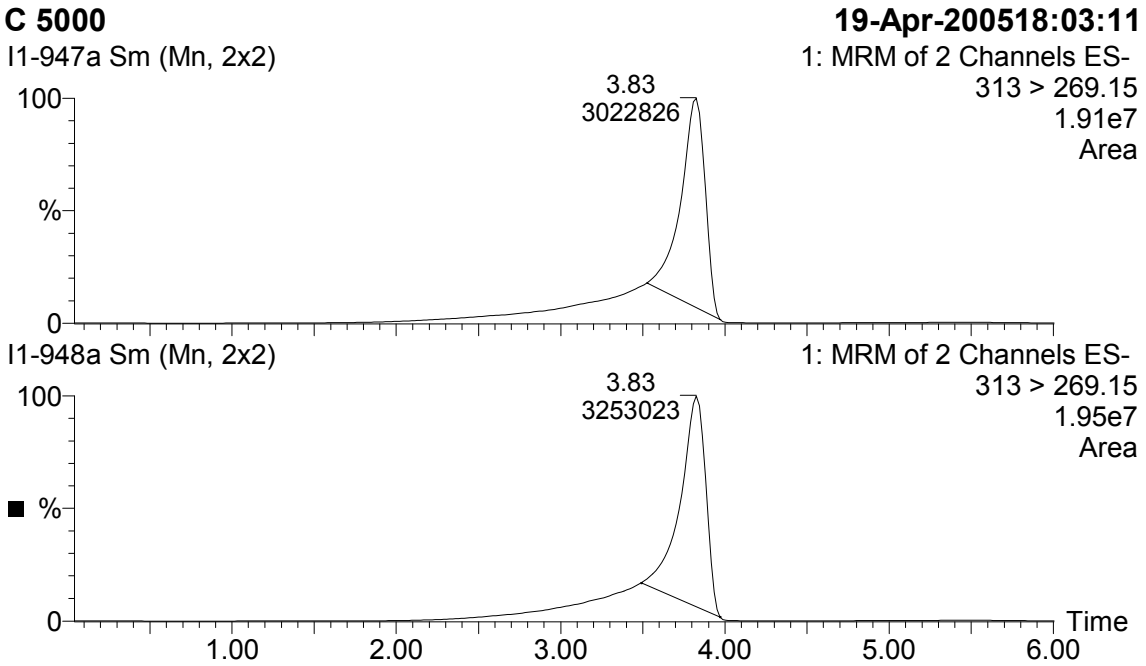

Figure 34: Representative Chromatogram Of 5000 ng/mL Urine Calibration Samples

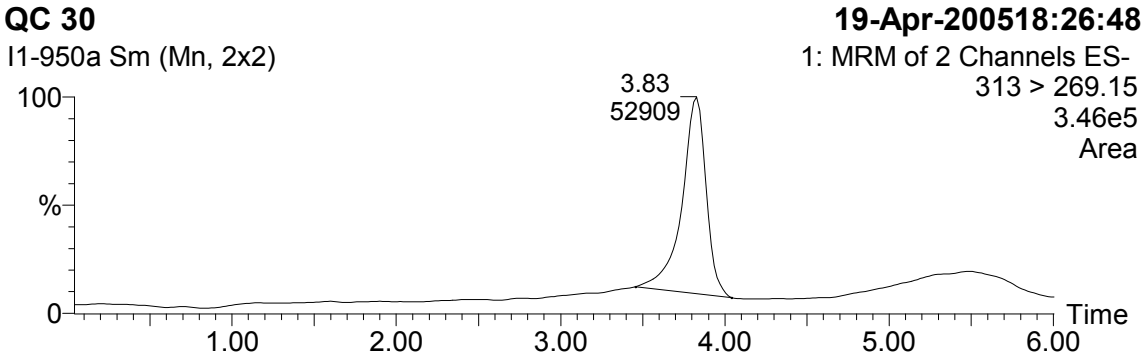

Figure 35: Representative Chromatogram Of A 30 ng/mL Quality Control Sample

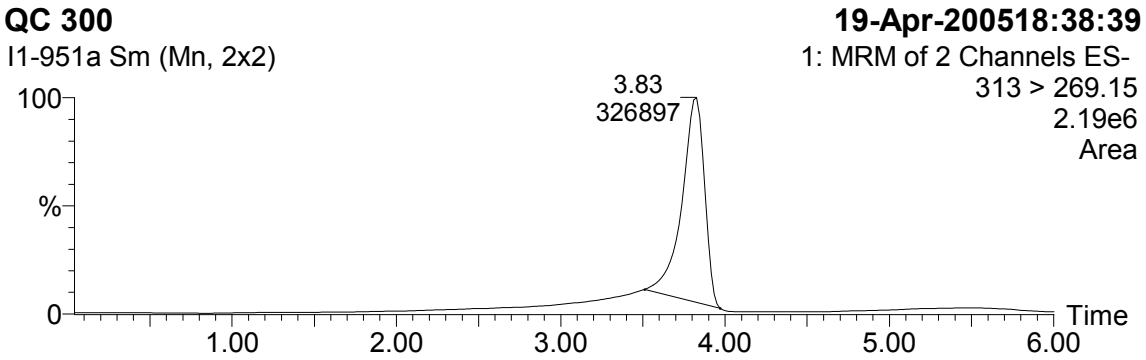

Figure 36: Representative Chromatogram Of A 300 ng/mL Quality Control Sample

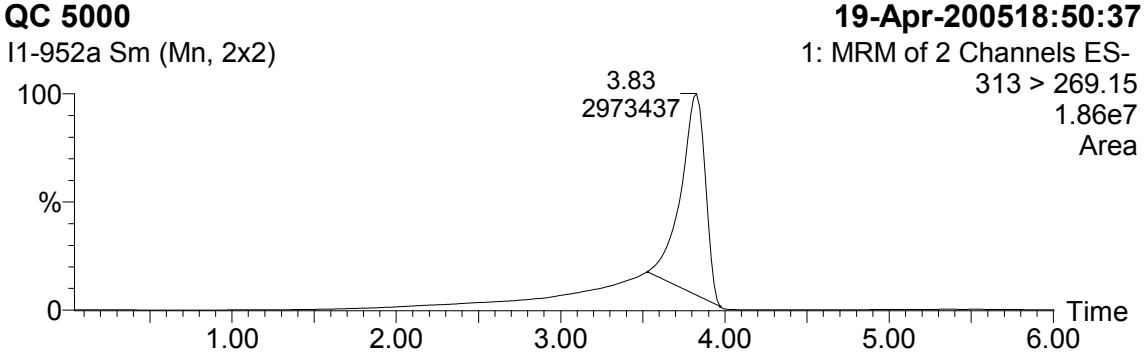

Figure 37: Representative Chromatogram Of A 5000 ng/mL Quality Control Sample

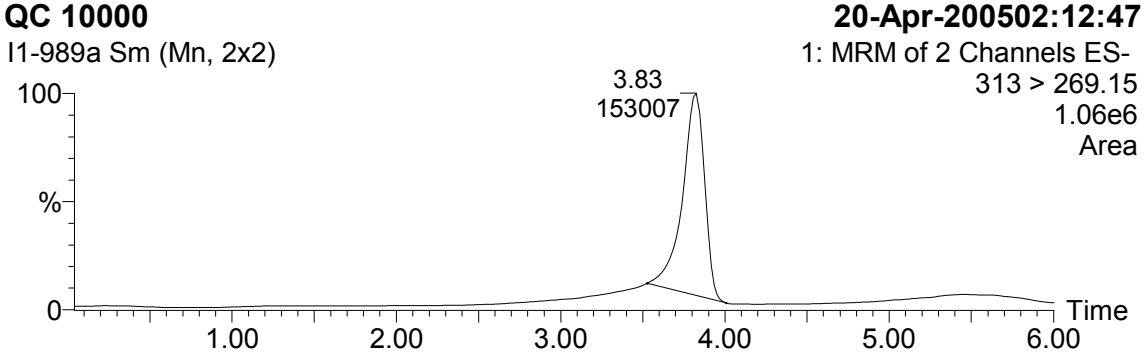

Figure 38: Representative Chromatogram Of A 10,000 ng/mL Quality Control Sample

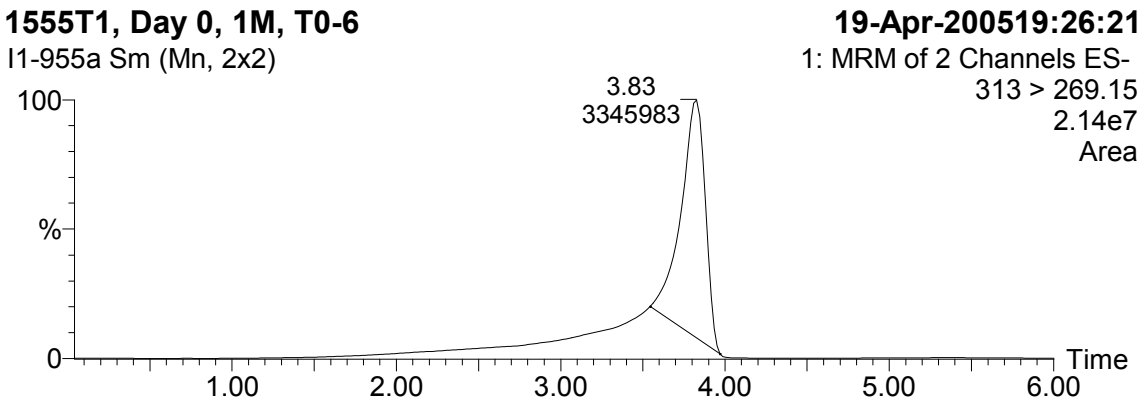

Figure 39: Chromatogram Of Animal No. 1555, Group T1, Male, 0 To 6 Hours Post-Dose Urine Sample

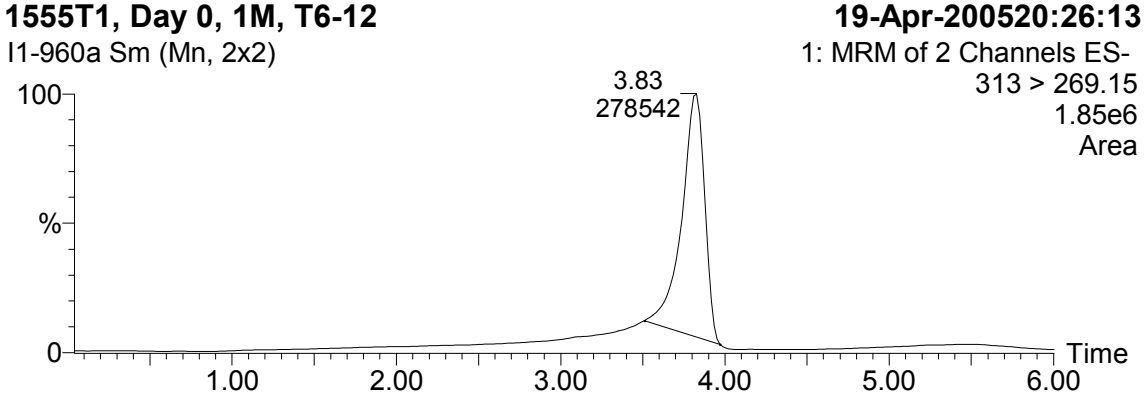

Figure 40: Chromatogram Of Animal No. 1555, Group T1, Male, 6 To 12 Hours Post-Dose Urine Sample

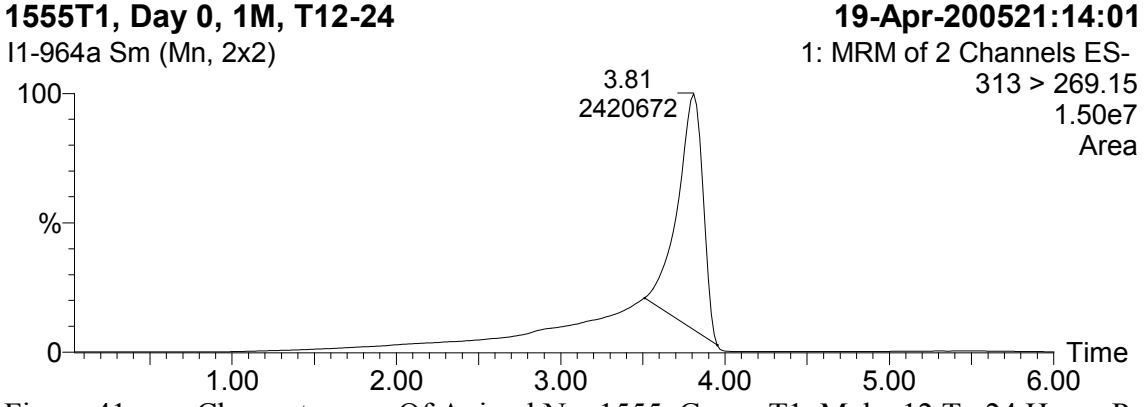

Figure 41: Chromatogram Of Animal No. 1555, Group T1, Male, 12 To 24 Hours Post-Dose Urine Sample

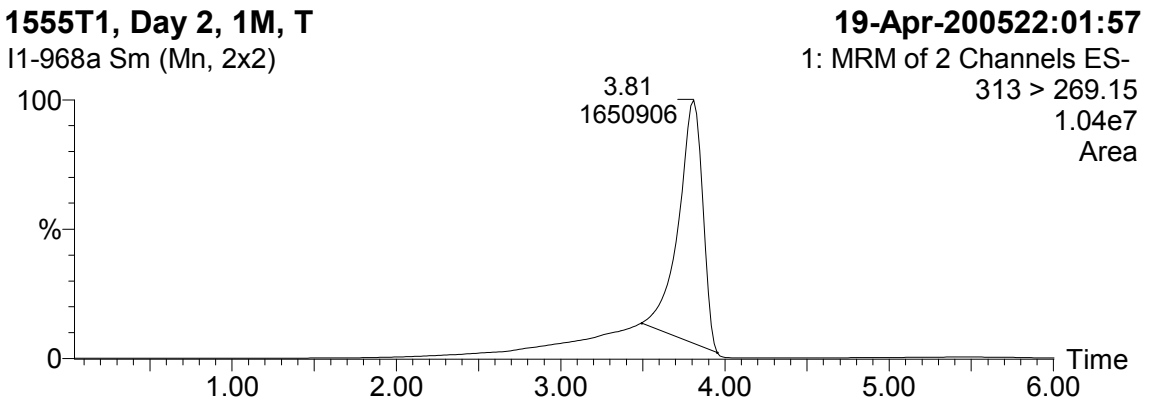

Figure 42: Chromatogram Of Animal No. 1555, Group T1, Male, Day 2 Urine Sample

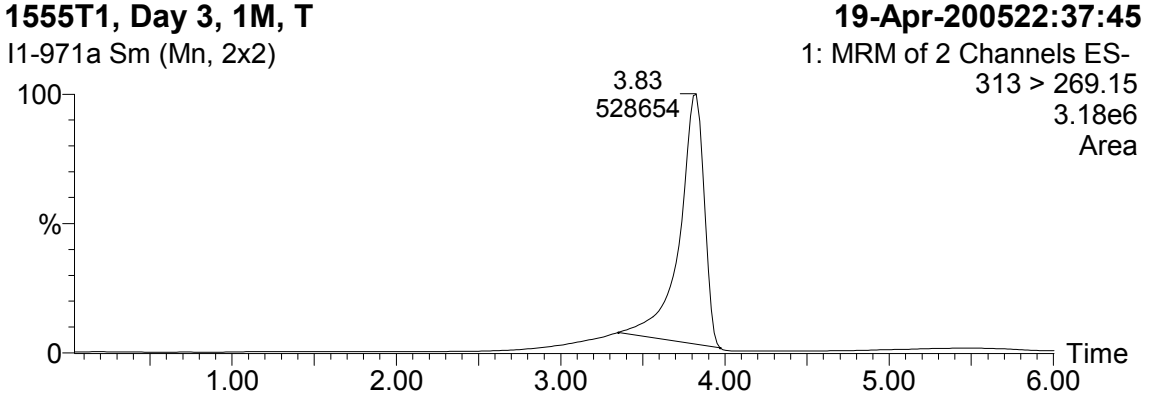

Figure 43: Chromatogram Of Animal No. 1555, Group T1, Male, Day 3 Urine Sample

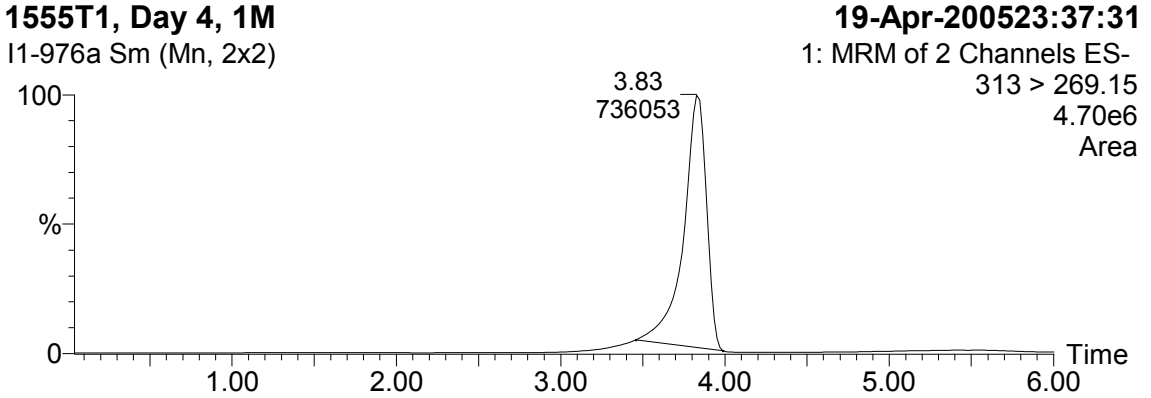

Figure 44: Chromatogram Of Animal No. 1555, Group T1, Male, Day 4 Urine Sample

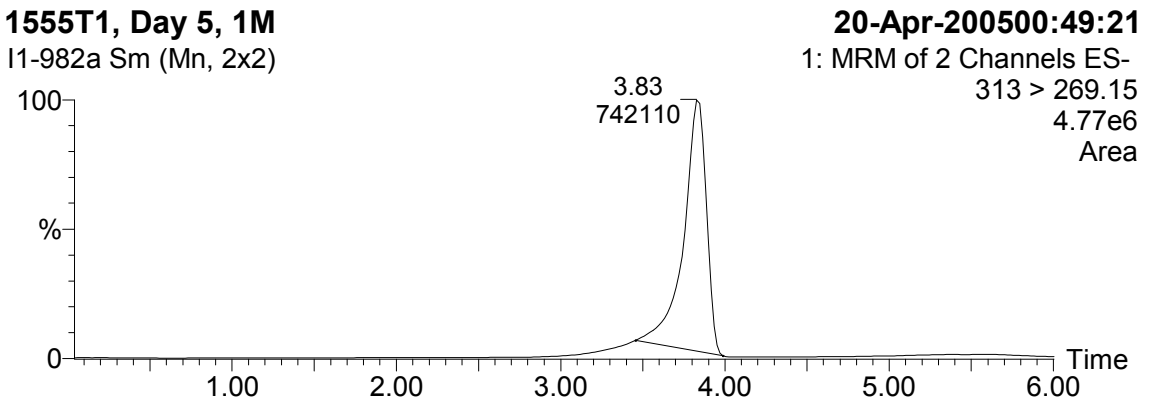

Figure 45: Chromatogram Of Animal No. 1555, Group T1, Male, Day 5 Urine Sample

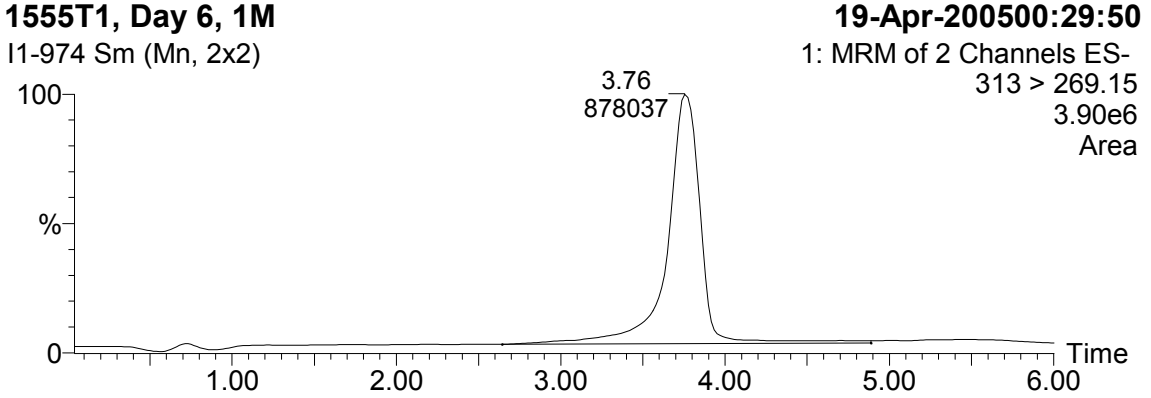

Figure 46: Chromatogram Of Animal No. 1555, Group T1, Male, Day 6 Urine Sample

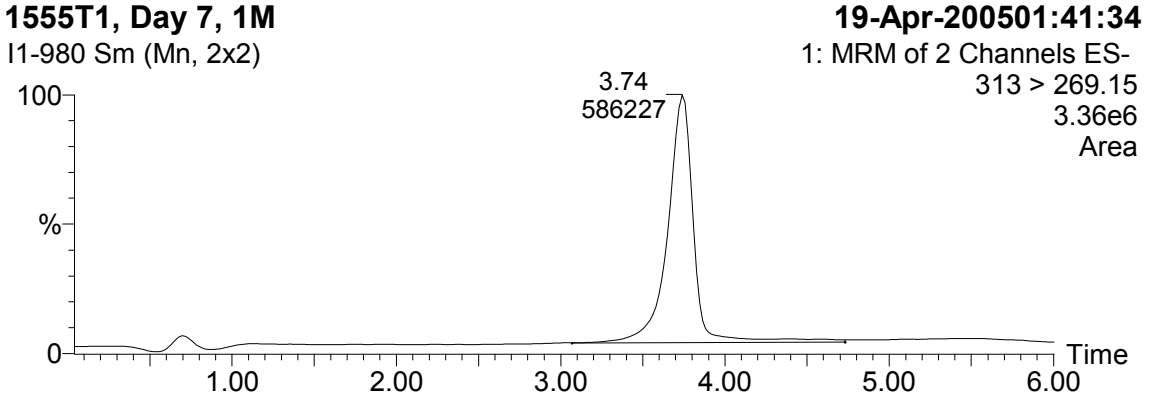

Figure 47: Chromatogram Of Animal No. 1555, Group T1, Male, Day 7 Urine Sample

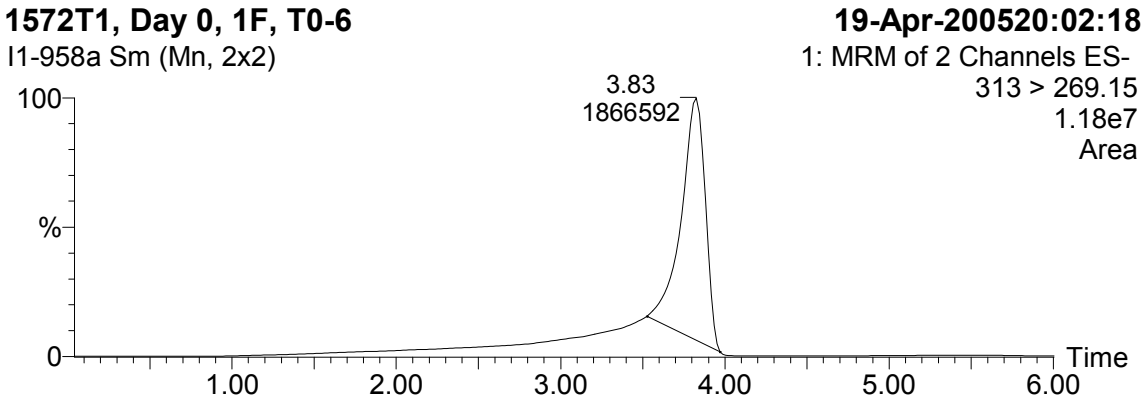

Figure 48: Chromatogram Of Animal No. 1572, Group T1, Female, 0 To 6 Hours Post-Dose Urine Sample

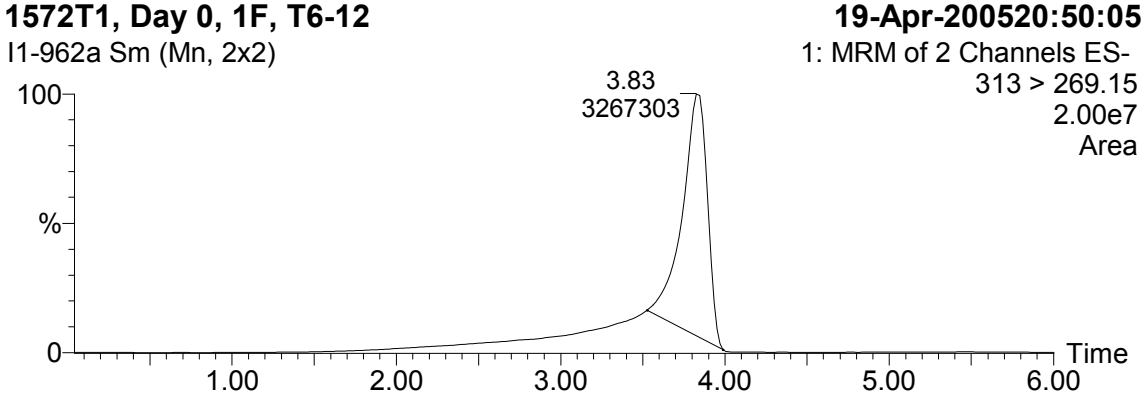

Figure 49: Chromatogram Of Animal No. 1572, Group T1, Female, 6 To 12 Hours Post-Dose Urine Sample

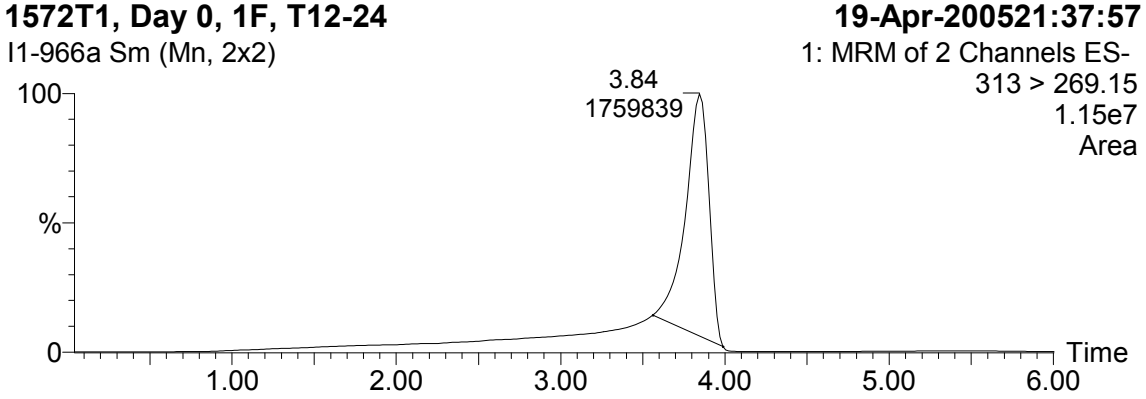

Figure 50: Chromatogram Of Animal No. 1572, Group T1, Female, 12 To 24 Hours Post-Dose Urine Sample

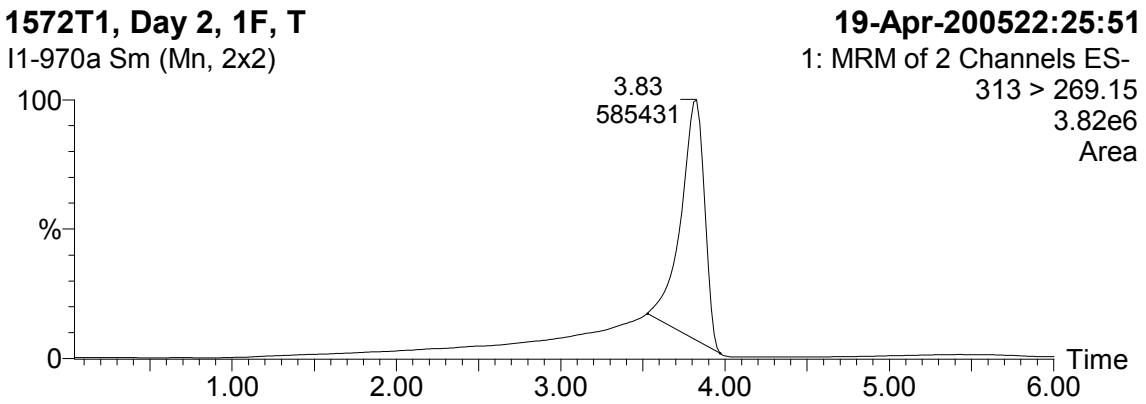

Figure 51: Chromatogram Of Animal No. 1572, Group T1, Female, Day 2 Urine Sample

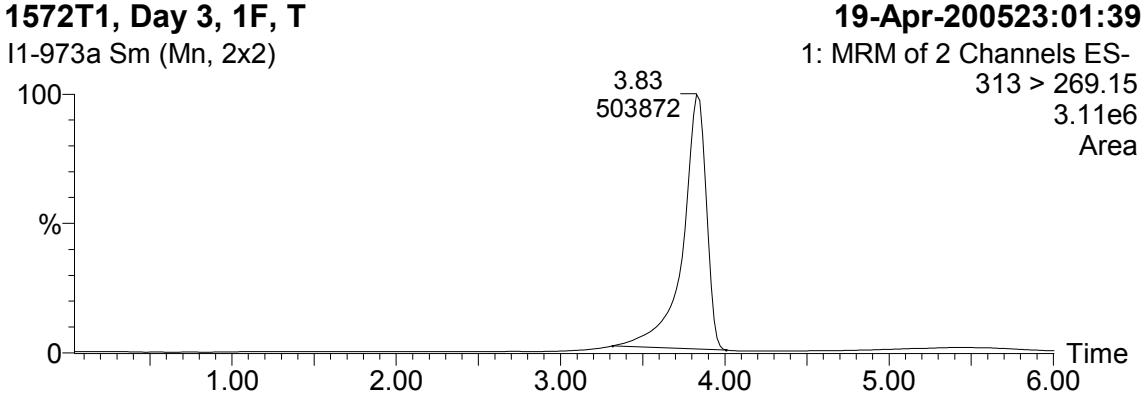

Figure 52: Chromatogram Of Animal No. 1572, Group T1, Female, Day 3 Urine Sample

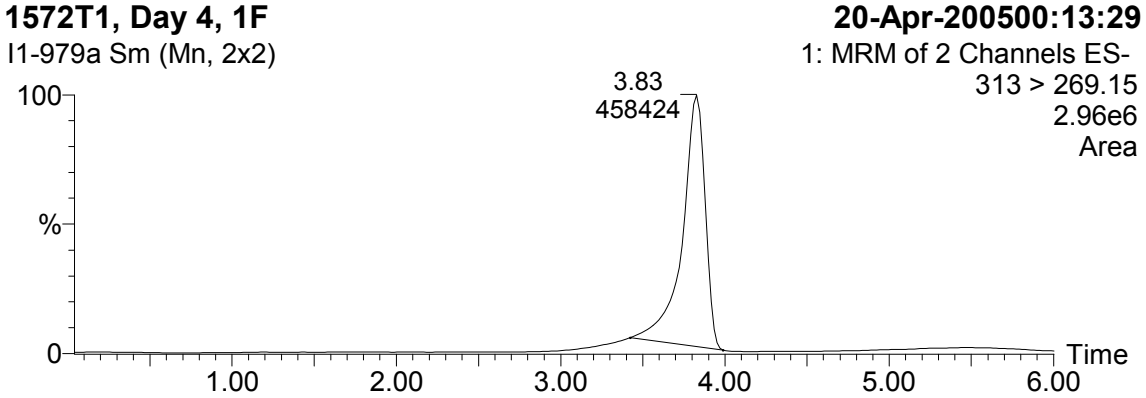

Figure 53: Chromatogram Of Animal No. 1572, Group T1, Female, Day 4 Urine Sample

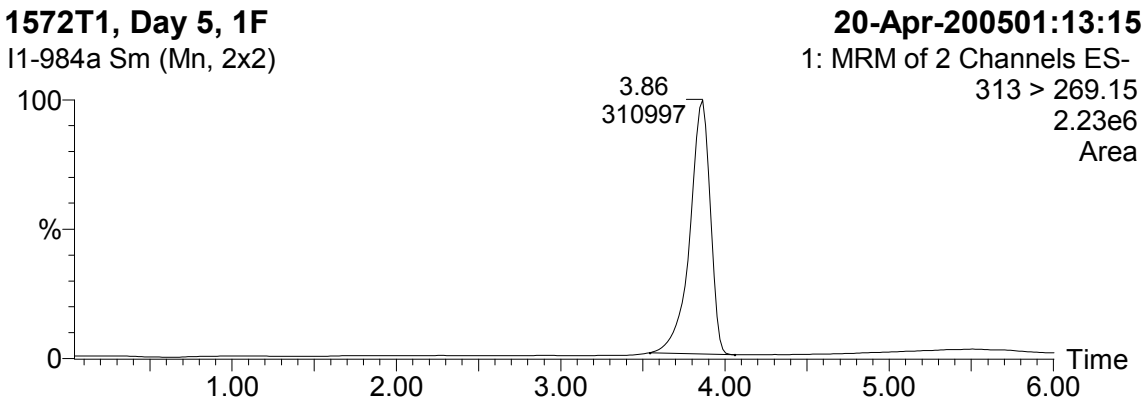

Figure 54: Chromatogram Of Animal No. 1572, Group T1, Female, Day 5 Urine Sample

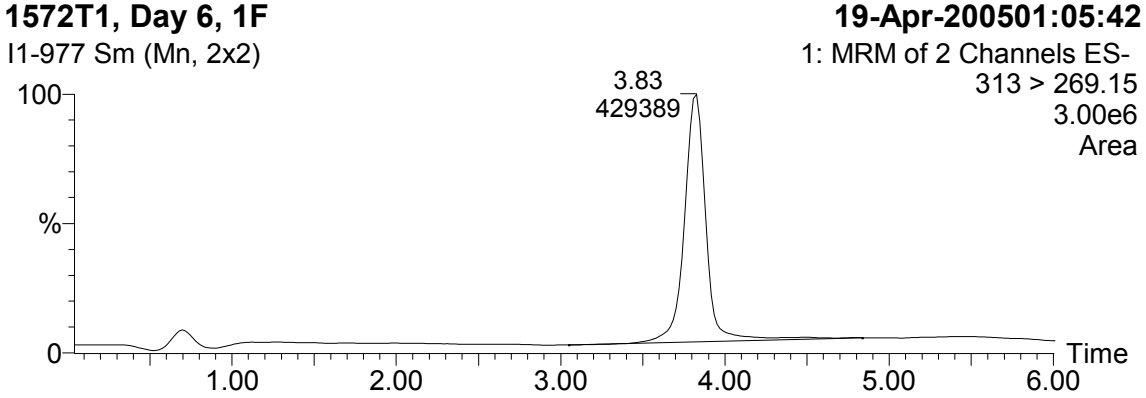

Figure 55: Chromatogram Of Animal No. 1572, Group T1, Female, Day 6 Urine Sample

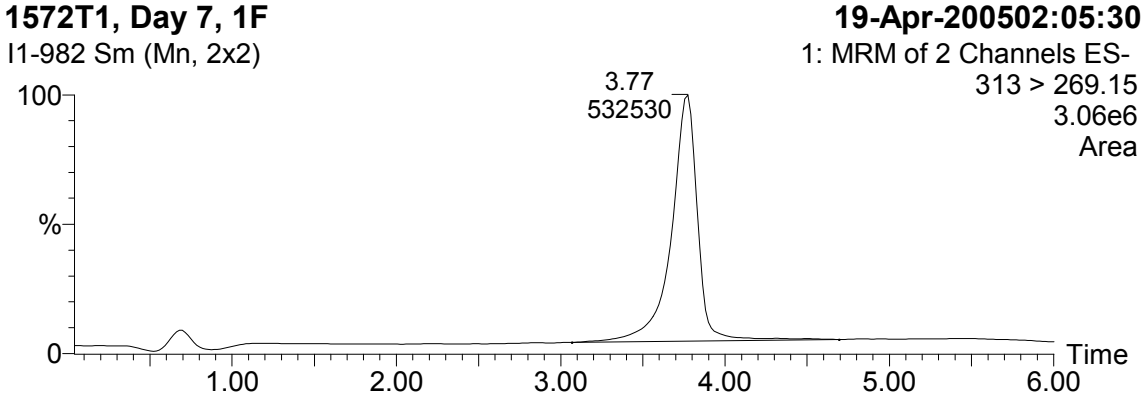

Figure 56: Chromatogram Of Animal No. 1572, Group T1, Female, Day 7 Urine Sample

ATTACHMENT I

Supporting Data

Table A-1: Monkey Serum Calibration and QC Sample Data of Sequence 534002(PFHxA)CMS
Validation Set I

Quantify Compound Summary Report

Sample List:C:\MASSLYNX\534002.PRO\SampleDB\534002(PFHxA)CMS
Last modified:Wed Mar 30 14:17:46 2005
Method:C:\MASSLYNX\534002.PRO\MethDB\534002 md9
Last modified:Fri Apr 01 12:06:42 2005
Job Code:

Printed:Mon Apr 04 08:35:47 2005

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Conc. (ng/mL)	% RE
I1-353		ACN	3.85	35985	bb	9.86	
I1-354	98-1	Solvent Blank	3.85	30650	bb	7.85	
I1-355	98-2	Monkey Serum Blank	3.85	35033	bb	9.49	
I1-356	98-3	Monkey Serum + IS	3.83	37721	bb	10.5	
I1-357	98-4	C 10	3.83	53440	bbX	17.2	72
I1-358	98-5	C 10	3.83	53649	bbX	17.3	73
I1-359	98-6	C 10	3.83	52294	bbX	16.7	67
I1-360	98-7	C 30	3.83	83846	bb	32.3	7.6
I1-361	98-8	C 30	3.83	78541	bb	29.5	-1.7
I1-362	98-9	C 30	3.83	79895	bb	30.2	0.64
I1-363	98-10	C 100	3.81	179397	bb	92.1	-7.9
I1-364	98-11	C 100	3.81	184876	bb	96.0	-4.0
I1-365	98-12	C 100	3.81	185889	bb	96.7	-3.3
I1-366	98-13	C 300	3.81	439985	bb	310	3.4
I1-367	98-14	C 300	3.83	437964	bb	308	2.8
I1-368	98-15	C 300	3.83	433883	bb	305	1.5
I1-369	98-16	C 1000	3.81	1099031	bb	1048	4.8
I1-370	98-17	C 1000	3.81	1087151	bb	1033	3.3
I1-371	98-18	C 1000	3.81	1060559	bb	1000	-0.020
I1-372	98-19	C 3000	3.83	2393671	bb	2899	-3.4
I1-373	98-20	C 3000	3.81	2441557	bb	2975	-0.85
I1-374	98-21	C 3000	3.83	2368176	bb	2859	-4.7
I1-375	98-22	C 5000	3.83	3478706	bb	4701	-6.0
I1-376	98-23	C 5000	3.81	4016357	bb	5656	13
I1-377	98-24	C 5000	3.81	3561184	bb	4845	-3.1
I1-378		ACN	3.85	40134	bb	11.5	
I1-379		ACN	3.85	39755	bb	11.4	
I1-380	99-1	QC 30	3.81	90275	bb	35.8	19
I1-381	99-2	QC 30	3.81	81482	bb	31.0	3.4
I1-382	99-3	QC 30	3.81	84064	bb	32.4	8.0
I1-383	99-4	QC 300	3.81	409408	bb	282	-6.1
I1-384	99-5	QC 300	3.81	418628	bb	290	-3.2
I1-385	99-6	QC 300	3.81	418861	bb	290	-3.2
I1-386	99-7	QC 5000	3.81	3266002	bb	4334	-13
I1-387	99-8	QC 5000	3.81	3198534	bb	4218	-16

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Conc. (ng/mL)	% RE
I1-388	99-9	QC 5000	3.81	3423292	bb	4605	-7.9
I1-389		ACN	3.85	38961	bb	11.0	
I1-390		ACN	3.85	40165	bb	11.5	

Table A-2: Monkey Serum Calibration and QC Sample Data of Sequence 534002(PFHxA)DMS1

Quantify Compound Summary Report

Sample List:C:\MASSLYNX\534002.PRO\SampleDB\534002(PFHxA)DMS1
Last modified:Thu Mar 31 17:25:51 2005
Method:C:\MASSLYNX\534002.PRO\MethDB\534002 md9
Last modified:Fri Apr 01 12:06:42 2005
Job Code:

Printed:Mon Apr 04 08:37:05 2005

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Conc. (ng/mL)	% RE
I1-391a		ACN	3.81	29653	bb	10.1	
I1-392a	100-1	Solvent Blank	3.83	19352	bb	6.04	
I1-393a	100-2	Monkey Serum Blank	3.78	53923	bb	21.0	
I1-394a	100-3	Monkey Serum + IS	3.78	35344	bb	12.5	
I1-395a	100-4	C 10	3.78	48084	bbX	18.3	83
I1-396a	100-5	C 10	3.78	47782	bbX	18.1	81
I1-397a	100-6	C 10	3.78	50576	bbX	19.4	94
I1-398a	100-7	C 30	3.78	76010	bb	32.1	6.9
I1-399a	100-8	C 30	3.78	71384	bb	29.7	-1.1
I1-400a	100-9	C 30	3.76	77114	bb	32.6	8.8
I1-401a	100-10	C 100	3.76	169722	bb	87.3	-13
I1-402a	100-11	C 100	3.78	183140	bb	96.1	-3.9
I1-403a	100-12	C 100	3.77	169246	bb	87.0	-13
I1-404a	100-13	C 300	3.76	470018	bb	318	6.1
I1-405a	100-14	C 300	3.76	452892	bb	304	1.2
I1-406a	100-15	C 300	3.76	454997	bb	305	1.8
I1-407a	100-16	C 1000	3.76	1187619	bb	1060	6.0
I1-408a	100-17	C 1000	3.76	1172097	bb	1042	4.2
I1-409a	100-18	C 1000	3.76	1192875	bb	1066	6.6
I1-410a	100-19	C 3000	3.76	2614869	bb	3011	0.38
I1-411a	100-20	C 3000	3.76	2688231	bb	3125	4.2
I1-412a	100-21	C 3000	3.76	2551763	bb	2915	-2.8
I1-413a	100-22	C 5000	3.76	3799671	bb	4971	-0.59
I1-414a	100-23	C 5000	3.76	3719066	bb	4829	-3.4
I1-415a	100-24	C 5000	3.76	3673558	bb	4750	-5.0
I1-416a		ACN	3.81	29060	bb	9.88	
I1-417a		ACN	3.81	30864	bb	10.6	
I1-418a	101-1	QC 30	3.78	82017	bb	35.2	17
I1-419a	101-2	QC 30	3.78	81091	bb	34.7	16
I1-420a	101-3	QC 30	3.76	79433	bb	33.9	13
I1-421a	101-4	QC 300	3.76	402769	bb	261	-13
I1-422a	101-5	QC 300	3.76	405574	bb	264	-12
I1-423a	101-6	QC 300	3.76	386821	bb	248	-17
I1-424a	101-7	QC 5000	3.76	3149361	bb	3862	-23
I1-425a	101-8	QC 5000	3.76	3459367	bb	4381	-12

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Conc. (ng/mL)	% RE
I1-426a	101-9	QC 5000	3.77	3471004	bb	4401	-12
I1-427a		ACN	3.81	28359	bb	9.59	
I1-428a	97-6	fresh solvent stock 500	3.79	939992	bb	781	56
I1-429a	97-8a	7- day solvent stock 500	3.77	798564	bb	631	26
I1-430a		ACN	3.81	27039	bb	9.05	
I1-431a	97-8b	7-day IS stock	3.83	14995	bb	4.45	
I1-432a	97-12	Fresh |S stock	3.83	15157	bb	4.51	
I1-433a		ACN	3.81	25665	bb	8.50	

Table A-3: Monkey Serum Calibration and QC Sample Data of Sequence 534002(PFHxA)EMS

Quantify Compound Summary Report
Sample List:C:\MASSLYNX\534002.PRO\SampleDB\534002(PFHxA)EMS
Last modified:Thu Mar 31 17:27:25 2005
Method:C:\MASSLYNX\534002.PRO\MethDB\534002 md9
Last modified:Fri Apr 01 12:06:42 2005
Job Code:

Printed:Mon Apr 04 08:28:57 2005

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Conc. (ng/mL)	% RE
I1-434	105-4	system suitability	3.8	39533	bb	16.6	
I1-435	105-4	system suitability	3.79	38090	bb	15.9	
I1-436	105-4	system suitability	3.79	38077	bb	15.9	
I1-437		ACN	3.81	31831	bb	12.9	
I1-438	105-1	Solvent Blank	3.83	23553	bb	9.09	
I1-439	105-2	Monkey Serum Blank	3.79	26624	bb	10.5	
I1-440	105-3	Monkey Serum + IS	3.8	22107	bb	8.45	
I1-441	105-4	C 10	3.79	39795	bbX	16.7	67
I1-442	105-5	C 10	3.79	40018	bbX	16.8	68
I1-443	105-6	C 10	3.79	39919	bbX	16.8	68
I1-444	105-7	C 30	3.78	70291	bb	32.8	9.3
I1-445	105-8	C 30	3.79	66862	bb	30.9	2.9
I1-446	105-9	C 30	3.78	67593	bb	31.3	4.3
I1-447	105-10	C 100	3.79	164690	bb	92.5	-7.5
I1-448	105-11	C 100	3.79	162447	bb	91.0	-9.0
I1-449	105-12	C 100	3.8	156482	bb	86.8	-13
I1-450	105-13	C 300	3.78	421573	bb	304	1.4
I1-451	105-14	C 300	3.78	422652	bb	305	1.7
I1-452	105-15	C 300	3.79	396187	bb	281	-6.5
I1-453	105-16	C 1000	3.78	1094867	bb	1074	7.4
I1-454	105-17	C 1000	3.78	1161398	bb	1163	16
I1-455	105-18	C 1000	3.78	1107878	bb	1091	9.1
I1-456	105-19	C 3000	3.78	2318962	bb	3025	0.84
I1-457	105-20	C 3000	3.78	2341843	bb	3067	2.2
I1-458	105-21	C 3000	3.78	2332278	bb	3050	1.7
I1-459	105-22	C 5000	3.78	3192615	bb	4764	-4.7
I1-460	105-23	C 5000	3.78	3150787	bb	4674	-6.5
I1-461	105-24	C 5000	3.76	3190711	bb	4759	-4.8
I1-462		ACN	3.81	28763	bb	11.4	
I1-463		ACN	3.81	32574	bb	13.2	
I1-464	106-1	QC 30	3.78	64390	bb	29.5	-1.6
I1-465	106-2	QC 30	3.78	64217	bb	29.4	-1.9
I1-466	106-3	QC 30	3.78	64419	bb	29.5	-1.6
I1-467	106-4	QC 300	3.78	394247	bb	279	-7.0
I1-468	106-5	QC 300	3.78	373675	bb	260	-13
I1-469	106-6	QC 300	3.78	382981	bb	269	-10

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Conc. (ng/mL)	% RE
I1-470	106-7	QC 5000	3.78	3208720	bb	4798	-4.0
I1-471	106-8	QC 5000	3.76	3272309	bb	4935	-1.3
I1-472	106-9	QC 5000	3.76	3300900	bb	4997	-0.061
I1-473		ACN	3.81	29887	bb	12.0	

