FINAL REPORT

STUDY TITLE

PHARMACOKINETIC (IN BLOOD) AND EXCRETION STUDY OF PERFLUOROHEXANOIC ACID AND NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

STUDY NUMBER

WIL-534004

STUDY DIRECTOR

Jeannie B. Kirkpatrick, MS

STUDY INITIATION DATE

1 April 2005

STUDY COMPLETION DATE

17 October 2005

PERFORMING LABORATORY

WIL Research Laboratories, LLC
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COMPLIANCE STATEMENT

This study, designated WIL-534004, 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

Jeannie B. Kirkpatrick, MS
Staff Toxicologist
Study Director

17 Oct 2005
Date

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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 rats.

1.2. STUDY DESIGN

For the pharmacokinetic phase (assigned to computer protocol WIL-534004A), the test articles, Perfluorohexanoic acid (PFHxA) in the vehicle, sterile water for injection, or nonafluoro-1-butanesulfonic acid (PFBS) in the vehicle, sterile water for injection, were administered as a single intravenous (bolus) injection to 2 groups (WIL-534004A; Groups 1 and 2) each consisting of 9 male and 9 female Crl:CD®(SD) rats. For the excretion phase (assigned to computer protocol WIL-534004B), the same test articles were administered as a single intravenous (bolus) injection to 2 groups (WIL-534004B; Groups 1 and 2) each consisting of 3 male and 3 female Crl:CD®(SD) rats. The dosage level was 10 mg/kg for all groups at a dosage volume 5 mL/kg.

All animals were observed twice daily for mortality and moribundity. Detailed physical examinations were performed during pretest. Individual body weights were recorded during pretest and on study day 0. Food consumption was recorded during pretest.

Blood samples for serum drug levels were collected from 3 animals/sex/group 0.5, 1, 1.5, 2, 4, 8 and 24 hours after dose administration on study day 0. All animals were euthanized and discarded following the final blood collection (study day 1). For urine excretion evaluation (WIL-534004B), animals were placed in plastic metabolism cages following dose administration on study day 0. Urine was collected on wet ice from all animals at 0-6, 6-12 and 12-24 hours post-dosing. All animals were euthanized and discarded following the final urine collection (study day 1).

Serum and urine concentrations of PFHxA or PFBS were measured using a validated LC-MS/MS method. The concentrations in serum and amounts excreted in urine were used for pharmacokinetic analysis.

1.3. RESULTS AND CONCLUSIONS

Systemic exposures to PFBS were approximately 2.5- to 3-fold higher than exposures to PFHxA at equivalent dosages. This is partially due to a terminal half-life for PFBS that is longer than that for PFHxA. Exposure to both PFBS and PFHxA was approximately 7- to 8-fold higher for male rats than for female rats. This may be attributable to several related factors such as the shorter half-life and higher apparent clearance and volume of distribution in females than in males. The terminal half-life of PFHxA in serum was about 2.5-fold shorter for female rats than for male rats (0.42 hours compared to 1.0 hours). The terminal half-life of PFBS in serum was approximately 3-fold shorter for female rats than male rats (0.64 hours compared to 2.1 hours). Apparent clearance of PFHxA and PFBS from the serum was approximately 7- to 8-fold higher for female rats than for male rats; apparent volume of distribution for PFHxA and PFBS in the serum was approximately 2.5- to 3-fold higher for female rats than for male rats. Low apparent volumes of distribution for both male and female rats may reflect the rapid clearance and elimination rather than distribution outside of the vasculature.

Approximately 80% of the administered dose of PFHxA and approximately 70% of the administered dose of PFBS was recovered in the urine during 24 hours post-dosing regardless of gender. The half-life for urinary elimination of PFHxA and PFBS ranged from approximately 2 to 3 hours, regardless of gender.

In conclusion, PFHxA treatment resulted in lower systemic exposure in Crl:CD®(SD) rats than PFBS at equivalent dosages. PFHxA and PFBS in the serum was cleared more rapidly in the female rats than the male rats and the females had a wider volume of distribution than the male rats. There were no gender differences between PFHxA and PFBS for urinary elimination.

2. INTRODUCTION

2.1. GENERAL STUDY INFORMATION

This report presents the data from "Pharmacokinetic (in Blood) and Excretion Study of Perfluorohexanoic Acid and Nonafluoro-1-Butanesulfonic Acid in Rats". Due to software spacing constraints, the study title appears as "Pharmacokinetic and Excretion Study in Rats" on the report tables.