Table A-4: Monkey Serum Calibration and QC Sample Data of Sequence 534002(PFHxA)FMS

Quantify Compound Summary Report

Sample List:C:\MASSLYNX\534002.PRO\SampleDB\534002(PFHxA)FMS
Last modified:Fri Apr 01 14:51:16 2005
Method:C:\MASSLYNX\534002.PRO\MethDB\534002 md9
Last modified:Fri Apr 01 12:06:42 2005
Job Code:

Printed:Mon Apr 04 08:27:30 2005

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Conc. (ng/mL)	% RE
I1-474	109-4	system suitability	3.78	47327	bb	17.5	
I1-475	109-4	system suitability	3.76	46128	bb	16.9	
I1-476	109-4	system suitability	3.76	46880	bb	17.2	
I1-477		ACN	3.83	11604	bb	2.54	
I1-478		ACN	3.81	11704	bb	2.57	
I1-479	109-1	Solvent Blank	3.81	19240	bb	5.12	
I1-480	109-2	Monkey Serum Blank	3.72	26424	bb	7.92	
I1-481	109-3	Monkey Serum + IS	3.74	27547	bb	8.38	
I1-482	109-4	C 10	3.76	45792	bbX	16.7	67
I1-483	109-5	C 10	3.76	45908	bbX	16.8	68
I1-484	109-6	C 10	3.76	43143	bbX	15.4	54
I1-485	109-7	C 30	3.72	67789	bb	28.3	-5.8
I1-486	109-8	C 30	3.74	73280	MM	31.4	4.5
I1-487	109-9	C 30	3.74	72674	bb	31.0	3.4
I1-488	109-10	C 100	3.72	174045	bb	98.1	-1.9
I1-489	109-11	C 100	3.74	175678	bb	99.3	-0.66
I1-490	109-12	C 100	3.72	178695	bb	102	1.6
I1-491	109-13	C 300	3.72	435922	bb	321	7.1
I1-492	109-14	C 300	3.69	357592	bb	249	-17
I1-493	109-15	C 300	3.74	437476	bb	323	7.6
I1-494	109-16	C 1000	3.65	853168	bb	754	-25
I1-495	109-17	C 1000	3.72	1205812	bb	1164	16
I1-496	109-18	C 1000	3.72	1181470	bb	1135	13
I1-497	109-19	C 3000	3.74	2883927	bb	3430	14
I1-498	109-20	C 3000	3.69	2469491	bb	2834	-5.5
I1-499	109-21	C 3000	3.72	2719057	bb	3191	6.4
I1-500	109-22	C 5000	3.72	4042899	bb	5187	3.7
I1-501	109-23	C 5000	3.69	3503190	bb	4354	-13
I1-502	109-24	C 5000	3.71	1770437	bbX	1878	-62
I1-503		ACN	3.81	9370	bb	1.89	
I1-504		ACN	3.81	9724	bb	1.99	
I1-505	110-1	QC 30	3.69	53339	bb	20.5	-32
I1-506	110-2	QC 30	3.69	57131	bb	22.5	-25
I1-507	110-3	QC 30	3.74	68987	bb	28.9	-3.6
I1-508	110-4	QC 300	3.71	367394	bb	258	-14
I1-509	110-5	QC 300	3.72	400933	bb	289	-3.7

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Conc. (ng/mL)	% RE
I1-510	110-6	QC 300	3.74	424609	bb	311	3.6
I1-511	110-7	QC 5000	3.7	3173879	bb	3858	-23
I1-512	110-8	QC 5000	3.7	3505617	bb	4358	-13
I1-513	110-9	QC 5000	3.72	4388737	bb	5733	15
I1-514		ACN	3.81	10094	bb	2.09	
I1-515		ACN	3.81	9469	bb	1.91	

Table A-5: Monkey Serum Calibration and QC Sample Data of Sequence 534002(PFHxA)GFMS

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534002.PRO\SampleDB\534002(PFHxA)GFMS
Last modified: Thu Apr 07 09:11:02 2005
Method: C:\MASSLYNX\534002.PRO\MethDB\534002 md9
Last modified: Fri Apr 01 12:06:42 2005
Job Code:

Printed: Mon Apr 11 09:20:05 2005

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-516a		system suitability	3.79	10514	bb	0	1.80	
I1-516b		system suitability	3.78	11341	bb	0	2.03	
I1-516c		system suitability	3.69	28114	bb	0	8.07	
I1-517						0		
I1-518						0		
I1-519						0		
I1-520		ACN	3.85	23258	bb	0	6.09	
I1-521	114-1	Solvent Blank	3.81	90023	bb	0	42.3	
I1-522	114-2	Monkey Serum Blank	3.79	29523	bb	0	8.67	
I1-523	114-3	Monkey Serum + IS	3.78	30314	bb	0	9.02	
I1-524	114-4	C 10	3.76	40867	bbX	0	13.9	39
I1-525	114-5	C 10	3.76	40840	bbX	0	13.9	39
I1-526	114-6	C 10	3.78	48897	bbX	0	18.0	80
I1-527	114-7	C 30	3.78	73212	bb	0	31.8	5.9
I1-528	114-8	C 30	3.78	72244	bb	0	31.2	4.0
I1-529	114-9	C 30	3.76	73970	bb	0	32.2	7.4
I1-530	114-10	C 100	3.76	168933	bb	0	98.9	-1.1
I1-531	114-11	C 100	3.76	158175	bb	0	90.6	-9.4
I1-532	114-12	C 100	3.76	151911	bb	0	85.9	-14
I1-533	114-13	C 300	3.76	381885	bb	0	285	-4.9
I1-534	114-14	C 300	3.76	390335	bb	0	294	-2.2
I1-535	114-15	C 300	3.76	377273	bb	0	281	-6.3
I1-536	114-16	C 1000	3.76	1197472	bb	0	1176	18
I1-537	114-17	C 1000	3.76	1117276	bb	0	1082	8.2
I1-538	114-18	C 1000	3.76	1120498	bb	0	1086	8.6
I1-539	114-19	C 3000	3.76	2691422	bb	0	3074	2.5
I1-540	114-20	C 3000	3.76	2623774	bb	0	2985	-0.52
I1-541	114-21	C 3000	3.76	2725782	bb	0	3120	4.0
I1-542	114-22	C 5000	3.76	3902350	bb	0	4726	-5.5
I1-543	114-23	C 5000	3.74	3920323	bb	0	4751	-5.0
I1-544	114-24	C 5000	3.76	3938713	bb	0	4777	-4.5
I1-545		ACN	3.83	25765	bb	0	7.09	
I1-546		ACN	3.83	26699	bb	0	7.48	
I1-547	115-1	QC 30	3.76	74275	bb	0	32.4	8.1
I1-548	115-2	QC 30	3.74	78012	bb	0	34.7	16
I1-549	115-3	QC 30	3.76	73244	bb	0	31.8	6.0

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-550	115-4	QC 300	3.76	398542	bb	0	301	0.46
I1-551	115-5	QC 300	3.76	374823	bb	0	279	-7.1
I1-552	115-6	QC 300	3.76	421615	bb	0	324	7.9
I1-553	115-7	QC 5000	3.94	38157	bb	0	12.6	-100
I1-554	115-8	QC 5000	3.79	33368	bb	0	10.4	-100
I1-555	115-9	QC 5000	3.78	37509	bb	0	12.3	-100
I1-608	115a-7	QC 5000	3.73	3991828	bb	0	4851	-3.0
I1-609	115a-8	QC 5000	3.72	4516794	bb	0	5588	12
I1-610	115a-9	QC 5000	3.72	4293990	bb	0	5274	5.5
I1-556		ACN	3.83	27521	bb	0	7.82	
I1-557		ACN	3.83	27288	bb	0	7.72	
I1-558	109-4	pss C 10	3.78	36662	bb	0	11.9	19
I1-559	109-7	pss C 30	3.76	65286	MM	0	27.1	-9.7
I1-560	109-10	pss C 100	3.76	148588	MM	0	83.4	-17
I1-561	109-13	pss C 300	3.74	421700	bb	0	324	7.9
I1-562	109-16	pss C 1000	3.76	902427	bb	0	834	-17
I1-563	109-19	pss C 3000	3.74	2963086	bb	0	3439	15
I1-564	109-22	pss C 5000	3.74	4226770	bb	0	5179	3.6
I1-565	110-1	pss QC 30	3.76	73254	bb	0	31.8	6.0
I1-566	110-4	pss QC 300	3.76	400553	bb	0	303	1.1
I1-567	110-7	pss QC 5000	3.74	4009309	bb	0	4875	-2.5
I1-568		ACN	3.83	26798	bb	0	7.52	

Table A-6: Monkey Serum Calibration and QC Sample Data of Sequence 534002(PFHxA)HMS

Quantify Compound Summary Report

Sample List:C:\MASSLYNX\534002.PRO\SampleDB\534002(PFHxA)HMS
Last modified:Fri Apr 08 08:21:53 2005
Method:C:\MASSLYNX\534002.PRO\MethDB\534002 md9
Last modified:Fri Apr 01 12:06:42 2005
Job Code:

Printed:Fri Apr 08 08:31:02 2005

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-569		sys suit	3.83	32520	bb	0	8.42	
I1-570		sys suit	3.83	22823	bb	0	4.83	
I1-571		sys suit	3.77	66828	bb	0	24.8	
I1-572		ACN	3.81	27055	bb	0	6.32	
I1-573	116-1	Solvent Blank	3.81	20131	bb	0	3.95	
I1-574	116-2	Monkey Serum Blank	3.78	33112	bb	0	8.66	
I1-575	116-3	Monkey Serum Blank	3.78	37285	bb	0	10.4	
I1-576	116-4	C 10	3.76	50820	bbX	0	16.6	66
I1-577	116-5	C 10	3.76	52010	bbX	0	17.2	72
I1-578	116-6	C 10	3.76	56991	bbX	0	19.6	96
I1-579	116-7	C 30	3.76	80922	bb	0	32.7	9.0
I1-580	116-8	C 30	3.74	83127	bb	0	34.0	13
I1-581	116-9	C 30	3.72	72602	bb	0	28.0	-6.7
I1-582	116-10	C 100	3.74	182676	bb	0	102	1.6
I1-583	116-11	C 100	3.74	165268	bb	0	88.7	-11
I1-584	116-12	C 100	3.74	163649	bb	0	87.5	-13
I1-585	116-13	C 300	3.72	409858	bb	0	294	-1.9
I1-586	116-14	C 300	3.74	419256	bb	0	303	0.99
I1-587	116-15	C 300	3.74	395732	bb	0	281	-6.2
I1-588	116-16	C 1000	3.73	1192433	bb	0	1110	11
I1-589	116-17	C 1000	3.73	1187600	bb	0	1105	10
I1-590	116-18	C 1000	3.72	1142823	bb	0	1055	5.5
I1-591	116-19	C 3000	3.72	2787813	bb	0	3022	0.74
I1-592	116-20	C 3000	3.72	2691123	bb	0	2901	-3.3
I1-593	116-21	C 3000	3.74	2828590	bb	0	3073	2.4
I1-594	116-22	C 5000	3.72	4242899	bb	0	4882	-2.4
I1-595	116-23	C 5000	3.74	4283742	bb	0	4935	-1.3
I1-596	116-24	C 5000	3.73	4144174	bb	0	4754	-4.9
I1-597		ACN	3.83	17807	bb	0	3.23	
I1-598		ACN	3.83	18104	bb	0	3.32	
I1-599	117-1	QC 30	3.74	71620	bb	0	27.4	-8.6
I1-600	117-2	QC 30	3.72	68585	MM	0	25.8	-14
I1-601	117-3	QC 30	3.74	69994	MM	0	26.5	-12
I1-602	117-4	QC 300	3.72	413125	bb	0	297	-0.89
I1-603	117-5	QC 300	3.72	386306	bb	0	273	-9.1
I1-604	117-6	QC 300	3.72	412676	bb	0	297	-1.0

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-605	117-7	QC 5000	3.72	3922241	bb	0	4466	-11
I1-606	117-8	QC 5000	3.72	4223304	bb	0	4857	-2.9
I1-607	117-9	QC 5000	3.72	4281091	bb	0	4932	-1.4
I1-611		ACN	3.82	18830	bb	0	3.54	
I1-612		ACN	3.81	18974	bb	0	3.58	
I1-613	117-10	LT stab 300	3.72	428913	bb	0	312	4.0
I1-614	117-11	LT stab 300	3.72	399466	bb	0	285	-5.1
I1-615	117-12	LT stab 300	3.73	416295	bb	0	300	0.081
I1-616	117-13	LT stab 5000	3.72	3338170	bb	0	3717	-26
I1-616a	115a-9	QC 5000	3.72	3579064	bb	0	4025	-20
I1-616b	117-9	QC 5000	3.69	3836889	bb	0	4356	-13
I1-617	117-14	LT stab 5000	3.69	4691211	bb	0	5468	9.4
I1-618	117-15	LT stab 5000	3.69	4286754	bb	0	4939	-1.2
I1-619		ACN	3.79	19112	bb	0	3.63	
I1-619a	117-13	LT stab 5000	3.7	4127977	bb	0	4733	-5.3

Table A-7: Monkey Urine Calibration and QC Sample Data of Sequence 534002(PFHxA)IMU

Quantify Compound Summary Report

Sample List:C:\MASSLYNX\534002.PRO\SampleDB\534002(PFHxA)IMU
Last modified:Fri Apr 08 08:31:44 2005
Method:C:\MASSLYNX\534002.PRO\MethDB\534002 md9
Last modified:Fri Apr 01 12:06:42 2005
Job Code:

Printed:Mon Apr 11 09:56:03 2005

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-620		sys suit	3.72	52806	bb	0	12.9	
I1-621		sys suit	3.71	63767	bb	0	17.6	
I1-622		sys suit	3.7	63092	bb	0	17.3	
I1-623		ACN	3.78	19378	bb	0	2.25	
I1-624	118-1	Solvent Blank	3.78	20811	bb	0	2.56	
I1-625	118-2	Monkey Urine Blank	3.73	43053	bb	0	9.20	
I1-626	118-3	Monkey Urine Blank	3.72	43497	bb	0	9.36	
I1-627	118-4	C 10	3.71	64300	bbX	0	17.8	78
I1-628	118-5	C 10	3.7	62520	bbX	0	17.1	71
I1-629	118-6	C 10	3.71	62670	bbX	0	17.1	71
I1-630	118-7	C 30	3.69	95400	bb	0	33.4	11
I1-631	118-8	C 30	3.69	97225	bb	0	34.4	15
I1-632	118-9	C 30	3.69	85141	bb	0	27.9	-6.9
I1-633	118-10	C 100	3.69	177387	bb	0	85.5	-14
I1-634	118-11	C 100	3.69	181905	bb	0	88.8	-11
I1-635	118-12	C 100	3.69	190625	bb	0	95.1	-4.9
I1-636	118-13	C 300	3.69	430714	bb	0	303	0.99
I1-637	118-14	C 300	3.69	429931	bb	0	302	0.74
I1-638	118-15	C 300	3.69	433967	bb	0	306	2.0
I1-639	118-16	C 1000	3.69	999645	bb	0	936	-6.4
I1-640	118-17	C 1000	3.69	1076797	bb	0	1030	3.0
I1-641	118-18	C 1000	3.69	1192894	bb	0	1176	18
I1-642	118-19	C 3000	3.69	2671846	bb	0	3239	8.0
I1-643	118-20	C 3000	3.69	2623818	bb	0	3168	5.6
I1-644	118-21	C 3000	3.69	2585500	bb	0	3111	3.7
I1-645	118-22	C 5000	3.69	3626474	bb	0	4696	-6.1
I1-646	118-23	C 5000	3.69	3576587	bb	0	4618	-7.6
I1-647	118-24	C 5000	3.69	3702818	bb	0	4816	-3.7
I1-648		ACN	3.78	16966	bb	0	1.75	
I1-649		ACN	3.76	17296	bb	0	1.82	
I1-650	119-1	QC 30	3.71	82191	bb	0	26.4	-12
I1-651	119-2	QC 30	3.7	83611	bb	0	27.2	-9.5
I1-652	119-3	QC 30	3.71	82796	bb	0	26.7	-11
I1-653	119-4	QC 300	3.69	437111	bb	0	309	3.1
I1-654	119-5	QC 300	3.69	454823	bb	0	327	8.9
I1-655	119-6	QC 300	3.69	424780	bb	0	297	-0.92

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-656	119-7	QC 5000	3.69	6526666	bb	0	9433	89
I1-657	119-8	QC 5000	3.69	6551039	bb	0	9474	89
I1-658	119-9	QC 5000	3.69	6298353	bb	0	9048	81
I1-659		ACN	3.78	17329	bb	0	1.82	
I1-660	113-6	stock 500 ng/mL	3.71	521341	bb	0	394	
I1-661	113-8a	stock stab 500 ng/mL	3.71	539902	bb	0	413	
I1-662		ACN	3.78	16670	bb	0	1.69	

Table A-8: Monkey Serum Calibration and QC Sample Data of Sequence 534002(PFHxA)JMS1

Quantify Compound Summary Report

Sample List:C:\MASSLYNX\534002.PRO\SampleDB\534002(PFHxA)JMS1
Last modified:Fri Apr 15 13:41:47 2005
Method:C:\MASSLYNX\534002.PRO\MethDB\534002 md9
Last modified:Fri Apr 01 12:06:42 2005
Job Code:

Printed:Thu Apr 21 12:24:35 2005

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-678a		sys suit	3.69	75600	bb	0	23.8	
I1-679a		sys suit	3.69	79040	bb	0	25.1	
I1-680a		sys suit	3.7	99663	bb	0	33.1	
I1-681a		ACN	3.76	23752	bb	0	6.32	
I1-682a	127-1	Solvent Blank	3.76	24650	bb	0	6.59	
I1-683a	127-2	Monkey Serum Blank	3.72	50386	bb	0	14.8	
I1-684a	127-3	Monkey Serum Blank	3.76	34661	bb	0	9.67	
I1-685a	127-4	C 10	3.74	57111	bbX	0	17.2	72
I1-686a	127-5	C 10	3.74	55282	bbX	0	16.5	65
I1-687a	127-6	C 10	3.72	65435	bbX	0	20.1	101
I1-688a	127-7	C 30	3.72	99684	bb	0	33.1	10
I1-689a	127-8	C 30	3.72	94433	bb	0	31.0	3.4
I1-690a	127-9	C 30	3.72	93282	bb	0	30.6	1.9
I1-691a	127-10	C 100	3.72	227211	bb	0	90.9	-9.1
I1-692a	127-11	C 100	3.72	218701	bb	0	86.7	-13
I1-693a	127-12	C 100	3.72	232444	bb	0	93.6	-6.5
I1-694a	127-13	C 300	3.72	563818	bb	0	294	-2.0
I1-695a	127-14	C 300	3.72	584421	bb	0	308	2.8
I1-696a	127-15	C 300	3.72	557567	bb	0	290	-3.5
I1-697a	127-16	C 1000	3.72	1522641	bb	0	1154	15
I1-698a	127-17	C 1000	3.72	1433804	bb	0	1059	5.9
I1-699a	127-18	C 1000	3.72	1476628	bb	0	1104	10
I1-700a	127-19	C 3000	3.7	3017635	bb	0	3146	4.9
I1-701a	127-20	C 3000	3.72	2919403	bb	0	2993	-0.23
I1-702a	127-21	C 3000	3.72	2946809	bb	0	3036	1.2
I1-703a	127-22	C 5000	3.72	3972374	bb	0	4787	-4.3
I1-704a	127-23	C 5000	3.7	3845966	bb	0	4554	-8.9
I1-705a	127-24	C 5000	3.7	3991190	bb	0	4822	-3.6
I1-706a		ACN	3.76	22833	bb	0	6.05	
I1-707a	128-1	QC 30	3.72	89461	bb	0	29.1	-3.0
I1-708a	128-4	QC 300	3.72	480493	bb	0	238	-21
I1-709a	128-7	QC 5000	3.72	3728605	bb	0	4342	-13
I1-710a		ACN	3.76	23079	bb	0	6.12	
I1-711a	129-1	1555T1, Day 0, 1M, T0	3.74	35407	bb	0	9.90	
I1-712a	129-2	1562T1, Day 0, 1M, T0	3.76	35136	bb	0	9.82	
I1-713a	129-3	1563T1, Day 0, 1M, T0	3.76	28989	bb	0	7.90	

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-714a	129-4	1572T1, Day 0, 1F, T0	3.74	33349	bb	0	9.25	
I1-715a	129-5	1576T1, Day 0, 1F, T0	3.76	34534	bb	0	9.62	
I1-716a	129-6	1573T1, Day 0, 1F, T0	3.74	33818	bb	0	9.40	
I1-717a	129-7	1555T1, Day 0, 1M, T1	3.7	11451585	bb	0	26801	
I1-718a	129-8	1562T1, Day 0, 1M, T1	3.7	13122406	bb	0	33927	
I1-719	129-9	1563T1, Day 0, 1M, T1	3.7	11335738	bb	0	26338	
I1-720	129-10	1572T1, Day 0, 1F, T1	3.7	11190497	bb	0	25762	
I1-721	129-11	1576T1, Day 0, 1F, T1	3.7	10510644	bb	0	23147	
I1-722	129-12	1573T1, Day 0, 1F, T1	3.7	8358802	bb	0	15750	
I1-723	129-13	1555T1, Day 0, 1M, T2	3.7	4141387	bb	0	5106	
I1-724	129-14	1562T1, Day 0, 1M, T2	3.7	6239138	bb	0	9760	
I1-725	129-15	1563T1, Day 0, 1M, T2	3.7	5486488	bb	0	7943	
I1-726	129-16	1572T1, Day 0, 1F, T2	3.7	5116630	bb	0	7111	
I1-727	129-17	1576T1, Day 0, 1F, T2	3.7	5407651	bb	0	7763	
I1-728	129-18	1573T1, Day 0, 1F, T2	3.7	4131240	bb	0	5086	
I1-729	129-19	1555T1, Day 0, 1M, T4	3.7	1754301	bb	0	1414	
I1-730	129-20	1562T1, Day 0, 1M, T4	3.7	3668678	bb	0	4235	
I1-731	129-21	1563T1, Day 0, 1M, T4	3.7	2285806	bb	0	2079	
I1-732		ACN	3.76	24163	bb	0	6.44	
I1-733	128-2	QC 30	3.7	92614	bb	0	30.3	1.1
I1-734	128-5	QC 300	3.7	518185	bb	0	263	-12
I1-735	128-8	QC 5000	3.7	4299516	bb	0	5412	8.2

Table A-9: Monkey Serum Calibration and QC Sample Data of Sequence 534002(PFHxA)JMS3

Quantify Compound Summary Report

Sample List:C:\MASSLYNX\534002.PRO\SampleDB\534002(PFHxA)JMS3
Last modified:Thu Apr 21 08:30:29 2005
Method:C:\MASSLYNX\534002.PRO\MethDB\534002 md9
Last modified:Fri Apr 01 12:06:42 2005
Job Code:

Printed:Thu Apr 21 10:16:15 2005

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1005		sys suit	3.9	38540	bb	0	24.4	
I1-1006		sys suit	3.85	40339	bb	0	25.6	
I1-1007		sys suit	3.85	49155	bb	0	31.5	
I1-1008						0		
I1-1009	145-1	Solvent Blank	3.92	5049	bb	0	3.16	
I1-1010	145-2	Monkey Serum Blank	3.92	5770	bb	0	3.59	
I1-1011	145-5	C 30	3.85	46476	bb	0	29.7	-0.94
I1-1012	145-6	C 30	3.85	50719	bb	0	32.6	8.7
I1-1013	145-7	C 100	3.85	123961	bb	0	86.8	-13
I1-1014	145-8	C 100	3.87	142117	bb	0	101	1.2
I1-1015	145-9	C 300	3.85	324428	bb	0	264	-12
I1-1016	145-10	C 300	3.87	385266	bb	0	324	8.0
I1-1017	145-11	C 1000	3.85	1069042	bb	0	1156	16
I1-1018	145-12	C 1000	3.85	941927	bb	0	982	-1.8
I1-1019	145-13	C 3000	3.85	2213581	bb	0	3046	1.5
I1-1020	145-14	C 3000	3.87	2429461	bb	0	3462	15
I1-1021	145-15	C 5000	3.85	2951533	bb	0	4541	-9.2
I1-1022	145-16	C 5000	3.85	2988287	bb	0	4621	-7.6
I1-1023		ACN				0		
I1-1024	146-1	QC 30	3.88	48104	bb	0	30.8	2.8
I1-1025	146-4	QC 300	3.87	351837	bb	0	290	-3.2
I1-1026	146-7	QC 10000	3.79	99858	MM	100	6819	-32
I1-1027		ACN				0		
I1-1028	148-2	FTS 1 5000ng	3.85	3195713	MM	0	5080	1.6
I1-1029	148-3	FTS 1 5000ng	3.85	3485000	bb	0	5746	15
I1-1030	148-4	FTS 1 5000ng	3.85	3357619	bb	0	5449	9.0
I1-1031	148-5	FTS 2 5000ng	3.85	3093958	MM	0	4853	-2.9
I1-1032	148-6	FTS 2 5000ng	3.85	2969017	MM	0	4579	-8.4
I1-1033	148-7	FTS 2 5000ng	3.85	3385508	bb	0	5514	10
I1-1034	148-8	FTS 3 5000ng	3.85	2973270	MM	0	4588	-8.2
I1-1035	148-9	FTS 3 5000ng	3.85	3086252	MM	0	4836	-3.3
I1-1036	148-10	FTS 3 5000ng	3.85	3159257	bb	0	4998	-0.038
I1-1037	148-11	LTS 300ng	3.85	322546	MM	0	262	-13
I1-1038	148-12	LTS 300ng	3.85	334664	bb	0	274	-8.8
I1-1039	148-13	LTS 300ng	3.85	389096	bb	0	328	9.3
I1-1040		ACN	3.94	3871	bb	0	2.45	

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1041	146-2	QC 30	3.86	40755	bb	0	25.9	-14
I1-1042	146-5	QC 300	3.85	339608	bb	0	278	-7.2
I1-1043	146-8	QC 10000	3.85	132165	bb	100	9328	-6.7
I1-1044		ACN	3.94	3980	bb	0	2.52	
I1-1045	147-1	1555T1, Day 0, 1M, T1	3.85	234621	bb	100	18004	
I1-1046	147-2	1562T1, Day 0, 1M, T1	3.85	320518	bb	100	25993	
I1-1047	147-3	1563T1, Day 0, 1M, T1	3.85	270397	bb	100	21259	
I1-1048	147-4	1572T1, Day 0, 1F, T1	3.85	311637	bb	100	25140	
I1-1049	147-5	1576T1, Day 0, 1F, T1	3.85	256739	bb	100	20004	
I1-1050	147-6	1573T1, Day 0, 1F, T1	3.85	228624	bb	100	17469	
I1-1051	147-7	1562T1, Day 0, 1M, T2	3.85	158009	bb	100	11419	
I1-1052	147-8	1563T1, Day 0, 1M, T2	3.85	126330	bb	100	8866	
I1-1053	147-9	1572T1, Day 0, 1F, T2	3.85	108094	bb	100	7447	
I1-1054	147-10	1576T1, Day 0, 1F, T2	3.85	113942	bb	100	7898	
I1-1055	147-11	1572T1, Day 0, 1F, T4	3.85	1819165	bb	0	2332	
I1-1056	147-12	1576T1, Day 0, 1F, T4	3.85	1331554	bb	2	3078	
I1-1057	147-13	1573T1, Day 0, 1F, T4	3.85	1626066	bb	0	2006	
I1-1058	147-14	1555T1, Day 0, 1M, T8	3.85	934398	bb	0	972	
I1-1059	147-15	1562T1, Day 0, 1M, T8	3.85	1562594	bb	0	1902	
I1-1060	147-16	1563T1, Day 0, 1M, T8	3.85	609139	bb	0	567	
I1-1061	147-17	1572T1, Day 0, 1F, T8	3.85	402389	bb	0	341	
I1-1062	147-18	1576T1, Day 0, 1F, T8	3.85	493622	bb	0	438	
I1-1063	147-19	1573T1, Day 0, 1F, T8	3.85	333003	bb	0	272	
I1-1064	147-20	1555T1, Day 0, 1M, T24	3.85	34352	bb	5	108	
I1-1065	147-21	1562T1, Day 0, 1M, T24	3.85	51020	bb	0	32.8	
I1-1066	147-22	1563T1, Day 0, 1M, T24	3.85	47339	bb	0	30.3	
I1-1067	147-23	1572T1, Day 0, 1F, T24	3.86	10423	bb	0	6.41	
I1-1068	147-24	1576T1, Day 0, 1F, T24	3.85	120802	bb	0	84.3	
I1-1069	147-25	1573T1, Day 0, 1F, T24	3.85	18378	bb	0	11.3	
I1-1070	147-26	1555T1, Day 0, 1M, T48	3.87	14943	bb	0	9.18	
I1-1071	147-27	1562T1, Day 0, 1M, T48	3.85	30190	bb	0	18.9	
I1-1072	147-28	1563T1, Day 0, 1M, T48	3.85	54946	bb	0	35.5	
I1-1073	147-29	1572T1, Day 0, 1F, T48	3.88	8963	bb	0	5.52	
I1-1074	147-30	1576T1, Day 0, 1F, T48	3.85	19429	bb	0	12.0	
I1-1075	148-1	1573T1, Day 0, 1F, T48	3.87	15176	bb	0	9.33	
I1-1076		ACN	3.92	4116	bb	0	2.60	
I1-1077	146-3	QC 30	3.81	32663	MM	0	20.5	-32
I1-1078	146-6	QC 300	3.83	338062	bb	0	277	-7.7
I1-1079	146-9	QC 10000	3.85	132315	bb	100	9340	-6.6
I1-1080		ACN	3.92	3599	bb	0	2.29	

Table A10: Monkey Urine Calibration, QC and Experimental Samples Sequence 534002(PFHxA)KMU1

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534002.PRO\SampleDB\534002(PFHxA)kMu1
Last modified: Thu Apr 21 10:02:36 2005
Method: C:\MASSLYNX\534002.PRO\MethDB\534002 md9
Last modified: Fri Apr 01 12:06:42 2005
Job Code:

Printed: Thu Apr 21 10:10:07 2005

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-931		sys suit	3.72	138572	bbI	0		
I1-932		sys suit	3.77	245658	bbI	0		
I1-933		sys suit	3.79	348471	bbI	0		
I1-934		ACN	3.81	339818	bbI	0		
I1-935	140-1	Solvent Blank	3.81	374938	bbI	0		
I1-936	140-2	Monkey Urine Blank	3.83	525852	bd	0	82.0	
I1-937	140-3	C 30	3.83	433172	MM	0	35.0	17
I1-938	140-4	C 30	3.83	414611	MM	0	26.3	-12
I1-939	140-5	C 100	3.83	552693	bd	0	97.1	-2.9
I1-940	140-6	C 100	3.83	572010	bd	0	108	8.3
I1-941	140-7	C 300	3.79	802263	bb	0	265	-12
I1-942	140-8	C 300	3.81	837276	bb	0	292	-2.7
I1-943	140-9	C 1000	3.77	1626838	bb	0	1020	2.0
I1-944	140-10	C 1000	3.77	1668727	bd	0	1063	6.3
I1-945	140-11	C 3000	3.76	3312669	bb	0	2897	-3.4
I1-946	140-12	C 3000	3.76	3620503	bb	0	3258	8.6
I1-947	140-13	C 5000	3.74	5009293	bb	0	4910	-1.8
I1-948	140-14	C 5000	3.74	4960055	bb	0	4851	-3.0
I1-949		ACN	3.81	403939	bb	0	21.2	
I1-950	141-1	QC 30	3.81	499704	bb	0	68	126
I1-951	141-4	QC 300	3.79	858893	bd	0	309	2.9
I1-952	141-7	QC 5000	3.74	4452969	MM	0	4245	-15
I1-953	141-10	QC 10000	3.79	576328	bb	100	11091	11
I1-954		ACN	3.81	393999	bb	0	15.9	
I1-955	137-13	FTS, 1, 300	3.77	848133	bb	0	300	
I1-956	137-14	FTS, 1, 300	3.78	876094	bb	0	322	
I1-957	137-15	FTS, 1, 300	3.78	827341	bb	0	284	
I1-958	137-16	FTS, 2, 300	3.77	878568	bb	0	324	
I1-959	137-17	FTS, 2, 300	3.81	851363	bb	0	303	
I1-960	137-18	FTS, 2, 300	3.79	867258	bb	0	315	
I1-961	137-19	FTS, 3, 300	3.7	899483	bb	0	341	
I1-962	137-20	FTS, 3, 300	3.7	884409	bb	0	329	
I1-963	137-21	FTS, 3, 300	3.72	866488	bb	0	315	
I1-964	137-22	FTS, 1, 5000	3.69	5463314	bb	0	5454	
I1-965	137-23	FTS, 1, 5000	3.72	4356035	bb	0	4129	
I1-966	137-24	FTS, 1, 5000	3.74	4956521	bb	0	4847	

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-967	137-25	FTS, 2, 5000	3.78	4993254	bb	0	4891	
I1-968	137-26	FTS, 2, 5000	3.76	5086359	bb	0	5002	
I1-969	137-27	FTS, 2, 5000	3.76	5067617	bb	0	4980	
I1-970	137-28	FTS, 3, 5000	3.74	5084375	bb	0	5000	
I1-971	137-29	FTS, 3, 5000	3.74	4907269	bb	0	4788	
I1-972	137-30	FTS, 3, 5000	3.72	4994411	bb	0	4892	
I1-973	137-1	1573T1, Day 5, 1F	3.81	550440	bd	0	95.8	
I1-974	137-2	1555T1, Day 6, 1M	3.76	878037	bd	0	324	
I1-975	137-3	1562T1, Day 6, 1M	3.74	1110698	bb	0	521	
I1-976	137-4	1563T1, Day 6, 1M	3.79	756949	bd	0	231	
I1-977	137-5	1572T1, Day 6, 1F	3.83	429389	bb	0	33.2	
I1-978	137-6	1576T1, Day 6, 1F	3.79	698290	bd	0	190	
I1-979	137-7	1573T1, Day 6, 1F	3.79	652456	bb	0	159	
I1-980	137-8	1555T1, Day 7, 1M	3.74	586227	bd	0	117	
I1-981	137-9	1563T1, Day 7, 1M	3.74	512482	bb	0	74.7	
I1-982	137-10	1572T1, Day 7, 1F	3.77	532530	bb	0	85.7	
I1-983	137-11	1576T1, Day 7, 1F	3.78	406265	bb	0	22.3	
I1-984	137-12	1573T1, Day 7, 1F	3.76	489788	bb	0	62.7	
I1-985		ACN	3.77	367188	bbI	0		
I1-986	141-2	QC 30	3.78	429418	bb	0	33.2	11
I1-987	141-5	QC 300	3.6	793262	bb	0	258	-14
I1-988	141-8	QC 5000	3.53	4479404	MM	0	4276	-14
I1-989	141-11	QC 10000	3.63	565493	bb	100	10450	4.5

Table A11: Monkey Urine Calibration, QC and Experimental Samples Sequence 534002(PFHxA)KMU2

Quantify Compound Summary Report

Sample List:C:\MASSLYNX\534002.PRO\SampleDB\534002(PFHxA)kMu2
Last modified:Tue Apr 19 08:26:20 2005
Method:C:\MASSLYNX\534002.PRO\MethDB\534002 md9
Last modified:Fri Apr 01 12:06:42 2005
Job Code:

Printed:Wed Apr 20 08:20:57 2005

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-931a		sys suit	3.88	31689	bb	0	18.0	
I1-932a		sys suit	3.85	39919	bb	0	23.3	
I1-933a		sys suit	3.83	40124	bb	0	23.4	
I1-934a						0		
I1-935a	140-1	Solvent Blank	3.9	8708	bb	0	4.34	
I1-936a	140-2	Monkey Urine Blank	3.83	67473	bb	0	42.4	
I1-937a	140-3	C 30	3.83	47539	bb	0	28.4	-5.3
I1-938a	140-4	C 30	3.83	55412	bb	0	33.8	13
I1-939a	140-5	C 100	3.83	128791	bb	0	90.1	-9.9
I1-940a	140-6	C 100	3.83	135806	bb	0	95.9	-4.1
I1-941a	140-7	C 300	3.83	354506	bb	0	304	1.2
I1-942a	140-8	C 300	3.83	354188	bb	0	303	1.1
I1-943a	140-9	C 1000	3.83	869522	bb	0	928	-7.2
I1-944a	140-10	C 1000	3.83	1073611	bb	0	1213	21
I1-945a	140-11	C 3000	3.83	2110402	bb	0	2915	-2.8
I1-946a	140-12	C 3000	3.83	2151103	bb	0	2989	-0.36
I1-947a	140-13	C 5000	3.83	3022826	bb	0	4696	-6.1
I1-948a	140-14	C 5000	3.83	3253023	bb	0	5181	3.6
I1-949a		ACN	3.93	5690	bb	0	2.75	
I1-950a	141-2	QC 30	3.83	52909	bb	0	32.1	7.0
I1-951a	141-5	QC 300	3.83	326897	bb	0	275	-8.3
I1-952a	141-8	QC 5000	3.83	2973437	bb	0	4593	-8.1
I1-953a	141-11	QC 10000	3.83	167633	bb	100	12306	23
I1-954a		ACN	3.93	5117	bb	0	2.45	
I1-955a	142-1	1555T1, Day 0, 1M, T0-6	3.83	3345983	bb	100	538105	
I1-956a	142-2	1562T1, Day 0, 1M, T0-6	3.83	3235650	bb	100	514407	
I1-957a	136-3	1563T1, Day 0, 1M, T0-6	3.86	195843	bb	0	148	
I1-958a	142-3	1572T1, Day 0, 1F, T0-6	3.83	1866592	bb	100	248101	
I1-959a	136-5	1573T1, Day 0, 1F, T0-6	3.86	1406822	bb	0	1717	
I1-960a	142-4	1555T1, Day 0, 1M, T6-12	3.83	278542	bb	100	22638	
I1-961a	142-5	1563T1, Day 0, 1M, T6-12	3.85	3191021	bb	2	10098	
I1-962a	142-6	1572T1, Day 0, 1F, T6-12	3.83	3267303	bb	2	10423	
I1-963a	136-9	1573T1, Day 0, 1F, T6-12	3.86	1158204	bb	0	1337	
I1-964a	142-7	1555T1, Day 0, 1M, T12-24	3.81	2420672	bb	2	6987	
I1-965a	136-11	1563T1, Day 0, 1M, T12-24	3.81	2422202	bb	0	3497	
I1-966a	136-12	1572T1, Day 0, 1F, T12-24	3.85	1759839	bb	0	2297	

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-967a	142-8	1573T1, Day 0, 1F, T12-24	3.83	3963612	bb	2	13526	
I1-968a	136-14	1555T1, Day 2, 1M, T	3.81	1650906	bb	0	2113	
I1-969a	136-15	1563T1, Day 2, 1M, T	3.81	1775512	bb	0	2324	
I1-970a	136-16	1572T1, Day 2, 1F, T	3.83	585431	bb	0	564	
I1-971a	136-17	1555T1, Day 3, 1M, T	3.83	528654	bb	0	497	
I1-972a	136-18	1563T1, Day 3, 1M, T	3.85	482766	bb	0	444	
I1-973a	136-19	1572T1, Day 3, 1F, T	3.83	503872	bb	0	468	
I1-974a	136-20	1576T1, Day 3, 1F, T	3.83	1325953	bb	0	1591	
I1-975a	136-21	1573T1, Day 3, 1F, T	3.86	192710	bb	0	145	
I1-976a	136-22	1555T1, Day 4, 1M	3.83	736053	bb	0	751	
I1-977a	136-23	1562T1, Day 4, 1M	3.83	722566	bb	0	734	
I1-978a	136-24	1563T1, Day 4, 1M	3.86	1280738	bb	0	1521	
I1-979a	136-25	1572T1, Day 4, 1F	3.83	458424	bb	0	416	
I1-980a	136-26	1576T1, Day 4, 1F	3.83	1225340	bb	0	1437	
I1-981a	136-27	1573T1, Day 4, 1F	3.83	604247	bb	0	587	
I1-982a	136-28	1555T1, Day 5, 1M	3.83	742110	bb	0	759	
I1-983a	136-29	1563T1, Day 5, 1M	3.86	694230	bb	0	698	
I1-984a	136-30	1572T1, Day 5, 1F	3.86	310997	bb	0	259	
I1-985a		ACN	3.92	5581	bb	0	2.69	
I1-986a	141-3	QC 30	3.83	57096	bb	0	35.0	17
I1-987a	141-6	QC 300	3.83	355103	bb	0	304	1.4
I1-988a	141-9	QC 5000	3.83	3279847	bb	0	5239	4.8
I1-989a	141-12	QC 10000	3.83	153007	bb	100	11042	10

Table A12: Monkey Serum Calibration, QC and Experimental Samples Sequence 534002(PFHxA)LMS1

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534002.PRO\SampleDB\534002(PFHxA)lMS1
Last modified: Thu Jul 07 08:15:38 2005
Method: C:\MASSLYNX\534002.PRO\MethDB\534002 PFHxA bio
Last modified: Thu Jul 07 07:58:16 2005
Job Code:

Printed: Fri Jul 08 16:04:35 2005

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1688a		sys suit	3.71	1154494	bb	0	210	
I1-1689a		sys suit	3.67	1261751	bb	0	238	
I1-1690a		sys suit	3.71	1426433	bb	0	282	
I1-1691a		ACN	3.74	125701	bb	0	11.4	
I1-1692a	235-1	Solvent Blank	3.78	95792	bb	0	8.12	
I1-1693a	235-2	Monkey Serum Blank	3.76	115951	bb	0	10.3	
I1-1694a	235-3	C 30	3.74	280640	bb	0	31.8	6.0
I1-1695a	235-4	C 30	3.74	263152	bb	0	29.3	-2.5
I1-1696a	235-5	C 100	3.74	628336	bb	0	92.0	-8.0
I1-1697a	235-6	C 100	3.73	648550	bb	0	96.0	-4.0
I1-1698a	235-7	C 300	3.74	1514276	bb	0	306	2.2
I1-1699a	235-8	C 300	3.74	1630009	bb	0	340	13
I1-1700a	235-9	C 1000	3.74	3571614	bb	0	1040	4.0
I1-1701a	235-10	C 1000	3.73	3276324	bb	0	918	-8.2
I1-1702a	235-11	C 3000	3.74	7187658	bb	0	2932	-2.3
I1-1703a	235-12	C 3000	3.72	7215258	bb	0	2949	-1.7
I1-1704a	235-13	C 5000	3.73	10809480	bb	0	5466	9.3
I1-1705a	235-14	C 5000	3.72	9829064	bb	0	4721	-5.6
I1-1706a		ACN	3.78	80705	bb	0	6.57	
I1-1707a	236-1	QC 30	3.74	273007	bb	0	30.7	2.3
I1-1708a	236-4	QC 300	3.72	1413924	bb	0	279	-7.1
I1-1709a	236-7	QC 5000	3.72	10730639	bb	0	5405	8.1
I1-1710a		ACN	3.76	129472	bb	0	11.8	
I1-1711a	237-1	FTS 1 300ng	3.72	1293900	bb	0	246	
I1-1712a	237-2	FTS 1 300ng	3.72	1251003	bb	0	235	
I1-1713a	237-3	FTS 1 300ng	3.72	1367230	bb	0	266	
I1-1714a	237-4	FTS 3 300ng	3.72	1177408	bb	0	216	
I1-1715a	237-5	FTS 3 300ng	3.72	1272596	bb	0	241	
I1-1716a	237-6	FTS 3 300ng	3.72	1177813	bb	0	216	
I1-1717a	237-7	FTS 3 300ng	3.73	1207796	bb	0	224	

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1718a	237-8	FTS 3 300ng	3.72	1088638	bb	0	194	
I1-1719a	237-9	FTS 3 300ng	3.72	1347417	bb	0	261	
I1-1720a	237-10	LTS 300ng	3.72	1340466	bb	0	259	
I1-1721a	237-11	LTS 300ng	3.72	1243805	bb	0	233	
I1-1722a	237-12	LTS 300ng	3.72	1292841	bb	0	246	
I1-1723a		ACN	3.74	118737	bb	0	10.6	
I1-1724a	236-2	QC 30	3.72	272348	bb	0	30.6	2.0
I1-1725a	236-5	QC 300	3.72	1476451	bb	0	296	-1.4
I1-1726a	236-8	QC 5000	3.72	10971363	bb	0	5593	12
I1-1727a		ACN	3.76	77159	bb	0	6.21	
I1-1728a	237-13	LTS 5000ng	3.71	10812214	bb	0	5468	
I1-1729a	237-14	LTS 5000ng	3.7	11105327	bb	0	5699	
I1-1730a	237-15	LTS 5000ng	3.71	11387165	bb	0	5924	
I1-1731a	237-16	4hrRTS 300ng	3.71	1210226	bb	0	225	
I1-1732a	237-17	4hrRTS 300ng	3.71	1241984	bb	0	233	
I1-1733a	237-18	4hrRTS 300ng	3.71	1262222	bb	0	238	
I1-1734a	237-19	4hrRTS 5000ng	3.71	9874598	bb	0	4755	
I1-1735a	237-20	4hrRTS 5000ng	3.7	10144165	bb	0	4956	
I1-1736a	237-21	4hrRTS 5000ng	3.71	10030087	bb	0	4871	
I1-1737a		ACN	3.76	74250	bb	0	5.93	
I1-1738a	236-3	QC 30	3.72	241690	bb	0	26.2	-13
I1-1739a	236-6	QC 300	3.71	1395169	bb	0	273	-8.9
I1-1740a	236-9	QC 5000	3.71	10893035	bb	0	5531	11
I1-1741a		ACN	3.75	139162	bb	0	13.0	

AGC Chemical

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534002.PRO\SampleDB\534002(PFHxA)MMU
Last modified: Thu Jul 07 13:48:14 2005
Method: C:\MASSLYNX\534002.PRO\MethDB\534002 PFHxA bio
Last modified: Mon Jul 18 10:05:15 2005
Job Code:

Printed: Fri Jul 22 10:10:31 2005

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.
I1-1765	242-1	LTS 300	3.71	1095795	bb	0
I1-1766	242-2	LTS 300	3.71	1101517	bb	0
I1-1767	242-3	LTS 300	3.69	1043513	bb	0
I1-1768	242-4	LTS 5000	3.71	7124059	bb	0
I1-1769	242-5	LTS 5000	3.71	6736695	bb	0
I1-1770	242-6	LTS 5000	3.7	7811020	bb	0

Table A14: Monkey Urine Calibration, QC and Experimental Samples Sequence 534002(PFHxA)MMU1

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534002.PRO\SampleDB\534002(PFHxA)MMU1
Last modified: Fri Jul 08 08:47:26 2005
Method: C:\MASSLYNX\534002.PRO\MethDB\534002 PFHxA bio
Last modified: Thu Jul 07 07:58:16 2005
Job Code:

Printed: Mon Jul 11 08:56:33 2005

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1742a		sys suit	3.7	153222	bb	0	25.3	
I1-1743a		sys suit	3.69	178601	bb	0	30.9	
I1-1744a		sys suit	3.69	190041	bb	0	33.5	
I1-1745a		ACN	3.72	97256	bb	0	14.1	
I1-1746a	240-1	Solvent Blank				0		
I1-1747a	240-2	Monkey Urine Blank	3.72	79001	bb	0	10.8	
I1-1748a	240-3	C 30	3.69	183116	bb	0	31.9	6.3
I1-1749a	240-4	C 30	3.69	177602	bb	0	30.6	2.1
I1-1750a	240-5	C 100	3.69	409325	bb	0	91.0	-9.0
I1-1751a	240-6	C 100	3.69	410075	bb	0	91.2	-8.8
I1-1752a	240-7	C 300	3.69	1053637	bb	0	316	5.3
I1-1753a	240-8	C 300	3.69	1020444	bb	0	303	0.92
I1-1754a	240-9	C 1000	3.69	2527967	bb	0	1011	1.1
I1-1755a	240-10	C 1000	3.69	2632823	bb	0	1067	6.7
I1-1756a	240-11	C 3000	3.67	5910103	bb	0	3157	5.2
I1-1757a	240-12	C 3000	3.69	5604800	bb	0	2939	-2.0
I1-1758a	240-13	C 5000	3.69	7855849	bb	0	4635	-7.3
I1-1759a	240-14	C 5000	3.67	8390533	bb	0	5067	1.3
I1-1760a		ACN				0		
I1-1761a	241-1	QC 30	3.69	186234	bb	0	32.6	8.6
I1-1762a	241-4	QC 300	3.67	1032559	bb	0	308	2.5
I1-1763a	241-7	QC 5000	3.69	7985722	bb	0	4739	-5.2
I1-1764a		ACN	3.7	93812	bb	0	13.4	
I1-1765a	242-1	LTS 300ng	3.69	1086927	bb	0	329	
I1-1766a	242-2	LTS 300ng	3.67	1018952	bb	0	302	
I1-1767a	242-3	LTS 300ng	3.67	1010015	bb	0	299	
I1-1768a	242-4	LTS 5000ng	3.67	8638501	bb	0	5271	
I1-1769a	242-5	LTS 5000ng	3.67	8993384	bb	0	5566	
I1-1770a	242-6	LTS 5000ng	3.67	8366723	bb	0	5047	
I1-1771a		ACN	3.71	89222	bb	0	12.6	

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1772a	241-2	QC 30	3.69	169569	bb	0	28.9	-3.8
I1-1773a	241-5	QC 300	3.67	948334	bb	0	275	-8.4
I1-1774a	241-8	QC 5000	3.67	7481773	bb	0	4339	-13
I1-1775a		ACN				0		
I1-1776a	242-7	4hrRTS 300ng	3.67	914757	bb	0	262	
I1-1777a	242-8	4hrRTS 300ng	3.67	875115	bb	0	247	
I1-1778a	242-9	4hrRTS 300ng	3.67	956467	bb	0	278	
I1-1779a	242-10	4hrRTS 5000ng	3.67	7496815	bb	0	4351	
I1-1780a	242-11	4hrRTS 5000ng	3.67	7545296	bb	0	4389	
I1-1781a	242-12	4hrRTS 5000ng	3.67	7538916	bb	0	4384	
I1-1782a		ACN				0		
I1-1783a	241-3	QC 30	3.69	162552	bb	0	27.3	-8.9
I1-1784a	241-6	QC 300	3.67	939106	bb	0	271	-9.6
I1-1785a	241-9	QC 5000	3.67	7210166	bb	0	4128	-17
I1-1786a		ACN	3.7	84321	bb	0	11.7	

Table A15: Monkey Urine Calibration, QC and Experimental Samples Sequence 534002(PFHxA)OMU1

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534002.PRO\SampleDB\534002(PFHxA)OMU1
Last modified: Mon Jul 18 10:23:09 2005
Method: C:\MASSLYNX\534002.PRO\MethDB\534002 PFHxA bio
Last modified: Mon Jul 18 10:05:15 2005
Job Code:

Printed: Thu Aug 04 10:20:52 2005

Compound 1: PFHxA

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1955a		sys suit	3.83	80190	bb	0	183	
I1-1956a		sys suit	3.69	5703	bd	0	19.3	
I1-1957a		sys suit	3.73	7205	bd	0	22.9	
I1-1958a		ACN	3.94	10788	bb	0	31.2	
I1-1959a	265-1	Solvent Blank	3.94	11828	bb	0	33.6	
I1-1960a	265-2	Monkey Urine Blank	3.78	3793	bd	0	14.3	
I1-1961a	265-3	C 30	3.74	12453	bdX	0	35.0	17
I1-1962a	265-4	C 30	3.74	14547	bdX	0	39.6	32
I1-1963a	265-5	C 100	3.74	42944	bb	0	100	0.38
I1-1964a	265-6	C 100	3.74	44134	bb	0	103	2.9
I1-1965a	265-7	C 300	3.74	113866	bb	0	263	-12
I1-1966a	265-8	C 300	3.75	130959	bd	0	306	2.1
I1-1967a	265-9	C 1000	3.75	351427	bd	0	1032	3.2
I1-1968a	265-10	C 1000	3.74	374464	bd	0	1129	13
I1-1969a	265-11	C 3000	3.74	657634	bb	0	2770	-7.7
I1-1970a	265-12	C 3000	3.75	682957	bb	0	2967	-1.1
I1-1971a	265-13	C 5000	3.74	831387	bb	0	4352	-13
I1-1972a	265-14	C 5000	3.75	955267	bb	0	5893	18
I1-1973a		ACN	3.78	7014	bd	0	22.5	
I1-1974a	266-1	QC 30	3.74	22436	bb	0	56.7	89
I1-1975a	266-4	QC 300	3.74	139158	bd	0	327	9.1
I1-1976a	266-7	QC 5000	3.74	960241	bb	0	5964	19
I1-1977a		ACN	3.78	5765	bb	0	19.4	
I1-1978a	267-1	LTS 300ng	3.73	139989	bd	0	330	
I1-1979a	267-2	LTS 300ng	3.72	133434	bd	0	313	
I1-1980a	267-3	LTS 300ng	3.73	136947	bd	0	322	
I1-1981a		ACN	3.78	4767	bb	0	16.9	
I1-1982a	266-2	QC 30	3.73	22010	bb	0	55.8	86
I1-1983a	266-5	QC 300	3.72	136934	bd	0	322	7.2
I1-1984a	266-8	QC 5000	3.73	890831	bb	0	5039	0.78

Compound 1: PFBS

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1985a		ACN	3.78	5291	bb	0	18.2	
I1-1986a	267-4	LTS 5000ng	3.73	919357	bb	0	5402	
I1-1987a	267-5	LTS 5000ng	3.73	928286	bb	0	5520	
I1-1988a	267-6	LTS 5000ng	3.73	968468	bb	0	6083	
I1-1989a		ACN	3.78	5576	bb	0	18.9	
I1-1990a	266-3	QC 30	3.73	19457	bb	0	50.3	68
I1-1991a	266-6	QC 300	3.73	133316	bb	0	312	4.1
I1-1992a	266-9	QC 5000	3.72	878784	bb	0	4893	-2.1
I1-1993a		ACN	3.78	5067	bb	0	17.7	

APPENDIX G

Bioanalytical Report (WIL Research Laboratories, LLC) [PFBS]

A PHARMACOKINETIC (IN BLOOD) AND EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Analyses Of PFBS In Serum And Urine Samples

Analytical Chemistry Department

WIL Research Laboratories, LLC

TABLE OF CONTENTS

Page

INDEX OF TABLES

Page

INDEX OF FIGURES

Page

Page

Page

Page

INDEX OF ATTACHMENTS

1. INTRODUCTION

This report provides a detailed description and validation of a high performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS) method in the negative electrospray ionization (ESI-) mode for the determination of nonafluoro-1-butanesulfonic acid (PFBS) in monkey serum. The method was cross-validated to determine PFBS in monkey urine. Method sensitivity, specificity/selectivity, calibration reproducibility, accuracy and precision were assessed and validated. Stability of PFBS in processed samples, in samples during frozen storage (-20°C), in samples after short term (4 hour) room temperature storage and in samples after the freeze-thaw process was evaluated.

This report details the analytical results from the determination of PFBS in monkey serum and urine samples. Analysis of serum samples resulted in levels ranging from not detected (ND) to 93,766 ng PFBS/mL. Analysis of urine samples resulted in levels ranging from less than the lower limit of quantitation (LLOQ) to 2,588,991 ng PFBS/mL.

2. BLANK MATRIX IDENTIFICATION

Blank monkey serum and urine were obtained from Bioreclamation, Inc., East Meadow, New York.

3. EXPERIMENTAL

3.1. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Instrument:	Hewlett Packard 1100 liquid chromatograph equipped with a diode array detector, autosampler, Micromass tandem quadrupole Quattro Ultima™ Mass Spectrometer and MassLynx™ software, or equivalent system
Column:	ACE C8 50 x 2.1 mm with a C8 guard cartridge, or equivalent
Column Temperature:	35°C
Mobile Phase:	A 10 mM ammonium acetate B Acetonitrile (ACN)

Gradient	Time (minutes)	Solvent A (%)	Solvent B (%)	Flow (mL/minute)
	0.00	80.0	20.0	0.3
	0.50	80.0	20.0	0.3
	1.50	10.0	90.0	0.3
	3.50	10.0	90.0	0.3
	3.60	80.0	20.0	0.4
	9.90	80.0	20.0	0.4
	10.0	80.0	20.0	0.3

Flow Rate: 0.3 mL/minute

Detector: Mass spectrometer with conditions as described in Section 3.2.

Injection Volume: 10 µL

Retention Time: Approximately 3.6 minutes for PFBS

Run Time: 10 minutes

Note: The retention times and run times varied depending on column performance.

3.2. MASS SPECTROMETRY

3.2.1. INSTRUMENT

A Micromass Quattro Ultima™ (or equivalent system) tandem mass spectrometer equipped with an ESI- interface was used in this study. Data acquisition and analysis were performed using MassLynx™ software version 3.4.

3.2.2. SOURCE PARAMETERS

Source:	ESI-
Capillary:	3.0 kV
Cone:	45 V
Hexapole 1:	0 V
Aperture 1:	0 V
Hexapole 2:	0 V
Source Block Temperature:	100°C
Desolvation Temperature:	300°C
Cone Gas Flow :	Approximately 100 L nitrogen/hour
Desolvation Gas Flow:	Approximately 500 L nitrogen/hour

Note: Settings varied depending on mass spectrometer performance.

3.2.3. ACQUISITION PARAMETERS

Function Type:	SIR (selected ion recording)
Precursor/Product Ion:	m/z 299
Dwell Time:	0.5 second

Note: Settings varied depending on mass spectrometer performance.

3.3. PREPARATION OF 10 MM AMMONIUM ACETATE

This solution was prepared by dissolving approximately 0.77 g of ammonium acetate in 1 L of deionized (DI) water. The solution was stirred to achieve complete dissolution and vacuum degassed. The preparation was scaled as needed, *i.e.*, if the volume of the preparation was doubled, then the stated amounts of any constituents were doubled.

3.4. PREPARATION OF PRIMARY STOCK SOLUTION

A stock solution of PFBS (WIL log no. 6396A) was prepared at a concentration of 1000 µg/mL in ACN. The solution was stirred to achieve complete dissolution.

3.5. PREPARATION OF CALIBRATION SAMPLES

An aliquot of the primary stock solution was diluted with ACN to yield a secondary stock solution at 25 µg PFBS/mL. Aliquots of this secondary stock solution were diluted with ACN to yield fortification solutions from 0.15 to 5 µg PFBS/mL.

Calibration samples containing 30 to 1,000 ng/mL PFBS were prepared by addition of 20 µL of the appropriate fortification solution to 0.1 mL of blank matrix in 1.5-mL conical tubes. The calibration samples were processed as described in Section 3.7. (Serum and Urine Sample Processing).

3.6. PREPARATION OF QUALITY CONTROL STOCK SOLUTIONS AND QUALITY CONTROL SAMPLES

An aliquot of the primary stock solution was diluted with ACN to yield a secondary stock solution at 25 µg PFBS/mL. Aliquots of this secondary stock solution were diluted with ACN to yield fortification solutions ranging from 0.15 to 3.75 µg PFBS/mL.

Quality control (QC) samples at concentrations of 30, 100 and 750 ng PFBS/mL were prepared by adding 20 µL of the appropriate fortification solution to 0.1 mL of blank matrix in 1.5-mL conical tubes. Dilutional QC samples were prepared at a concentration of 10,000 ng PFBS/mL by adding 10 µL of the primary stock solution to 990 µL of blank matrix. A 10-µL aliquot of the dilutional QC was diluted to 1 mL with blank matrix. The QC samples were processed as described in Section 3.7 (Serum and Urine Sample Processing).

3.7. SERUM AND URINE SAMPLE PROCESSING

Aliquots (0.1 mL) of the experimental samples were transferred into 1.5-mL conical tubes. ACN (20 µL) was added to the experimental samples to simulate the analyte

fortification step of the standards and QC samples. The 1.5-mL conical tubes containing the calibration, QC and experimental samples were capped and mixed with vortex action for approximately 10 seconds. ACN (880 µL) was added to each tube. The tubes were capped, mixed with vortex action for approximately 10 seconds and centrifuged at a minimum of 3650 rpm for approximately 10 minutes at 4°C. A portion of each supernatant fraction was transferred to an autosampler vial for analysis.

3.8. CONCENTRATION QUANTITATION

An external standard method of quantitation was used for determination of PFBS in serum and urine. A calibration curve was constructed for each set of analyses. Using the Quantify program in MassLynx™, the peak area of PFBS (y) and the theoretical concentrations of the calibration standards (x) were fit with least-squares regression analysis to the ln-quadratic function (excluding zero):

$$\ln (y) = a \times [\ln (x)]^2 + b \times \ln (x) + c$$

Concentrations were back-calculated from the results of the regression analysis using the Quantify program in the MassLynx™ software.

4. RESULTS AND DISCUSSION

4.1. METHOD VALIDATION AND CROSS-VALIDATION

A method was developed and validated for the determination of PFBS in monkey serum. Method sensitivity, specificity/selectivity, calibration reproducibility, accuracy and precision were assessed and validated. The monkey serum assay was then cross-validated for the determination of PFBS in monkey urine in a single validation session. The results of the monkey serum assay validation and the subsequent cross-validation are summarized in Tables 1 and 2 (calibration samples) and 3 and 4 (quality control samples). Stability of PFBS in processed samples, in samples during frozen storage (-20°C), in samples after short-term (4 hour) room temperature storage and in samples after the freeze-thaw process was evaluated.

Under the described chromatographic conditions, the retention time of PFBS was approximately 3.6 minutes. The total run time for each analysis was approximately 10 minutes.

Figures 1 through 25 illustrate typical chromatograms for a processed solvent blank (Figure 1), a processed serum blank (Figure 2), processed serum calibration samples (Figures 3 through 7), processed serum QC samples (Figures 8 through 11) and experimental serum samples (Figures 12 through 25).

Figures 26 through 52 illustrate typical chromatograms for a processed solvent blank (Figure 26), a processed urine blank (Figure 27), processed urine calibration samples (Figures 28 through 32), processed urine QC samples (Figures 33 through 36) and experimental urine samples (Figures 37 through 52).

4.2. SENSITIVITY

According to WIL SOP, the lower limit of quantitation (LLOQ) can be defined as the lowest calibration concentration that meets the validation acceptance criteria, *i.e.,* percent relative standard deviation (%RSD) ≤ 20% and percent relative error (%RE) within ± 20%. As shown in Tables 1 and 2, the LLOQ was 30 ng/mL for PFBS in monkey serum and urine. The monkey serum and urine validation sessions resulted in mean %RSD and %RE values at the LLOQ for each analyte as indicated in the following table.

	%RE	%RSD
Serum	2.8	3.7
Urine	1.4	5.8

4.3. SPECIFICITY/SELECTIVITY

Assay specificity/selectivity refers to the ability of the assay chromatography to specifically detect and quantitate the analyte(s) of interest from potentially interfering compounds. Blank serum and urine from 6 individual animals (3 male and 3 female)

were analyzed for PFBS. The lowest calibration samples had area responses that were greater than 3 times the response of the 6 individual blank samples that were analyzed (Supporting Data Tables B5 and B9). Assay specificity/selectivity was confirmed when assessment of the assay accuracy and precision met the acceptance criteria.

4.4. CALIBRATION ACCEPTABILITY

During each validation session, triplicate calibration samples at each concentration level were prepared and analyzed as described previously. Single injections were made for each processed calibration sample. The resulting peak area versus concentration data were fit to the ln-quadratic function using least-squares regression analysis, excluding zero. The regression equation was used to back-calculate the corresponding concentrations from the peak areas. The reproducibility of the calibration curve data was considered valid when 1) the inter-session variability (RSD) of the back-calculated concentrations at each concentration level was ≤ 15%, except at the lowest concentration level where ≤ 20% was acceptable; and 2) the mean back-calculated concentration at each concentration level was within ± 15% of the theoretical values (%RE within ± 15%), except at the lowest concentration level where %RE within ± 20% was acceptable.

The back-calculated concentration values and the associated intra- and inter-session statistics for PFBS calibration samples used during the five validation sessions for monkey serum are shown in Table 1. The intra-session variability of back-calculated concentrations at each level used for the cross-validation to urine is shown in Table 2. The intra- and inter-session statistics for the calibration samples are summarized in the following table.

	%RE Range	%RSD Range
Serum	-3.7 to 4.0	1.2 to 3.7
Urine	-2.6 to 1.4	1.4 to 5.8

Based on the results in Tables 1 and 2, the reproducibility of the calibration data was acceptable for the determination of PFBS concentration in monkey serum and urine.

4.5. ACCURACY AND PRECISION

During each validation session, triplicate QC samples at each concentration level were prepared and analyzed as described previously. Single injections were made of each processed QC sample. The regression equation was used to calculate the corresponding concentrations from the QC peak area data. The variability (RSD) of calculated QC concentration data was used as a measure of assay precision. For the serum and urine assays, the precision of the method was considered acceptable when the intra- or inter-session RSD of the calculated concentrations at each QC concentration level was $\leq 15\%$, except at the lowest concentration level where $\leq 20\%$ was acceptable. The accuracy of the method was considered acceptable when intra- or inter-session concentration means of the calculated concentrations at each QC concentration level had %RE values within ± 15%, except at the lowest concentration level where $\leq 20\%$ was acceptable.

The back-calculated concentration values and the associated intra- and inter-session statistics for the PFBS QC samples used during the five validation sessions for monkey serum are shown in Table 3. The intra-session variability of back-calculated concentrations at each level used for the cross-validation to urine are shown in Table 4. The intra- and inter-session statistics for the QC samples are summarized in the following table.

	%RE Range	%RSD Range
Serum	-3.6 to 6.0	2.0 to 4.0
Urine	-7.6 to 13	1.6 to 18

Based on the results in Tables 3 and 4, the accuracy and precision of the QC sample data were acceptable for the determination of PFBS concentration in monkey serum and urine.

4.6. STABILITY

Stability of PFBS in processed samples, in samples during frozen storage (-20°C), in samples after short-term (4 hour) room temperature storage and in samples after the freeze-thaw process was evaluated. According to WIL SOP, stability is indicated if the mean measured post-storage (or treatment) analyte(s) concentration is not less than 90% of the corresponding time-zero concentration.

4.6.1. STABILITY OF PFBS IN PROCESSED SERUM AND URINE SAMPLES

Stability of PFBS in processed monkey serum and urine samples was evaluated. The samples were re-injected after refrigerated or room temperature storage to evaluate the test article stability in processed samples. The mean concentrations of the reanalyzed samples were compared to the corresponding time-zero PFBS concentrations.

The mean measured concentrations in serum samples after 2 days of room temperature storage ranged from 90.8% to 94.5% of the corresponding time-zero concentrations (Table 5). Since post-storage concentrations of PFBS met the specified criteria, PFBS in monkey serum samples was considered to be stable through 2 days of room temperature storage.

The mean measured concentrations in urine samples after 7 days of refrigerated storage ranged from 54.5% to 97.1% of the corresponding time-zero concentrations (Table 6). Since the post-storage concentrations did not meet the specified citeria (except at the 100 ng PFBS/mL level), samples should be analyzed as soon as possible after sample processing.

4.6.2. LONG TERM FROZEN STABILITY OF PFBS IN SERUM AND URINE SAMPLES

Frozen (-20°C) storage stability of PFBS in monkey serum and urine was assessed. Chemical degradation usually occurs at a rate described by a monoexponential decay function: $C_t = C_0 \times e^{(kt)}$. The natural logarithm transformation of this function is: $\ln(C_t) = \ln(C_0) + kt$. Here, C_0 is the analyte concentration at $t = 0$; and C_t is the analyte

concentration after storage time = t days. Tables 7 and 8 summarize and graphically represent the ln (concentration) vs storage time in days for monkey serum and urine. The data were fit using least-squares regression analysis to the monoexponential function yielding degradation constants (k), summarized in the following table.

	Serum	Urine
100 ng/mL	$-1.93e^{-03}$	$1.23e^{-03}$
750 ng/mL	$-8.67e^{-04}$	$9.22e^{-04}$

When fit to the exponential function shown previously, the urine samples resulted in positive degradation constants (k), indicating no detectable loss during the storage of the samples. The largest serum degradation constant (-0.00193) would result in a 10% loss in 55 days, 50% in 359 days, etc.

4.6.3. STABILITY OF PFBS IN SERUM AND URINE SAMPLES AT ROOM TEMPERATURE

Serum and urine samples were fortified with PFBS. Triplicate samples were used to evaluate the stability of the analyte after 4-hours of room temperature storage. The mean test article concentrations of the stored samples were compared to the corresponding time-zero PFBS concentrations.

The mean measured concentrations in serum samples after 4-hours of room temperature storage were 96.3% and 92.9% of the corresponding time-zero concentrations (Table 9). Since post-storage concentrations of PFBS met the specified criteria, PFBS in monkey serum samples was considered to be stable after 4-hours of room temperature storage.

The mean measured concentrations in urine samples after 4-hours of room temperature storage were 109% and 102% of the corresponding time-zero concentrations (Table 10). Since post-storage concentrations of PFBS met the specified criteria, PFBS in monkey urine samples was considered to be stable after 4-hours of room temperature storage.

4.6.4. FREEZE/THAW STABILITY OF PFBS IN SERUM AND URINE SAMPLES

The freeze-thaw stability of PFBS in monkey serum and urine was evaluated. Serum and urine samples were fortified at 100 and 750 ng PFBS/mL. Triplicate samples from each concentration level were used to evaluate the stability of the analytes after each of 3 freeze-thaw cycles. The samples were frozen and thawed (1 cycle), and the process repeated 2 more times (cycles 2 and 3) for the analysis of freeze thaw stability.

The mean measured concentrations in serum samples for up to 3 freeze-thaw cycles ranged from 94.7% to 118% of the corresponding time-zero concentrations (Table 11). Since post-storage concentrations of PFBS met the specified criteria, PFBS in monkey serum samples was considered to be stable through 3 freeze-thaw cycles.

The mean measured concentrations in urine samples for up to 3 freeze-thaw cycles ranged from 92.1% to 96.8% of the corresponding time-zero concentrations (Table 12). Since post-storage concentrations of PFBS met the specified criteria, PFBS in monkey urine samples was considered to be stable through 3 freeze-thaw cycles.

4.7. ANALYSIS OF EXPERIMENTAL SAMPLES

Monkey serum and urine samples were analyzed for PFBS and the results are summarized in Tables 13 and 14, respectively. In addition to the experimental (unknown) samples, each set of analyses consisted of at least duplicate calibration samples, one solvent blank, one blank matrix sample and at least triplicate QC samples at each concentration level. For an analytical run for serum or urine samples to be considered valid, at least two-thirds of the QC samples, with at least one at each concentration level, could not deviate more than ± 15% from the QC target concentration values, except at the lowest concentration level where ≤ 20% was acceptable. Based on the stated criteria, all the PFBS analyses were acceptable.

5. CONCLUSION

An HPLC/MS/MS ESI- method for the determination of PFBS in monkey serum was developed and validated. The validated monkey serum assay was cross-validated for the determination of PFBS in monkey urine. Stability of PFBS in processed samples, in samples during frozen storage (-20°C), in samples after short-term (4 hour) room temperature storage and in samples after the freeze-thaw process was evaluated. Analysis of serum samples resulted in levels ranging from ND to 93,766 ng PFBS/mL. Analysis of urine samples resulted in levels ranging LLOQ to 2,588,991 ng PFBS/mL.

6. KEY STUDY PERSONNEL AND REPORT SUBMISSION

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TABLES 1-14

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 1: PFBS Back-Calculated Concentrations And Intra-Session And Inter-Session Statistics Of Calibration Samples In Monkey Serum

Theo. Conc. (ng/mL)	30	60	100	300	1000
Set 1 (5/16/05), 534002(PFBS)AMS, analyst SMS2					
Samp 1	30.4	57.5	98.3	303	996
Samp 2	29.8	57.1	99.2	326	970
Samp 3	32.4	57.9	99.1	307	993
Mean	30.9	57.5	98.9	312	986
SD	1.3	0.41	0.50	12	14
RSD	4.4	0.71	0.51	4.0	1.4
%RE	2.9	-4.1	-1.1	4.1	-1.4
Set 2 (5/17/05), 534002(PFBS)BMS, analyst SMS2					
Samp 1	30.1	58.8	97.0	314	998
Samp 2	30.4	57.9	99.2	303	973
Samp 3	31.4	59.0	96.6	321	989
Mean	30.7	58.6	97.6	312	987
SD	0.68	0.61	1.4	8.9	12
RSD	2.2	1.0	1.4	2.9	1.3
%RE	2.2	-2.4	-2.4	4.2	-1.3
Set 3 (5/17/05), 534002(PFBS)CMS, analyst SMS2					
Samp 1	29.7	57.0	94.3	311	977
Samp 2	30.4	56.5	99.8	318	984
Samp 3	33.3	58.1	98.8	322	985
Mean	31.1	57.2	97.6	317	982
SD	1.9	0.80	2.9	5.4	4.6
RSD	6.0	1.4	3.0	1.7	0.47
%RE	3.7	-4.7	-2.4	5.6	-1.8

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 1: PFBS Back-Calculated Concentrations And Intra-Session And Inter-Session Statistics Of Calibration Samples In Monkey Serum

Theo. Conc. (ng/mL)	**30**	**60**	**100**	**300**	**1000**
Set 4 (5/17/05), 534002(PFBS)DMS, analyst SMS2					
Samp 1	32.3	58.6	99.4	321	975
Samp 2	31.8	54.6	99.0	322	985
Samp 3	30.3	55.3	95.1	314	980
Mean	31.5	56.1	97.8	319	980
SD	1.0	2.2	2.4	4.5	5.0
RSD	3.3	3.9	2.4	1.4	0.51
%RE	4.9	-6.4	-2.2	6.3	-2.0
Set 5 (5/18/05), 534002(PFBS)EMS, analyst KLK					
Samp 1	30.9	58.4	103	301	1016
Samp 2	30.1	59.4	99.9	301	996
Samp 3	29.3	60.4	99.4	296	989
Mean	30.1	59.4	101	300	1000
SD	0.78	0.98	2.1	2.9	14
RSD	2.6	1.7	2.1	0.97	1.4
%RE	0.33	-1.0	0.89	-0.14	0.0078
Interset Statistics					
n	15	15	15	15	15
Mean	30.8	57.8	98.6	312	987
SD	1.1	1.5	2.2	9.5	12
RSD	3.7	2.6	2.2	3.0	1.2
%RE	2.8	-3.7	-1.4	4.0	-1.3

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 2: PFBS Back-Calculated Concentrations And Intra-Session Statistics Of Calibration Samples In Monkey Urine

Theo. Conc. (ng/mL)	**30**	**60**	**100**	**300**	**1000**
Set 1 (5/18/05), 534002(PFBS)FMU, analyst KLK					
Samp 1	32.5	56.9	*	309	979
Samp 2	29.7	60.3	104	302	998
Samp 3	29.2	58.1	98.2	301	1006
Intra-set Statistics					
n	3	3	2	3	3
Mean	30.4	58.5	101	304	995
SD	1.8	1.7	3.7	4.2	14
RSD	5.8	3.0	3.7	1.4	1.4
%RE	1.4	-2.6	0.89	1.3	-0.52

* Data point excluded due to suspected sample preparation error and outlier test.

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 3: PFBS Concentrations And Intra-Session And Inter-Session Statistics Of Quality Control Samples In Monkey Serum

Theo. Conc. (ng/mL)	**30**	**100**	**750**
Set 1 (5/16/05), 534002(PFBS)AMS, analyst SMS2			
Samp 1	33.2	97.3	781
Samp 2	33.1	96.4	787
Samp 3	31.3	98.6	783
Mean	32.6	97.5	783
SD	1.1	1.1	3.2
RSD	3.3	1.1	0.41
%RE	8.5	-2.5	4.4
Set 2 (5/17/05), 534002(PFBS)BMS, analyst SMS2			
Samp 1	31.8	99.1	790
Samp 2	31.9	95.5	809
Samp 3	31.4	96.4	786
Mean	31.7	97.0	795
SD	0.25	1.8	12
RSD	0.77	1.9	1.5
%RE	5.6	-3.0	6.1
Set 3 (5/17/05), 534002(PFBS)CMS, analyst SMS2			
Samp 1	30.5	98.9	812
Samp 2	30.8	94.0	825
Samp 3	31.8	95.3	831
Mean	31.0	96.0	823
SD	0.70	2.5	10
RSD	2.3	2.6	1.2
%RE	3.4	-4.0	9.7

Table 3: PFBS Concentrations And Intra-Session And Inter-Session Statistics Of Quality Control Samples In Monkey Serum

Theo. Conc. (ng/mL)	**30**	**100**	**750**
Set 4 (5/17/05), 534002(PFBS)DMS, analyst SMS2			
Samp 1	30.3	97.1	786
Samp 2	28.8	97.1	805
Samp 3	30.1	92.9	793
Mean	29.7	95.7	795
SD	0.79	2.4	9.7
RSD	2.7	2.5	1.2
%RE	-0.85	-4.3	6.0
Set 5 (5/18/05), 534002(PFBS)EMS, analyst KLK			
Samp 1	30.8	93.7	791
Samp 2	29.5	97.7	788
Samp 3	30.0	95.6	753
Mean	30.1	95.7	777
SD	0.65	2.0	21
RSD	2.1	2.1	2.7
%RE	0.37	-4.3	3.6
Interset Statistics			
n	15	15	15
Mean	31.0	96.4	795
SD	1.2	1.9	19
RSD	4.0	2.0	2.4
%RE	3.4	-3.6	6.0

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 4: PFBS Concentrations And Intra-Session Statistics Of Quality Control Samples In Monkey Urine

Theo. Conc. (ng/mL)	**30**	**100**	**750**
Set 1 (5/18/05), 534002(PFBS)FMU, analyst KLK			
Samp 1	29.9	96.3	775
Samp 2	30.6	91.5	788
Samp 3	41.0	89.5	800
Intra-set Statistics			
n	3	3	3
Mean	33.8	92.4	788
SD	6.2	3.5	13
RSD	18	3.8	1.6
%RE	13	-7.6	5.0

534002 PFBS bio tables.xls Summary II (PFBS)MU
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A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 5: 2-Day Room Temperature PFBS Stability Analysis Of Processed Monkey Serum Samples

Area Count Comparison of Quality Control Samples

Time Point	Date Analyzed	Theo. Conc (ng/mL)	Ref # (534002 -)	Run #	Area	Percent of Time Zero (%)
Quality Control Samples						
T = 0	5/16/2005	100	161 - 4	I1-1110	2320141	
2-Day	5/18/2005		161 - 4	I1-1247	2192432	94.5
T = 0	5/16/2005	100	161 - 5	I1-1111	2303087	
2-Day	5/18/2005		161 - 5	I1-1248	2126830	92.3
T = 0	5/16/2005	100	161 - 6	I1-1112	2344681	
2-Day	5/18/2005		161 - 6	I1-1249	2128541	90.8

534002 PFBS bio tables.xls 2-day RT pss
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A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 6: 7-Day Refrigerated PFBS Stability Analysis Of Processed Monkey Urine Samples

Time Point	Date Analyzed	Theo. Conc (ng/mL)	Ref # (534002 -)	Run #	Conc.	Mean Conc.	Percent of Time Zero (%)
Quality Control Samples							
T = 0	7/8/2005	30	250 - 1	I1-1849	30.8	32.5	---
			250 - 2	I1-1860	32.8		
			250 - 3	I1-1871	33.9		
7-Day	7/15/2005		250 - 1	I1-1936b	16.8	17.7	54.5
			250 - 2	I1-1937b	18.0		
			250 - 3	I1-1938b	18.3		
T = 0	7/8/2005	100	250 - 4	I1-1850	100	100	---
			250 - 5	I1-1861	98.9		
			250 - 6	I1-1872	101		
7-Day	7/15/2005		250 - 4	I1-1939b	89.7	97.1	97.1
			250 - 5	I1-1940b	94.9		
			250 - 6	I1-1946b	107		
T = 0	7/8/2005	750	250 - 7	I1-1851	798	795	---
			250 - 8	I1-1862	747		
			250 - 9	I1-1873	839		
7-Day	7/15/2005		250 - 7	I1-1947b	694	686	86.3
			250 - 8	I1-1948b	689		
			250 - 9	I1-1949b	677		

534002 PFBS bio tables.xls PSS(PFBS) MU
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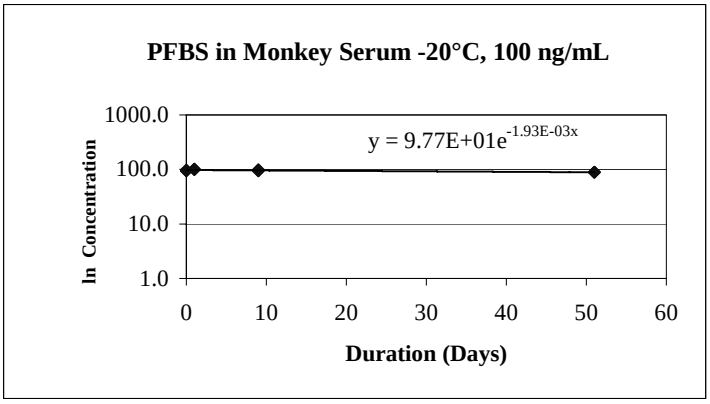
A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 7: Stability Of PFBS In Monkey Serum - Long-Term Frozen Storage At -20°C

Theo. Conc. (ng/mL)	Storage Duration (Days)	Run# (534002-)	Assay Conc. (ng/mL)	Mean Conc. (ng/mL)	k
100	0	I1-1240	93.7	95.7	-1.93E-03
	0	I1-1241	97.7		
	0	I1-1242	95.6		
	1	I1-1377	98.8	100	
	1	I1-1378	99.9		
	1	I1-1379	101		
	9	I1-1669	93.4	95.7	
	9	I1-1670	94.6		
	9	I1-1671	99.2		
	51	I1-1809	88.6	88.6	
	51	I1-1810	88.6		
	51	I1-1811	88.6		



534002 PFBS bio tables.xls LT (PFBS) MS
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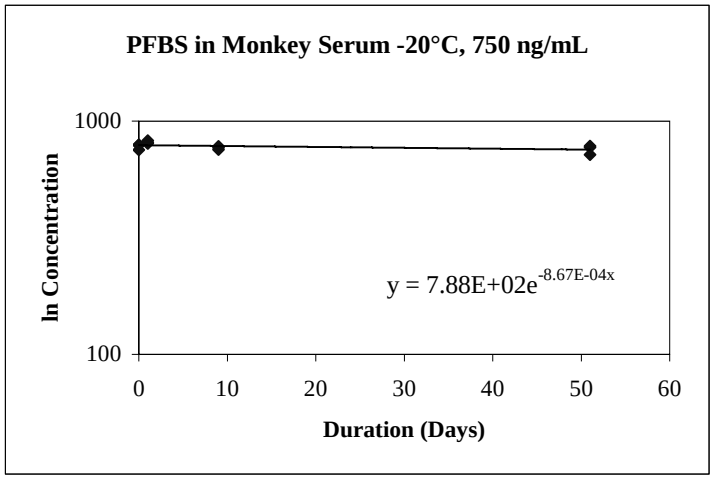
A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 7: Stability Of PFBS In Monkey Serum - Long-Term Frozen Storage At -20°C

Theo. Conc. (ng/mL)	Storage Duration (Days)	Run# (534002-)	Assay Conc. (ng/mL)	Mean Conc. (ng/mL)	k
750	0	I1-1243	791	777	-8.67E-04
	0	I1-1244	788		
	0	I1-1245	753		
	1	I1-1380	826	813	
	1	I1-1381	810		
	1	I1-1382	802		
	9	I1-1678	769	767	
	9	I1-1679	754		
	9	I1-1680	779		
	51	I1-1812	781	757	
	51	I1-1813	717		
	51	I1-1814	773		



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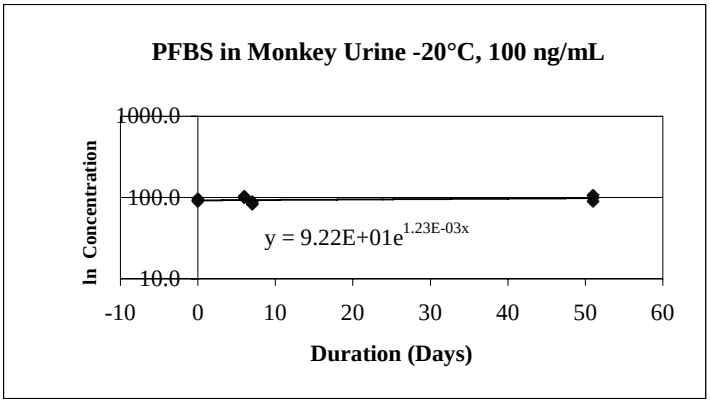
A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 8: Stability Of PFBS In Monkey Urine - Long-Term Frozen Storage At -20°C

Theo. Conc. (ng/mL)	Storage Duration (Days)	Run# (534002-)	Assay Conc. (ng/mL)	Mean Conc. (ng/mL)	k
100	0	I1-1278	96.3	92.4	1.23E-03
	0	I1-1279	91.5		
	0	I1-1280	89.5		
	6	I1-1465	99.9	101	
	6	I1-1466	104		
	6	I1-1467	100		
	7	I1-1570	82.6	85.1	
	7	I1-1571	88.7		
	7	I1-1572	84.0		
	51	I1-1853	107	98.6	
	51	I1-1854	99.3		
	51	I1-1855	89.9		



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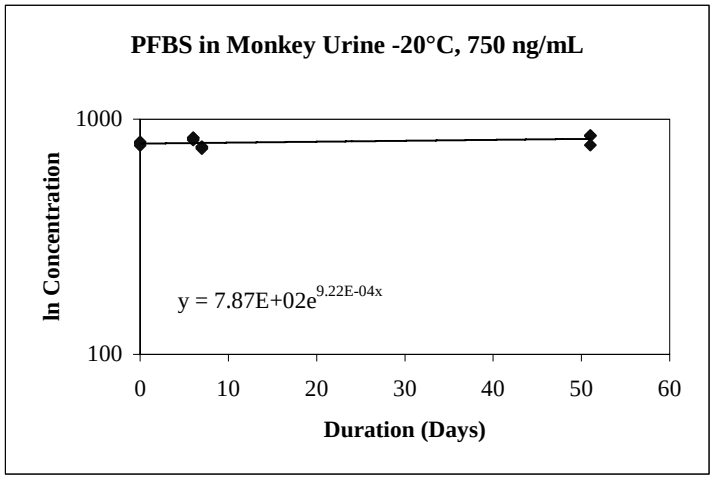
A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 8: Stability Of PFBS In Monkey Urine - Long-Term Frozen Storage At -20°C

Theo. Conc. (ng/mL)	Storage Duration (Days)	Run# (534002-)	Assay Conc. (ng/mL)	Mean Conc. (ng/mL)	k
750	0	I1-1281	775	788	9.22E-04
	0	I1-1282	788		
	0	I1-1283	800		
	6	I1-1468	834	827	
	6	I1-1469	831		
	6	I1-1470	815		
	7	I1-1579	749	758	
	7	I1-1580	765		
	7	I1-1581	759		
	51	I1-1856	777	827	
	51	I1-1857	851		
	51	I1-1858	852		



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A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 9: 4-Hour Room Temperature Stability Of PFBS In Monkey Serum

Theo. Conc. (ng/mL)	Time hr (hr)	Run # (534002-)	Analyzed Conc (ng/mL)	% Target (%)	Mean Conc (ng/mL)	RSD (%)	Mean % Target (%)	% of Time Zero (%)
100	0	I1-1809	88.6	88.6	88.6	0.025	88.6	***
		I1-1810	88.6	88.6				
		I1-1811	88.6	88.6				
	4	I1-1820	88.2	88.2	85.3	4.7	85.3	96.3
		I1-1821	80.7	80.7				
		I1-1822	87.0	87.0				
750	0	I1-1812	781	104	757	4.6	101	***
		I1-1813	717	95.6				
		I1-1814	773	103				
	4	I1-1823	739	98.5	704	4.5	93.8	92.9
		I1-1824	695	92.7				
		I1-1825	677	90.3				

534002 PFBS bio tables.xls 4hr RTS (PFBS) MS
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A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 10: 4-Hour Room Temperature Stability Of PFBS In Monkey Urine

Theo. Conc. (ng/mL)	Time hr (hr)	Run # (534002-)	Analyzed Conc (ng/mL)	% Target (%)	Mean Conc (ng/mL)	RSD (%)	Mean % Target (%)	% of Time Zero (%)
100	0	I1-1853	107	107	98.6	8.492	98.6	***
		I1-1854	99.3	99.3				
		I1-1855	89.9	89.9				
	4	I1-1864	111	111	107.8	3.0	108	109
		I1-1865	104	104				
		I1-1866	108	108				
750	0	I1-1856	777	104	827	5.2	110	***
		I1-1857	851	113				
		I1-1858	852	114				
	4	I1-1867	760	101	840	9.1	112	102
		I1-1868	911	121				
		I1-1869	848	113				

534002 PFBS bio tables.xls 4hr RTS (PFBS) MU
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A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 11: Freeze-Thaw Stability Of PFBS In Monkey Serum

Theo. Conc. (ng/mL)	# of Cycles	Run # (534002-)	Analyzed Conc (ng/mL)	% Target (%)	Mean Conc (ng/mL)	RSD (%)	Mean % Target (%)	% of Time Zero (%)
Monkey Serum								
100	0	I1-1240	93.7	93.7	95.7	2.1	95.7	---
		I1-1241	97.7	97.7				
		I1-1242	95.6	95.6				
	1	I1-1669	93.4	93.4	95.7	3.2	95.7	100
		I1-1670	94.6	94.6				
		I1-1671	99.2	99.2				
	2	I1-1672	99.4	99.4	101	4.0	101	105
		I1-1673	105	105				
		I1-1674	97.2	97.2				
	3	I1-1675	109	109	112	9.3	112	118
		I1-1676	124	124				
		I1-1677	104	104				
750	0	I1-1243	791	105	777	2.7	104	---
		I1-1244	788	105				
		I1-1245	753	100				
	1	I1-1678	769	103	767	1.6	102	98.7
		I1-1679	754	101				
		I1-1680	779	104				
	2	I1-1681	781	104	766	2.0	102	98.6
		I1-1682	750	100				
		I1-1683	768	102				
	3	I1-1684	745	99.3	736	5.5	98.2	94.7
		I1-1685	693	92.4				
		I1-1686	772	103				

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 12: Freeze-Thaw Stability Of PFBS In Monkey Urine

Theo. Conc. (ng/mL)	# of Cycles	Run # (534002-)	Analyzed Conc (ng/mL)	% Target (%)	Mean Conc (ng/mL)	RSD (%)	Mean % Target (%)	% of Time Zero (%)
Monkey Urine								
100	0	I1-1278	96.3	96.3	92.4	3.8	92.4	---
		I1-1279	91.5	91.5				
		I1-1280	89.5	89.5				
	1	I1-1570	82.6	82.6	85.1	3.8	85.1	92.1
		I1-1571	88.7	88.7				
		I1-1572	84.0	84.0				
	2	I1-1573	89.7	89.7	89.5	4.1	89.5	96.8
		I1-1574	93.0	93.0				
		I1-1575	85.7	85.7				
	3	I1-1576	82.1	82.1	86.4	4.3	86.4	93.5
		I1-1577	88.4	88.4				
		I1-1578	88.8	88.8				
750	0	I1-1281	775	103	788	1.6	105	---
		I1-1282	788	105				
		I1-1283	800	107				
	1	I1-1579	749	100	758	1.1	101	96.2
		I1-1580	765	102				
		I1-1581	759	101				
	2	I1-1582	722	96.2	734	1.4	97.8	93.1
		I1-1583	740	98.7				
		I1-1584	739	98.5				
	3	I1-1585	714	95.2	729	2.1	97.2	92.5
		I1-1586	728	97.0				
		I1-1587	745	99.3				

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A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 13: Monkey Serum Experimental Sample PFBS Concentrations

Run #	Ref # (534002-)	Animal #	Sex	Timept. (hrs)	PFBS (ng/mL)
I1-1313	185-1	1555T2	m	0	ND
I1-1625	205-1	1555T2	m	1	77987
I1-1631	205-7	1555T2	m	2	51795
I1-1636	205-12	1555T2	m	4	28978
I1-1650	205-18	1555T2	m	8	18468
I1-1656	205-24	1555T2	m	24	4371
I1-1357	185-37	1555T2	m	48	1180
I1-1314	185-2	1562T2	m	0	ND
I1-1626	205-2	1562T2	m	1	93766
I1-1632	205-8	1562T2	m	2	73703
I1-1637	205-13	1562T2	m	4	50733
I1-1651	205-19	1562T2	m	8	45348
I1-1657	205-25	1562T2	m	24	22244
I1-1661	205-29	1562T2	m	48	16069
I1-1315	185-3	1563T2	m	0	ND
I1-1627	205-3	1563T2	m	1	74930
		1563T2	m	2	ISV
I1-1638	205-14	1563T2	m	4	27354
I1-1652	205-20	1563T2	m	8	20140
I1-1658	205-26	1563T2	m	24	5134
I1-1359	185-39	1563T2	m	48	1205
I1-1316	185-4	1572T2	f	0	ND
I1-1628	205-4	1572T2	f	1	69087
I1-1633	205-9	1572T2	f	2	48722
I1-1639	205-15	1572T2	f	4	27485
I1-1653	205-21	1572T2	f	8	15634
I1-1659	205-27	1572T2	f	24	3441
I1-1360	185-40	1572T2	f	48	1027
I1-1318	185-6	1573T2	f	0	ND
I1-1630	205-6	1573T2	f	1	54860
I1-1635	205-11	1573T2	f	2	31648
I1-1649	205-17	1573T2	f	4	16324
I1-1655	205-23	1573T2	f	8	7707
I1-1356	185-36	1573T2	f	24	787
I1-1362	185-42	1573T2	f	48	79.8
I1-1317	185-5	1576T2	f	0	ND
I1-1629	205-5	1576T2	f	1	75915
I1-1634	205-10	1576T2	f	2	53442
I1-1648	205-16	1576T2	f	4	35453
I1-1654	205-22	1576T2	f	8	21094
I1-1660	205-28	1576T2	f	24	4680
I1-1361	185-41	1576T2	f	48	1098

ISV = insufficient sample volume to re-analyze
ND = not detected

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 14: Monkey Urine Experimental Sample PFBS Concentrations

Run #	Ref # (534002-)	Animal #	Sex	Timept. (hrs)	Day	PFBS ng/mL	Urine Vol (mL)	Total PFBS (µg)
I1-1546	201-1	1555T2	M	0-6	---	476434	2	953
I1-1550	201-4	1555T2	M	6-12	---	192869	2	386
I1-1551	201-5	1555T2	M	12-24	---	15739	50	787
I1-1555	201-9	1555T2	M	24-48	2	5877	38	223
I1-1561	201-15	1555T2	M	48-72	3	2199	36	79.2
I1-1501	196-22	1555T2	M	72-96	4	431	73	31.5
I1-1506	196-28	1555T2	M	96-120	5	234	56	13.1
I1-1512	196-34	1555T2	M	120-144	6	109	59	6.46
I1-1517	196-39	1555T2	M	144-168	7	31.2	290	9.04
		1562T2	M	0-6	---	no sample provided		
		1562T2	M	6-12	---	no sample provided		
		1562T2	M	12-24	---	no sample provided		
I1-1556	201-10	1562T2	M	24-48	2	15873	22	349
I1-1562	201-16	1562T2	M	48-72	3	19409	5	97.0
I1-1566	201-20	1562T2	M	72-96	4	14288	16	229
I1-1507	196-29	1562T2	M	96-120	5	726	35	25.4
		1562T2	M	120-144	6	no sample provided		
I1-1518	196-40	1562T2	M	144-168	7	219	400	87.8
		1563T2	M	0-6	---	no sample provided		
I1-1476	196-5	1563T2	M	6-12	---	108882	5	544
I1-1552	201-6	1563T2	M	12-24	---	36394	60	2184
I1-1557	201-11	1563T2	M	24-48	2	9301	79	735
I1-1563	201-17	1563T2	M	48-72	3	3305	62	205
I1-1503	196-24	1563T2	M	72-96	4	1017	68	69.1
I1-1508	196-30	1563T2	M	96-120	5	420	46	19.3
I1-1513	196-35	1563T2	M	120-144	6	739	78	57.6
I1-1519	196-41	1563T2	M	144-168	7	907	80	72.5
I1-1547	201-2	1572T2	F	0-6	---	1980921	2	3962
		1572T2	F	6-12	---	no sample provided		
I1-1553	201-7	1572T2	F	12-24	---	296106	22	6514
I1-1558	201-12	1572T2	F	24-48	2	6952	55	382
I1-1564	201-18	1572T2	F	48-72	3	2995	43	129
		1572T2	F	72-96	4	sample broke		
I1-1509	196-31	1572T2	F	96-120	5	231	50	11.6
I1-1514	196-36	1572T2	F	120-144	6	360	48	17.3
I1-1520	196-42	1572T2	F	144-168	7	247	41	10.1
		1573T2	F	0-6	---	no sample provided		
		1573T2	F	6-12	---	no sample provided		
I1-1554	201-8	1573T2	F	12-24	---	86571	11	952
I1-1560	201-14	1573T2	F	24-48	2	3922	14	54.9
I1-1500	196-21	1573T2	F	48-72	3	904	15	13.6
I1-1505	196-27	1573T2	F	72-96	4	305	6	1.83
I1-1511	196-33	1573T2	F	96-120	5	281	41	11.5
I1-1516	196-38	1573T2	F	120-144	6	<LLOQ	27	<LLOQ
I1-1522	196-44	1573T2	F	144-168	7	89.6	40	3.59
I1-1548	201-3	1576T2	F	0-6	---	2588991	1	2589

A PHARMACOKINETIC (IN BLOOD) AND
EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Table 14: Monkey Urine Experimental Sample PFBS Concentrations

Run #	Ref #	Animal #	Sex	Timept.	Day	PFBS	Urine Vol	Total PFBS
	(534002-)			(hrs)		ng/mL	(mL)	(µg)
		1576T2	F	6-12	---	no sample provided		
		1576T2	F	12-24	---	no sample provided		
I1-1559	201-13	1576T2	F	24-48	2	59170	2	118
I1-1565	201-19	1576T2	F	48-72	3	8412	10	84.1
I1-1504	196-26	1576T2	F	72-96	4	761	33	25.1
I1-1510	196-32	1576T2	F	96-120	5	91.8	270	24.8
I1-1515	196-37	1576T2	F	120-144	6	<LLOQ	249	<LLOQ
I1-1521	196-43	1576T2	F	144-168	7	98.4	160	15.7

< LLOQ = less than the lower limit of quantitation (30 ng/mL)

FIGURES 1-52

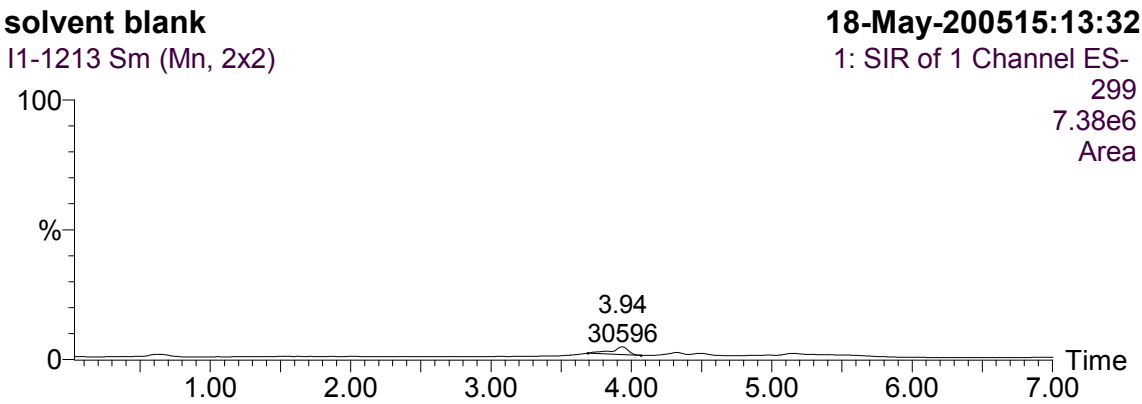

Figure 1: Representative Chromatogram Of A Processed Solvent Blank (Serum Assay)

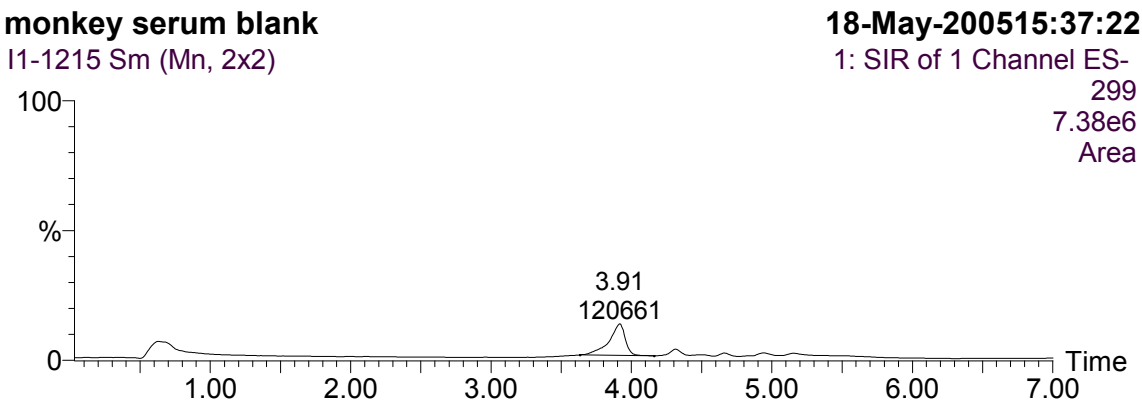

Figure 2: Representative Chromatogram Of A Processed Blank Serum Sample

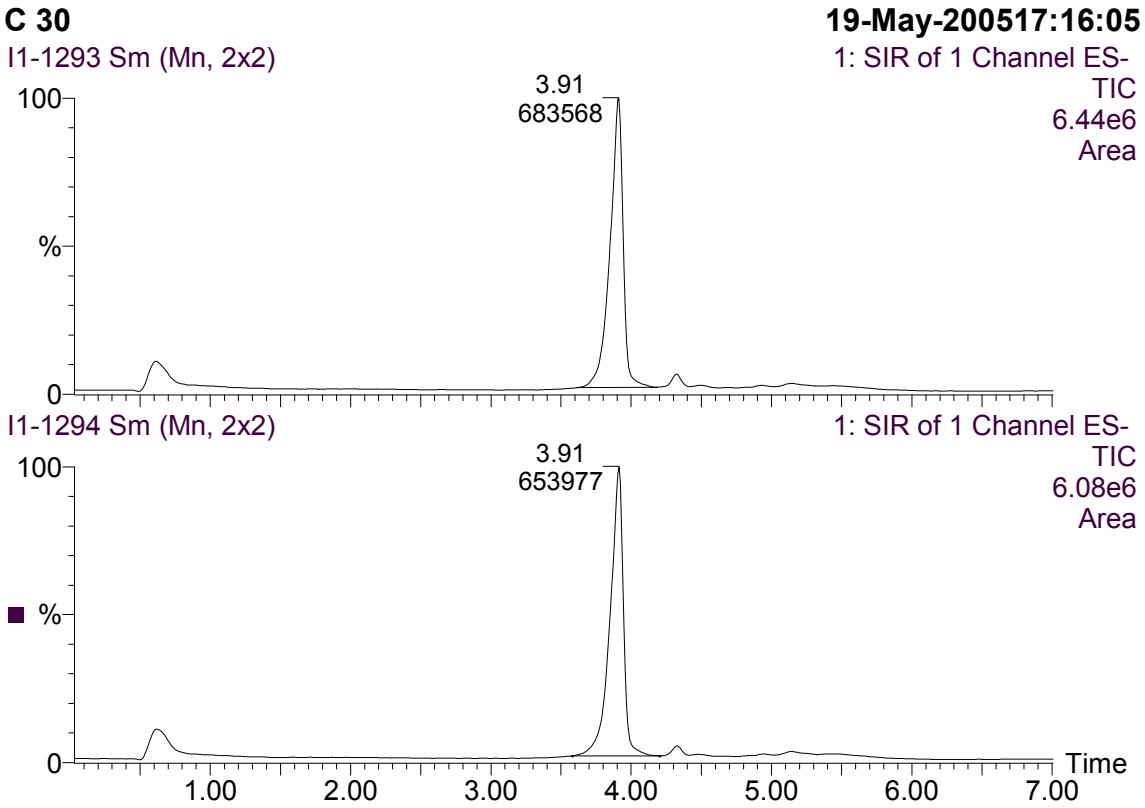

Figure 3: Representative Chromatograms Of 30 ng/mL Serum Calibration Samples

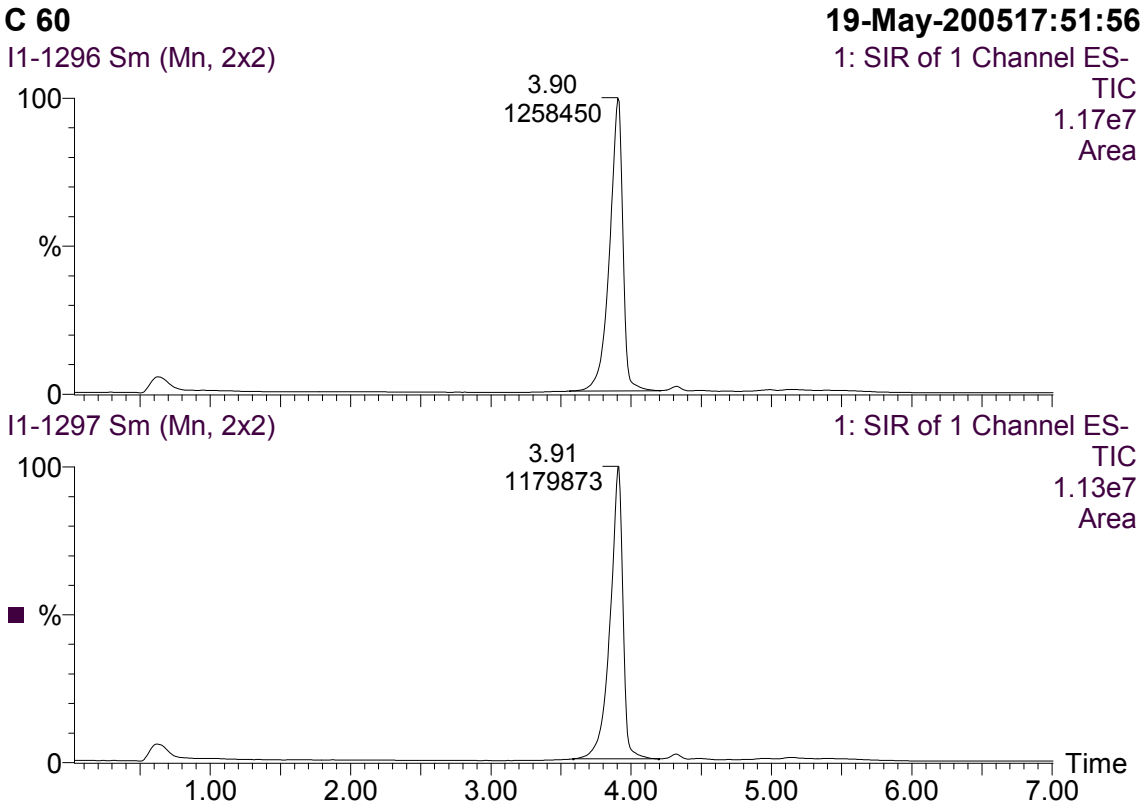

Figure 4: Representative Chromatograms Of 60 ng/mL Serum Calibration Samples

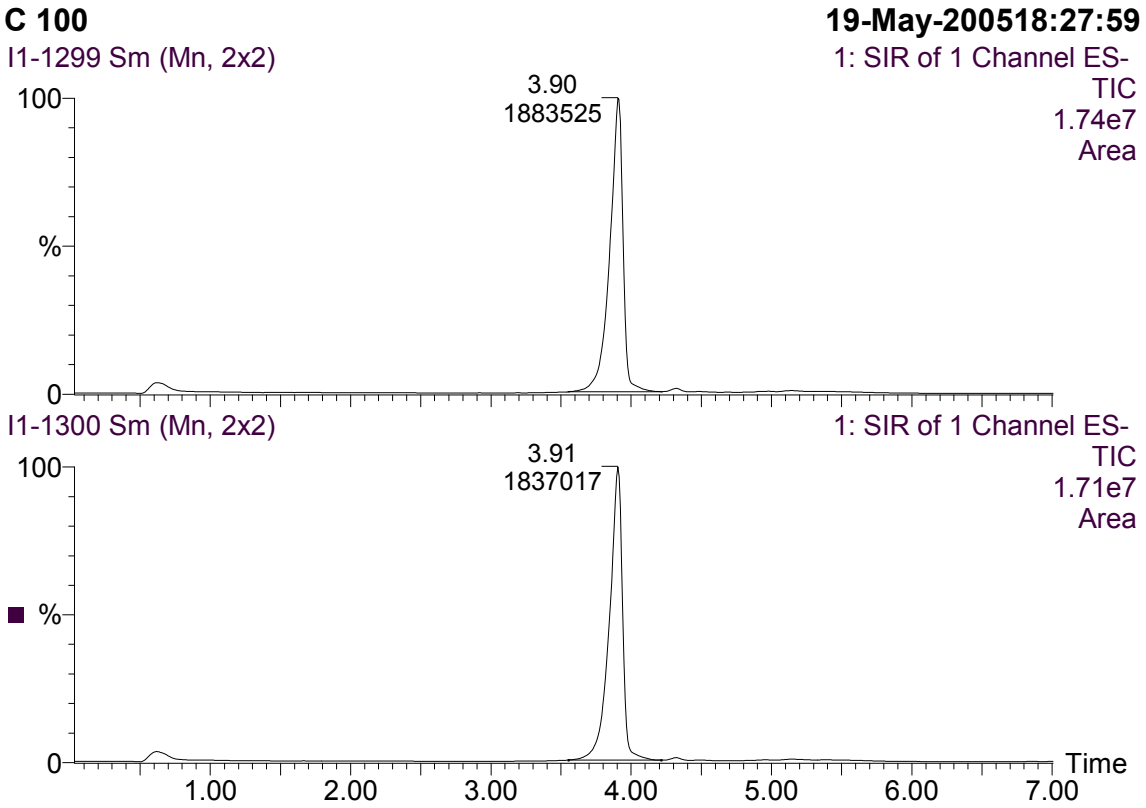

Figure 5: Representative Chromatograms Of 100 ng/mL Serum Calibration Samples

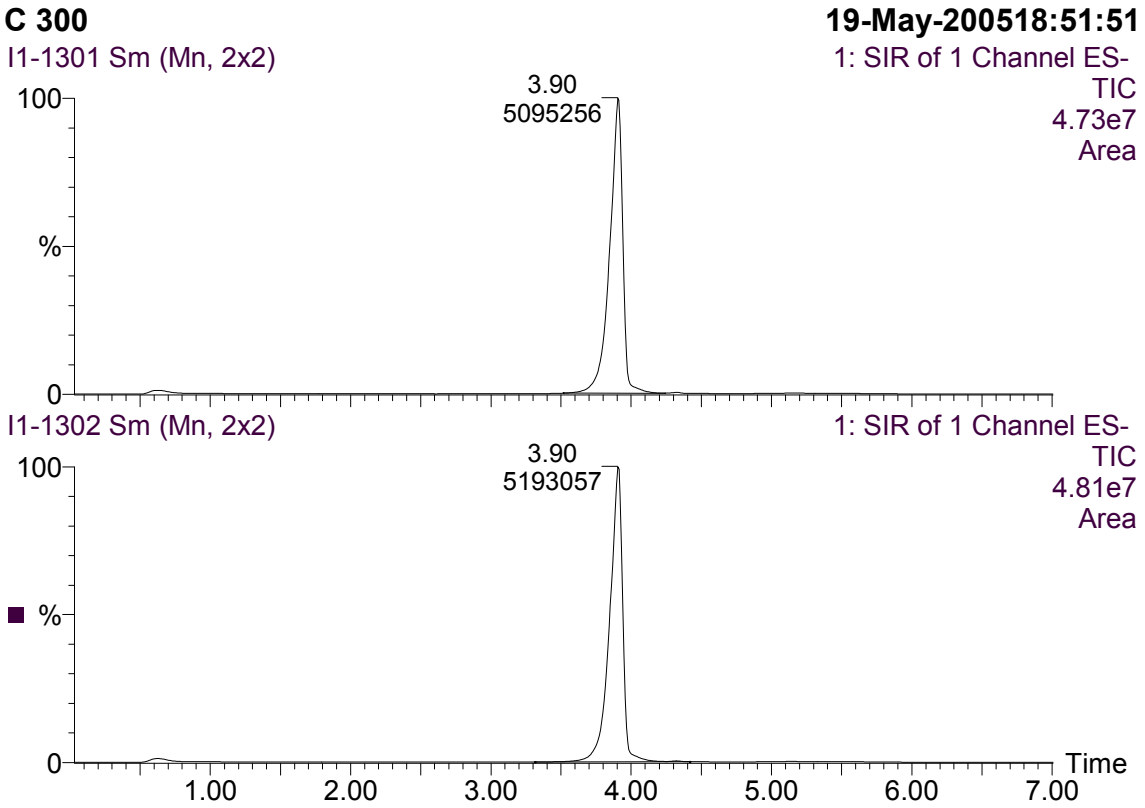

Figure 6: Representative Chromatograms Of 300 ng/mL Serum Calibration Samples

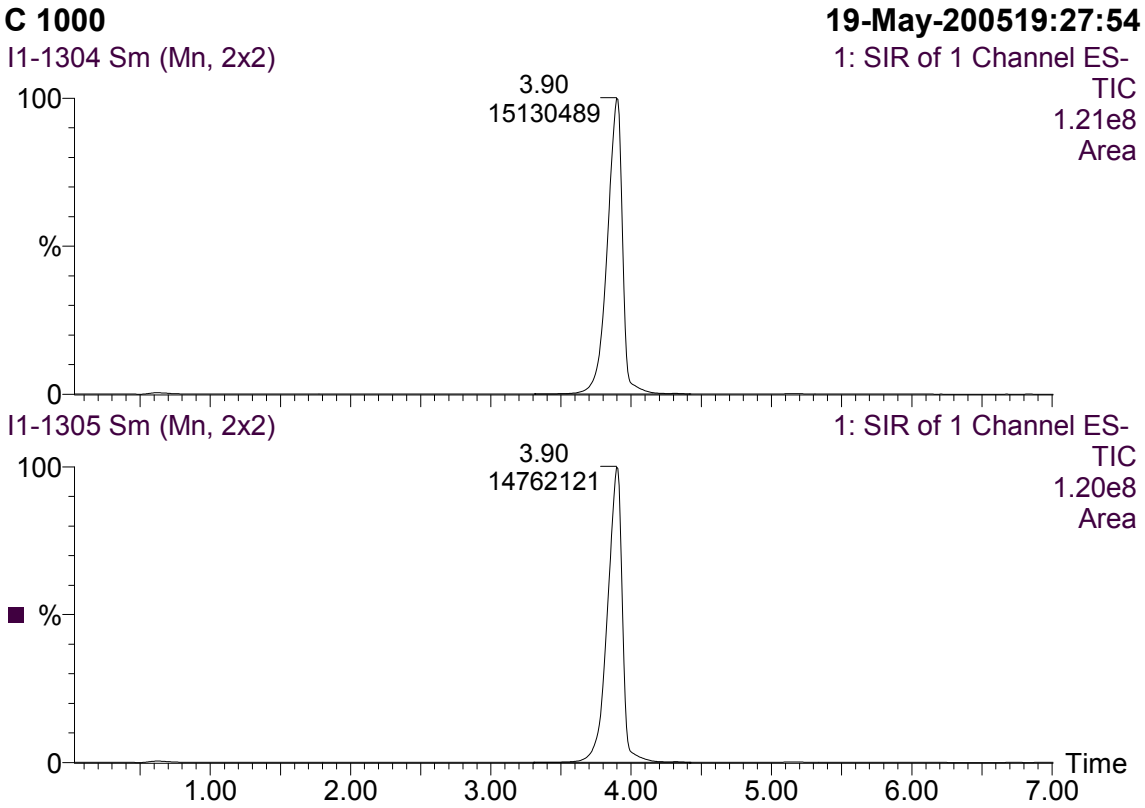

Figure 7: Representative Chromatograms Of 1000 ng/mL Serum Calibration Samples

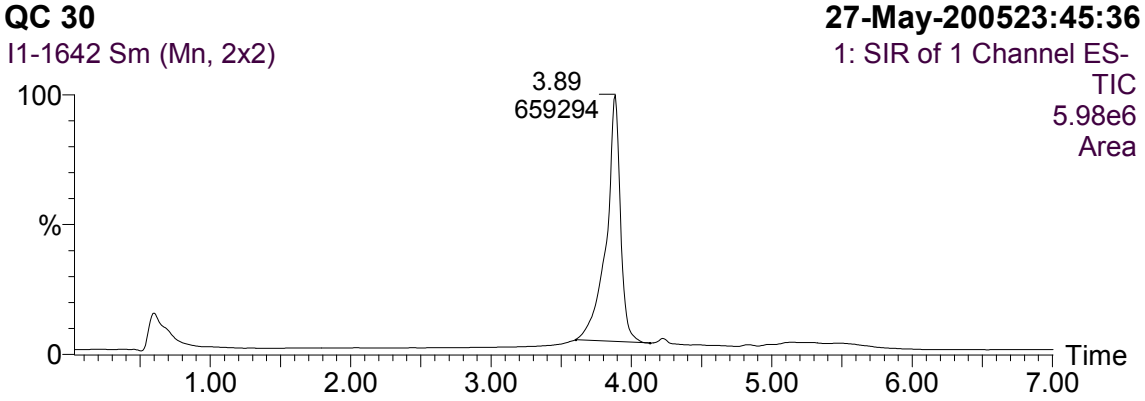

Figure 8: Representative Chromatogram Of A 30 ng/mL Serum QC Sample

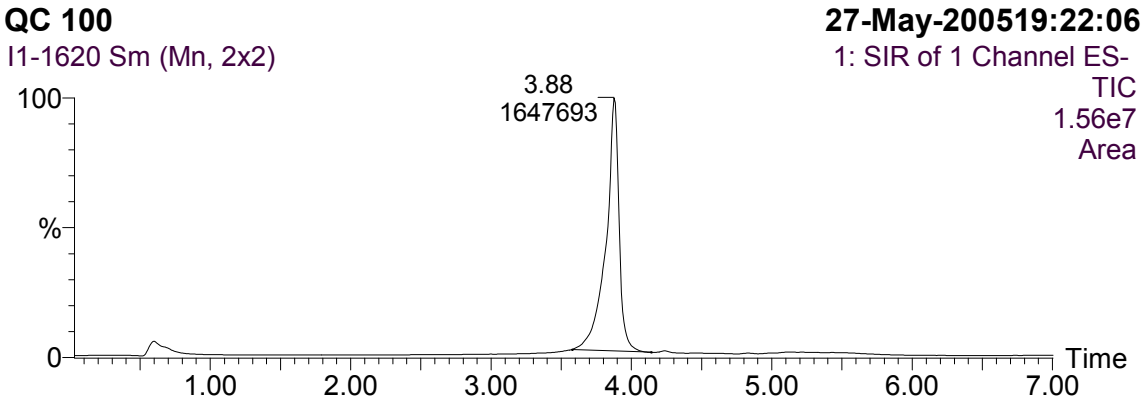

Figure 9: Representative Chromatogram Of A 100 ng/mL Serum QC Sample

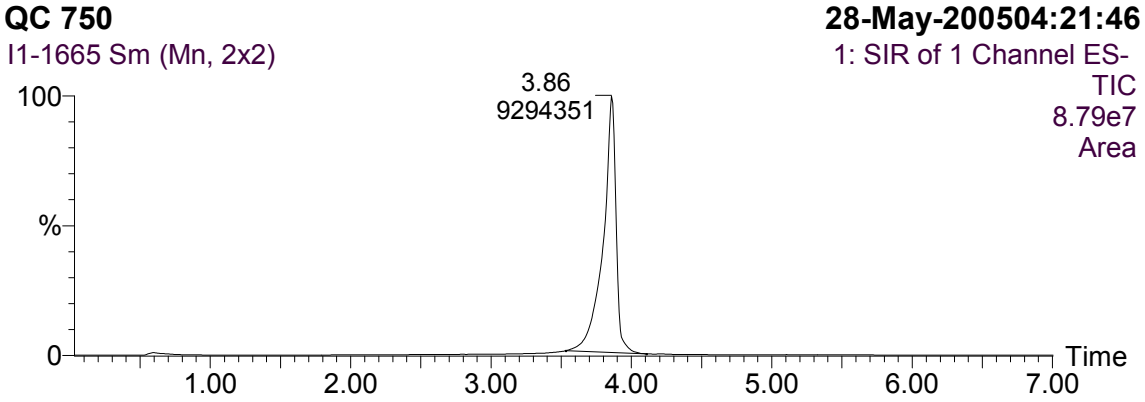

Figure 10: Representative Chromatogram Of A 750 ng/mL Serum QC Sample

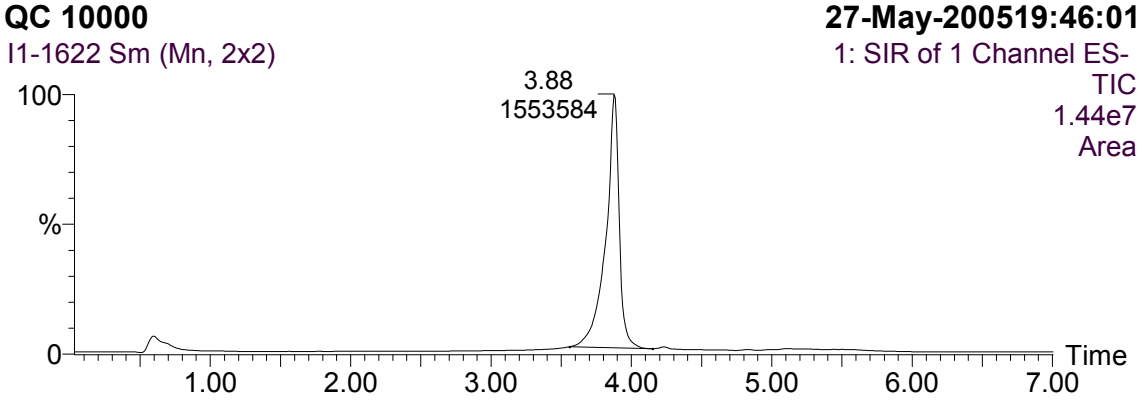

Figure 11: Representative Chromatogram Of A 10,000 ng/mL Serum QC Sample

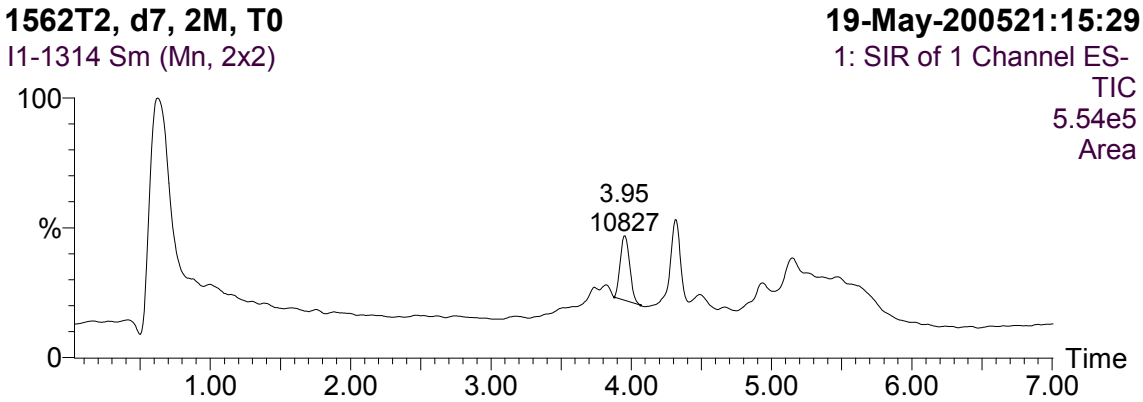

Figure 12: Chromatogram Of Animal No. 1562, Group T2, Male, Pre-Dose Serum Sample

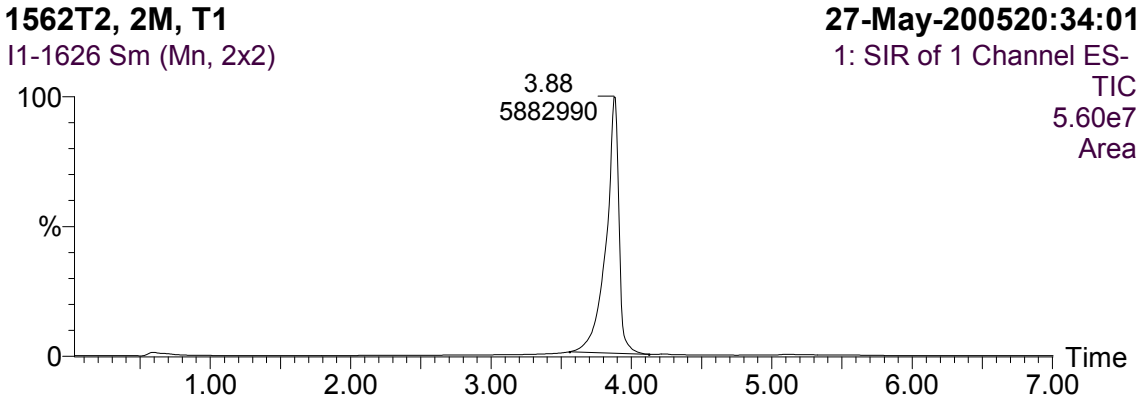

Figure 13: Chromatogram Of Animal No. 1562, Group T2, Male, 1 Hour Post-Dose Serum Sample

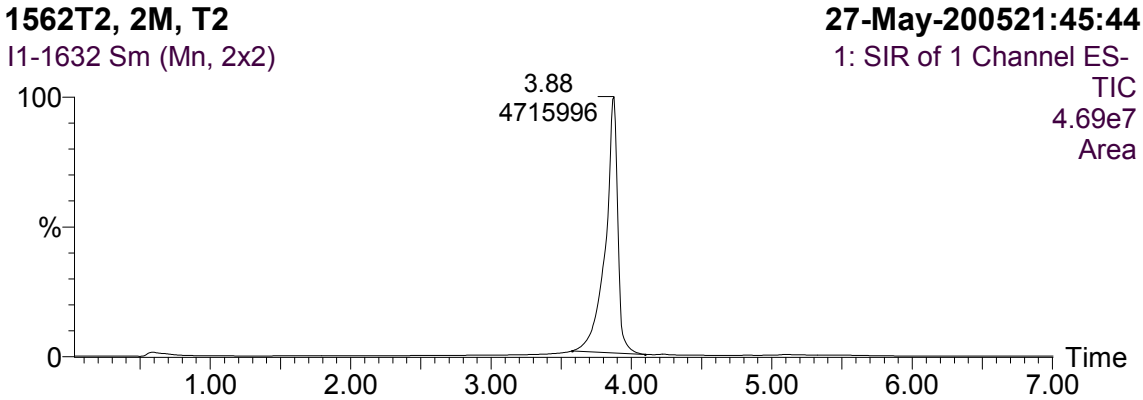

Figure 14: Chromatogram Of Animal No. 1562, Group T2, Male, 2 Hours Post-Dose Serum Sample

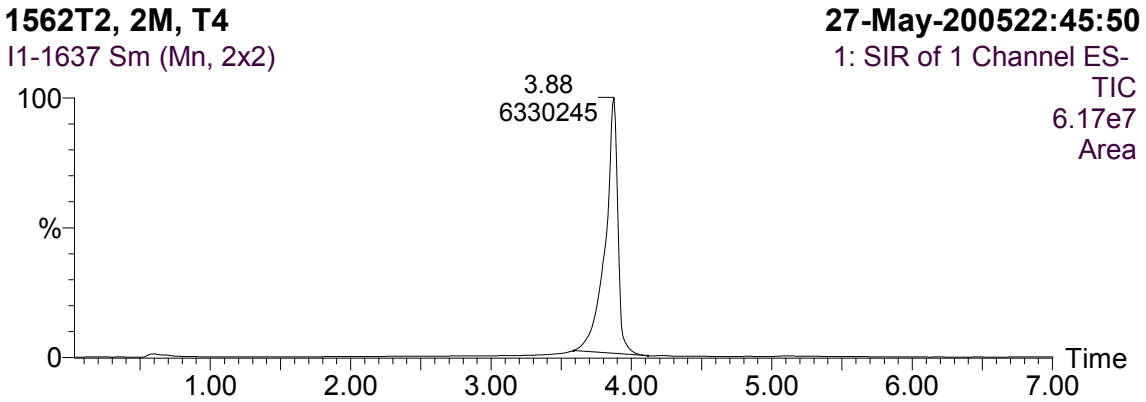

Figure 15: Chromatogram Of Animal No. 1562, Group T2, Male, 4 Hours Post-Dose Serum Sample

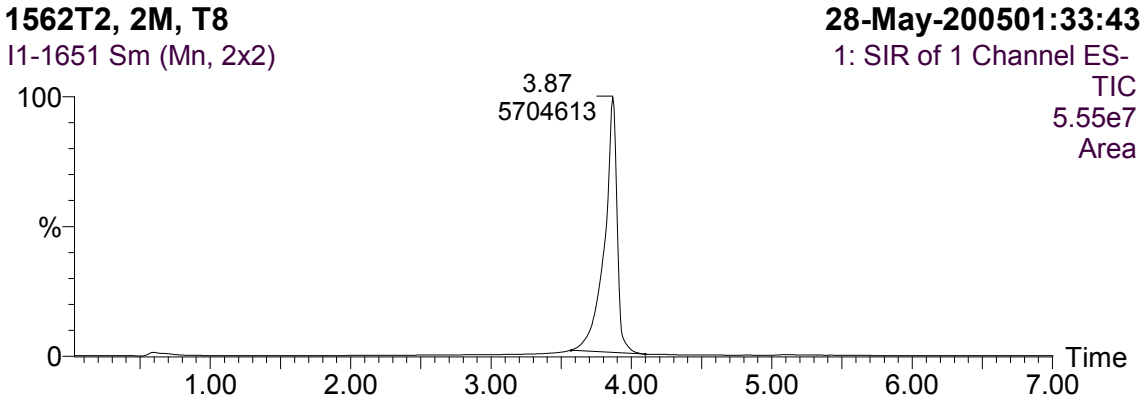

Figure 16: Chromatogram Of Animal No. 1562, Group T2, Male, 8 Hours Post-Dose Serum Sample

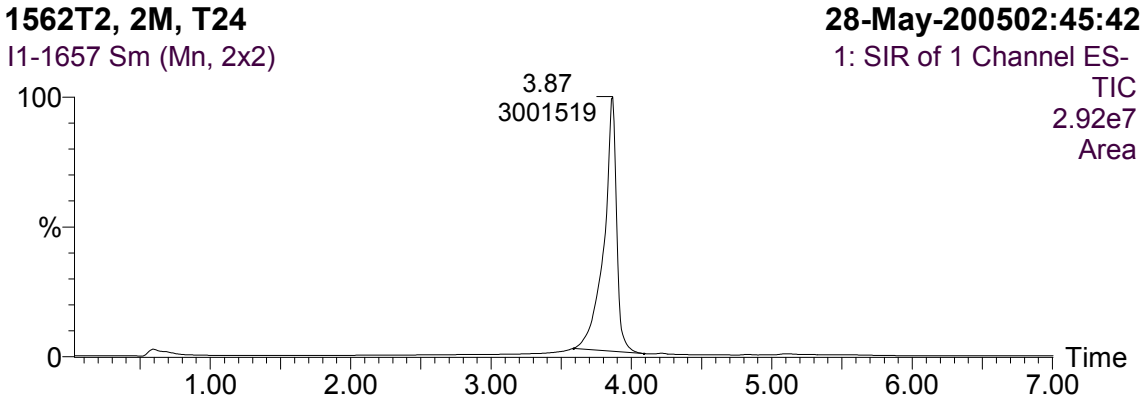

Figure 17: Chromatogram Of Animal No. 1562, Group T2, Male, 24 Hours Post-Dose Serum Sample

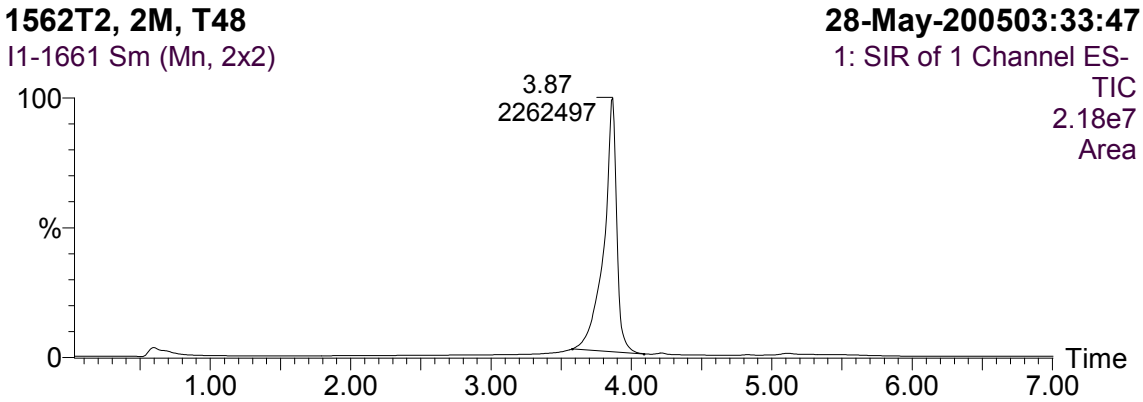

Figure 18: Chromatogram Of Animal No. 1562, Group T2, Male, 48 Hours Post-Dose Serum Sample

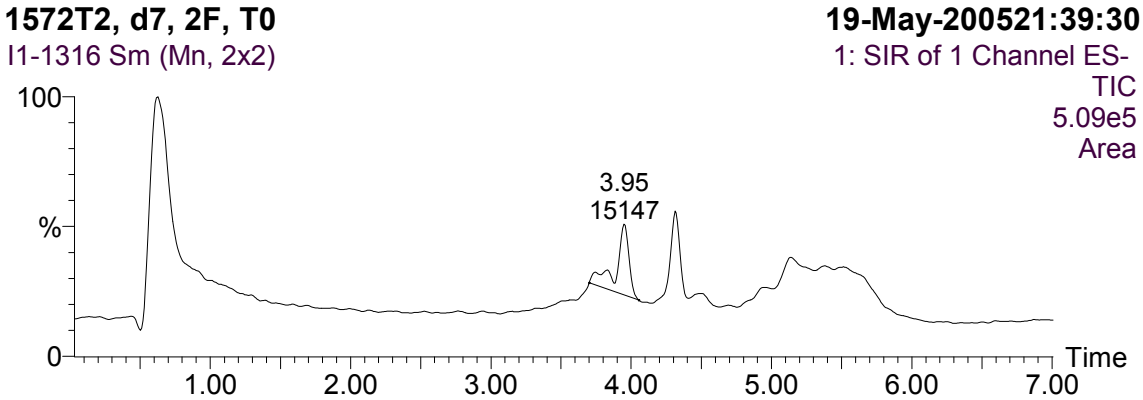

Figure 19: Chromatogram Of Animal No. 1572, Group T2, Female, Pre-Dose Serum Sample

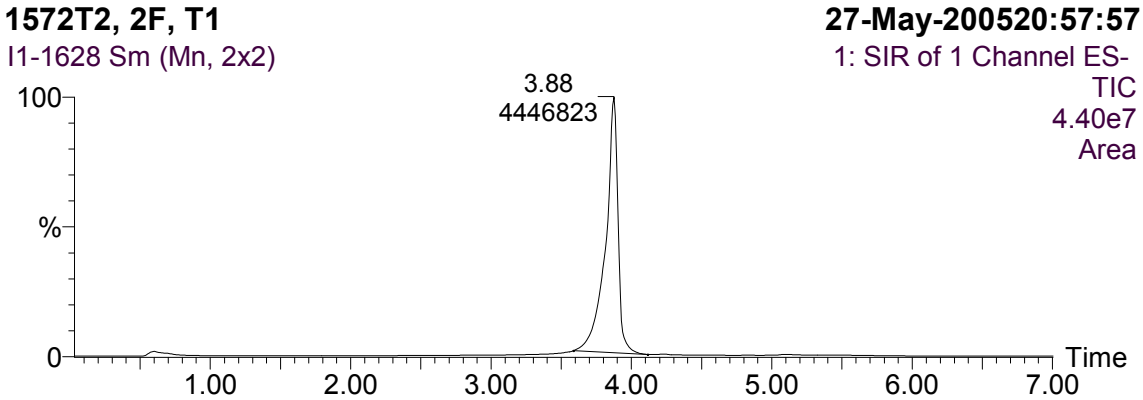

Figure 20: Chromatogram Of Animal No. 1572, Group T2, Female, 1 Hour Post-Dose Serum Sample

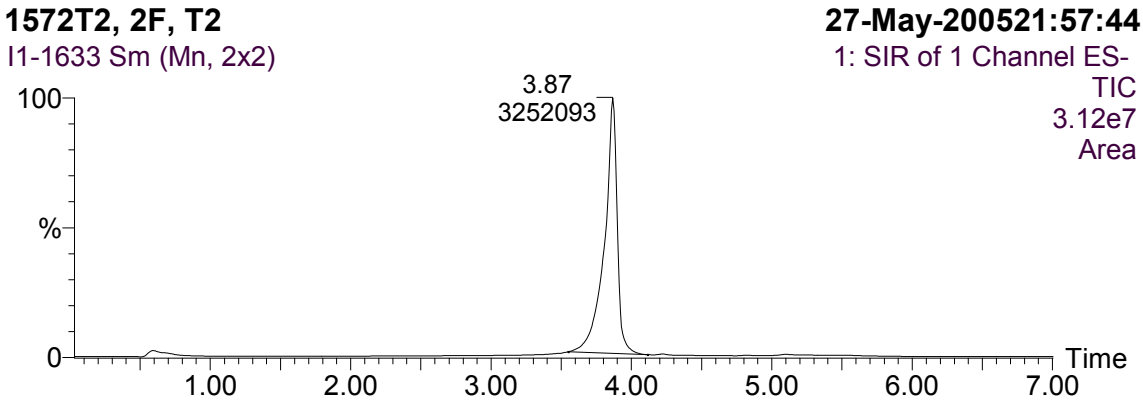

Figure 21: Chromatogram Of Animal No. 1572, Group T2, Female, 2 Hours Post-Dose Serum Sample

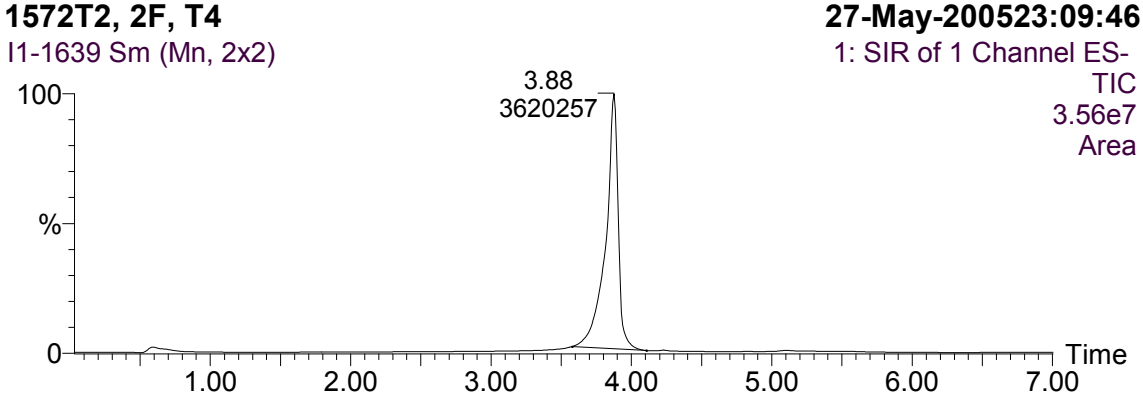

Figure 22: Chromatogram Of Animal No. 1572, Group T2, Female, 4 Hours Post-Dose Serum Sample

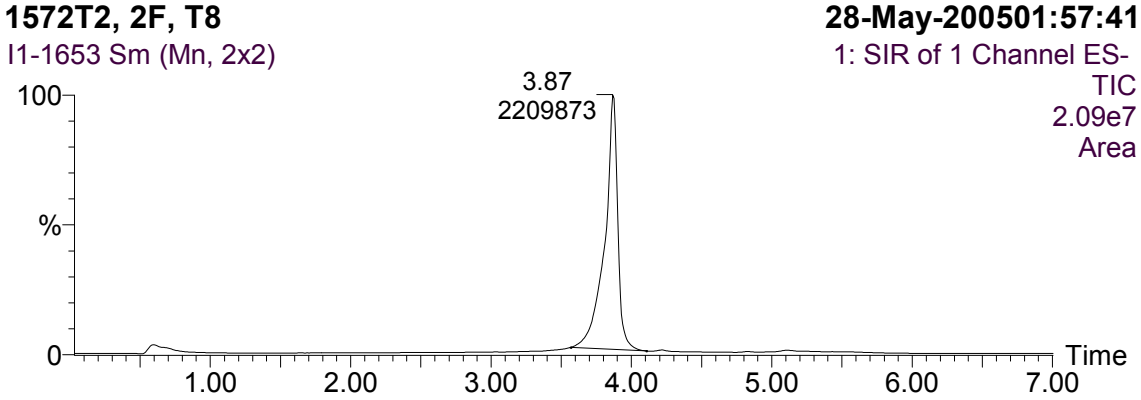

Figure 23: Chromatogram Of Animal No. 1572, Group T2, Female, 8 Hours Post-Dose Serum Sample

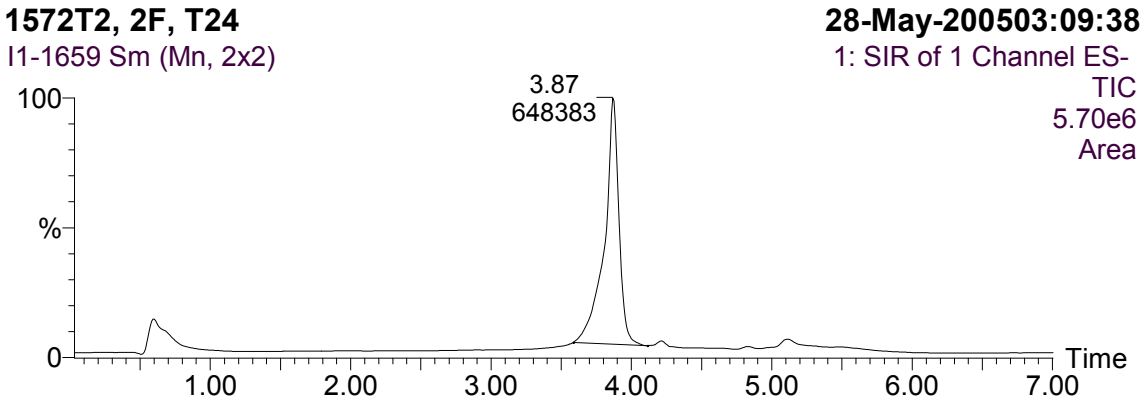

Figure 24: Chromatogram Of Animal No. 1572, Group T2, Female, 24 Hours Post-Dose Serum Sample

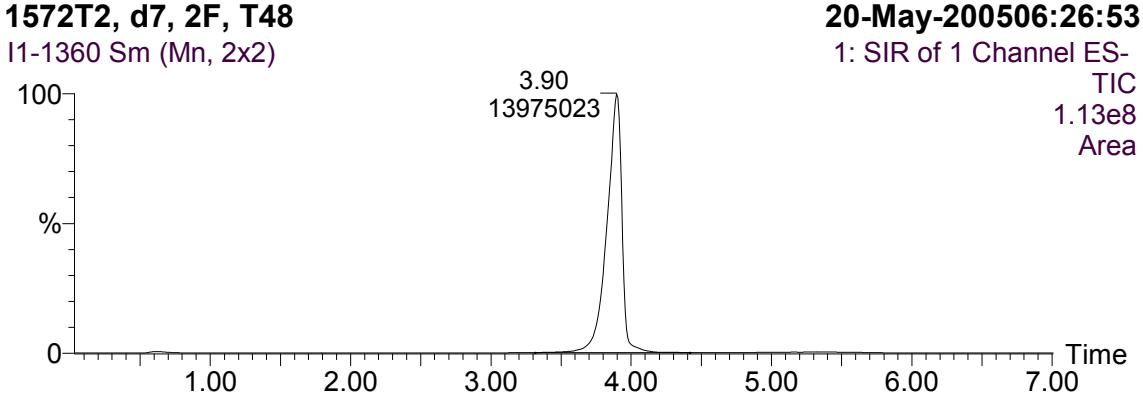

Figure 25: Chromatogram Of Animal No. 1572, Group T2, Female, 48 Hours Post-Dose Serum Sample

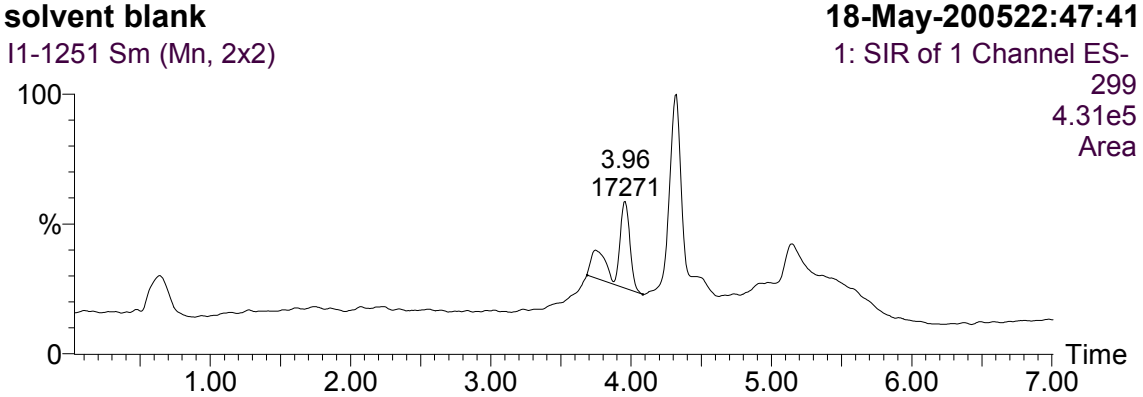

Figure 26: Representative Chromatogram Of A Processed Solvent Blank (Urine Assay)

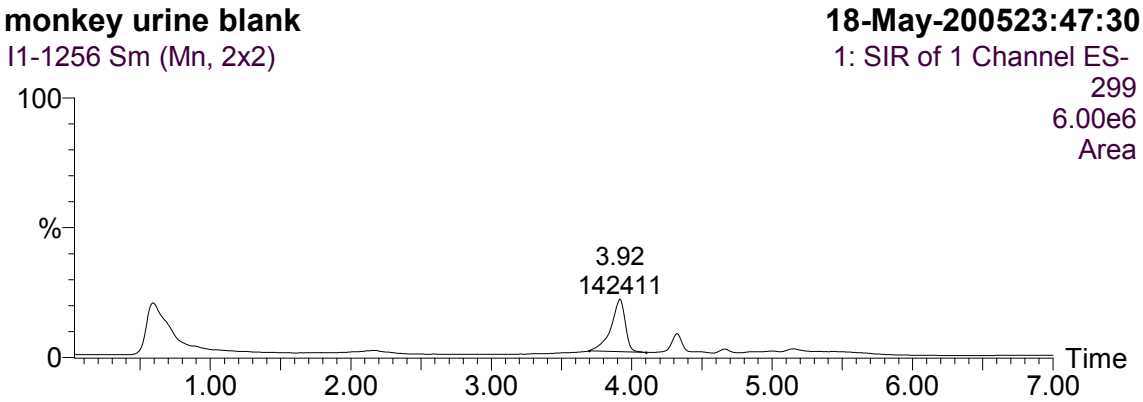

Figure 27: Representative Chromatogram Of A Processed Blank Urine Sample

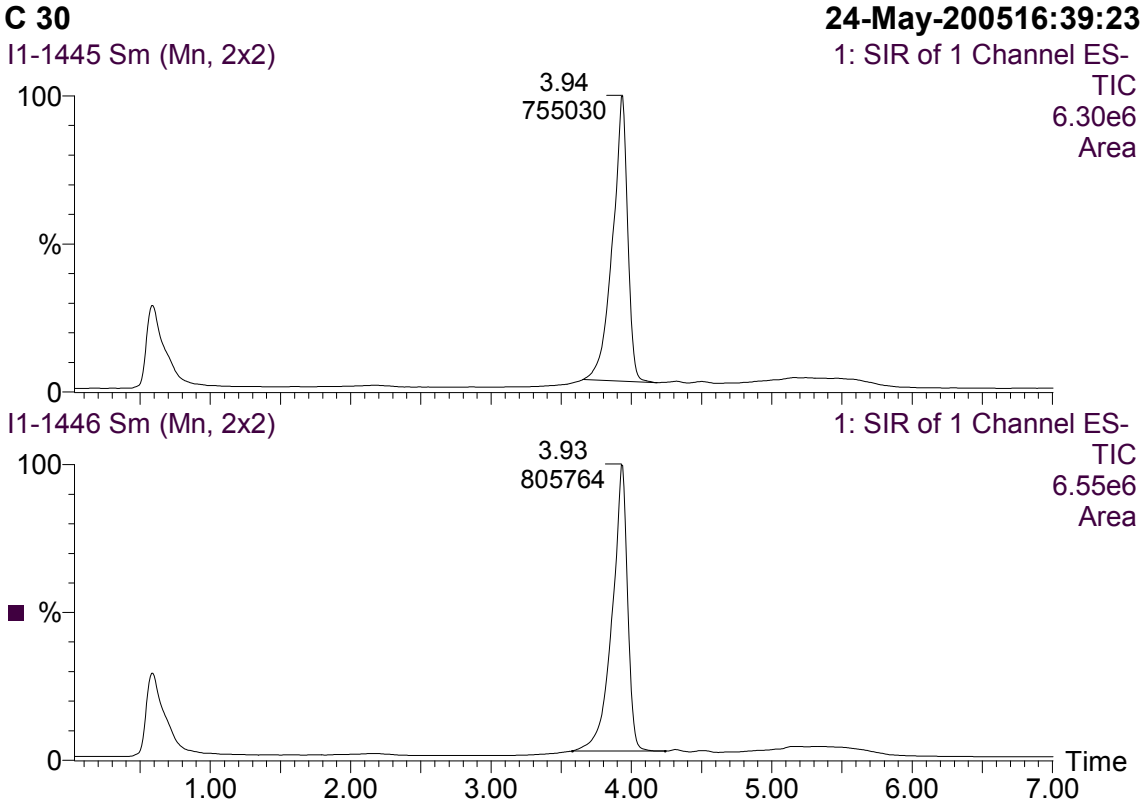

Figure 28: Representative Chromatograms of 30 ng/mL Urine Calibration Samples

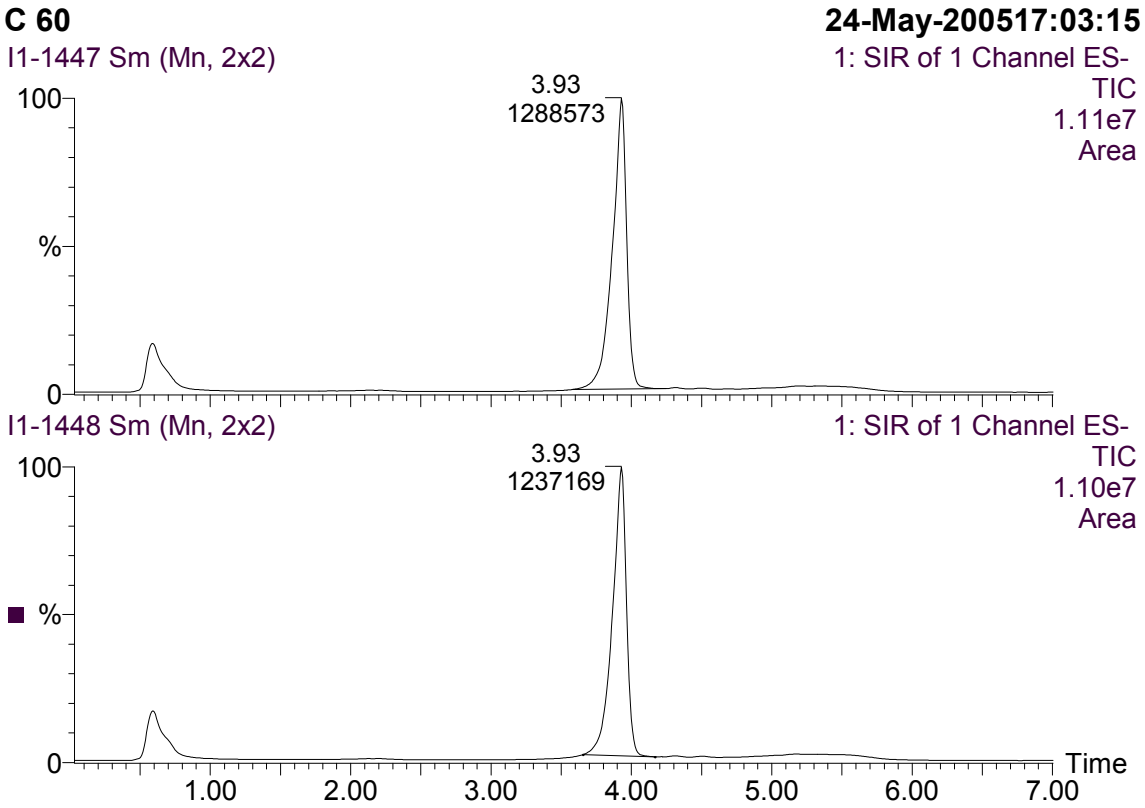

Figure 29: Representative Chromatograms of 60 ng/mL Urine Calibration Samples

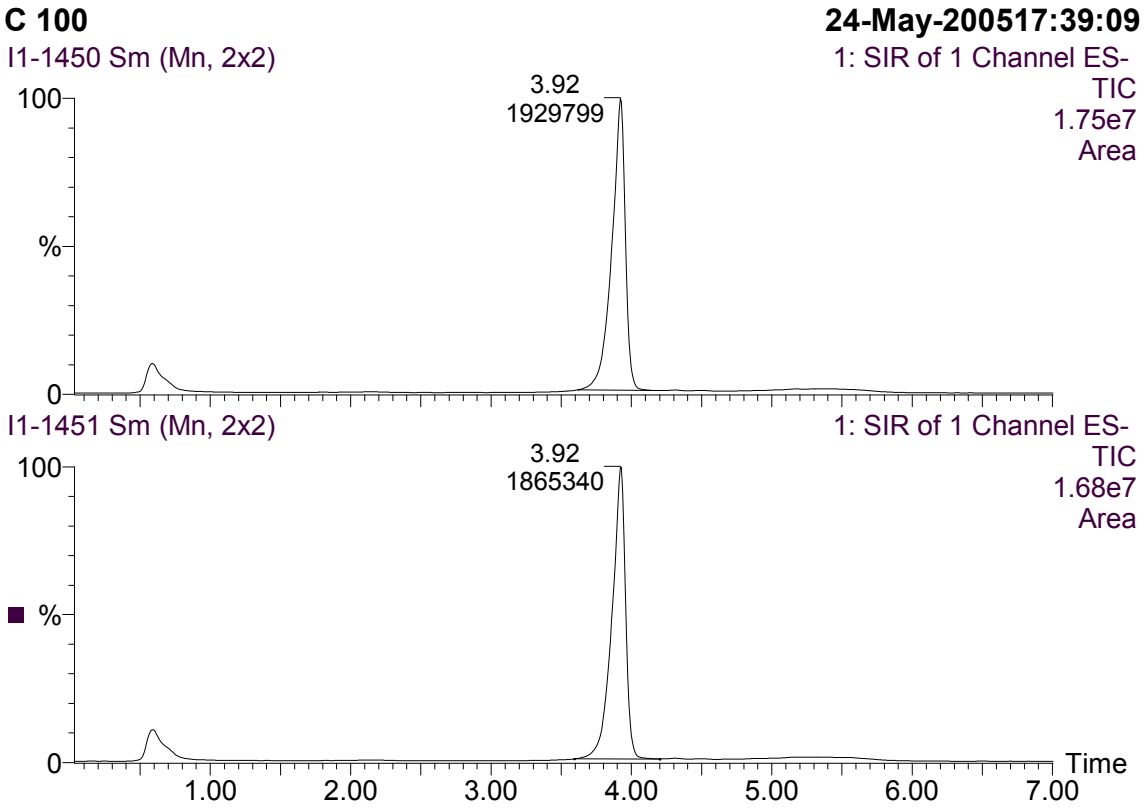

Figure 30: Representative Chromatograms of 100 ng/mL Urine Calibration Samples

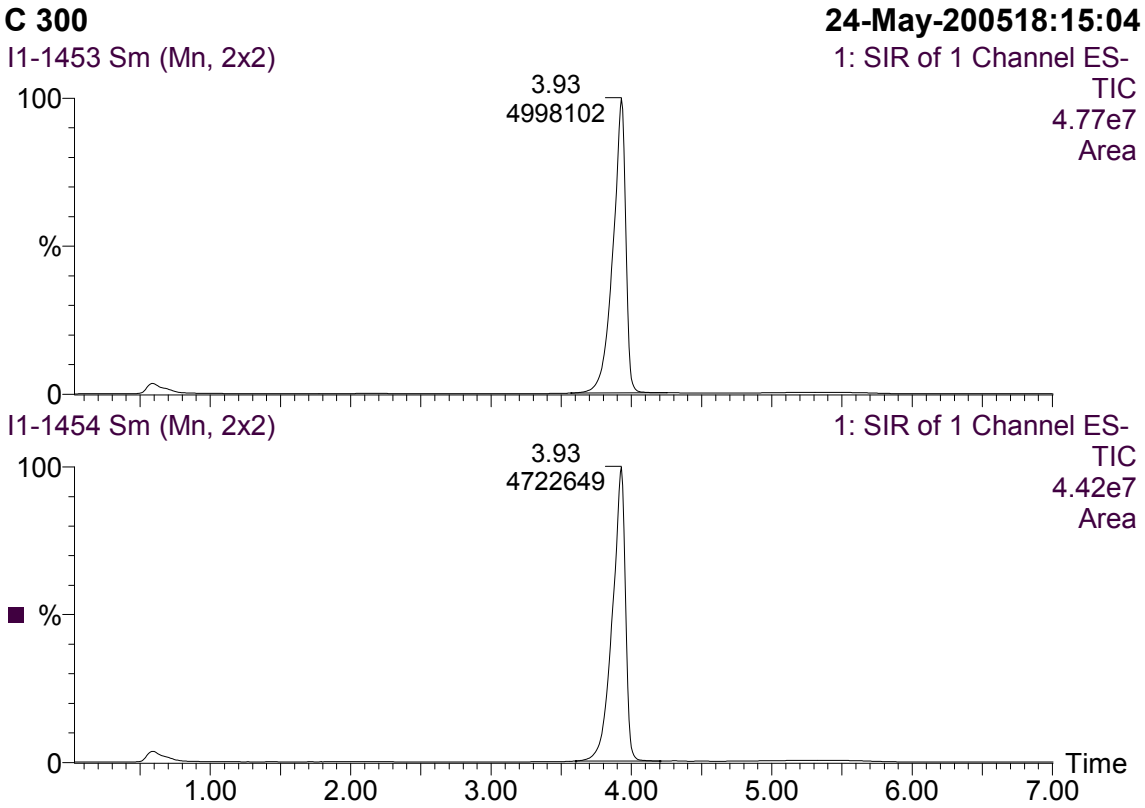

Figure 31: Representative Chromatograms of 300 ng/mL Urine Calibration Samples

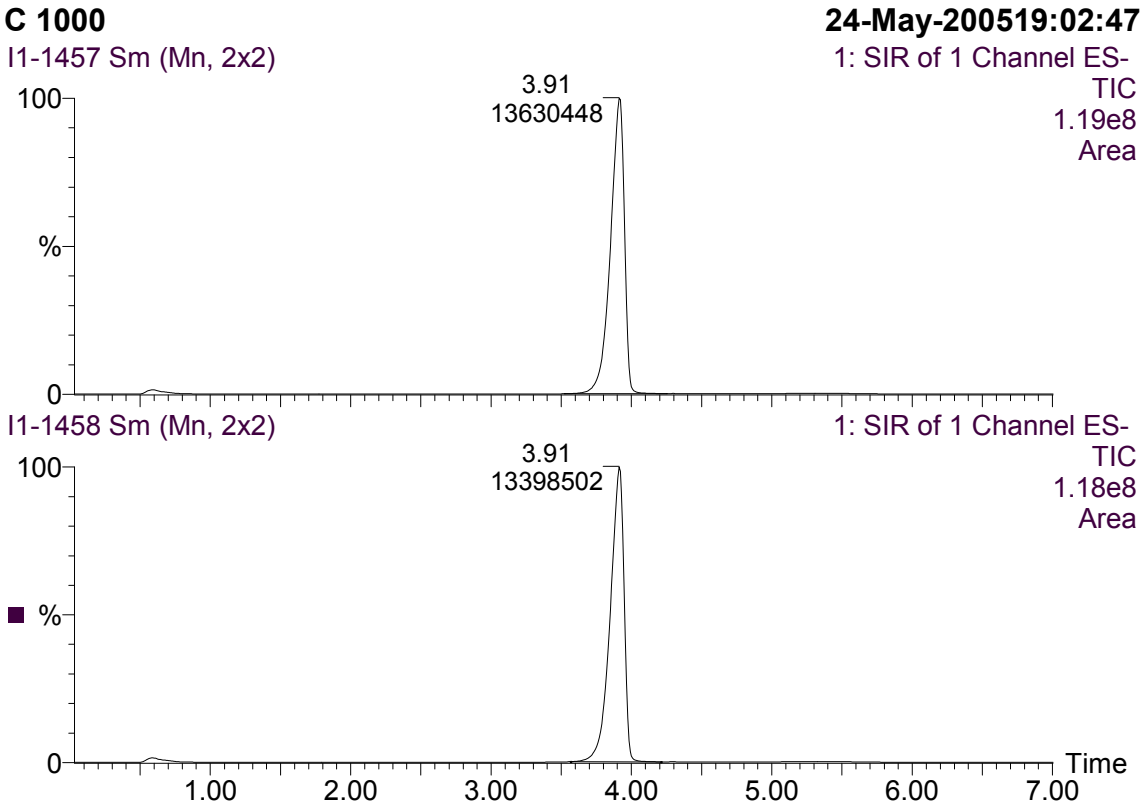

Figure 32: Representative Chromatograms of 1000 ng/mL Urine Calibration Samples

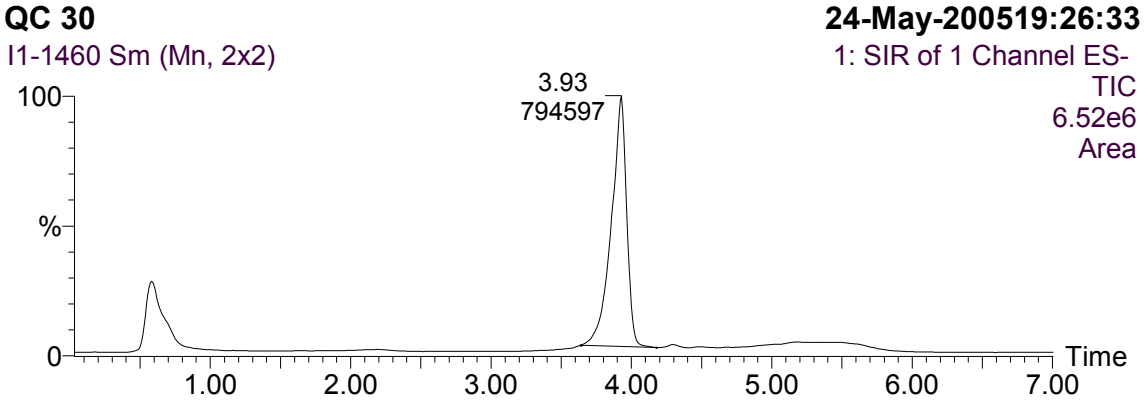

Figure 33: Representative Chromatogram Of A 30 ng/mL Urine QC Sample

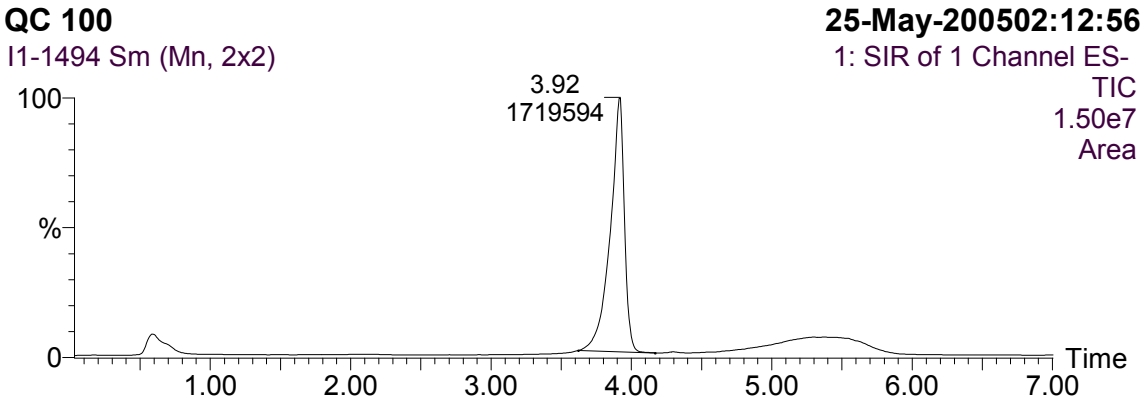

Figure 34: Representative Chromatogram Of A 100 ng/mL Urine QC Sample

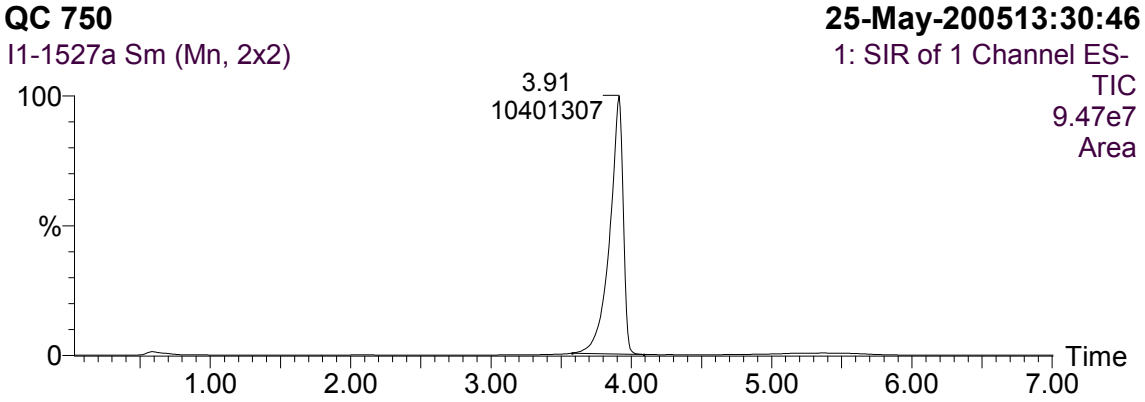

Figure 35: Representative Chromatogram Of A 750 ng/mL Urine QC Sample

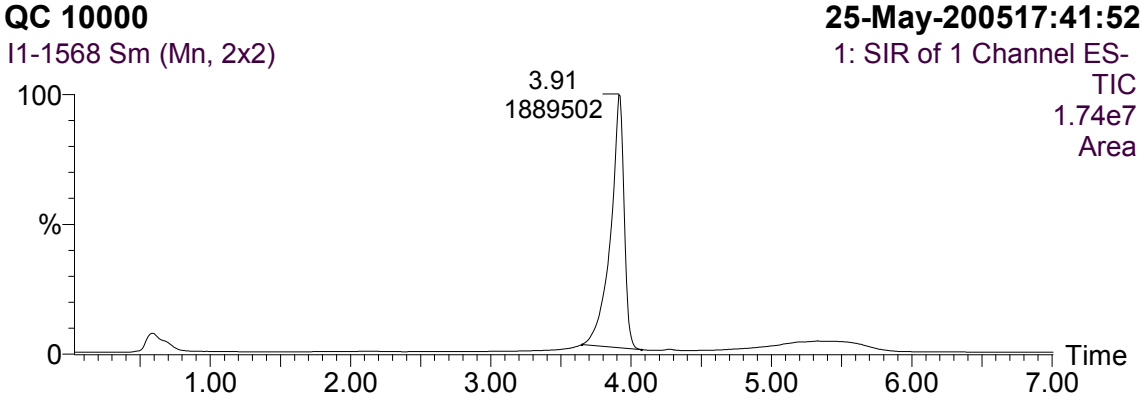

Figure 36: Representative Chromatogram Of A 10,000 ng/mL Urine QC Sample

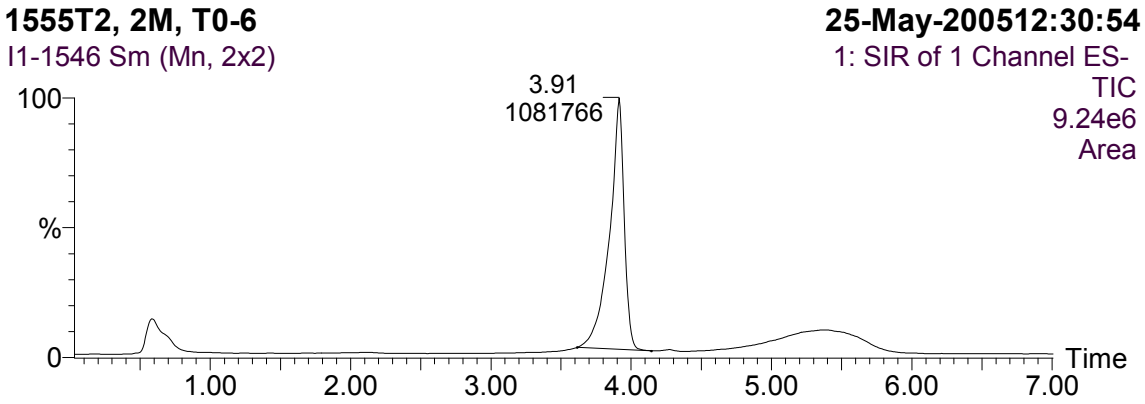

Figure 37: Chromatogram Of Animal No. 1555, Group T2, Male, 0 To 6 Hours Post-Dose Urine Sample

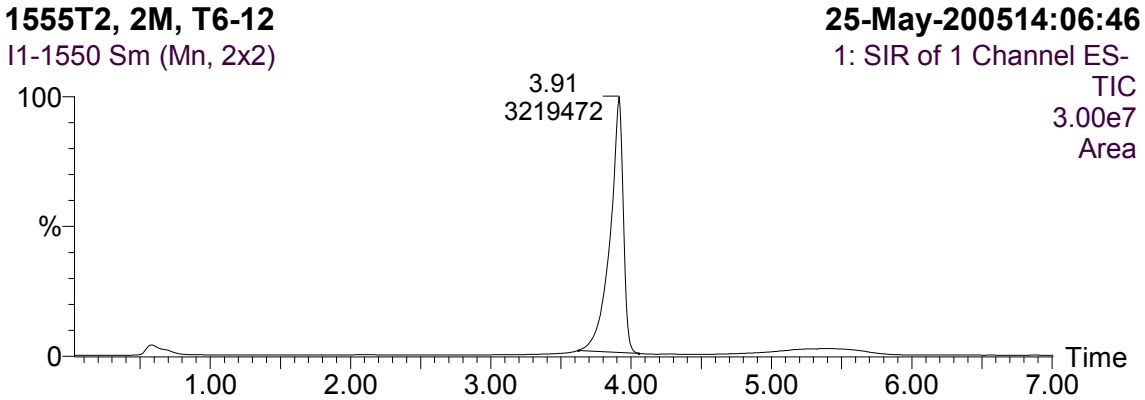

Figure 38: Chromatogram Of Animal No. 1555, Group T2, Male, 6 To 12 Hours Post-Dose Urine Sample

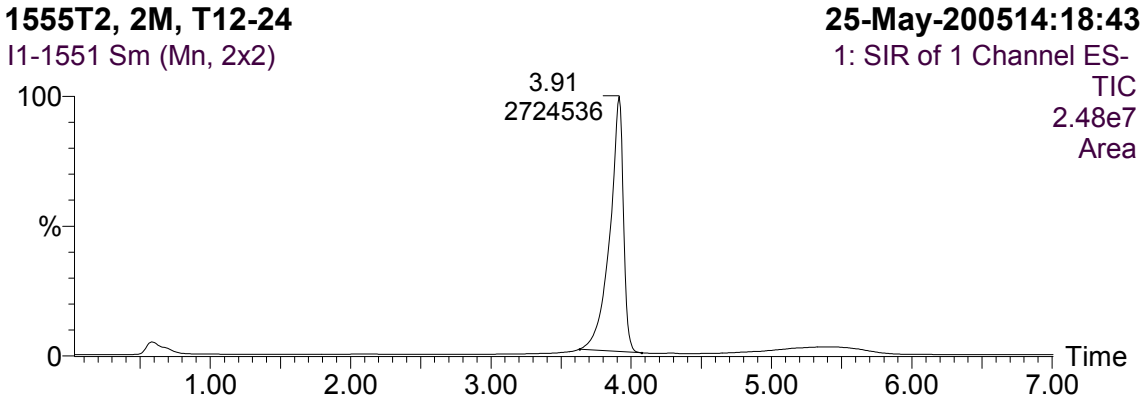

Figure 39: Chromatogram Of Animal No. 1555, Group T2, Male, 12 To 24 Hours Post-Dose Urine Sample

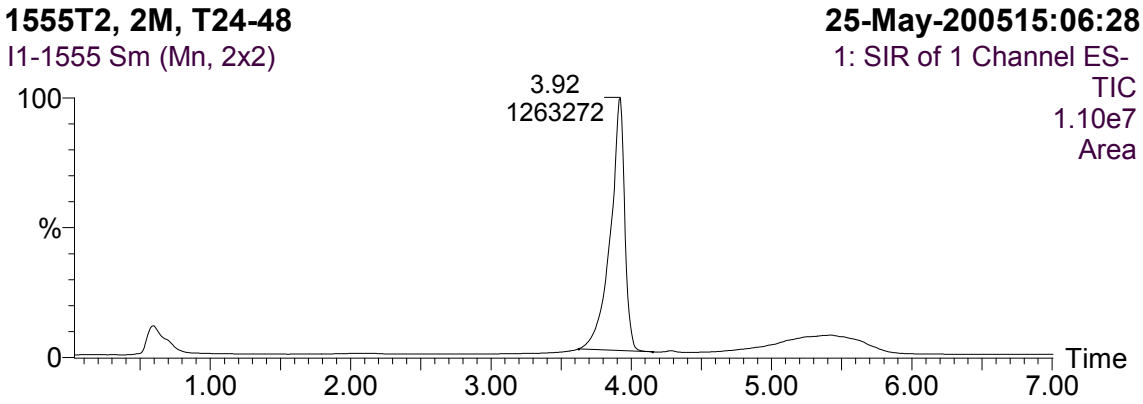

Figure 40: Chromatogram Of Animal No. 1555, Group T2, Male, Day 2 Urine Sample

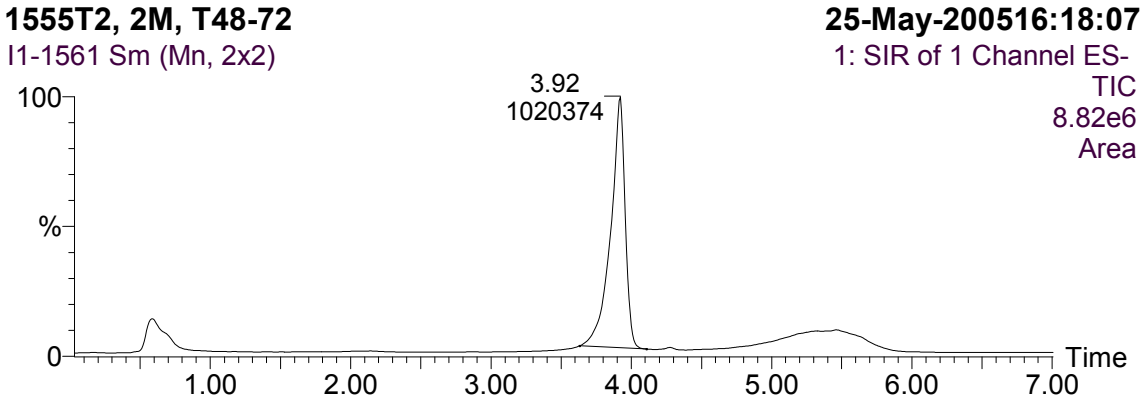

Figure 41: Chromatogram Of Animal No. 1555, Group T2, Male, Day 3 Urine Sample

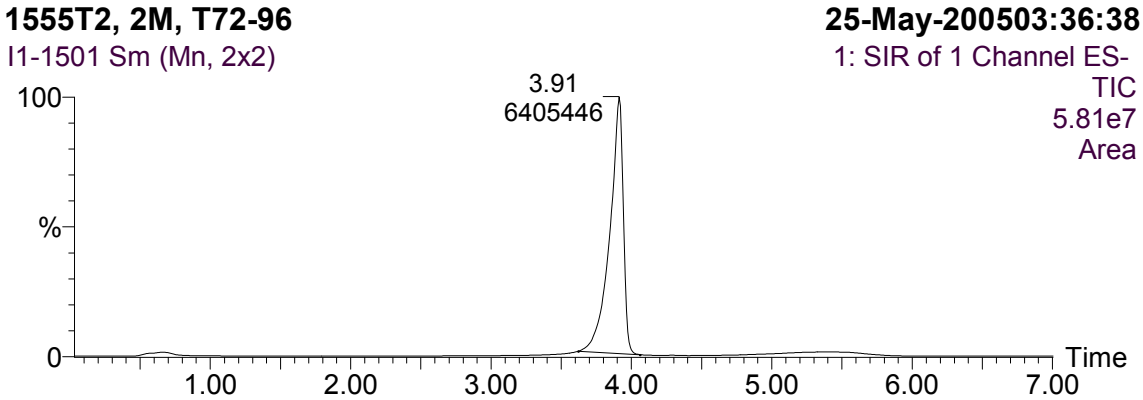

Figure 42: Chromatogram Of Animal No. 1555, Group T2, Male, Day 4 Urine Sample

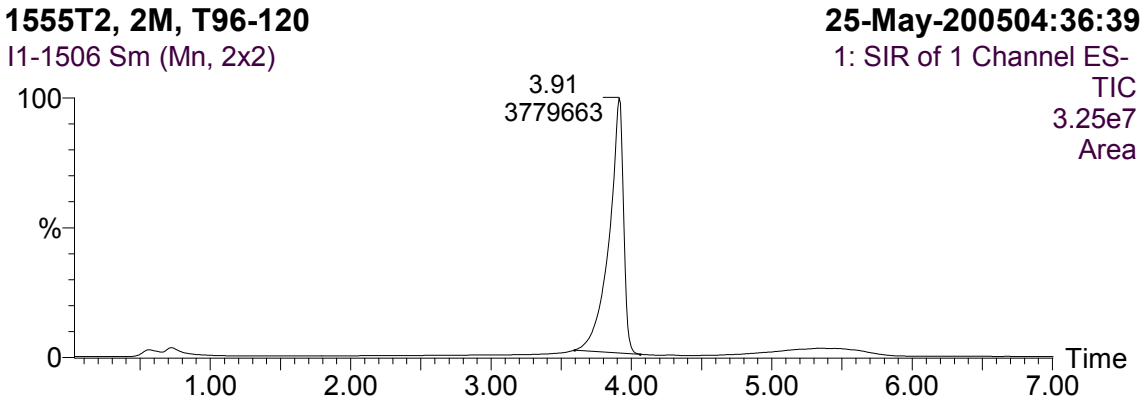

Figure 43: Chromatogram Of Animal No. 1555, Group T2, Male, Day 5 Urine Sample

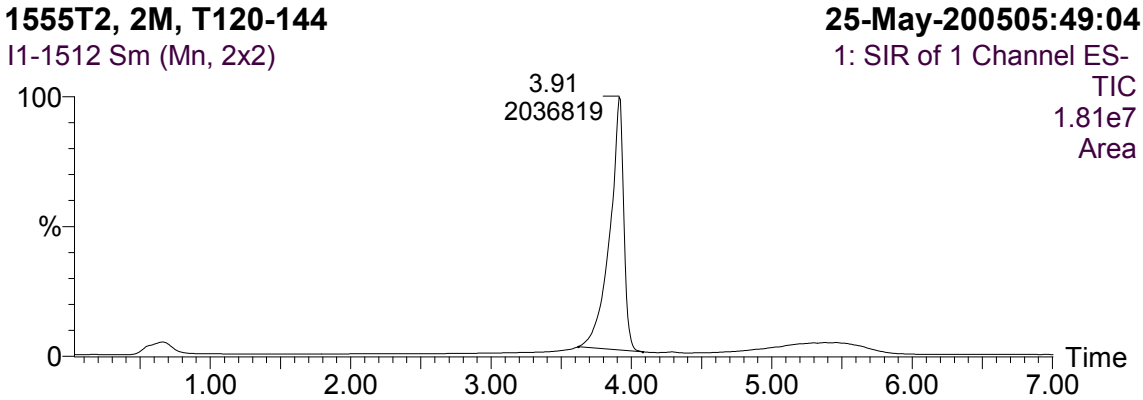

Figure 44: Chromatogram Of Animal No. 1555, Group T2, Male, Day 6 Urine Sample

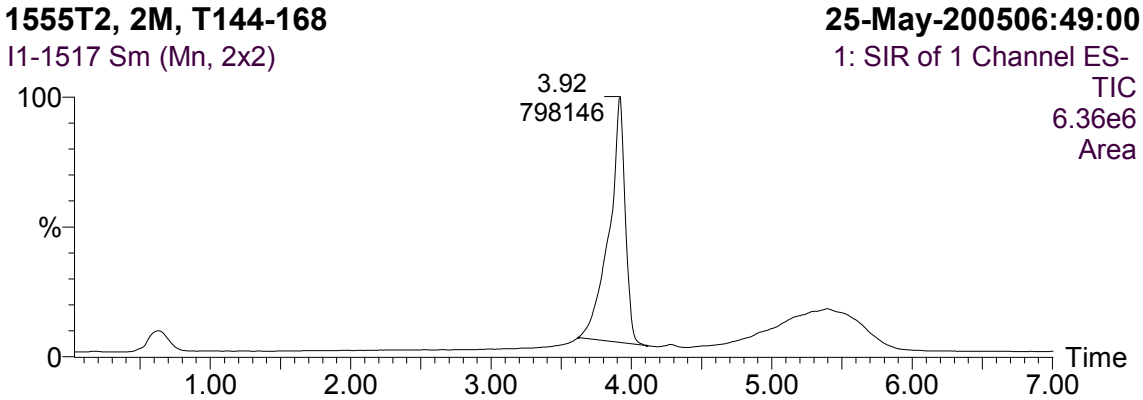

Figure 45: Chromatogram Of Animal No. 1555, Group T2, Male, Day 7 Urine Sample

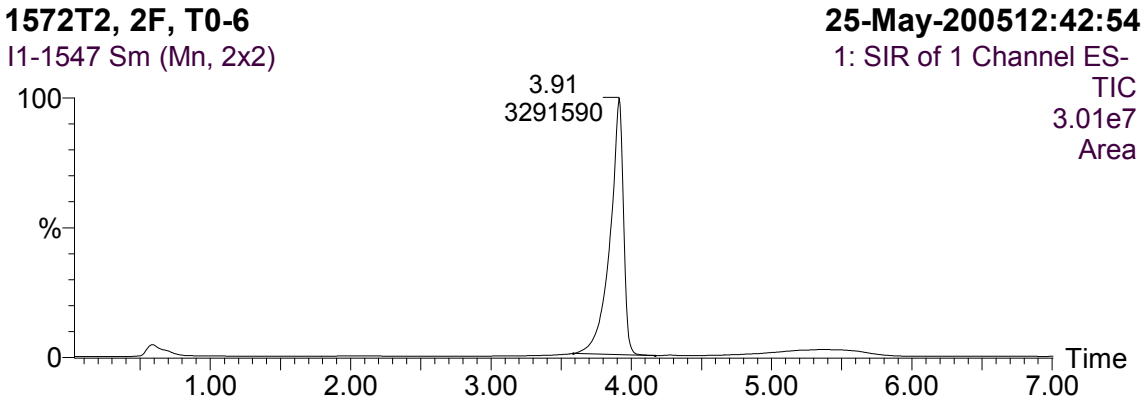

Figure 46: Chromatogram Of Animal No. 1572, Group T2, Female, 0 To 6 Hours Post-Dose Urine Sample

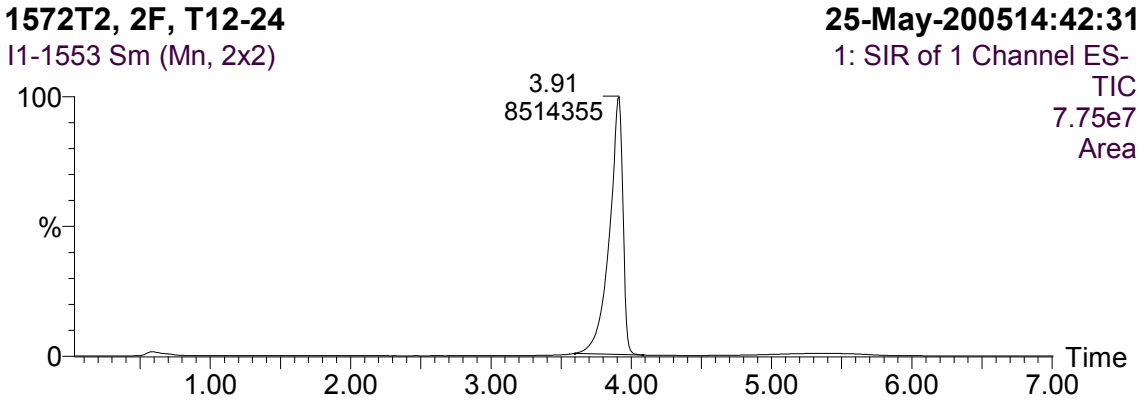

Figure 47: Chromatogram Of Animal No. 1572, Group T2, Female, 12 To 24 Hours Post-Dose Urine Sample

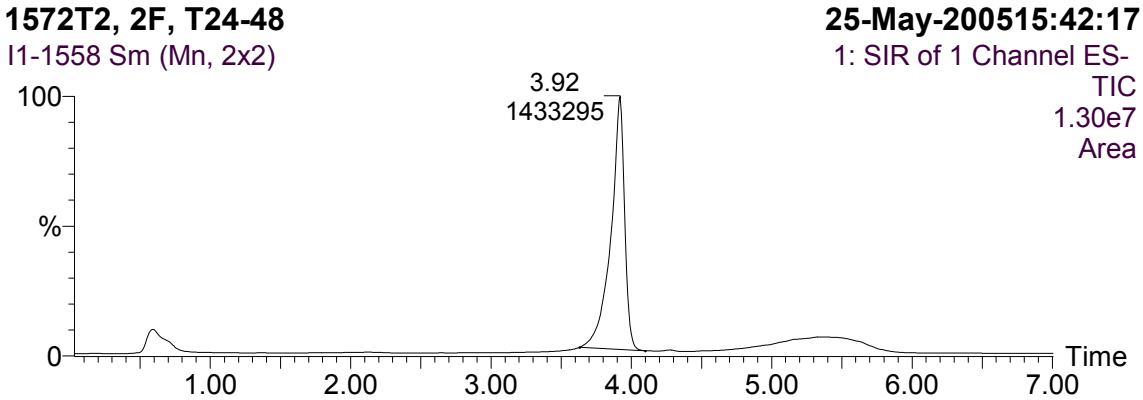

Figure 48: Chromatogram Of Animal No. 1572, Group T2, Female, Day 2 Urine Sample

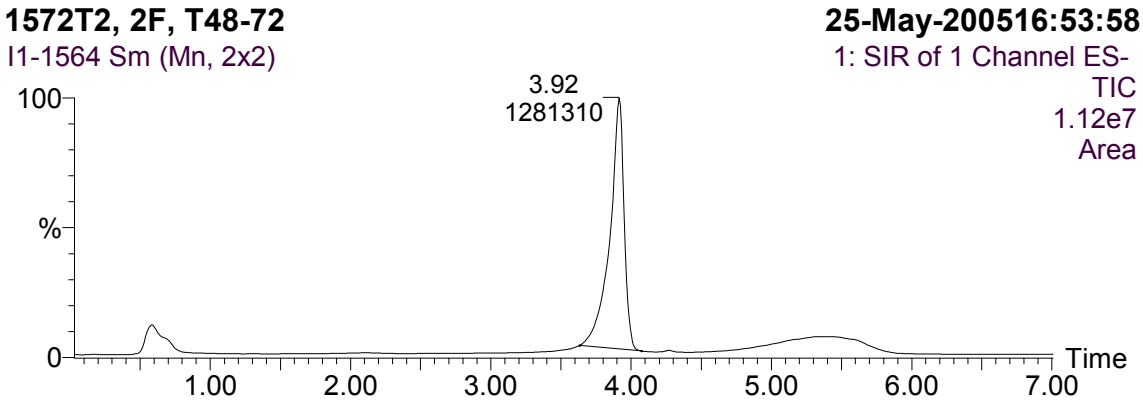

Figure 49: Chromatogram Of Animal No. 1572, Group T2, Female, Day 3 Urine Sample

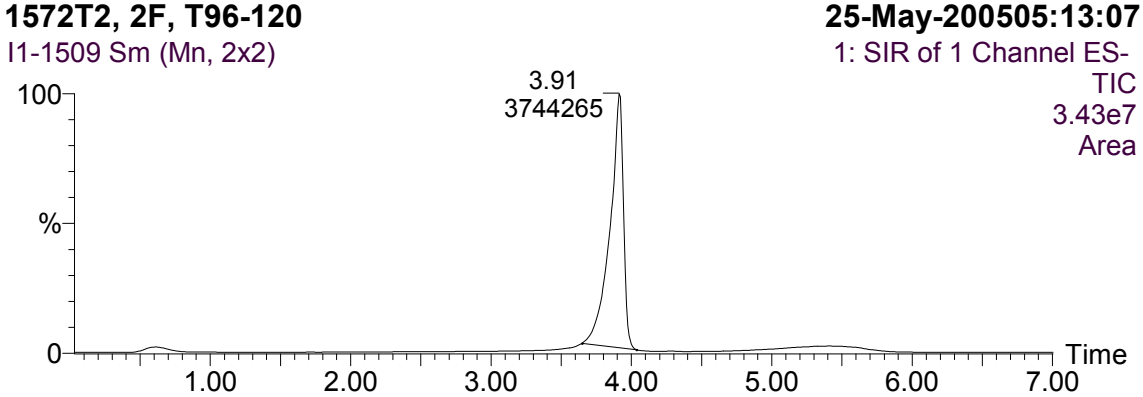

Figure 50: Chromatogram Of Animal No. 1572, Group T2, Female, Day 5 Urine Sample

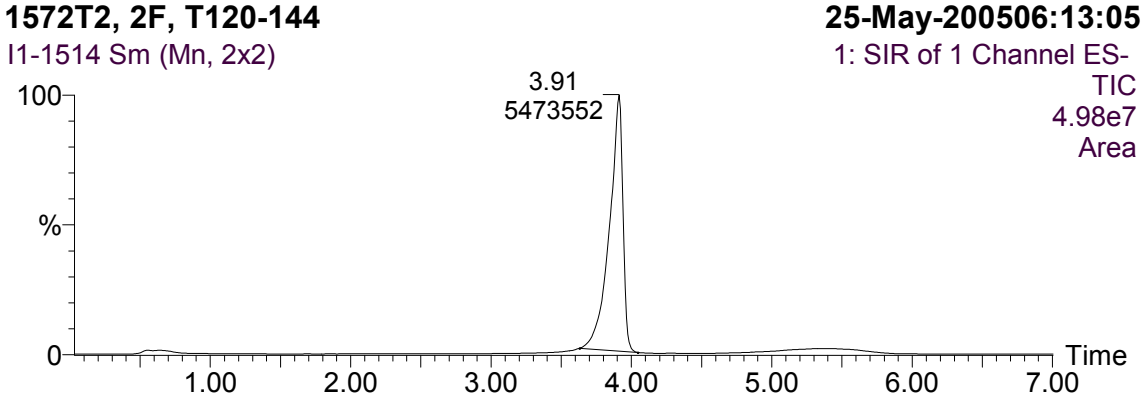

Figure 51: Chromatogram Of Animal No. 1572, Group T2, Female, Day 6 Urine Sample

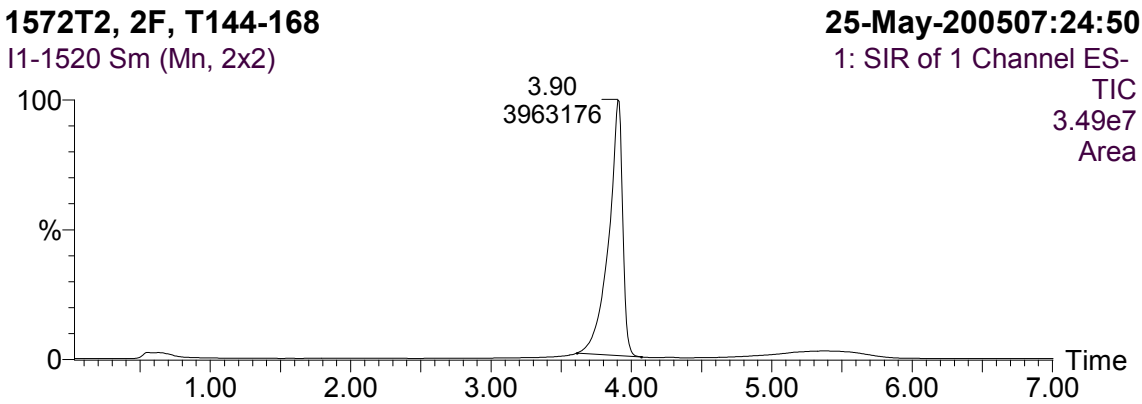

Figure 52: Chromatogram Of Animal No. 1572, Group T2, Female, Day 7 Urine Sample

ATTACHMENT I

Supporting Data

Table B1: Calibration and Quality Control samples for Sequence 534002(PFBS)AMS

Quantify Compound Summary Report

Sample List:C:\MASSLYNX\534002.PRO\SampleDB\534002(PFBS)AMS
Last modified:Mon May 16 12:01:50 2005
Method:C:\MASSLYNX\534002.PRO\MethDB\534002 PFBS bio
Last modified:Thu May 12 15:20:32 2005
Job Code:

Printed:Tue May 17 09:56:31 2005

Compound 1: PFBS

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1084	159-10	sys suit	3.75	7331770	bb	0	395	
I1-1085	159-10	sys suit	3.78	5270496	bb	0	266	
I1-1086	159-10	sys suit	3.85	11412764	bb	0	669	
I1-1087		ACN	3.92	77692	bb	0	1.10	
I1-1088		ACN	3.94	83675	bb	0	1.22	
I1-1089	160-1	solvent blank	3.94	76621	bb	0	1.08	
I1-1090	160-2	monkey serum blank	3.92	278415	bb	0	6.34	
I1-1091	160-3	C 30	3.9	922320	bb	0	30.4	1.4
I1-1092	160-4	C 30	3.91	907270	bb	0	29.8	-0.71
I1-1093	160-5	C 30	3.91	968275	bb	0	32.4	7.9
I1-1094	160-6	C 60	3.9	1522624	bb	0	57.5	-4.2
I1-1095	160-7	C 60	3.9	1515075	bb	0	57.1	-4.8
I1-1096	160-8	C 60	3.9	1532191	bb	0	57.9	-3.4
I1-1097	160-9	C 100	3.9	2338696	bb	0	98.3	-1.7
I1-1098	160-10	C 100	3.9	2355722	bb	0	99.2	-0.82
I1-1099	160-11	C 100	3.91	2354862	bb	0	99.1	-0.86
I1-1100	160-12	C 300	3.91	5879274	bb	0	303	1.1
I1-1101	160-13	C 300	3.9	6251189	bb	0	326	8.8
I1-1102	160-14	C 300	3.9	5937480	bb	0	307	2.3
I1-1103	160-15	C 1000	3.9	16027253	bb	0	996	-0.37
I1-1104	160-16	C 1000	3.9	15668597	bb	0	970	-3.0
I1-1105	160-17	C 1000	3.9	15980169	bb	0	993	-0.72
I1-1106		ACN	3.95	107268	bb	0	1.73	
I1-1107	161-1	QC 30	3.91	988579	bb	0	33.2	11
I1-1108	161-2	QC 30	3.91	986068	bb	0	33.1	10
I1-1109	161-3	QC 30	3.91	943214	bb	0	31.3	4.4
I1-1110	161-4	QC 100	3.9	2320141	bb	0	97.3	-2.7
I1-1111	161-5	QC 100	3.91	2303087	bb	0	96.4	-3.6
I1-1112	161-6	QC 100	3.91	2344681	bb	0	98.6	-1.4
I1-1113	161-7	QC 750	3.9	13013934	bb	0	781	4.1
I1-1114	161-8	QC 750	3.9	13102704	bb	0	787	4.9
I1-1115	161-9	QC 750	3.91	13042604	bb	0	783	4.3
I1-1116		ACN	3.95	108371	bb	0	1.75	

Table B2: Calibration and Quality Control samples for Sequence 534002(PFBS)BMS

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534002.PRO\SampleDB\534002(PFBS)BMS
Last modified: Tue May 17 11:56:03 2005
Method: C:\MASSLYNX\534002.PRO\MethDB\534002 PFBS bio
Last modified: Thu May 12 15:20:32 2005
Job Code:

Printed: Wed May 18 08:08:09 2005

Compound 1: PFBS

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1117	163-10	sys suit	3.89	13581449	bb	0	869	
I1-1118	163-10	sys suit	3.87	16524385	bb	0	1095	
I1-1119	163-10	sys suit	3.87	16919534	bb	0	1125	
I1-1120		ACN	3.92	92811	bb	0	1.67	
I1-1121		ACN	3.92	89247	MM	0	1.58	
I1-1122	164-1	solvent blank	3.94	74144	MM	0	1.23	
I1-1123	164-2	monkey serum blank	3.91	317599	bb	0	8.41	
I1-1124	164-3	C 30	3.9	862520	bb	0	30.1	0.49
I1-1125	164-4	C 30	3.9	868528	bb	0	30.4	1.4
I1-1126	164-5	C 30	3.9	891484	bb	0	31.4	4.8
I1-1127	164-6	C 60	3.9	1470536	bb	0	58.8	-2.0
I1-1128	164-7	C 60	3.9	1451740	bb	0	57.9	-3.5
I1-1129	164-8	C 60	3.9	1474800	bb	0	59.0	-1.6
I1-1130	164-9	C 100	3.89	2203214	bb	0	97.0	-3.0
I1-1131	164-10	C 100	3.9	2243193	bb	0	99.2	-0.83
I1-1132	164-11	C 100	3.9	2195590	bb	0	96.6	-3.4
I1-1133	164-12	C 300	3.9	5783489	bb	0	314	4.7
I1-1134	164-13	C 300	3.9	5611849	bb	0	303	0.96
I1-1135	164-14	C 300	3.9	5881758	bb	0	321	6.8
I1-1136	164-15	C 1000	3.9	15275102	bb	0	998	-0.21
I1-1137	164-16	C 1000	3.9	14953873	bb	0	973	-2.7
I1-1138	164-17	C 1000	3.89	15160161	bb	0	989	-1.1
I1-1139		ACN	3.95	72429	MM	0	1.19	
I1-1140	165-1	QC 30	3.9	898911	bb	0	31.8	5.9
I1-1141	165-2	QC 30	3.9	901383	bb	0	31.9	6.2
I1-1142	165-3	QC 30	3.9	890840	bb	0	31.4	4.7
I1-1143	165-4	QC 100	3.9	2241170	bb	0	99.1	-0.94
I1-1144	165-5	QC 100	3.9	2176153	bb	0	95.5	-4.5
I1-1145	165-6	QC 100	3.9	2192018	bb	0	96.4	-3.6
I1-1146	165-7	QC 750	3.9	12536870	bb	0	790	5.4
I1-1147	165-8	QC 750	3.9	12790317	bb	0	809	7.9
I1-1148	165-9	QC 750	3.9	12483573	bb	0	786	4.9
I1-1149		ACN	3.95	70670	MM	0	1.15	

Table B3: Calibration and Quality Control samples for Sequence 534002(PFBS)CMS

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534002.PRO\SampleDB\534002(PFBS)CMS
Last modified: Tue May 17 13:39:04 2005
Method: C:\MASSLYNX\534002.PRO\MethDB\534002 PFBS bio
Last modified: Thu May 12 15:20:32 2005
Job Code:

Printed: Wed May 18 08:31:16 2005

Compound 1: PFBS

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1150		ACN	3.95	73178	MM	0	0.766	
I1-1151	167-1	solvent blank	3.95	64746	MM	0	0.628	
I1-1152	167-2	monkey serum blank	3.92	365399	bb	0	8.59	
I1-1153	167-3	C 30	3.91	902227	bb	0	29.7	-1.0
I1-1154	167-4	C 30	3.91	918682	bb	0	30.4	1.4
I1-1155	167-5	C 30	3.91	982419	bb	0	33.3	11
I1-1156	167-6	C 60	3.9	1481802	bb	0	57.0	-5.1
I1-1157	167-7	C 60	3.9	1473476	bb	0	56.5	-5.8
I1-1158	167-8	C 60	3.9	1504677	bb	0	58.1	-3.2
I1-1159	167-9	C 100	3.9	2198486	bb	0	94.3	-5.7
I1-1160	167-10	C 100	3.9	2298798	bb	0	99.8	-0.18
I1-1161	167-11	C 100	3.91	2279446	bb	0	98.8	-1.24
I1-1162	167-12	C 300	3.9	5786429	bb	0	311	3.7
I1-1163	167-13	C 300	3.9	5889900	bb	0	318	5.9
I1-1164	167-14	C 300	3.9	5951225	bb	0	322	7.2
I1-1165	167-15	C 1000	3.9	15342602	bb	0	977	-2.3
I1-1166	167-16	C 1000	3.89	15447684	bb	0	984	-1.6
I1-1167	167-17	C 1000	3.9	15456362	bb	0	985	-1.5
I1-1168		ACN	3.95	72080	MM	0	0.747	
I1-1169	168-1	QC 30	3.9	919639	bb	0	30.5	1.5
I1-1170	168-2	QC 30	3.9	927221	bb	0	30.8	2.7
I1-1171	168-3	QC 30	3.9	950088	bb	0	31.8	6.0
I1-1172	168-4	QC 100	3.9	2281158	bb	0	98.9	-1.1
I1-1173	168-5	QC 100	3.9	2191687	bb	0	94.0	-6.0
I1-1174	168-6	QC 100	3.9	2215146	bb	0	95.3	-4.7
I1-1175	168-7	QC 750	3.9	13062899	bb	0	812	8.2
I1-1176	168-8	QC 750	3.9	13249481	bb	0	825	10
I1-1177	168-9	QC 750	3.9	13335701	bb	0	831	11
I1-1178		ACN	3.96	76724	MM	0	0.826	

Table B4: Calibration and Quality Control samples for Sequence 534002(PFBS)DMS

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534002.PRO\SampleDB\534002(PFBS)DMS
Last modified: Tue May 17 14:36:14 2005
Method: C:\MASSLYNX\534002.PRO\MethDB\534002 PFBS bio
Last modified: Thu May 12 15:20:32 2005
Job Code:

Printed: Wed May 18 08:48:33 2005

Compound 1: PFBS

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1179		ACN	3.96	74740	MM	0	0.271	
I1-1180	170-1	solvent blank	3.95	170827	MM	0	1.71	
I1-1181	170-2	monkey serum blank	3.91	475443	bb	0	10.4	
I1-1182	170-3	C 30	3.9	1002120	bb	0	32.3	7.7
I1-1183	170-4	C 30	3.91	990405	bb	0	31.8	5.9
I1-1184	170-5	C 30	3.9	958935	bb	0	30.3	1.1
I1-1185	170-6	C 60	3.9	1534123	bb	0	58.6	-2.3
I1-1186	170-7	C 60	3.9	1455879	bb	0	54.6	-9.1
I1-1187	170-8	C 60	3.9	1469382	bb	0	55.3	-7.9
I1-1188	170-9	C 100	3.9	2281561	bb	0	99.4	-0.60
I1-1189	170-10	C 100	3.9	2273657	bb	0	99.0	-1.0
I1-1190	170-11	C 100	3.9	2205661	bb	0	95.1	-4.9
I1-1191	170-12	C 300	3.91	5873564	bb	0	321	7.1
I1-1192	170-13	C 300	3.91	5879533	bb	0	322	7.3
I1-1193	170-14	C 300	3.9	5756138	bb	0	314	4.6
I1-1194	170-15	C 1000	3.9	15583550	bb	0	975	-2.5
I1-1195	170-16	C 1000	3.9	15728397	bb	0	985	-1.5
I1-1196	170-17	C 1000	3.9	15648902	bb	0	980	-2.0
I1-1197		ACN	3.96	69173	MM	0	0.221	
I1-1198	171-1	QC 30	3.9	957437	bb	0	30.3	0.85
I1-1199	171-2	QC 30	3.91	926113	bb	0	28.8	-3.9
I1-1200	171-3	QC 30	3.9	955083	bb	0	30.1	0.49
I1-1201	171-4	QC 100	3.9	2240142	bb	0	97.1	-2.9
I1-1202	171-5	QC 100	3.9	2240303	bb	0	97.1	-2.9
I1-1203	171-6	QC 100	3.9	2165502	bb	0	92.9	-7.1
I1-1204	171-7	QC 750	3.9	12806256	bb	0	786	4.8
I1-1205	171-8	QC 750	3.9	13089416	bb	0	805	7.3
I1-1206	171-9	QC 750	3.9	12917994	bb	0	793	5.8
I1-1207		ACN	3.96	68800	MM	0	0.218	

Table B5: Calibration and Quality Control samples for Sequence 534002(PFBS)EMS

Quantify Compound Summary Report

Sample List:C:\MASSLYNX\534002.PRO\SampleDB\534002(PFBS)EMS
Last modified:Wed May 18 14:01:07 2005
Method:C:\MASSLYNX\534002.PRO\MethDB\534002 PFBS bio
Last modified:Thu May 12 15:20:32 2005
Job Code:

Printed:Thu May 19 08:10:37 2005

Compound 1: PFBS

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1208	174-10	sys suit	3.91	2108967	bb	0	92.0	
I1-1209	174-10	sys suit	3.9	2315421	bb	0	103	
I1-1210	174-10	sys suit	3.9	2177413	bb	0	95.6	
I1-1211		ACN	3.95	11432	MM	0	0.143	
I1-1212		ACN	3.95	25272	MM	0	0.396	
I1-1213	174-1	solvent blank	3.94	30596	bb	0	0.505	
I1-1214	174-2	monkey serum blank	3.91	245003	bb	0	6.79	
I1-1215	174-18	female monkey serum blank	3.91	120661	bb	0	2.83	
I1-1216	174-19	female monkey serum blank	3.94	34776	bb	0	0.594	
I1-1217	174-20	female monkey serum blank	3.92	101382	bb	0	2.28	
I1-1218	174-21	male monkey serum blank	3.92	51548	bb	0	0.976	
I1-1219	174-22	male monkey serum blank	3.91	258614	bb	0	7.26	
I1-1220	174-23	male monkey serum blank	3.91	174987	bb	0	4.48	
I1-1221	174-3	C 30	3.91	849178	bb	0	30.9	2.9
I1-1222	174-4	C 30	3.91	831658	bb	0	30.1	0.36
I1-1223	174-5	C 30	3.91	813454	bb	0	29.3	-2.3
I1-1224	174-6	C 60	3.91	1442244	bb	0	58.4	-2.7
I1-1225	174-7	C 60	3.91	1463033	bb	0	59.4	-0.99
I1-1226	174-8	C 60	3.91	1482578	bb	0	60.4	0.60
I1-1227	174-9	C 100	3.91	2324765	bb	0	103	3.3
I1-1228	174-10	C 100	3.91	2260639	bb	0	99.9	-0.082
I1-1229	174-11	C 100	3.91	2251632	bb	0	99.4	-0.56
I1-1230	174-12	C 300	3.91	5744075	bb	0	301	0.43
I1-1231	174-13	C 300	3.91	5742669	bb	0	301	0.40
I1-1232	174-14	C 300	3.91	5661729	bb	0	296	-1.3
I1-1233	174-15	C 1000	3.9	16257968	bb	0	1016	1.6
I1-1234	174-16	C 1000	3.9	15986019	bb	0	996	-0.41
I1-1235	174-17	C 1000	3.91	15888332	bb	0	989	-1.1
I1-1236		ACN	3.96	11467	bb	0	0.144	
I1-1237	175-1	QC 30	3.91	847790	bb	0	30.8	2.7
I1-1238	175-2	QC 30	3.91	818694	bb	0	29.5	-1.5
I1-1239	175-3	QC 30	3.91	828665	bb	0	30.0	-0.077
I1-1240	175-4	QC 100	3.91	2141048	bb	0	93.7	-6.3
I1-1241	175-5	QC 100	3.91	2218724	bb	0	97.7	-2.3
I1-1242	175-6	QC 100	3.9	2179173	bb	0	95.6	-4.4
I1-1243	175-7	QC 750	3.91	13105219	bb	0	791	5.4

Compound 1: PFBS

Run #	Ref. #	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1244	175-8	QC 750	3.91	13071845	bb	0	788	5.1
I1-1245	175-9	QC 750	3.9	12571422	bb	0	753	0.44
I1-1246		ACN	3.96	11967	bb	0	0.152	
I1-1247	161-4	QC 100	3.9	2192432	bb	0	96.3	
I1-1248	161-5	QC 100	3.9	2126830	bb	0	92.9	
I1-1249	161-6	QC 100	3.91	2128541	bb	0	93.0	

Table B6: Calibration and Quality Control samples for Sequence 534002(PFBS)GMS

Quantify Compound Summary Report

Sample List:C:\MASSLYNX\534002.PRO\SampleDB\534002(PFBS)GMS
Last modified:Tue May 31 09:06:56 2005
Method:C:\MASSLYNX\534002.PRO\MethDB\534002 PFBSbio1
Last modified:Tue May 31 08:31:36 2005
Job Code:

Printed:Tue May 31 09:20:32 2005

Compound 1: PFBS

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1285	182-10	sys suit	3.88	13218780	bb	0	867	
I1-1286	182-10	sys suit	3.89	16388707	bb	0	1100	
I1-1287	182-10	sys suit	3.89	16563326	bb	0	1113	
I1-1288		ACN	3.94	23241	bb	0	0.631	
I1-1289		ACN	3.93	29903	db	0	0.847	
I1-1290	183-1	solvent blank	3.93	60765	db	0	1.93	
I1-1291	183-2	monkey serum blank	3.91	135328	bb	0	4.88	
I1-1292	183-3	C 30	3.9	659842	bb	0	30.0	-0.16
I1-1293	183-4	C 30	3.91	683568	bb	0	31.2	3.9
I1-1294	183-5	C 30	3.91	653977	bb	0	29.6	-1.2
I1-1295	183-6	C 60	3.91	1221684	bb	0	60.2	0.37
I1-1296	183-7	C 60	3.9	1258450	bb	0	62.3	3.8
I1-1297	183-8	C 60	3.91	1179873	bb	0	57.9	-3.5
I1-1298	183-9	C 100	3.9	1874062	bb	0	97.6	-2.4
I1-1299	183-10	C 100	3.9	1883525	bb	0	98.2	-1.8
I1-1300	183-11	C 100	3.9	1837017	bb	0	95.5	-4.5
I1-1301	183-12	C 300	3.9	5095256	bd	0	300	-0.025
I1-1302	183-13	C 300	3.9	5193057	bb	0	306	2.1
I1-1303	183-14	C 300	3.9	5395939	bb	0	320	6.6
I1-1304	183-15	C 1000	3.9	15130489	bb	0	1007	0.67
I1-1305	183-16	C 1000	3.9	14762121	bb	0	980	-2.0
I1-1306	183-17	C 1000	3.9	14875621	bb	0	988	-1.2
I1-1307		ACN	3.95	17576	bb	0	0.455	
I1-1308	184-1	QC 30	3.9	688874	bb	0	31.5	4.8
I1-1309	184-4	QC 100	3.9	1962947	bd	0	103	2.9
I1-1310	184-7	QC 750	3.9	12020223	bb	0	780	4.0
I1-1311	189-2	QC 1000	3.9	8367241	bb	2	1043	4.3
I1-1312		ACN	3.96	11166	bb	0	0.267	
I1-1313	185-1	1555T2, d7, 2M, T0	3.95	15432	bb	0	0.39	
I1-1314	185-2	1562T2, d7, 2M, T0	3.95	10827	bb	0	0.257	
I1-1315	185-3	1563T2, d7, 2M, T0	3.95	10493	bb	0	0.248	
I1-1316	185-4	1572T2, d7, 2F, T0	3.95	15147	bb	0	0.382	
I1-1317	185-5	1576T2, d7, 2F, T0	3.95	18278	bb	0	0.476	
I1-1318	185-6	1573T2, d7, 2F, T0	3.96	14450	bb	0	0.361	
I1-1319	185-7	1555T2, d7, 2M, T1	3.77	62287016	bb	0	4783	
I1-1320	185-8	1562T2, d7, 2M, T1	3.76	63523820	bb	0	4887	

Compound 1: PFBS

Run #	Ref. #	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1321	185-9	1563T2, d7, 2M, T1	3.77	62156292	bb	0	4772	
I1-1322	185-10	1572T2, d7, 2F, T1	3.77	60959964	bb	0	4672	
I1-1323	185-11	1576T2, d7, 2F, T1	3.77	61157520	bb	0	4688	
I1-1324	185-12	1573T2, d7, 2F, T1	3.77	59481420	bb	0	4548	
I1-1325	185-13	1555T2, d7, 2M, T2	3.78	58440848	bb	0	4461	
I1-1326	185-14	1562T2, d7, 2M, T2	3.77	63562356	bb	0	4891	
I1-1327	185-15	1563T2, d7, 2M, T2	3.82	47449988	bb	2	7099	
I1-1328	185-16	1572T2, d7, 2F, T2	3.78	93977	bb	0	3.21	
I1-1329	185-17	1576T2, d7, 2F, T2	3.79	57729744	bb	0	4401	
I1-1330	185-18	1573T2, d7, 2F, T2	3.81	51487228	bb	0	3882	
I1-1331	185-19	1555T2, d7, 2M, T4	3.79	53579704	bb	0	4056	
I1-1332	185-20	1562T2, d7, 2M, T4	3.77	60906780	bb	0	4667	
I1-1333	185-21	1563T2, d7, 2M, T4	3.81	49317468	bb	0	3703	
I1-1334		ACN	3.95	105284	bb	0	3.66	
I1-1335		ACN	3.95	83999	bb	0	2.82	
I1-1336	184-2	QC 30	3.91	739802	bb	0	34.1	14
I1-1337	184-5	QC 100	3.91	2017649	bb	0	106	6.1
I1-1338	184-8	QC 750	3.9	11545641	bb	0	746	-0.54
I1-1339	189-3	QC 1000	3.9	7901175	bb	2	979	-2.1
I1-1340		ACN	3.95	50006	bb	0	1.54	

Table B7: Calibration and Quality Control samples for Sequence 534002(PFBS)GMS1

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534002.PRO\SampleDB\534002(PFBS)GMS1
Last modified: Tue May 31 09:13:15 2005
Method: C:\MASSLYNX\534002.PRO\MethDB\534002 PFBSbio1
Last modified: Tue May 31 08:31:36 2005
Job Code:

Printed: Mon Jun 20 14:24:21 2005

Compound 1: PFBS

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1335		ACN	3.95	83999	bb	0	2.82	
I1-1336	184-2	QC 30	3.91	739802	bb	0	34.1	14
I1-1337	184-5	QC 100	3.91	2017649	bb	0	106	6.1
I1-1338	184-8	QC 750	3.9	11545641	bb	0	746	-0.54
I1-1339	189-3	QC 1000	3.9	7901175	bb	2	979	-2.2
I1-1340		ACN	3.95	50006	bb	0	1.54	
I1-1341		ACN	3.95	42181	bb	0	0.802	
I1-1342	185-22	1572T2, d7, 2F, T4	3.82	49481808	bb	0	3584	
I1-1343	185-23	1576T2, d7, 2F, T4	3.8	52231584	bb	0	3774	
I1-1344	185-24	1573T2, d7, 2F, T4	3.83	40762872	bb	0	2973	
I1-1345	185-25	1555T2, d7, 2M, T8	3.82	43873588	bb	0	3192	
I1-1346	185-26	1562T2, d7, 2M, T8	3.79	55951560	bb	0	4031	
I1-1347	185-27	1563T2, d7, 2M, T8	3.82	44331640	bb	0	3224	
I1-1348	185-28	1572T2, d7, 2F, T8	3.82	41232044	bb	0	3006	
I1-1349	185-29	1576T2, d7, 2F, T8	3.82	44030932	bb	0	3203	
I1-1350	185-30	1573T2, d7, 2F, T8	3.85	32047676	bb	0	2352	
I1-1351	185-31	1555T2, d7, 2M, T24	3.87	27959466	bb	0	2057	
I1-1352	185-32	1562T2, d7, 2M, T24	3.81	46163924	bb	0	3352	
I1-1353	185-33	1563T2, d7, 2M, T24	3.87	28936188	bb	0	2128	
I1-1354	185-34	1572T2, d7, 2F, T24	3.89	25359814	bb	0	1868	
I1-1355	185-35	1576T2, d7, 2F, T24	3.87	28851626	bb	0	2122	
I1-1356	185-36	1573T2, d7, 2F, T24	3.9	10771571	bb	0	787	
I1-1357	185-37	1555T2, d7, 2M, T48	3.89	16014136	bb	0	1180	
I1-1358	185-38	1562T2, d7, 2M, T48	3.82	42797904	bb	0	3116	
I1-1359	185-39	1563T2, d7, 2M, T48	3.9	16354969	bb	0	1205	
I1-1360	185-40	1572T2, d7, 2F, T48	3.9	13975023	bb	0	1027	
I1-1361	185-41	1576T2, d7, 2F, T48	3.9	14916829	bb	0	1098	
I1-1362	185-42	1573T2, d7, 2F, T48	3.9	1327618	bb	0	79.8	
I1-1363		ACN	3.95	21912	bb	0	0.276	
I1-1364		ACN	3.95	20467	bb	0	0.246	
I1-1365	184-3	QC 30	3.9	589110	bb	0	30.2	0.68
I1-1366	184-6	QC 100	3.9	1630290	bd	0	101	1.1
I1-1367	184-9	QC 750	3.91	11034084	bb	0	807	7.5
I1-1368	189-5	QC 1000	3.9	7399716	bb	2	1064	6.4
I1-1369		ACN	3.95	14495	bb	0	0.134	
I1-1370		ACN	3.95	14575	bb	0	0.135	

I1-1371	186-1	STS 4hr 100 ng/mL	3.91	1744947	bd	0	109	
I1-1372	186-2	STS 4hr 100 ng/mL	3.9	1646864	bd	0	102	
I1-1373	186-3	STS 4hr 100 ng/mL	3.9	1645487	bd	0	102	
I1-1374	186-4	STS 4hr 750 ng/mL	3.9	10274645	bb	0	749	
I1-1375	186-5	STS 4hr 750 ng/mL	3.9	11004258	bb	0	804	
I1-1376	186-6	STS 4hr 750 ng/mL	3.9	10302869	bb	0	751	
I1-1377	187-1	LTS d1 100ng/mL	3.9	1597946	bd	0	98.8	
I1-1378	187-2	LTS d1 100ng/mL	3.9	1613595	bb	0	99.9	
I1-1379	187-3	LTS d1 100ng/mL	3.9	1633992	bb	0	101	
I1-1380	187-4	LTS d1 750ng/mL	3.9	11291288	bb	0	826	
I1-1381	187-5	LTS d1 750ng/mL	3.89	11079511	bb	0	810	
I1-1382	187-6	LTS d1 750ng/mL	3.9	10972770	bb	0	802	
I1-1383	161-4	PSS d3 100 ng/mL	3.9	1792432	bd	0	113	
I1-1384	161-5	PSS d3 100 ng/mL	3.9	1734982	bb	0	109	
I1-1385	161-6	PSS d3 100 ng/mL	3.9	1754596	bd	0	110	
I1-1386		ACN	3.94	9485	bb	0	0.0610	
I1-1387		ACN	3.94	10822	bb	0	0.0780	
I1-1388	183-3	C 30	3.9	584750	bb	0	29.9	-0.24
I1-1389	183-6	C 60	3.9	1043037	bb	0	60.1	0.20
I1-1390	183-9	C 100	3.9	1619773	bd	0	100	0.37
I1-1391	183-12	C 300	3.9	4306223	bd	0	299	-0.48
I1-1392	183-15	C 1000	3.9	13628745	bb	0	1001	0.15
I1-1393		ACN	3.94	19104	bb	0	0.218	
I1-1394		ACN	3.95	9780	bb	0	0.0640	

Table B8: Calibration and Quality Control samples for Sequence 534002(PFBS)IMS

Quantify Compound Summary Report

Sample List:C:\MASSLYNX\534002.PRO\SampleDB\534002(PFBS)IMS
Last modified:Fri May 27 14:37:11 2005
Method:C:\MASSLYNX\534002.PRO\MethDB\534002 PFBSbio1
Last modified:Tue May 31 08:31:36 2005
Job Code:

Printed: Tue May 31 08:33:02 2005

Compound 1: PFBS

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1596		sys suit	3.88	10168420	bb	0	837	
I1-1597		sys suit	3.88	7823790	bb	0	636	
I1-1598		sys suit	3.89	9029096	bb	0	739	
I1-1599		ACN	3.93	94625	db	0	1.98	
I1-1600		ACN	3.92	89011	db	0	1.78	
I1-1601	203-1	solvent blank	3.93	87421	db	0	1.73	
I1-1602	203-2	monkey serum blank	3.92	216193	bb	0	7.41	
I1-1603	203-3	C 30	3.89	602670	bb	0	31.3	4.2
I1-1604	203-4	C 30	3.89	582519	bb	0	29.9	-0.40
I1-1605	203-5	C 30	3.89	600833	bb	0	31.1	3.8
I1-1606	203-6	C 60	3.89	1007737	bb	0	60.5	0.91
I1-1607	203-7	C 60	3.89	924400	bb	0	54.3	-9.5
I1-1608	203-8	C 60	3.89	975714	bb	0	58.1	-3.1
I1-1609	203-9	C 100	3.89	1403468	bd	0	91.1	-8.9
I1-1610	203-10	C 100	3.89	1580006	bd	0	105	5.1
I1-1611	203-11	C 100	3.89	1563701	bb	0	104	3.8
I1-1612	203-12	C 300	3.89	3982746	bd	0	306	1.9
I1-1613	203-13	C 300	3.88	3907594	bb	0	299	-0.22
I1-1614	203-14	C 300	3.89	4148535	bb	0	320	6.6
I1-1615	203-15	C 1000	3.88	12130918	bb	0	1005	0.46
I1-1616	203-16	C 1000	3.88	11978556	bb	0	992	-0.84
I1-1617	203-17	C 1000	3.88	11799286	bb	0	976	-2.4
I1-1618		ACN	3.92	124839	bb	0	3.14	
I1-1619	204-1	QC 30	3.89	704192	bb	0	38.3	28
I1-1620	204-4	QC 100	3.88	1647693	bd	0	111	11
I1-1621	204-7	QC 750	3.88	9503636	bb	0	780	4.0
I1-1622	204-10	QC 10000	3.88	1553584	bd	100	10300	3.0
I1-1623		ACN	3.92	124654	bb	0	3.13	
I1-1624		ACN	3.92	145453	dd	0	4.02	
I1-1625	205-1	1555T2, 2M, T1	3.88	4965586	bb	200	77987	
I1-1626	205-2	1562T2, 2M, T1	3.88	5882990	bb	200	93766	
I1-1627	205-3	1563T2, 2M, T1	3.88	4787515	bb	200	74930	
I1-1628	205-4	1572T2, 2F, T1	3.88	4446823	bb	200	69087	
I1-1629	205-5	1576T2, 2F, T1	3.88	4844928	bb	200	75915	
I1-1630	205-6	1573T2, 2F, T1	3.88	3613783	bb	200	54860	
I1-1631	205-7	1555T2, 2M, T2	3.88	3433419	bb	200	51795	

I1-1632	205-8	1562T2, 2M, T2	3.88	4715996	bb	200	73703	
I1-1633	205-9	1572T2, 2F, T2	3.87	3252093	bb	200	48722	
I1-1634	205-10	1576T2, 2F, T2	3.87	3530379	bb	200	53442	
I1-1635	205-11	1573T2, 2F, T2	3.88	2232823	bb	200	31648	
I1-1636	205-12	1555T2, 2M, T4	3.88	3795558	bb	100	28978	
I1-1637	205-13	1562T2, 2M, T4	3.88	6330245	bb	100	50733	
I1-1638	205-14	1563T2, 2M, T4	3.87	3604814	bb	100	27354	
I1-1639	205-15	1572T2, 2F, T4	3.88	3620257	bb	100	27485	
I1-1640		ACN	3.92	149612	dd	0	4.20	
I1-1641		ACN	3.91	148690	dd	0	4.16	
I1-1642	204-2	QC 30	3.89	659294	bd	0	35.2	17
I1-1643	204-5	QC 100	3.88	1590387	bd	0	106	5.9
I1-1644	204-8	QC 750	3.88	9331050	bb	0	765	2.0
I1-1645	204-11	QC 10000	3.88	1685217	bd	100	11354	14
I1-1646		ACN	3.91	146428	dd	0	4.06	
I1-1647		ACN	3.91	147595	dd	0	4.11	
I1-1648	205-16	1576T2, 2F, T4	3.88	4552888	bb	100	35453	
I1-1649	205-17	1573T2, 2F, T4	3.88	2293343	bb	100	16324	
I1-1650	205-18	1555T, 2M, T8	3.87	2551344	bb	100	18468	
I1-1651	205-19	1562T2, 2M, T8	3.87	5704613	bb	100	45348	
I1-1652	205-20	1563T2, 2M, T8	3.87	2751158	bb	100	20140	
I1-1653	205-21	1572T2, 2F, T8	3.87	2209873	bb	100	15634	
I1-1654	205-22	1576T2, 2F, T8	3.87	2864826	bb	100	21094	
I1-1655	205-23	1573T2, 2F, T8	3.88	1224038	bd	100	7707	
I1-1656	205-24	1555T2, 2M, T24	3.87	779590	bb	100	4371	
I1-1657	205-25	1562T2, 2M, T24	3.87	3001519	bb	100	22244	
I1-1658	205-26	1563T2, 2M, T24	3.87	884221	bd	100	5134	
I1-1659	205-27	1572T2, 2F, T24	3.87	648383	bd	100	3441	
I1-1660	205-28	1576T2, 2F, T24	3.87	822214	bb	100	4680	
I1-1661	205-29	1562T2, 2M, T48	3.87	2262497	bb	100	16069	
I1-1662		ACN	3.9	149940	dd	0	4.22	
I1-1663	204-3	QC 30	3.88	638221	bd	0	33.7	12
I1-1664	204-6	QC 100	3.87	1557224	bd	0	103	3.3
I1-1665	204-9	QC 750	3.86	9294351	bb	0	762	1.6
I1-1666	204-12	QC 10000	3.86	1593070	bd	100	10615	6.2
I1-1667		ACN	3.9	137178	bd	0	3.66	
I1-1668		ACN	3.89	120788	db	0	2.98	
I1-1669	205-30	FZ Thaw 1x 100ng	3.86	1432337	bd	0	93.4	-6.6
I1-1670	205-31	FZ Thaw 1x 100ng	3.86	1447856	bd	0	94.6	-5.4
I1-1671	205-32	FZ Thaw 1x 100ng	3.86	1505362	bd	0	99.2	-0.84
I1-1672	205-33	FZ Thaw 2x 100ng	3.86	1508473	bd	0	99.4	-0.59
I1-1673	205-34	FZ Thaw 2x 100ng	3.86	1578383	bd	0	105	5.0
I1-1674	205-35	FZ Thaw 2x 100ng	3.86	1480839	bd	0	97.2	-2.8
I1-1675	205-36	FZ Thaw 3x 100ng	3.85	1626867	bd	0	109	8.9
I1-1676	205-37	FZ Thaw 3x 100ng	3.86	1817756	bb	0	124	24
I1-1677	205-38	FZ Thaw 3x 100ng	3.86	1569545	bd	0	104	4.3
I1-1678	205-39	FZ Thaw1x 750ng	3.85	9373232	bb	0	769	2.5
I1-1679	205-40	FZ Thaw1x 750ng	3.84	9200610	bb	0	754	0.56
I1-1680	205-41	FZ Thaw1x 750ng	3.84	9489065	bb	0	779	3.9
I1-1681	205-42	FZ Thaw 2x 750ng	3.84	9514321	bb	0	781	4.1
I1-1682	205-43	FZ Thaw 2x 750ng	3.84	9150453	bb	0	750	-0.016
I1-1683	205-44	FZ Thaw 2x 750ng	3.82	9364971	bb	0	768	2.4

I1-1684	205-45	FZ Thaw 3x 750ng	3.82	9093781	bb	0	745	-0.67
I1-1685	205-46	FZ Thaw 3x 750ng	3.77	8484684	bb	0	693	-7.6
I1-1686	205-47	FZ Thaw 3x 750ng	3.7	9404466	MM	0	772	2.9
I1-1687		ACN	3.63	126757	bb	0	3.22	

Table B9: Calibration and Quality Control samples for Sequence 534002(PFBS)FMU

Quantify Compound Summary Report

Sample List:C:\MASSLYNX\534002.PRO\SampleDB\534002(PFBS)FMU
Last modified:Wed May 18 14:01:30 2005
Method:C:\MASSLYNX\534002.PRO\MethDB\534002 PFBS bio
Last modified:Thu May 12 15:20:32 2005
Job Code:

Printed:Thu May 19 08:15:07 2005

Compound 1: PFBS

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1250		ACN	3.95	11764	bb	0	0.280	
I1-1251	178-1	solvent blank	3.96	17271	bb	0	0.427	
I1-1252	178-2	monkey urine blank	3.92	151640	bb	0	4.67	
I1-1253	178-18	female monkey urine blank	3.92	233309	bb	0	7.55	
I1-1254	178-19	female monkey urine blank	3.91	167792	bb	0	5.23	
I1-1255	178-20	female monkey urine blank	3.92	179891	bb	0	5.65	
I1-1256	178-21	male monkey urine blank	3.92	142411	bb	0	4.36	
I1-1257	178-22	male monkey urine blank	3.91	166118	bb	0	5.17	
I1-1258	178-23	male monkey urine blank	3.92	49784	bb	0	1.36	
I1-1259	178-3	C 30	3.91	858338	bb	0	32.5	8.2
I1-1260	178-4	C 30	3.91	792315	bb	0	29.7	-1.1
I1-1261	178-5	C 30	3.91	780290	bb	0	29.2	-2.8
I1-1262	178-6	C 60	3.91	1412254	bb	0	56.9	-5.2
I1-1263	178-7	C 60	3.91	1487484	bb	0	60.3	0.56
I1-1264	178-8	C 60	3.91	1439408	bb	0	58.1	-3.1
I1-1265	178-9	C 100	3.88	7414624	bbX	0	375	275
I1-1266	178-10	C 100	3.91	2396810	bb	0	104	3.5
I1-1267	178-11	C 100	3.9	2288543	bb	0	98.2	-1.8
I1-1268	178-12	C 300	3.91	6262031	bb	0	309	3.0
I1-1269	178-13	C 300	3.91	6131937	bb	0	302	0.52
I1-1270	178-14	C 300	3.91	6130563	bb	0	301	0.49
I1-1271	178-15	C 1000	3.9	17128856	bb	0	979	-2.1
I1-1272	178-16	C 1000	3.91	17415298	bb	0	998	-0.16
I1-1273	178-17	C 1000	3.9	17538202	bb	0	1006	0.65
I1-1274		ACN	3.95	12013	bb	0	0.287	
I1-1275	179-1	QC 30	3.91	798178	bb	0	29.9	-0.30
I1-1276	179-2	QC 30	3.91	813511	bb	0	30.6	1.9
I1-1277	179-3	QC 30	3.9	1055766	bb	0	41.0	37
I1-1278	179-4	QC 100	3.91	2247847	bb	0	96.3	-3.7
I1-1279	179-5	QC 100	3.91	2148684	bb	0	91.5	-8.5
I1-1280	179-6	QC 100	3.91	2107163	bb	0	89.5	-11
I1-1281	179-7	QC 750	3.91	13968147	bb	0	775	3.3
I1-1282	179-8	QC 750	3.9	14183969	bb	0	788	5.1
I1-1283	179-9	QC 750	3.9	14365329	bb	0	800	6.7
I1-1284		ACN	3.95	12832	bb	0	0.308	

Table B10: Calibration, QC and Experimental Urine Samples from Sequence 534002(PFBS)HMU

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534002.PRO\SampleDB\534002(PFBS)HMU
Last modified: Thu May 26 08:11:33 2005
Method: C:\MASSLYNX\534002.PRO\MethDB\534002 PFBSbio1
Last modified: Wed May 25 15:44:19 2005
Job Code:

Printed: Thu May 26 08:49:38 2005

Compound 1: PFBS

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1437		sys suit	3.95	13055250	MM	0	942	-91
I1-1438		sys suit	3.93	14603358	MM	0	1061	
I1-1439		sys suit	3.93	13383952	MM	0	967	
I1-1440		ACN	3.97	134777	bb	0	1.61	
I1-1441		ACN	3.97	124055	bb	0	1.36	
I1-1442	194-1	solvent blank	3.97	129253	MM	0	1.48	
I1-1443	194-2	monkey urine blank	3.96	345748	MM	0	8.76	
I1-1444	194-3	C 30	3.94	801072	bb	0	31.3	4.5
I1-1445	194-4	C 30	3.94	755030	bb	0	28.8	-4.0
I1-1446	194-5	C 30	3.93	805764	bb	0	31.6	5.3
I1-1447	194-6	C 60	3.93	1288573	bb	0	60.4	0.59
I1-1448	194-7	C 60	3.93	1237169	bb	0	57.1	-4.8
I1-1449	194-8	C 60	3.93	1266584	bb	0	59.0	-1.7
I1-1450	194-9	C 100	3.92	1929799	bb	0	102	2.2
I1-1451	194-10	C 100	3.92	1865340	bb	0	97.9	-2.1
I1-1452	194-11	C 100	3.94	1814994	bb	0	94.5	-5.5
I1-1453	194-12	C 300	3.93	4998102	bb	0	324	8.1
I1-1454	194-13	C 300	3.93	4722649	bb	0	304	1.2
I1-1455	194-14	C 300	3.92	4659592	bb	0	299	-0.34
I1-1456	194-15	C 1000	3.91	14070622	bb	0	1020	2.0
I1-1457	194-16	C 1000	3.91	13630448	bb	0	986	-1.4
I1-1458	194-17	C 1000	3.91	13398502	bb	0	968	-3.2
I1-1459		ACN	3.96	135872	bb	0	1.64	
I1-1460	195-1	QC 30	3.93	794597	bb	0	31.0	3.3
I1-1461	195-4	QC 100	3.92	1830213	bb	0	95.5	-4.5
I1-1462	195-7	QC 750	3.91	11264030	bb	0	804	7.2
I1-1463	194-10	QC 10000	3.92	2014739	bb	100	10799	8.0
I1-1464		ACN	3.96	126685	bb	0	1.42	
I1-1465	197-1	LT Stab 100	3.92	1895514	bb	0	99.9	
I1-1466	197-2	LT Stab 100	3.92	1954128	bb	0	104	
I1-1467	197-3	LT Stab 100	3.92	1896437	bb	0	100	
I1-1468	197-4	LT Stab 750	3.91	11650200	bb	0	834	
I1-1469	197-5	LT Stab 750	3.91	11615565	bb	0	831	
I1-1470	197-6	LT Stab 750	3.92	11414038	bb	0	815	
I1-1471		ACN	3.96	140433	bb	0	1.75	
I1-1472	196-1	1555T2, 2M, T0-6	3.86	30193674	bb	100	224013	

Compound 1: PFBS

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1473	196-2	1572T2, 2F, T0-6	3.8	44798684	MM	100	330967	
I1-1474	196-3	1576T2, 2F, T0-6	3.79	48525464	MM	100	357748	
I1-1475	196-4	1555T2, 2M, T6-12	3.9	20514414	bb	100	151236	
I1-1476	196-5	1563T2, 2M, T6-12	3.9	14969929	bb	100	108882	
I1-1477	196-6	1555T2, 2M, T12-24	3.85	34646352	MM	2	5139	
I1-1478	196-7	1563T2, 2M, T12-24	3.82	46536528	MM	2	6870	
I1-1479	196-8					2		
I1-1480	196-9	1573T2, 2F, T12-24	3.76	58905324	MM	2	8627	
I1-1481	196-10	1555T2, 2M, T24-48	3.88	27767596	bb	0	2059	
I1-1482	196-11	1562T2, 2M, T24-48	3.82	34773272	bb	0	2579	
I1-1483	196-12	1563T2, 2M, T24-48	3.86	29724296	bb	0	2205	
I1-1484	196-13	1572T2, 2F, T24-48	3.87	31664646	MM	0	2349	
I1-1485	196-14	1576T2, 2F, T24-48	3.74	73076456	MM	0	5297	
I1-1486	196-15	1573T2, 2F, T24-48	3.89	25203356	bb	0	1867	
I1-1487	196-16	1555T2, 2M, T48-72	3.9	21575242	bb	0	1593	
I1-1488	196-17	1562T2, 2M, T48-72	3.82	42537036	MM	0	3146	
I1-1489	196-18	1563T2, 2M, T48-72	3.89	25240296	bb	0	1870	
I1-1490	196-19	1572T2, 2F, T48-72	3.89	24431812	bb	0	1809	
I1-1491		ACN	3.96	149234	bb	0	1.97	
I1-1492		ACN	3.96	142629	bb	0	1.80	
I1-1493	195-2	QC 30	3.92	745201	bb	0	28.3	-5.8
I1-1494	195-5	QC 100	3.92	1719594	bb	0	88.2	-12
I1-1495	195-8	QC 750	3.91	9602575	bb	0	676	-9.9
I1-1496	195-11	QC 10000	3.91	1832031	bb	100	9566	-4.3
I1-1497		ACN	3.96	139186	bb	0	1.72	
I1-1498		ACN	3.96	132002	bb	0	1.55	
I1-1499	196-20	1576T2, 2F, T48-72	3.87	31293340	bb	0	2322	
I1-1500	196-21	1573T2, 2F, T48-72	3.91	12571813	bb	0	904	
I1-1501	196-22	1555T2, 2M, T72-96	3.91	6405446	bb	0	431	
I1-1502	196-23	1562T2, 2M, T72-96	3.83	36058432	MM	0	2674	
I1-1503	196-24	1563T2, 2M, T72-96	3.91	14032891	bb	0	1017	
I1-1504	196-26	1576T2, 2F, T72-96	3.91	10702679	bb	0	761	
I1-1505	196-27	1573T2, 2F, T72-96	3.91	4738666	bb	0	305	
I1-1506	196-28	1555T2, 2M, T96-120	3.91	3779663	bb	0	234	
I1-1507	196-29	1562T2, 2M, T96-120	3.91	10249184	MM	0	726	
I1-1508	196-30	1563T2, 2M, T96-120	3.91	6261574	MM	0	420	
I1-1509	196-31	1572T2, 2F, T96-120	3.91	3744265	bb	0	231	
I1-1510	196-32	1576T2, 2F, T96-120	3.92	1774418	bb	0	91.8	
I1-1511	196-33	1573T2, 2F, T96-120	3.91	4424466	bb	0	281	
I1-1512	196-34	1555T2, 2M, T120-144	3.91	2036819	bb	0	109	
I1-1513	196-35	1563T2, 2M, T120-144	3.91	10421141	bb	0	739	
I1-1514	196-36	1572T2, 2F, T120-144	3.91	5473552	bb	0	360	
I1-1515	196-37	1576T2, 2F, T120-144	3.92	643911	bb	0	22.9	
I1-1516	196-38	1573T2, 2F, T120-144	3.96	272699	MM	0	5.9	
I1-1517	196-39	1555T2, 2M, T144-168	3.92	798146	bb	0	31.2	
I1-1518	196-40	1562T2, 2M, T144-168	3.91	3584750	MM	0	219	
I1-1519	196-41	1563T2, 2M, T144-168	3.91	12599026	bb	0	907	
I1-1520	196-42	1572T2, 2F, T144-168	3.9	3963176	bb	0	247	

Compound 1: PFBS

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1521	196-43	1576T2, 2F, T144-168	3.91	1873212	MM	0	98.4	
I1-1522	196-44	1573T2, 2F, T144-168	3.91	1741595	bb	0	89.6	
I1-1523		ACN	3.96	130128	bb	0	1.50	
I1-1524		ACN	3.96	120122	bb	0	1.27	
I1-1525a	195-3	QC 30	3.92	741007	bb	0	28.0	-6.6
I1-1526a	195-6	QC 100	3.91	1787812	bb	0	92.7	-7.3
I1-1527a	195-9	QC 750	3.91	10401307	bb	0	737	-1.7
I1-1528a	195-12	QC 10000	3.92	1727358	bb	100	8869	-11
I1-1529		ACN	3.97	124610	db	0	1.37	
I1-1530		ACN	3.96	132407	bb	0	1.55	
I1-1531		ACN	3.96	129005	bb	0	1.48	
I1-1532		ACN	3.96	134446	bb	0	1.60	
I1-1533		ACN	3.96	129888	bb	0	1.50	
I1-1534		ACN	3.96	127848	bb	0	1.45	
I1-1535		ACN	3.96	135100	bb	0	1.62	
I1-1536		ACN	3.96	133244	bb	0	1.57	
I1-1537		ACN	3.96	136527	bb	0	1.65	
I1-1538		ACN	3.96	131706	bb	0	1.54	
I1-1539		ACN	3.96	126851	bb	0	1.43	
I1-1540		ACN	3.96	132590	bb	0	1.56	
I1-1541		ACN	3.96	133551	bb	0	1.58	
I1-1542		ACN	3.96	130967	bb	0	1.52	
I1-1543		ACN	3.97	123393	bb	0	1.35	
I1-1544	199-10	QC 10000	3.91	2043451	bb	100	10995	9.9
I1-1545		ACN	3.96	135793	db	0	1.64	
I1-1546	201-1	1555T2, 2M, T0-6	3.91	1081766	bb	10000	476434	
I1-1547	201-2	1572T2, 2F, T0-6	3.91	3291590	bb	10000	1980921	
I1-1548	201-3	1576T2, 2F, T0-6	3.92	4120822	bb	10000	2588991	
I1-1549		ACN	3.96	127848	bb	0	1.45	
I1-1550	201-4	1555T2, 2M, T6-12	3.91	3219472	bb	1000	192869	
I1-1551	201-5	1555T2, 2M, T12-24	3.91	2724536	bb	100	15739	
I1-1552	201-6	1563T2, 2M, T12-24	3.91	5523526	bb	100	36394	
I1-1553	201-7	1572T2, 2F, T12-24	3.91	8514355	bb	500	296106	
I1-1554	201-8	1573T2, 2F, T12-24	3.91	12068119	bb	100	86571	
I1-1555	201-9	1555T2, 2M, T24-48	3.92	1263272	bb	100	5877	
I1-1556	201-10	1562T2, 2M, T24-48	3.91	2743316	bb	100	15873	
I1-1557	201-11	1563T2, 2M, T24-48	3.91	1792338	bb	100	9301	
I1-1558	201-12	1572T2, 2F, T24-48	3.92	1433295	bb	100	6952	
I1-1559	201-13	1576T2, 2F, T24-48	3.91	2166066	bb	500	59170	
I1-1560	201-14	1573T2, 2F, T24-48	3.92	939454	bb	100	3922	
I1-1561	201-15	1555T2, 2M, T48-72	3.92	1020374	bb	50	2199	
I1-1562	201-16	1562T2, 2M, T48-72	3.92	932535	bb	500	19409	
I1-1563	201-17	1563T2, 2M, T48-72	3.91	1379650	bb	50	3305	
I1-1564	201-18	1572T2, 2F, T48-72	3.91	1281310	bb	50	2995	
I1-1565	201-19	1576T2, 2F, T48-72	3.91	2876788	bb	50	8412	
I1-1566	201-20	1562T2, 2M, T72-96	3.92	4482258	bb	50	14288	
I1-1567		ACN	3.96	129075	bb	0	1.48	
I1-1568	199-11	QC 10000	3.91	1889502	bb	100	9952	-0.48

Compound 1: PFBS

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1569		ACN	3.96	130842	bb	0	1.52	-98
I1-1570	201-21	FZ Thaw 1x 100ng	3.91	1634704	bb	0	82.6	-17
I1-1571	201-22	FZ Thaw 1x 100ng	3.91	1728291	bb	0	88.7	-11
I1-1572	201-23	FZ Thaw 1x 100ng	3.92	1655851	bb	0	84.0	-16
I1-1573	201-24	FZ Thaw 2x 100ng	3.91	1743265	bb	0	89.7	-10
I1-1574	201-25	FZ Thaw 2x 100ng	3.91	1792615	bb	0	93.0	-7.0
I1-1575	201-26	FZ Thaw 2x 100ng	3.91	1681783	bb	0	85.7	-14
I1-1576	201-27	FZ Thaw 3x 100ng	3.91	1627941	MM	0	82.1	-18
I1-1577	201-28	FZ Thaw 3x 100ng	3.91	1722285	bb	0	88.4	-12
I1-1578	201-29	FZ Thaw 3x 100ng	3.91	1728498	bb	0	88.8	-11
I1-1579	201-30	FZ Thaw 1x 750ng	3.91	10552118	MM	0	749	-0.14
I1-1580	201-31	FZ Thaw 1x 750ng	3.91	10758112	MM	0	765	2.0
I1-1581	201-32	FZ Thaw 1x 750ng	3.9	10687765	MM	0	759	1.3
I1-1582	201-33	FZ Thaw 2x 750ng	3.91	10196387	bb	0	722	-3.8
I1-1583	201-34	FZ Thaw 2x 750ng	3.91	10435371	MM	0	740	-1.3
I1-1584	201-35	FZ Thaw 2x 750ng	3.9	10423448	MM	0	739	-1.5
I1-1585	201-36	FZ Thaw 3x 750ng	3.9	10096760	MM	0	714	-4.8
I1-1586	201-37	FZ Thaw 3x 750ng	3.91	10276159	MM	0	728	-3.0
I1-1587	201-38	FZ Thaw 3x 750ng	3.9	10496550	MM	0	745	-0.71
I1-1588		ACN	3.95	116283	bb	0	1.19	
I1-1589	199-12	QC 10000	3.91	1735446	MM	100	8922	-11
I1-1590		ACN	3.96	125188	db	0	1.39	

Table B11: Calibration, QC and Experimental Urine Samples from Sequence 534002(PFBS)JMS

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534002.PRO\SampleDB\534002(PFBS)JMS
Last modified: Fri Jul 08 18:54:23 2005
Method: C:\MASSLYNX\534002.PRO\MethDB\534002 PFBSbio1
Last modified: Tue May 31 08:31:36 2005
Job Code:

Printed: Mon Jul 11 08:34:35 2005

Compound 1: PFBS

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1787		sys suit	3.87	1368850	bb	0	58.4	
I1-1788		sys suit	3.85	977832	bd	0	39.2	
I1-1789		sys suit	3.85	930793	bb	0	37.0	
I1-1790		ACN	3.88	221331	bb	0	7.20	
I1-1791		ACN	3.88	133338	bb	0	4.14	
I1-1792	245-1	solvent blank	3.88	91354	bb	0	2.75	
I1-1793	245-2	monkey serum blank	3.86	190574	bb	0	6.11	
I1-1794	245-3	C 30	3.84	829756	bb	0	32.3	7.8
I1-1795	245-4	C 30	3.84	732372	MM	0	28.0	-6.8
I1-1796	245-5	C 60	3.84	1427418	bb	0	61.4	2.4
I1-1797	245-6	C 60	3.85	1456763	bb	0	62.9	4.9
I1-1798	245-7	C 100	3.84	2062237	MM	0	95.8	-4.2
I1-1799	245-8	C 100	3.84	1966613	bb	0	90.4	-9.6
I1-1800	245-9	C 300	3.85	5468157	MM	0	324.196	8.1
I1-1801	245-10	C 300	3.84	5222469	bd	0	305.668	1.9
I1-1802	245-11	C 1000	3.84	12859511	bd	0	996.064	-0.39
I1-1803	245-12	C 1000	3.83	12666795	bd	0	976.048	-2.4
I1-1804		ACN	3.88	46082	bb	0	1.34	
I1-1805	246-1	QC 30	3.84	640057	bb	0	23.9	-20
I1-1806	246-4	QC 100	3.85	1892007	bb	0	86.3	-14
I1-1807	246-7	QC 750	3.84	11255659	bd	0	833	11
I1-1808		ACN	3.89	41261	bb	0	1.19	
I1-1809	247-1	LT STB 100ng	3.84	1934052	bb	0	88.6	
I1-1810	247-2	LT STB 100ng	3.85	1933283	bb	0	88.6	
I1-1811	247-3	LT STB 100ng	3.84	1933826	bb	0	88.6	
I1-1812	247-4	LT STB 750ng	3.84	10725343	bb	0	781	
I1-1813	247-5	LT STB 750ng	3.84	10053447	bb	0	717	
I1-1814	247-6	LT STB 750ng	3.84	10635864	bb	0	773	
I1-1815		ACN	3.88	27638	bb	0	0.789	
I1-1816	246-2	QC 30	3.85	634269	bd	0	23.7	-21

Compound 1: PFBS

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1817	246-5	QC 100	3.84	1867862	bb	0	85.0	-15
I1-1818	246-8	QC 750	3.84	10758841	bd	0	785	4.6
I1-1819		ACN	3.88	23829	bb	0	0.678	
I1-1820	248-1	4Hr RT STB 100ng	3.84	1925925	bb	0	88.2	
I1-1821	248-2	4Hr RT STB 100ng	3.85	1790566	bb	0	80.7	
I1-1822	248-3	4Hr RT STB 100ng	3.85	1904454	bb	0	87.0	
I1-1823	248-4	4Hr RT STB 750ng	3.84	10282977	bd	0	739	
I1-1824	248-5	4Hr RT STB 750ng	3.84	9819544	bb	0	695	
I1-1825	248-6	4Hr RT STB 750ng	3.84	9625772	bb	0	677	
I1-1826		ACN	3.88	21948	bb	0	0.624	
I1-1827	246-3	QC 30	3.85	596092	bb	0	22.0	-27
I1-1828	246-6	QC 100	3.85	1774713	bb	0	79.9	-20
I1-1829	246-9	QC 750	3.84	10524571	bd	0	762	1.6
I1-1830		ACN	3.88	20466	bb	0	0.581	

Table B12: Calibration, QC and Experimental Urine Samples from Sequence 534002(PFBS)KMU

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534002.PRO\SampleDB\534002(PFBS)KMU
Last modified: Fri Jul 08 18:54:44 2005
Method: C:\MASSLYNX\534002.PRO\MethDB\534002 PFBSbio1
Last modified: Tue May 31 08:31:36 2005
Job Code:

Printed: Mon Jul 11 08:48:54 2005

Compound 1: PFBS

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1831		sys suit	3.84	567315	bb	0	33.3	
I1-1832		sys suit	3.84	529409	bb	0	31.1	
I1-1833		sys suit	3.84	522198	bb	0	30.6	
I1-1834		ACN	3.88	16169	bb	0	1.27	
I1-1835		ACN	3.88	17469	bb	0	1.35	
I1-1836	249-1	solvent blank	3.89	7721	MM	0	0.683	
I1-1837	249-2	monkey urine blank	3.84	84956	MM	0	5.44	
I1-1838	249-3	C 30	3.84	538854	bb	0	31.6	5.4
I1-1839	249-4	C 30	3.84	532131	bb	0	31.2	4.0
I1-1840	249-5	C 60	3.84	941515	bb	0	55.7	-7.1
I1-1841	249-6	C 60	3.85	993521	bb	0	58.9	-1.8
I1-1842	249-7	C 100	3.84	1495236	bd	0	90.4	-9.6
I1-1843	249-8	C 100	3.84	1578844	bd	0	95.8	-4.2
I1-1844	249-9	C 300	3.84	4910463	bd	0	337	12
I1-1845	249-10	C 300	3.85	4893370	bd	0	335	12
I1-1846	249-11	C 1000	3.84	12194969	bd	0	997	-0.34
I1-1847	249-12	C 1000	3.84	11482713	MM	0	925	-7.5
I1-1848		ACN	3.88	13640	bb	0	1.10	
I1-1849	250-1	QC 30	3.85	525493	bb	0	30.8	2.8
I1-1850	250-4	QC 100	3.85	1645055	bd	0	100	0.126
I1-1851	250-7	QC 750	3.84	10178973	MM	0	798	6.4
I1-1852		ACN	3.87	16801	bb	0	1.31	
I1-1853	251-1	LT STB 100ng	3.85	1744942	bb	0	107	
I1-1854	251-2	LT STB 100ng	3.85	1632813	bb	0	99.3	
I1-1855	251-3	LT STB 100ng	3.84	1487393	bb	0	89.9	
I1-1856	251-4	LT STB 750ng	3.84	9955920	MM	0	777	
I1-1857	251-5	LT STB 750ng	3.84	10726726	MM	0	851	
I1-1858	251-6	LT STB 750ng	3.84	10740250	MM	0	852	
I1-1859		ACN	3.88	15406	bb	0	1.22	
I1-1860	250-2	QC 30	3.84	558602	bb	0	32.8	9.2

Compound 1: PFBS

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1861	250-5	QC 100	3.85	1625675	bb	0	98.9	-1.1
I1-1862	250-8	QC 750	3.84	9642852	bb	0	747	-0.35
I1-1863		ACN	3.88	13999	bb	0	1.12	
I1-1864	252-1	4Hr RT STB 100ng	3.84	1807534	bb	0	111	
I1-1865	252-2	4Hr RT STB 100ng	3.84	1709051	bb	0	104	
I1-1866	252-3	4Hr RT STB 100ng	3.84	1772903	bb	0	108	
I1-1867	252-4	4Hr RT STB 750ng	3.84	9772903	MM	0	760	
I1-1868	252-5	4Hr RT STB 750ng	3.84	11341258	bd	0	911	
I1-1869	252-6	4Hr RT STB 750ng	3.83	10694468	bd	0	848	
I1-1870		ACN	3.88	15024	bb	0	1.19	
I1-1871	250-3	QC 30	3.85	577384	bb	0	33.9	13
I1-1872	250-6	QC 100	3.84	1658981	bd	0	101	1.0
I1-1873	250-9	QC 750	3.84	10606260	bd	0	839	12
I1-1874		ACN	3.88	14296	bb	0	1.14	

Table B13: Calibration, QC and Experimental Urine Samples from Sequence 534002(PFBS)LMU1

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534002.PRO\SampleDB\534002(PFBS)LMU1
Last modified: Fri Jul 15 11:17:14 2005
Method: C:\MASSLYNX\534002.PRO\MethDB\534002 PFBSbio2
Last modified: Fri Jul 15 14:42:03 2005
Job Code:

Printed: Fri Jul 22 16:51:50 2005

Compound 1: PFBS

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1914b		sys suit	4.02	608901	bb	0	37.8	
I1-1915b		sys suit	4.00	831660	bb	0	64.8	
I1-1916b		sys suit	4.00	567938	bb	0	33.0	
I1-1917b		ACN	4.05	94273	bbI	0		
I1-1918b		ACN	4.05	214011	bbI	0		
I1-1919b	260-1	solvent blank	4.04	180698	dbI	0		
I1-1920b	260-2	monkey urine blank	4.07	215834	bbI	0		
I1-1921b	260-3	C 30	4.04	498511	bb	0	24.9	-17
I1-1922b	260-4	C 30	4.04	600101	bb	0	36.8	23
I1-1923b	260-5	C 60	4.00	1075745	bbX	0	94.9	58
I1-1924b	260-6	C 60	4.00	1055135	bbX	0	92.4	54
I1-1925b	260-7	C 100	4.00	1068758	bb	0	94.1	-5.9
I1-1926b	260-8	C 100	3.99	1105197	bb	0	98.6	-1.4
I1-1927b	260-9	C 300	4.00	2710450	bb	0	289	-3.6
I1-1928b	260-10	C 300	4.01	3066621	bb	0	329	9.7
I1-1929b	260-11	C 1000	3.98	10361629	bb	0	1007	0.75
I1-1930b	260-12	C 1000	3.98	10006450	bb	0	979	-2.14
I1-1931b		ACN	4.05	126230	bbI	0		
I1-1932b	261-1	QC 30	4.00	565716	MM	0	32.7	9.1
I1-1933b	261-4	QC 100	3.99	1335647	bb	0	127	27
I1-1934b	261-7	QC 750	3.99	5877881	bb	0	616	-18
I1-1935b		ACN	4.05	97386	bbI	0		
I1-1936b	250-1	QC 30	4.00	426762	bb	0	16.8	
I1-1937b	250-2	QC 30	3.99	436869	bb	0	18.0	
I1-1938b	250-3	QC 30	4.00	439825	bb	0	18.3	
I1-1939b	250-4	QC 100	4.00	1033292	bb	0	89.7	
I1-1940b	250-5	QC 100	4.02	1075041	bb	0	94.9	
I1-1941b		ACN	4.07	97529	bbI	0		
I1-1942b	261-2	QC 30	3.99	463543	bb	0	21.0	-30
I1-1943b	261-5	QC 100	4.00	1173448	bb	0	107	7.0

Compound 1: PFBS

Run # (534002-)	Ref. # (534002-)	Sample Text	RT	Area	Flags	Mult.	Conc. (ng/mL)	% RE
I1-1944b	261-8	QC 750	4.01	6566972	bb	0	681	-9.2
I1-1945b		ACN	4.07	97827	bbI	0		
I1-1946b	250-6	QC 100	4.00	1170786	bb	0	107	
I1-1947b	250-7	QC 750	4.00	6707671	bb	0	694	
I1-1948b	250-8	QC 750	4.01	6652618	bd	0	689	
I1-1949b	250-9	QC 750	4.00	6525956	bd	0	677	
I1-1950b		ACN	4.05	108742	bbI	0		
I1-1951b	261-3	QC 30	4.00	510405	bb	0	26.3	-12
I1-1952b	261-6	QC 100	4.02	1221893	MM	0	113	13
I1-1953b	261-9	QC 750	4.00	7084124	bb	0	728	-2.9
I1-1954b		ACN	4.06	99322	bbI	0		

APPENDIX H

Toxicokinetic Report (WIL Research Laboratories, LLC)

STUDY TITLE

A PHARMACOKINETIC (IN BLOOD) AND EXCRETION STUDY IN CYNOMOLGUS MONKEYS

REPORT TITLE

PHARMACOKINETICS OF PFHxA AND PFBS IN SERUM AND URINE FOLLOWING A SINGLE INTRAVENOUS DOSE TO CYNOMOLGUS MONKEYS

REPORT DATE

2 September 2005

Sponsor

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1.0 SUMMARY

Three male and three female Cynomolgus monkeys were administered a single intravenous dose of perfluorohexanoic acid (PFHxA) followed 7 days later by a single intravenous dose of nona-1-butanesulfonic acid (PFBS). The dosage level for both test articles was 10 mg/kg. Blood samples were collected from each animal at 0 (prior to dosing), 1, 2, 4, 8, 24, and 48 hours following each dose. Urine was collected from each animal over the following intervals: 0-6, 6-12, and 12-24 hours post-dosing and then daily until 7 days post-dosing. Serum and urine concentrations of PFHxA or PFBS were measured using a validated LC-MS/MS method. The serum concentration immediately following the intravenous dose was estimated based on the first two measured values. The concentrations in serum and amounts excreted in urine were used for pharmacokinetic analysis.

The mean pharmacokinetic parameters for PFHxA and PFBS are summarized in the following table:

MEAN PHARMACOKINETIC RESULTS

10 mg/kg Intravenous Dose	SERUM					URINE	
	C_0* (ng/mL)	$AUC_{0-\infty}$ (ng•h/mL)	Half-life (h)**	Cl (L/h/kg)	V_d (L/kg)	Half-life (h)***	% of Dose Eliminated
PFHxA							
Males	57877	84002	5.3	0.122	0.989	21	11.7
Females	65178	75157	2.4	0.136	0.474	23	12.8
PFBS							
Males	113852	1114762	15	0.0124	0.209	30	9.18
Females	100301	488859	8.1	0.0229	0.248	25	19.3

* Values were estimated.
** For the terminal elimination phase.
*** For urinary elimination.

Systemic exposures to PFBS were approximately an order of magnitude higher than exposures to PFHxA at equivalent dosages. The half-life of PFHxA in serum was shorter than the half-life of PFBS in serum. Apparent systemic clearance and volume of distribution were approximately an order of magnitude higher for PFHxA than for PFBS. The lower apparent volume of distribution for PFBS than for PFHxA suggests that PFBS is sequestered outside the vasculature. On average, only about 10-20% of the administered dose of either compound was recovered in the urine during the 7 days post-dosing. The low percentage of dose recovered may be due to incomplete capture of the urine; therefore, estimations of the half-life for urinary elimination of PFHxA and PFBS may not be meaningful. There did not appear to be any gender differences in the pharmacokinetics of PFHxA. For PFBS, male monkeys appeared to have higher

exposure and longer half-lives than female monkeys, but the mean values were greatly influenced by one male with high exposure and a long half-life.

2.0 INTRODUCTION

Three male and three female Cynomolgus monkeys were administered a single intravenous dose of perfluorohexanoic acid (PFHxA) followed 7 days later by a single intravenous dose of nona-1-butanesulfonic acid (PFBS). The dosage level for both test articles was 10 mg/kg. Blood samples were collected from each animal at 0 (prior to dosing), 1, 2, 4, 8, 24, and 48 hours following each dose. Blood samples were collected from a femoral vein into chilled tubes containing no anticoagulant. Serum was separated using a refrigerated centrifuge. Urine was collected from each animal over the following intervals: 0-6, 6-12, and 12-24 hours post-dosing and then daily until 7 days post-dosing. However, due to the limitations of the test chamber design, quantitative recovery of urine may not have been attained. Some urine may also have been adsorbed to the feces. Urine samples were maintained on wet ice during collection. All samples were stored at approximately -70°C until analysis with the following exception: serum samples collected at 48 hours post-dosing were stored at -20°C. Serum and urine concentrations of PFHxA or PFBS were measured by the Analytical Chemistry Department at WIL Research Laboratories, LLC using a validated LC-MS/MS method. The serum concentration immediately following the intravenous dose was estimated based on the

first two measured values. The concentrations in serum and amounts excreted in urine were used for pharmacokinetic analysis.

3.0 EXPERIMENTAL

3.1 Data Processing

All calculations were performed using Microsoft® Excel 2000 on a Microsoft® Windows 2000 platform. Graphical presentations were created using DeltaGraph® 5.4.1.

3.2 Bioanalysis

Serum and urine concentrations of PFHxA and PFBS were measured using a validated LC-MS/MS method by the Analytical Chemistry Department at WIL Research Laboratories, LLC. A detailed description of the analytical method and the results for each sample may be found in Appendices F and G. The lower limit of quantitation (LLOQ) for the analytes was 30 ng/mL for both test articles in both matrices.

3.3 Toxicokinetic Evaluation & Statistical Analysis

All toxicokinetic parameters were calculated from the individual serum or urine concentration data as follows:

C_0	The estimated concentration of the compound in serum immediately following intravenous administration. The values were set equal to the y-intercept of the linear regression based on the log concentration of the first two measured values.

AUC_{0-48}	The area under the serum concentration vs. time curve from 0 to 48 h post-dosing. The values were calculated by linear trapezoidal summation using the equation: $AUC_{0-48} = \Sigma\ (0.5 \bullet (y_1 + y_2) \bullet \Delta\ t)$ where y_1 and y_2 are successive serum concentrations and Δt is the sampling interval, in hours, between y_1 and y_2.
$AUC_{0-\infty}$	The estimate of the area under the serum concentration vs. time curve from 0 h to infinity. The values were calculated using the formula: $AUC_{0-\infty} = AUC_{0-48} + (\text{serum concentration at 48 h}/K_{el})$ where AUC_{0-48} is defined previously and K_{el} is defined subsequently.
ARE	The amount remaining to be eliminated by a given route. The values were calculated using the formula: ARE = Total amount eliminated via route– Amount eliminated in previous interval(s)
K_{el}	The terminal elimination rate constant for the compound in serum or urine. The values were calculated using the equation: $K_{el} = -\ln[10] \times b$ where b is the slope of the least-squares linear regression line of the log serum concentrations from 4 h post-dosing to the last value >LLOQ or the log ARE values over the intervals indicated on the tables.
Half-life	The half-life for the compound in serum or the half-life of urinary elimination. The values were calculated using the formula: $\text{Half-life} = -\ln[0.5]/K_{el}$ where K_{el} is defined previously.

Cl	The apparent systemic clearance for the compound in serum. The values were calculated using the formula: $Cl = Dosage / AUC_{0-\infty}$ where $AUC_{0-\infty}$ is defined previously.
V_d	The apparent volume of distribution for the compound in serum. The values were calculated using the formula: $V_d = Cl/K_{el}$ where Cl and K_{el} are defined previously.
Urinary Elimination as % Dose	The total amount eliminated in urine expressed as a percentage of the analyte dose. The value was calculated using the equation: Total as % Dose = 100 * ARE at 0 h/(Mean BW * Dosage) where ARE is defined previously and BW is the body weight used to calculate the administered dose. Body weights were obtained from Table 9 of the main report.

In the calculation of the toxicokinetic parameters, samples were assigned a value of zero if the concentration was below the LLOQ. For urine samples, a value of zero was also assigned if no sample or insufficient sample was available for analysis.

4.0 RESULTS AND DISCUSSION

4.1 Serum Concentration Data

4.1.1 PFHxA in Male Monkeys

Individual serum concentrations of PFHxA following a single intravenous dosage of 10 mg PFHxA/kg to male monkeys are presented in Table 1 and illustrated in Figure 1.

Table 1. Individual Concentrations (ng/mL) of PFHxA in Serum of Male Monkeys following Intravenous Administration of 10 mg PFHxA/kg

Hours Post-Dosing	Animal No. 1555	Animal No. 1562	Animal No. 1563
0*	63487	59170	50975
1	18004	25993	21259
2	5106	11419	8866
4	1414	4235	2079
8	972	1902	567
24	108	32.8	30.3
48	0.00	0.00	35.5

*Estimated values.

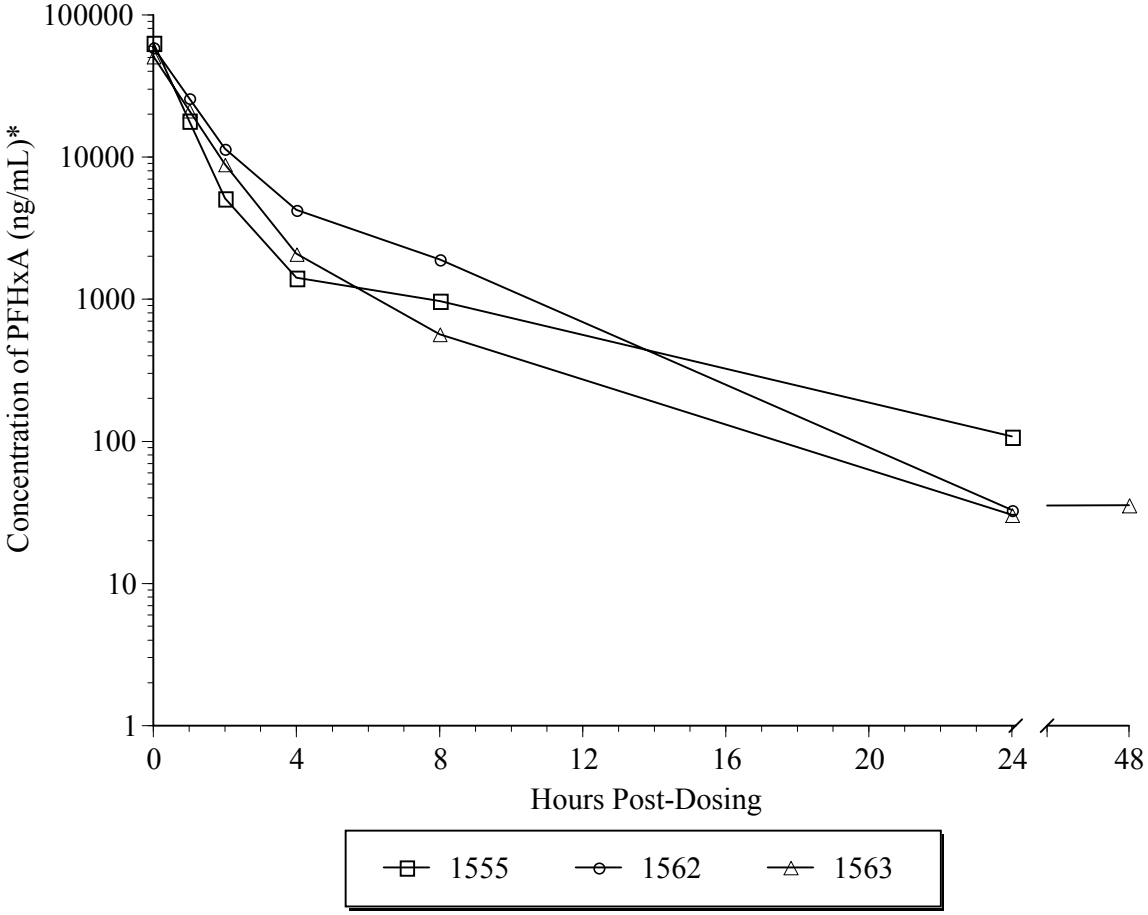

Figure 1. Individual Concentrations of PFHxA in Serum of Male Monkeys following Intravenous Administration of 10 mg PFHxA/kg

*Concentration at 0 h estimated.

The concentrations of PFHxA in serum of male monkeys were similar among the replicate animals. Except for one animal, the concentration of PFHxA was below the LLOQ (30 ng/mL) by 48 hours post-dosing.

4.1.2 PFHxA in Female Monkeys

Individual serum concentrations of PFHxA following a single intravenous dosage of 10 mg PFHxA/kg to female monkeys are presented in Table 2 and illustrated in Figure 2.

Table 2. Individual Concentrations (ng/mL) of PFHxA in Serum of Female Monkeys following Intravenous Administration of 10 mg PFHxA/kg

Hours Post-Dosing	Animal No. 1572	Animal No. 1573	Animal No. 1576
0*	84870	59998	50665
1	25140	17469	20004
2	7447	5086	7898
4	2332	2006	3078
8	341	272	438
24	0.00	0.00	84.3
48	0.00	0.00	0.00

*Estimated values.

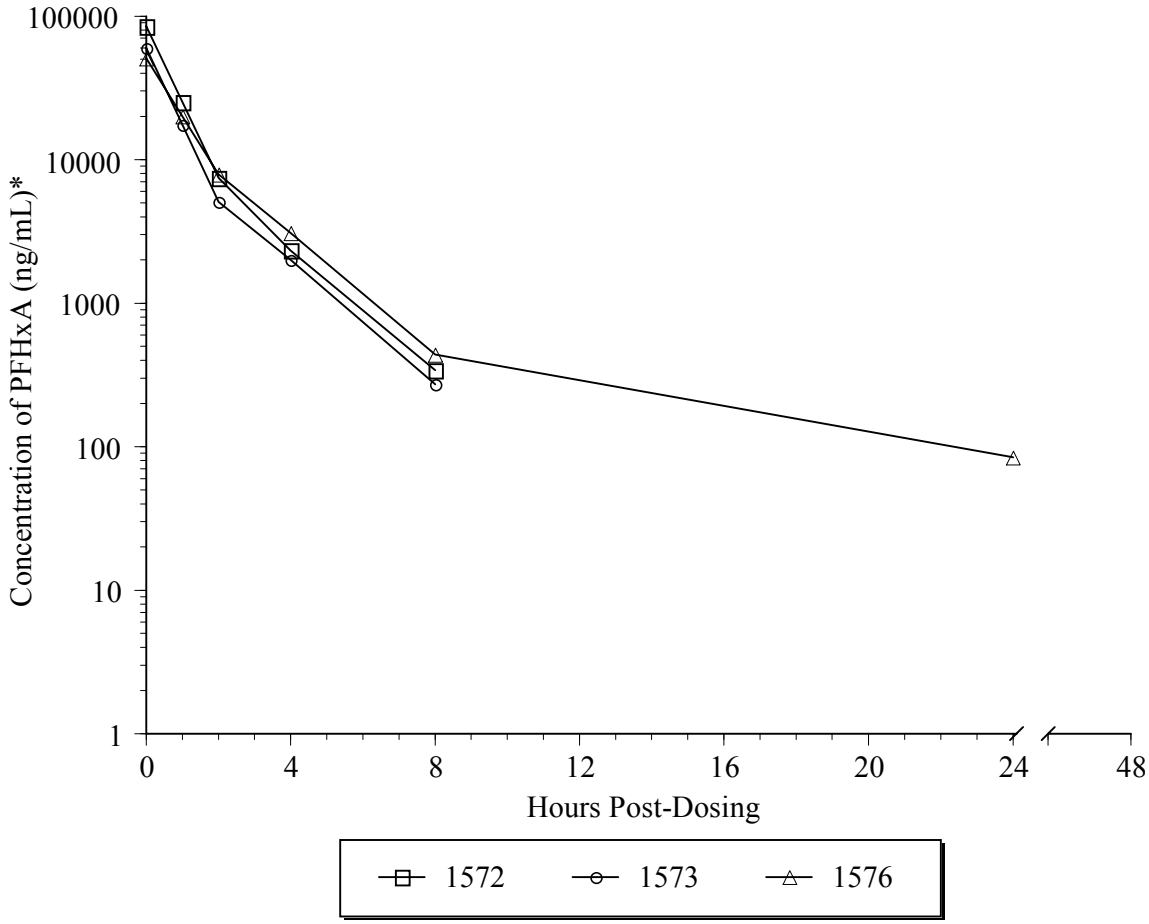

Figure 2. Individual Concentrations of PFHxA in Serum of Female Monkeys following Intravenous Administration of 10 mg PFHxA/kg

*Concentration at 0 h estimated.

The concentrations of PFHxA in serum of female monkeys were similar among the replicate animals. The concentration of PFHxA in serum of two of the animals was below the LLOQ (30 ng/mL) by 24 hours post-dosing and the concentration of PFHxA in the other animal was below the LLOQ by 48 hours post-dosing.

4.1.3 PFBS in Male Monkeys

Individual serum concentrations of PFBS following a single intravenous dosage of 10 mg PFBS/kg to male monkeys are presented in Table 3 and illustrated in Figure 3.

Table 3. Individual Concentrations (ng/mL) of PFBS in Serum of Male Monkeys following Intravenous Administration of 10 mg PFBS/kg

Hours Post-Dosing	Animal No. 1555	Animal No. 1562	Animal No. 1563
0*	117424	119291	104842
1	77987	93766	74930
2	51795	73703	ISV
4	28978	50733	27354
8	18468	45348	20140
24	4371	22244	5134
48	1180	16069	1205

*Estimated values.
ISV=Insufficient volume for analysis.

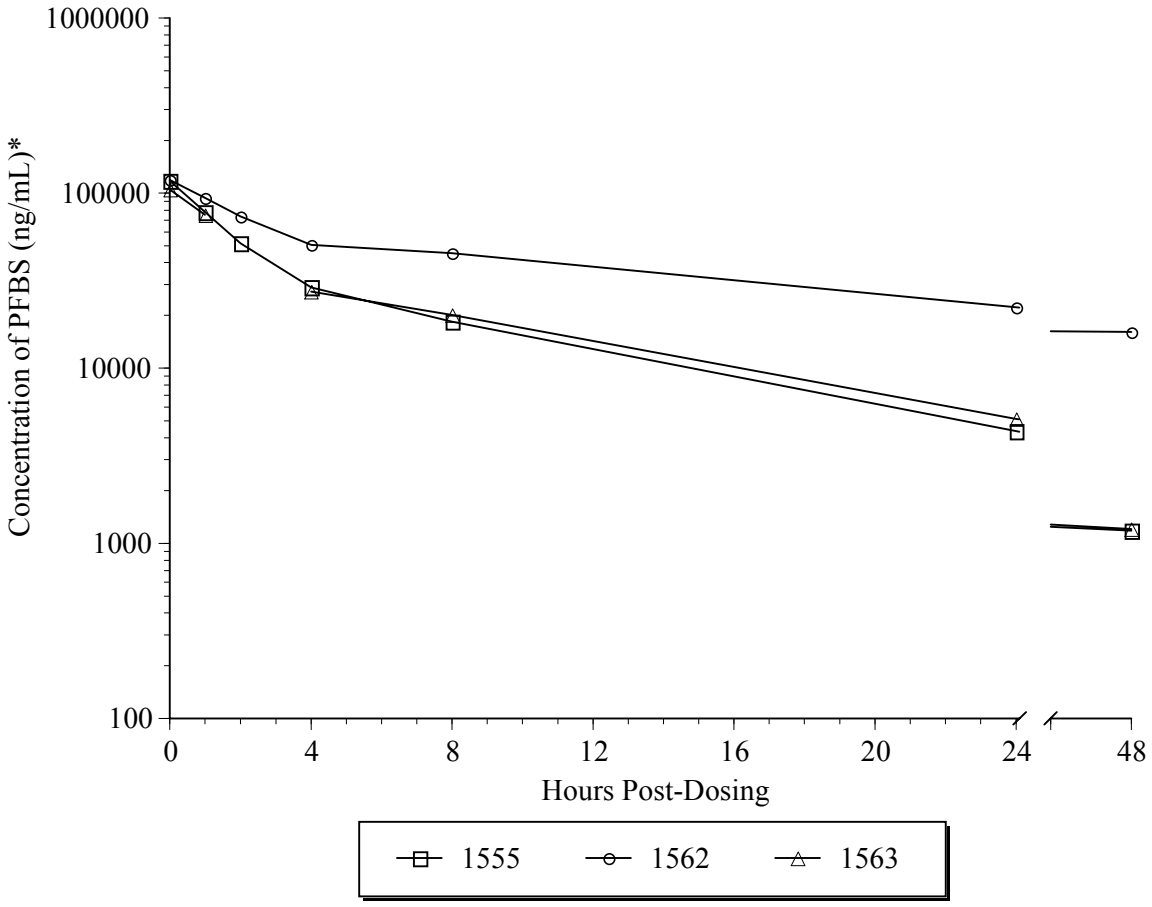

Figure 3. Individual Concentrations of PFBS in Serum of Male Monkeys following Intravenous Administration of 10 mg PFBS/kg

*Concentration at 0 h estimated.

The concentrations of PFBS in serum of male monkeys were similar between two of the replicate animals, but the concentrations remained elevated in the third animal (Animal No. 1562). The concentrations of PFBS in serum were still appreciable at 48 hours post-dosing.

4.1.4 PFBS in Female Monkeys

Individual serum concentrations of PFBS following a single intravenous dosage of 10 mg PFBS/kg to female monkeys are presented in Table 4 and illustrated in Figure 4.

Table 4. Individual Concentrations (ng/mL) of PFBS in Serum of Female Monkeys following Intravenous Administration of 10 mg PFBS/kg

Hours Post-Dosing	Animal No. 1572	Animal No. 1573	Animal No. 1576
0*	97966	95098	107839
1	69087	54860	75915
2	48722	31648	53442
4	27485	16324	35453
8	15634	7707	21094
24	3441	787	4680
48	1027	79.8	1098

*Estimated values.

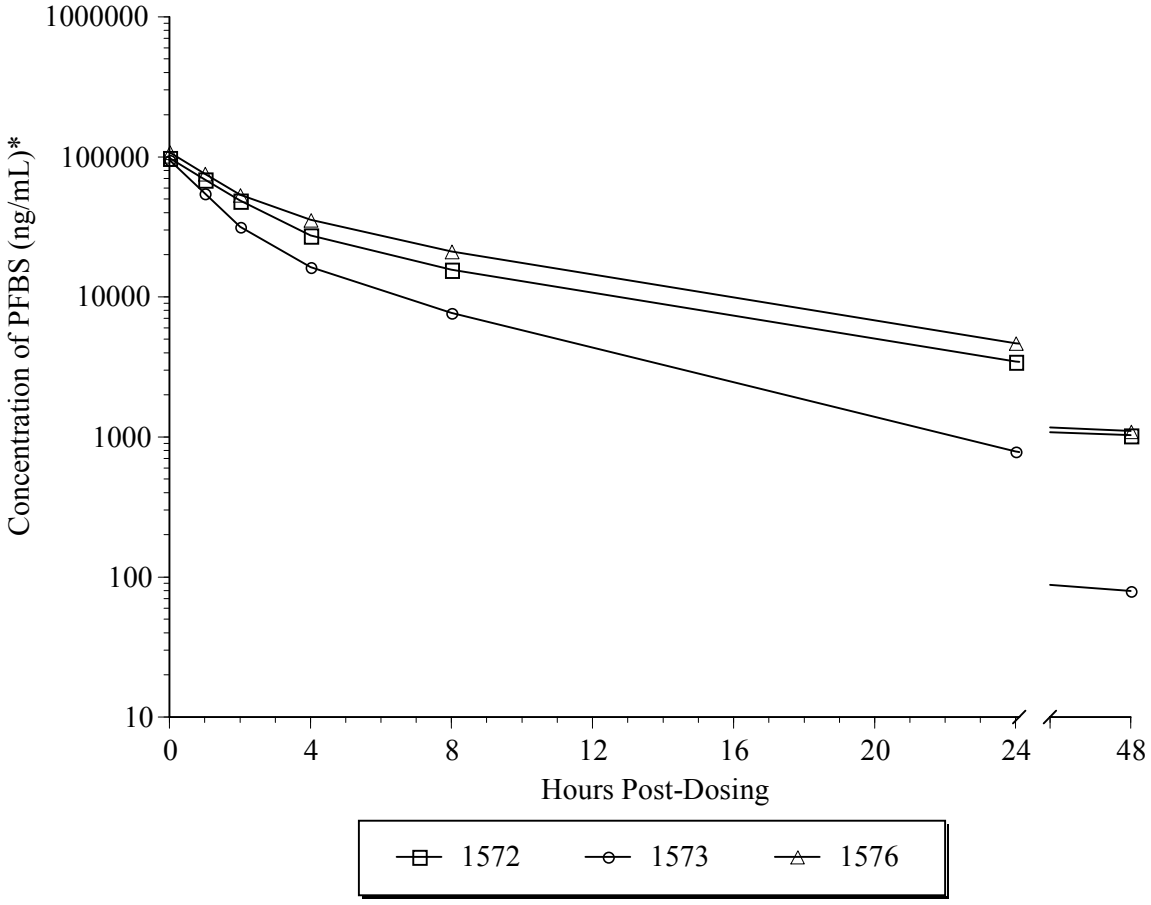

Figure 4. Individual Concentrations of PFBS in Serum of Female Monkeys following Intravenous Administration of 10 mg PFBS/kg

*Concentration at 0 h estimated.

The concentrations of PFBS in serum of female monkeys were generally similar among the replicate animals, although the concentrations decreased faster for one animal (Animal No. 1573) than for the other two animals. The concentrations of PFBS in serum were still appreciable at 48 hours post-dosing.

4.2 Serum Pharmacokinetics

4.2.1 PFHxA Pharmacokinetics

The individual pharmacokinetic parameters for PFHxA in serum of male and female monkeys are presented in Table 5.

Table 5. PFHxA Serum Pharmacokinetic Parameters following Intravenous Administration of 10 mg PFHxA/kg to Male and Female Monkeys

	C_0* (ng/mL)	$AUC_{0-\infty}$ (ng•h/mL)	K_{el} (1/h)**	Half-life (h)**	Cl (L/h/kg)	V_d (L/kg)
Males						
Animal No. 1555	63487	73528	0.131	5.3	0.136	1.04
Animal No. 1562	59170	105090	0.246	2.8	0.0952	0.387
Animal No. 1563	50975	73387	0.0883	7.8	0.136	1.54
Mean:	57877	84002	0.155	5.3	0.122	0.989
SD:	6355	18263	0.0816	2.5	0.0237	0.579
Females						
Animal No. 1572	84870	89156	0.480	1.4	0.112	0.233
Animal No. 1573	59998	63836	0.500	1.4	0.157	0.314
Animal No. 1576	50665	72480	0.158	4.4	0.138	0.874
Mean:	65178	75157	0.379	2.4	0.136	0.474
SD:	17681	12871	0.192	1.7	0.0223	0.349

* Values were estimated.
** For the terminal elimination phase.

The pharmacokinetic parameters for PFHxA were generally similar between the genders. The terminal half-life of PFHxA in serum tended to be longer for male monkeys than for female monkeys, but the parameter was variable. Half of the animals (two males and one

female) had apparent volumes of distribution that were near or in excess of unity, suggesting that distribution was mainly in the vasculature. The lower apparent volume of distribution for the other three monkeys resulted from a higher estimation of the terminal elimination rate constant (shorter half-life).

4.2.2 PFBS Pharmacokinetics

The individual pharmacokinetic parameters for PFBS in serum of male and female monkeys are presented in Table 6.

Table 6. PFBS Serum Pharmacokinetic Parameters following Intravenous Administration of 10 mg PFBS/kg to Male and Female Monkeys

	C_0* (ng/mL)	$AUC_{0-\infty}$ (ng•h/mL)	K_{el} (1/h)**	Half-life (h)**	Cl (L/h/kg)	V_d (L/kg)
Males						
Animal No. 1555	117424	603949	0.0721	9.6	0.0166	0.230
Animal No. 1562	119291	2106890	0.0268	26	0.00475	0.177
Animal No. 1563	104842	633448	0.0714	9.7	0.0158	0.221
Mean:	113852	1114762	0.0568	15	0.0124	0.209
SD:	7859	859335	0.0259	9.4	0.00661	0.0282
Females						
Animal No. 1572	97966	525126	0.0733	9.5	0.0190	0.260
Animal No. 1573	95098	293287	0.120	5.8	0.0341	0.285
Animal No. 1576	107839	648164	0.0779	8.9	0.0154	0.198
Mean:	100301	488859	0.0902	8.1	0.0229	0.248
SD:	6684	180197	0.0255	2.0	0.00990	0.0448

* Values were estimated.
** For the terminal elimination phase.

The pharmacokinetic parameters for PFBS were generally similar between the genders. The terminal half-life of PFBS in serum was appreciably longer for one male monkey (Animal No. 1562) than for the other monkeys. All of the animals had low apparent volumes of distribution, suggesting that distribution occurred outside of the vasculature.

4.3 Urinary Elimination

Concentrations of PFHxA and PFBS in individual urine samples can be found in the tables of the bioanalytical report (Appendices F and G).

4.3.1 PFHxA Urinary Elimination in Male Monkeys

Individual amounts of PFHxA eliminated in urine and urinary pharmacokinetic parameters following a single intravenous dosage of 10 mg PFHxA/kg to male monkeys are presented in Table 7, with the amount remaining to be eliminated illustrated in Figure 5.

Table 7. Individual Amounts (µg) of PFHxA in Urine of Male Monkeys following Intravenous Administration of 10 mg PFHxA/kg

Hours Post-Dosing	Animal No. 1555	Animal No. 1562	Animal No. 1563
0-6	4305	2572	1.19
6-12	226	0.00	151
12-24	273	0.00	332
24-48	137	0.00	246
48-72	36.3	0.00	79.9
72-96	27.1	9.54	71.5
96-120	8.35	0.00	34.9
120-144	11.3	0.521	19.0
144-168	3.51	0.00	4.26
Total Eliminated as a % of Dose	20.9	10.8	3.36
Elimination Rate Constant (1/h)*	0.0351	0.0279	0.0357
Half-life (h)*	20	25	19

*Based on 6-144 h except 6-120 h for Animal No. 1562.

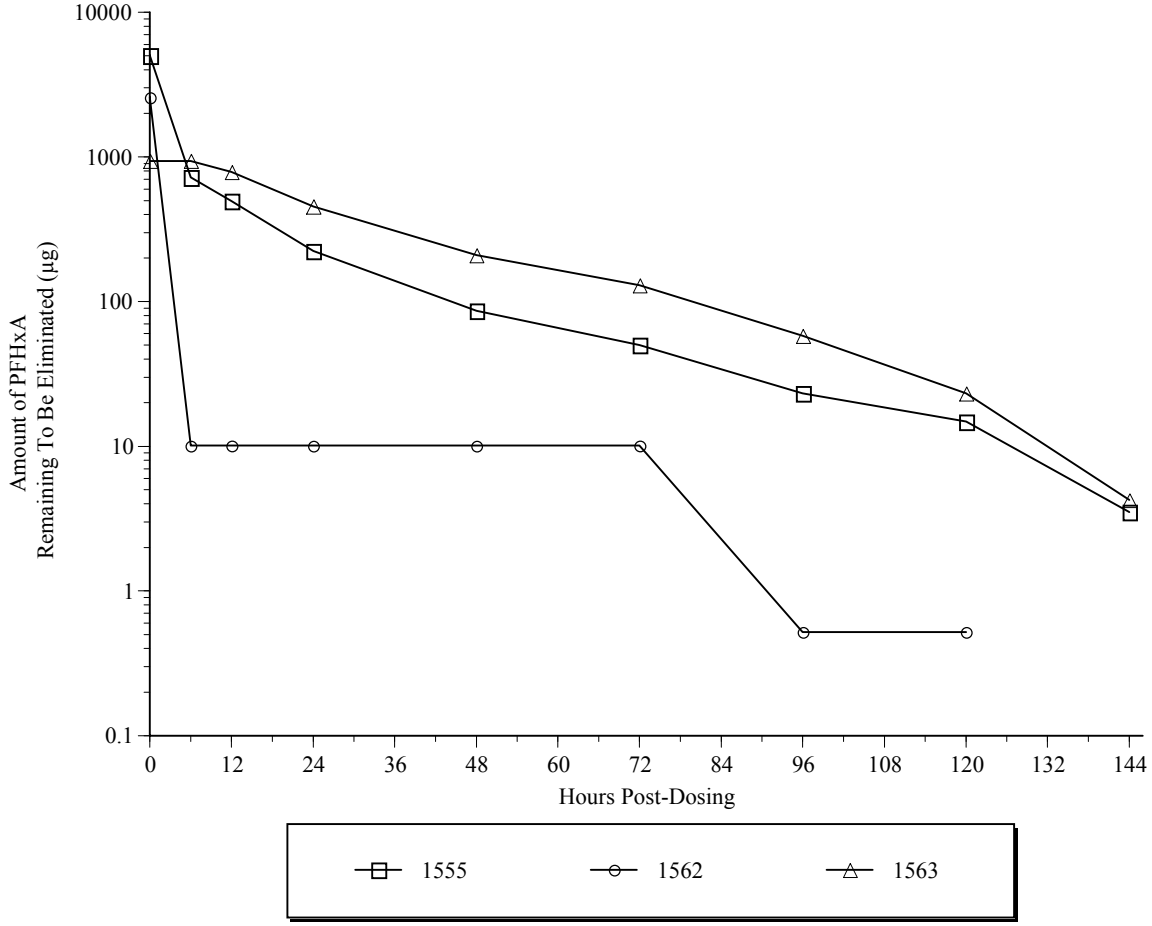

Figure 5. Individual Amounts of PFHxA Remaining To Be Eliminated in Urine of Male Monkeys following Intravenous Administration of 10 mg PFHxA/kg

The amount of PFHxA recovered in the urine of male monkeys was low and variable among the replicate animals. Recovery in the urine accounted for 3.4% to 21% of the administered dose. The low percentage of dose recovered may be due to incomplete capture of the urine; therefore, the calculated half-life for the urinary elimination of PFHxA may not be meaningful.

4.3.2 PFHxA Urinary Elimination in Female Monkeys

Individual amounts of PFHxA eliminated in urine and urinary pharmacokinetic parameters following a single intravenous dosage of 10 mg PFHxA/kg to female monkeys are presented in Table 8, with the amount remaining to be eliminated illustrated in Figure 6.

Table 8. Individual Amounts (µg) of PFHxA in Urine of Female Monkeys following Intravenous Administration of 10 mg PFHxA/kg

Hours Post-Dosing	Animal No. 1572	Animal No. 1573	Animal No. 1576
0-6	9428	5.15	0.00
6-12	62.5	6.68	0.00
12-24	209	54.1	0.00
24-48	45.1	0.00	0.00
48-72	35.1	3.49	49.3
72-96	21.2	1.17	1.44
96-120	7.51	1.92	0.00
120-144	0.730	5.08	1.14
144-168	4.28	2.01	0.00
Total Eliminated as a % of Dose	37.7	0.318	0.208
Elimination Rate Constant (1/h)*	0.0337	0.0219	0.0385
Half-life (h)*	21	32	18

*Based on 6-144 h, 0-144 h, and 0-120 h for Animal Nos. 1572, 1573, and 1576, respectively.

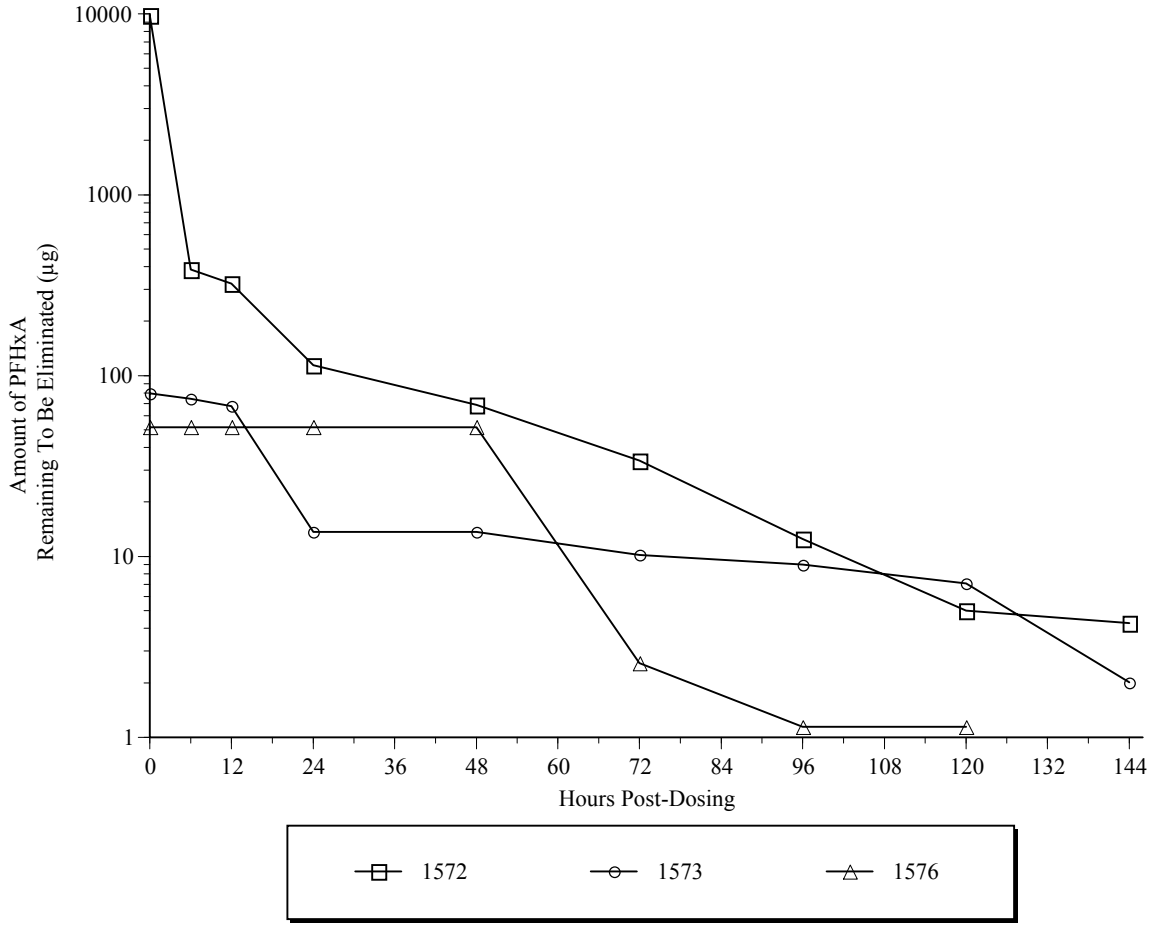

Figure 6. Individual Amounts of PFHxA Remaining To Be Eliminated in Urine of Female Monkeys following Intravenous Administration of 10 mg PFHxA/kg

The amount of PFHxA recovered in the urine of female monkeys was low and variable among the replicate animals. Recovery in the urine accounted for 0.2% to 38% of the administered dose. The low percentage of dose recovered may be due to incomplete

capture of the urine; therefore, the calculated half-life for the urinary elimination of PFHxA may not be meaningful.

4.3.3 PFBS Urinary Elimination in Male Monkeys

Individual amounts of PFBS eliminated in urine and urinary pharmacokinetic parameters following a single intravenous dosage of 10 mg PFBS/kg to male monkeys are presented in Table 9, with the amount remaining to be eliminated illustrated in Figure 7.

Table 9. Individual Amounts (µg) of PFBS in Urine of Male Monkeys following Intravenous Administration of 10 mg PFBS/kg

Hours Post-Dosing	Animal No. 1555	Animal No. 1562	Animal No. 1563
0-6	953	0.00	0.00
6-12	386	0.00	544
12-24	787	0.00	2184
24-48	223	349	735
48-72	79.2	97.0	205
72-96	31.5	229	69.1
96-120	13.1	25.4	19.3
120-144	6.46	0.00	57.6
144-168	9.04	87.8	72.5
Total Eliminated as a % of Dose	10.4	3.28	13.9
Elimination Rate Constant (1/h)*	0.0307	0.0201	0.0212
Half-life (h)*	23	34	33

*Based on 24-144 h.

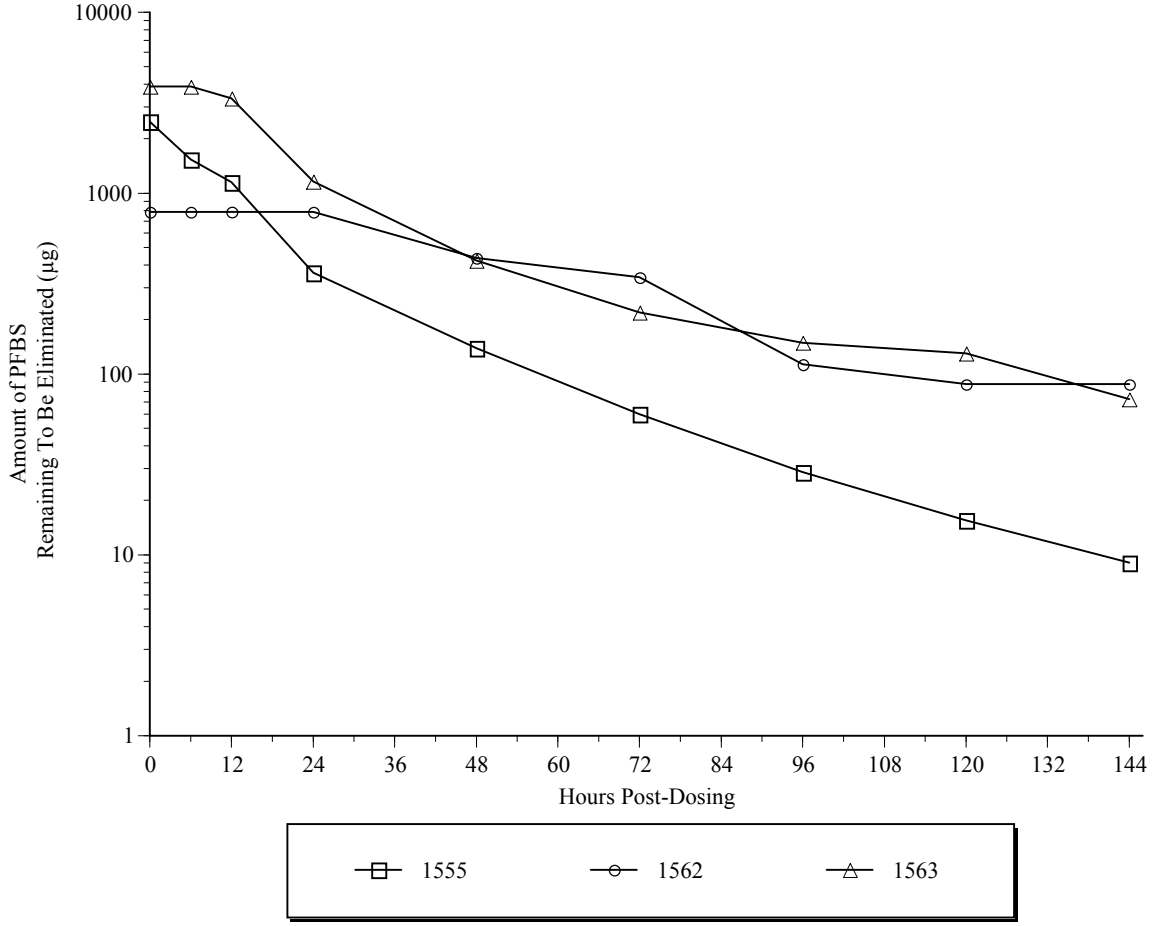

Figure 7. Individual Amounts of PFBS Remaining To Be Eliminated in Urine of Male Monkeys following Intravenous Administration of 10 mg PFBS/kg

The amount of PFBS recovered in the urine of male monkeys was low, but relatively consistent among the replicate animals. Recovery in the urine accounted for 3% to 14% of the administered dose. The low percentage of dose recovered may be due to incomplete capture of the urine; therefore, the calculated half-life for the urinary elimination of PFBS may not be meaningful.

4.3.4 PFBS Urinary Elimination in Female Monkeys

Individual amounts of PFBS eliminated in urine and urinary pharmacokinetic parameters following a single intravenous dosage of 10 mg PFBS/kg to female monkeys are presented in Table 10, with the amount remaining to be eliminated illustrated in Figure 8.

Table 10. Individual Amounts (µg) of PFBS in Urine of Female Monkeys following Intravenous Administration of 10 mg PFBS/kg

Hours Post-Dosing	Animal No. 1572	Animal No. 1573	Animal No. 1576
0-6	3962	0.00	2589
6-12	0.00	0.00	0.00
12-24	6514	952	0.00
24-48	382	54.9	118
48-72	129	13.6	84.1
72-96	*	1.83	25.1
96-120	11.6	11.5	24.8
120-144	17.3	0.00	0.00
144-168	10.1	3.59	15.7
Total Eliminated as a % of Dose	42.4	4.15	11.4
Elimination Rate Constant (1/h)*	0.0302	0.0267	0.0255
Half-life (h)**	23	26	27

* Sample broke.
** Based on 24-144 h.

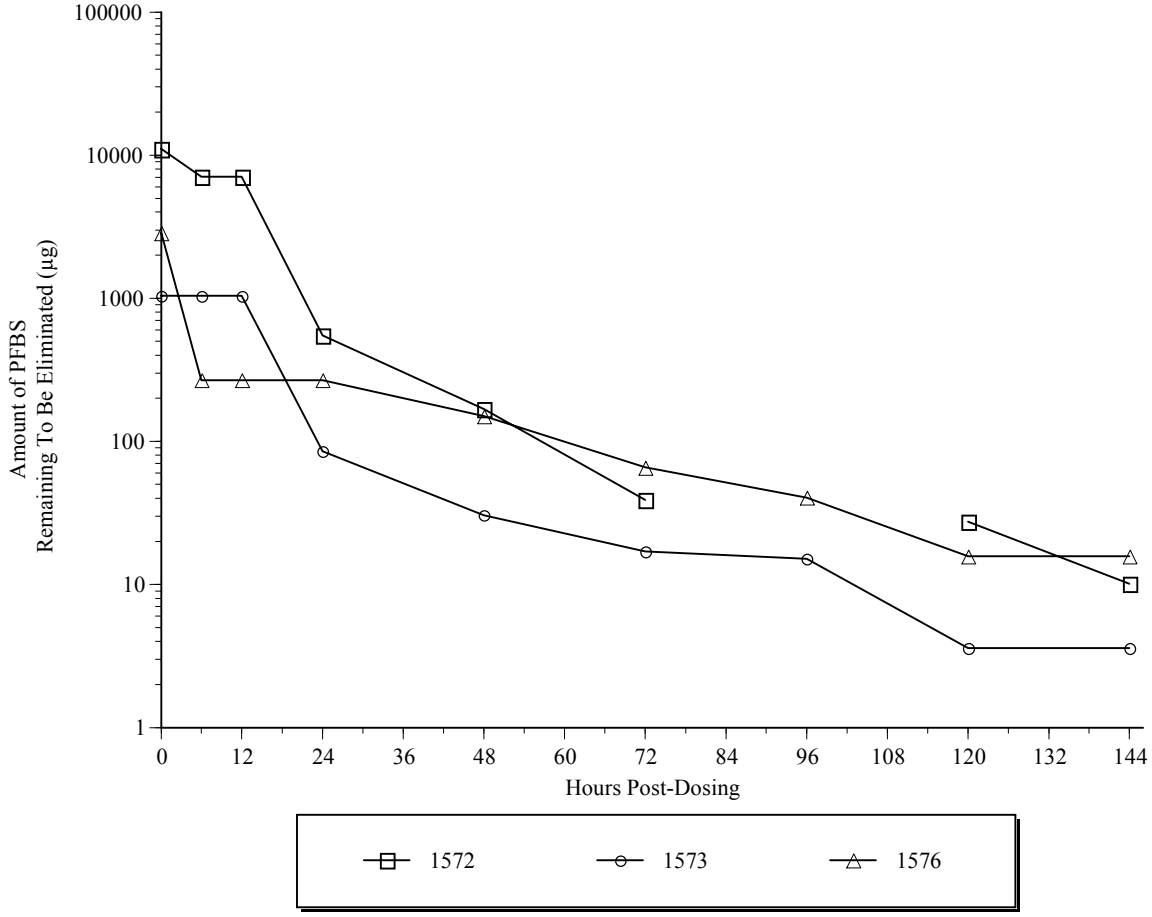

Figure 8. Individual Amounts of PFBS Remaining To Be Eliminated in Urine of Female Monkeys following Intravenous Administration of 10 mg PFBS/kg

The amount of PFBS recovered in the urine of female monkeys was low, but relatively consistent among the replicate animals. Recovery in the urine accounted for 4% to 42% of the administered dose. The low percentage of dose recovered may be due to incomplete capture of the urine; therefore, the calculated half-life for the urinary elimination of PFBS may not be meaningful.

5.0 CONCLUSIONS

Systemic exposures to PFBS were approximately an order of magnitude higher than exposures to PFHxA at equivalent dosages. The half-life of PFHxA in serum was shorter than the half-life of PFBS in serum. Apparent systemic clearance and volume of distribution were approximately an order of magnitude higher for PFHxA than for PFBS. The lower apparent volume of distribution for PFBS than for PFHxA suggests that PFBS is sequestered outside the vasculature. On average, only about 10-20% of the administered dose of either compound was recovered in the urine during the 7 days post-dosing. The low percentage of dose recovered may be due to incomplete capture of the urine; therefore, estimations of the half-life for urinary elimination of PFHxA and PFBS may not be meaningful. There did not appear to be any gender differences in the pharmacokinetics of PFHxA. For PFBS, male monkeys appeared to have higher exposure and longer half-lives than female monkeys, but the mean values were greatly influenced by one male with high exposure and a long half-life.

6.0 RESPONSIBLE PERSONNEL

Report Prepared By:

Daniel W. Sved, Ph.D.
Director, Metabolism and Analytical Chemistry

2 September 2005
Date

Report Reviewed By:

Jeannie B. Kirkpatrick, M.S.
Staff Toxicologist;
Study Director

2 September 2005
Date

APPENDIX I

Study Protocol

Study Number: WIL-534002

PROTOCOL AMENDMENT II

Sponsor: AGC Chemical

A. Title of Study:

A Pharmacokinetic (in Blood) and Excretion Study in Cynomolgus Monkeys

B. Protocol Modification:

3 STUDY SCHEDULE:

1) "Proposed Study Completion Date:" is changed to "Proposed Experimental Termination (Completion) Date:". This date is July 18, 2005 (date of last bioanalytical data collection).

2) Proposed Audited Report Date: August 11, 2005

C. Reason for Protocol Modification:

1) OECD Good Laboratory Practice (GLP) standards require the Experimental Completion Date and EPA GLP standards require the Experimental Termination Date. The date of the last data collection is added.

2) The proposed audited report date is added.

Approved By:

AGC Chemical

Motoki Shinohara
Sponsor Representative

29 August, 2005
Date

Prepared By:

WIL Research Laboratories, LLC

Jeannie B. Kirkpatrick, M.S.
Study Director

29 August 2005
Date

Christopher P. Chengelis, Ph.D., DABT
Director, Toxicology

30 Aug 2005
Date

Study Number: WIL-534002

PROTOCOL AMENDMENT I

Sponsor: AGC Chemical

A. Title of Study:

A Pharmacokinetic (in Blood) and Excretion Study in Cynomolgus Monkeys

B. Protocol Modification:

1) **4.1.9 Vehicle:**

In accordance with the study director notification issued January 31, 2005, the vehicle is changed to sterile water for injection.

2) **4.2.9 Vehicle:**

In accordance with the study director notification issued January 31, 2005, the vehicle is changed to sterile water for injection.

3) **7.1.2 Testing Facility:**

The first sentence in the third paragraph is changed to the following:

The animals will be allowed a pretest week (as part of the acclimation period) during which body weights will be recorded and food consumption and general health will be monitored, but they will not receive the test article.

4) **7.3 Route and Rationale of Test Article Administration:**

In accordance with the study director notification issued January 31, 2005, the dosing route is changed to intravenous (bolus) injection.

5) **7.4.1 Organization of Test Groups:**

In accordance with the study director notification dated January 31, 2005, the dose level is changed to 10 mg/kg for both test articles and the dose volume is changed to 5 mL/kg. The concentration of the test articles in the vehicle will be 2 mg/mL.

6) **7.5.1 Test Article Preparation:**

In accordance with the study director notification issued February 7, 2005, the dosing formulations will be sterile filtered.

7.5.2 Analysis of Test Article Formulations:

7) In accordance with the study director notification dated February 7, 2005, homogeneity assessments will not be performed.

8) In accordance with the study director notification dated February 15, 2005, samples for analysis will be collected after overnight refrigerated storage.

9) In accordance with the study director notification dated February 15, 2005, formulation stability will be documented for at least two days.

10) **7.5.3 Concentration Analysis:**

In accordance with the study director notification issued February 18, 2005, samples will be collected using the same IV dosing apparatus as used in the study, and will be analyzed, comparing the results to those from samples collected using standard sampling procedures.

11) **8.5 Sample Collection for Determination of Serum Drug Levels:**

In accordance with the study director notification issued February 7, 2005, blood samples for toxicokinetic analysis will be collected at predose and at 1, 2, 4, 8, 24 and 48 hours after dosing.

12) **8.6 Toxicokinetics for Elimination:**

In accordance with the study director notification dated February 10, 2005, total volumes will be recorded for all toxicokinetic urine collections.

C. Reason for Protocol Modification:

1,2) Change of vehicle due to change in route of dosing.

3) Clarification added that food consumption will be monitored but not recorded.

4) Change of dosing route at the request of the Sponsor.

5) Change in dose level at the request of the Sponsor. The dose volume change is the result of the change in dosing routes.

6) Addition of filter sterilization during preparation of dosing formulations due to change in dosing route.

7) Removal of homogeneity assessment due to filter sterilization during formulation preparation.

8) Overnight refrigeration before sampling improves the consistency of the analytical results obtained.

9) The interval duration for stability evaluations of test article formations is revised.

10) Addition of instructions for alternate method of collection of formulation samples for analysis in order to determine possible test article adherence to the dosing apparatus.

11) Addition of time points for TK blood collection requested by the Sponsor.

12) Addition of volume measurements for TK urines to allow calculation of total test article amount excreted in urine.

Approved By:
AGC Chemical

Motoki Shinohara — 19 July, 2005
Motoki Shinohara — Date
Sponsor Representative

Prepared By:
WIL Research Laboratories, LLC

Jeannie B. Kirkpatrick — 14 July 2005
Jeannie B. Kirkpatrick, M.S. — Date
Study Director

Christopher P. Chengelis — 14 July 2005
Christopher P. Chengelis, Ph.D., DABT — Date
Director, Toxicology

PROTOCOL

A PHARMACOKINETIC (IN BLOOD) AND EXCRETION STUDY IN CYNOMOLGUS MONKEYS

Submitted To:

AGC Chemical
Asahi Glass Company, Ltd.
10 Goikaigan, Ichihara-shi
Chiba 290-8566, JAPAN

WIL Research Laboratories, LLC
1407 George Road
Ashland, OH 44805-9281

1 OBJECTIVE:

The objective of this study is to evaluate the pharmacokinetic (in blood) and excretion profiles of the test article in cynomolgus monkeys.

This protocol has been designed and the study will be conducted in compliance with the U.S. Environmental Protection Agency, CFR Part 792, and the Organisation for Economic Cooperation and Development [C(97)186/Final] Good Laboratory Practice (GLP) Regulations.

2 PERSONNEL INVOLVED IN THE STUDY:

2.1 Sponsor Representative:

Motoki Shinohara
Safety Manager
Environments & Safety Office

2.2 WIL Study Director:

Jeannie B. Kirkpatrick, M.S.
Staff Toxicologist
Tel: (419)-289-8700
Fax: (419) 289-3650
Email: jkirkpatrick@wilresearch.com

2.3 WIL Deputy Director:

Jozef J.W.M. Mertens, Ph.D., D.A.B.T.
Associate Director, General Toxicology

2.4 WIL Departmental Responsibilities:

Christopher P. Chengelis, Ph.D., D.A.B.T.
Director, Toxicology

Teresa D. Morris, B.S.
Operations Manager, Toxicology

Sally A. Keets, A.S.
Senior Operations Manager, Vivarium

Theresa M. Rafeld
Group Supervisor, Formulations Laboratory

Lisa T. Snyder, D.V.M.
Clinical Veterinarian

Susan C. Haley, B.S.
Manager, Clinical Pathology

Carol A. Kopp, B.S., L.A.T.
Manager, Gross Pathology and
Developmental Toxicology Laboratory

Philip L. Stetson, M.D., Ph.D.
Associate Director, Analytical Chemistry

Daniel W. Sved, Ph.D.
Director, Metabolism and Analytical Chemistry

Heather L. Osborn, B.S., RQAP-GLP
Manager, Quality Assurance

Lewis E. Kaufman, M.S., RAC, RQAP-GLP
Director, Regulatory Affairs and Services

3 STUDY SCHEDULE:

Proposed Stock Animal Selection Date:	January 12, 2005
Proposed Experimental Start Date:	January 13, 2005
Proposed Study Completion Date:	To be determined.
Proposed Audited Report Date:	To be determined.

4 TEST ARTICLE INFORMATION:

4.1 Test Article 1:

4.1.1 Identification:

Perfluorohexanoic acid (PFHxA)

4.1.2 Lot Number:

To be provided by the Sponsor.

4.1.3 Purity:

To be provided by the Sponsor. The purity will be considered to be 100% for the purpose of dosage calculations.

4.1.4 Stability:

Stability data are the responsibility of the Sponsor.

4.1.5 Physical Description:

To be documented by WIL Research Laboratories, LLC.

4.1.6 Storage Conditions:

To be provided by the Sponsor.

4.1.7 Reserve Samples:

Retention samples will be collected and stored in accordance with Standard Operating Procedures.

4.1.8 Personnel Safety:

To be provided by the Sponsor. It is the responsibility of the Sponsor to notify the testing facility of any special handling requirements for the test article.

4.1.9 Vehicle:

0.5% aqueous methylcellulose.

4.2 Test Article 2:

4.2.1 Identification:

Nonafluoro-1-butanesulfonic acid (PFBS)

4.2.2 Lot Number:

To be provided by the supplier. The PFBS will be purchased by WIL Research Laboratories, LLC.

4.2.3 Purity:

To be provided by the supplier. The purity will be considered to be 100% for the purpose of dosage calculations.

4.2.4 Stability:

Stability data are the responsibility of the supplier.

4.2.5 Physical Description:

To be documented by WIL Research Laboratories, LLC.

4.2.6 Storage Conditions:

To be provided by the supplier.

4.2.7 Reserve Samples:

Retention samples will be collected and stored in accordance with Standard Operating Procedures.

4.2.8 Personnel Safety:

To be provided by the supplier or the Sponsor. It is the responsibility of the Sponsor to notify the testing facility of any special handling requirements for the test article.

4.2.9 Vehicle:

0.5% aqueous methylcellulose

5 TEST SYSTEM:

5.1 Species:

Cynomolgus monkey (Macaca fascicularis)

5.2 Source:

Covance Research Products, Inc.
Alice, Texas

5.3 Number of Animals:

Three males and three females will be selected from available stock and assigned to the study.

5.4 Approximate Age and Weight:

Animals will be young adults (estimated to be 2-2.5 years old) at initiation of dosing. Animals are anticipated to weigh approximately 2-5 kg at initiation of dosing.

5.5 Identification System:

Each animal will be uniquely identified by a tattoo. Individual cage cards will be affixed to each cage and will display the animal number, group number, tattoo identification and study number.

5.6 Justification for Selection and Number of Animals:

This species of animal is recognized to be appropriate for both single- and repeated-dose toxicity studies. The cynomolgus monkey was chosen because it is a widely used species for which significant control data are available. The number of animals utilized on study is the minimum number required to yield scientifically meaningful data and is consistent with agency expectations.

6 SPECIFIC MAINTENANCE SCHEDULE:

6.1 Animal Housing:

The animals will be housed individually, due to urine collection requirements, in clean stainless steel cages in an environmentally controlled room at the time of receipt. The cages will be elevated above stainless steel flush pans which will be cleaned daily. The facilities at WIL Research Laboratories, LLC are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

6.2 Environmental Conditions:

Controls will be set to maintain an average daily temperature of 71 ± 5ºF (22 ± 3ºC) and an average daily relative humidity of 50 ± 20%. Temperature and relative humidity will be monitored continuously. Data for these two parameters will be scheduled for automatic collection on an hourly basis. Fluorescent lighting controlled by light timers will provide illumination for a 12-hour light/dark photoperiod. The light/dark period may be interrupted to

accommodate protocol-specified activities. The ventilation rate will be set at a minimum of 10 room air changes per hour, 100% fresh air.

6.3 Drinking Water:

Reverse osmosis-treated tap water will be available *ad libitum*. Filters servicing the automatic watering system will be changed regularly. Municipal water supplying the laboratory will be analyzed for contaminants according to WIL Standard Operating Procedures to ascertain that none are present at concentrations that would be expected to affect the outcome of the study.

6.4 Diet:

Approximately 7 to 9 PMI Nutrition International, LLC Certified Monkey LabDiet® 5048 biscuits will be offered twice daily. Vitamin tablets will be used as appropriate to supplement the diet. Analyses of the certified feed for the presence of contaminants will be provided by the manufacturer to ensure that none are present at concentrations that would be expected to affect the outcome of the study.

Diet will be supplemented with other items such as raisins or fresh fruits, which are presented to the animal as part of an environmental enrichment program and provide additional nutrients. Offering supplements or items known to cause diarrhea should be avoided. Nutritional supplements will not be analyzed.

6.5 Psychological Enrichment:

All animals will be regularly presented with an enriched environment in accordance with Standard Operating Procedures and the Animal Welfare Act. This may include interactions with the technicians, playing of music, TV and/or recorded sounds, popcorn and/or fruit treats, Kong toys, foraging boards and mirrors.

7 EXPERIMENTAL DESIGN:

7.1 Animal Receipt and Acclimation:

7.1.1 Supplier:

Animals were tested for Simian Immunodeficiency Virus (SIV), Simian T Lymphotropic virus (STLV1), Simian Retrovirus (SRV) [including PCR for SRV-1 and SRV-2] and Herpes B, were tuberculosis free and were vaccinated for measles and Hepatitis A.

7.1.2 Testing Facility:

Each animal was inspected by a veterinarian upon receipt. Animals judged to be in good health were placed in quarantine and acclimated for at least 5 weeks. All animals were weighed and assigned a permanent animal number. During the acclimation period, each animal was observed twice daily for changes in general appearance and behavior. Stool samples were collected during the acclimation period and checked for parasites. Three tuberculin (TB) tests were administered approximately 2 weeks apart, one by the supplier prior to shipment and two by WIL Research Laboratories prior to initiation of dosing. There were no animals that were TB reactors. Data collected from the time of receipt until just prior to initiation of pretest will be maintained in the stock colony records.

During the acclimation period animals will be acclimated to the handling procedures required for dosing and blood sampling. The acclimation procedures will be documented.

The animals will be allowed a pretest week (as part of the acclimation period) during which body weight and food consumption will be recorded and general health will be monitored, but they will not receive the test article. All animals will receive a detailed physical examination approximately one week prior to initiation of dosing and at the time of animal selection for assignment to study.

7.2 Randomization:

The monkeys will be arbitrarily assigned to the study.

7.3 Route and Rationale of Test Article Administration:

The route of administration will be oral (via nasogastric intubation) since this is the intended route of human exposure.

7.4 Organization of Test Groups, Dosage Levels and Treatment Regimen:

7.4.1 Organization of Test Groups:

The following table presents the study group arrangement.

Group Number	No. of Animals Per Sex	Treatment No.[a]	Test Article	Dose (mg/kg)	Concentration (mg/mL)	Dose Volume (mL/kg)
1	3	1	PFHxA	300	30	10
	3	2	PFBS	300	30	10

a =Animals will be dosed once per test article. There will be a 7-day nondosing observation period between treatments. After the last dose is administered, animals will be monitored for at least 7 days before being assigned to the WIL Stock Monkey Colony.

7.4.2 Treatment Regimen:

The dosing formulations will be administered to the stomach using a nasogastric tube. Administration of the dosing formulation will be followed by a 5-mL flush using deionized water. The first day of dosing is day 0; the first week of dosing is week 0. One dose of each test article will be administered, each followed by 7 days of observation and blood/urine collection.

7.4.3 Adjustment of Doses:

Individual doses will be adjusted weekly based on the most recent body weights. Adjusted doses will become effective the day after the weekly body weight collection.

7.5 Preparation and Analysis of Dosing Formulations:

7.5.1 Test Article Preparation:

The test article will be prepared for dosing as weight-to-volume mixtures in an appropriate vehicle. No correction for purity will be made. A complete description of the method of test article preparation will be documented in the study records and described in the final report. Test article preparations will be prepared within 1 week of use for dosing and stored refrigerated. Frequency of preparations and storage conditions may be adjusted based on stability results.

7.5.2 Analysis of Test Article Formulations:

Analyses to demonstrate the stability and homogeneity of the test article formulations will be conducted concurrently with use for dosing using a

validated method. For stability assessments, test article formulations will be prepared, stored refrigerated (protected from light), and sampled over a minimum of 3 days. The samples will be analyzed to evaluate stability. Additional aspects of stability (e.g., stability of frozen samples) may also be investigated. For homogeneity assessments, samples will be collected from the top, middle and bottom strata of the formulations prepared for dosing and the samples analyzed to assess the homogeneity of the test article in the mixtures. If the test article formulations will be stored prior to use, the formulations will be stored under suitable conditions for up to the maximum length of time acceptable. After remixing for a minimum of 10 minutes using a magnetic stirrer, samples will be collected from the top and bottom strata of the formulations and analyzed to assess the homogeneity after storage and resuspension.

7.5.3 Concentration Analysis:

Concentration will be confirmed during the dosing period. Samples will be drawn from each test article formulation. These will be submitted to the WIL Research Analytical Chemistry Laboratory and analyzed for test material concentration.

The Analytical Chemistry report will be appended to the final report for this study.

8 PARAMETERS TO BE EVALUATED:

8.1 Viability Observations:

All animals will be observed for mortality/moribundity twice daily, once in the morning and once in the afternoon. Moribund animals will be anesthetized with ketamine and euthanized by barbiturate overdose and necropsied as described in Section 8.7.1.

8.2 Clinical Observations:

8.2.1 Daily Observations:

Test animals will be observed twice daily on dosing days for clinical signs and mortality, once just prior to each dosing and again at approximately 1 to 2 hours after each dose administration. Observations will include, but are not limited to, changes in the skin sites, fur, eyes and mucous membranes; respiratory, circulatory, autonomic and central nervous systems; somatomotor activity and behavior patterns. Positive findings noted at the clinical examinations will be recorded. The

absence of signs will be noted in a general comment. The animals will be observed once daily on non-dosing days between doses and for 7 days following the last dose.

8.2.2 Detailed Physical Examinations:

All animals will receive a detailed physical examination at least weekly during the acclimation period and dosing phases.

8.2.3 Unscheduled Observations:

A separate computer protocol will be used to collect any findings outside the above-specified observations. Only the presence of findings will be recorded; the absence of findings will not be recorded.

8.3 Individual Body Weights:

The body weights of each animal will be determined at least weekly, starting at least one week prior to initiation of dosing. Animals will be weighed on the day prior to each dosing. Body weights will also be recorded approximately one week after the last dose.

8.4 Individual Food Consumption:

Food consumption will not be determined. Any decrease in appetite will be recorded as part of the daily observations when applicable.

8.5 Sample Collection for Determination of Serum Drug Levels:

Approximately 0.5 mL of blood will be obtained from each animal prior to each dosing and at four time points (to be provided by the Sponsor) after completion of each dose administration. The blood samples will be collected via a femoral vein (or a site other than the femoral vein, if needed) into chilled tubes containing no anticoagulant. Serum will be separated using a refrigerated centrifuge. The maximum volume possible of serum will be transferred into sample tubes labeled with type of sample, animal identification number, WIL study number, time point and study day and date of collection. Serum samples will be frozen with minimal delay at approximately -20°C and stored at approximately -20°C until transferred to the Analytical Chemistry Department for analysis.

8.6 Toxicokinetics for Elimination:

After each dose administration, urine will be collected on wet ice over the following intervals: 0-6, 6-12 and 12-24 hours post-dosing and then once daily for the next 6 days (until 7 days post-dosing). Urine samples will be frozen with minimal delay at approximately -20°C and stored at approximately -20°C until transferred to the Analytical Chemistry Department for analysis.

Serum and urine samples will be analyzed for PFHxA or PFBS by the Analytical Chemistry Department at WIL Research Laboratories, LLC, using a validated LC/MS/MS method.

Subsequently, pertinent toxicokinetic parameters, such as C_{max}, AUC, and elimination half-life, will be determined as data permit, for each of the analytes following a single dose of the test material.

8.7 Anatomic Pathology:

8.7.1 Macroscopic Examination:

A formal necropsy examination will not be conducted as part of this study, unless an animal is found dead or euthanized *in extremis*. Animals *in extremis* will be anesthetized by intramuscular ketamine and euthanized by intravenous barbiturate overdose. If conducted, necropsy will include examination of the external surface, all orifices and the cranial, thoracic, abdominal and pelvic cavities including viscera. Gross lesions will be collected and placed in 10% neutral-buffered formalin.

8.7.2 Microscopic Examination:

Microscopic examination of tissues will not be scheduled, except by amendment to the protocol.

9 STATISTICAL METHODS:

Because of the small group sizes, statistical inferences will not be calculated.

10 QUALITY ASSURANCE:

The study will be audited by the WIL Quality Assurance Unit while in progress to assure compliance with EPA and OECD Good Laboratory Practice regulations, adherence to the protocol and to WIL Standard Operating Procedures. The raw data for the draft final report will be audited by the WIL Quality Assurance Unit to assure that the final report accurately describes the conduct and the findings of the study.

This is a regulated study and will be included on the WIL master list of regulated studies.

11 RECORDS TO BE MAINTAINED:

All original raw data records, as defined by WIL SOPs and the applicable GLPs, will be stored in the Archives at WIL Research Laboratories, LLC as described in Section 12.

12 WORK PRODUCT:

Sponsor will have title to all documentation records, raw data, specimens or other work product generated during the performance of the study. Any remaining formulation, clinical pathology (serum, whole blood and/or plasma) and/or toxicokinetic samples will be discarded after issuance of the final report. All work product including raw paper data, pertinent electronic storage media and specimens, will be retained in the Archives at WIL Research Laboratories, LLC at no charge for a period of 10 years following issuance of the final report in the Archives at WIL Research Laboratories, LLC. Thereafter, WIL Research Laboratories will charge a monthly archiving fee for retention of all work product. All work product will be stored in compliance with regulatory requirements.

Any work product, including documents, specimens and samples that are required by this protocol, its amendments, or other written instructions of the Sponsor, to be shipped by WIL Research Laboratories, LLC to another location will be appropriately packaged and labeled as defined by WIL's SOPs and delivered to a common carrier for shipment. WIL Research Laboratories, LLC will not be responsible for shipment following delivery to the common carrier.

13 REPORTS:

The final report will contain a summary, test article data, methods and procedures, appropriate individual animal and summary data tables, a copy of the protocol and amendments (if any) and an interpretation and discussion of the study results. The report will contain all information necessary to conform with current FDA specifications. The report will be comprehensive and shall attempt to define the level(s) including toxic effects, as well as "no-effect" level(s) under the conditions of this investigation.

WIL Research Laboratories will provide one (1) copy of an Audited Draft Report, submitted in a timely manner upon completion of the study prior to issuance of the final report. One (1) revision will be permitted as part of the cost of the study, from which Sponsor's reasonable revisions and suggestions will be incorporated into the Final Report, as appropriate. Additional changes or revisions may be made, at extra cost. It is expected that the Sponsor will review the draft report and provide

comments to WIL within a two (2) month time frame following submission. WIL will submit the Final Report within one (1) month following receipt of comments. If the Sponsor's comments and/or authorization to finalize the report have not been received at WIL within one year following submission of the draft report, WIL may elect to finalize the report following appropriate written notification to the Sponsor. Two (2) electronic copies of the Final Report on CD-R will be provided; requests for additional electronic or paper copies of the Final Report may result in additional charges.

14 PROTOCOL MODIFICATION:

Modification of the protocol may be accomplished during the course of this investigation. However, no changes will be made in the study design without the verbal or written permission of the Sponsor. In the event that the Sponsor verbally requests or approves a change in the protocol, such changes will be made by appropriate documentation in the form of a protocol amendment. All alterations of the protocol and reasons for the modification(s) will be signed by the Study Director and the Sponsor Representative.

15 ANIMAL WELFARE ACT COMPLIANCE:

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR). The Sponsor should make particular note of the following:

- The Sponsor Representative's signature, on this protocol, documents for the Study Director the Sponsor's assurance that the study described in this protocol does not unnecessarily duplicate previous experiments.

- Whenever possible, procedures used in this study have been designated to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study protocol or in written laboratory Standard Operating Procedures.

- Animals that experience severe or chronic pain or distress that cannot be relieved will be painlessly euthanized as deemed appropriate by the veterinary staff and Study Director. The Sponsor will be advised by the Study Director of all circumstances which could lead to this action in as timely a manner as possible.

- Methods of euthanasia used during this study are in conformance with the above-referenced regulation.

- The Sponsor/Study Director has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals and has provided a written narrative description (AWA covered species) of the methods and sources used to determine that alternatives are not available.

16 PROTOCOL APPROVAL:

Sponsor approval received via **email** on **December 27, 2004**.

AGC Chemical

Motoki Shinohara — 1/13/2005
Motoki Shinohara — Date
Sponsor Representative

WIL Research Laboratories, LLC

Jeannie B. Kirkpatrick — 1/5/2005
Jeannie B. Kirkpatrick, M.S. — Date
Staff Toxicologist

5 January 2005
Christopher P. Chengelis, Ph.D., D.A.B.T. — Date
Director, Toxicology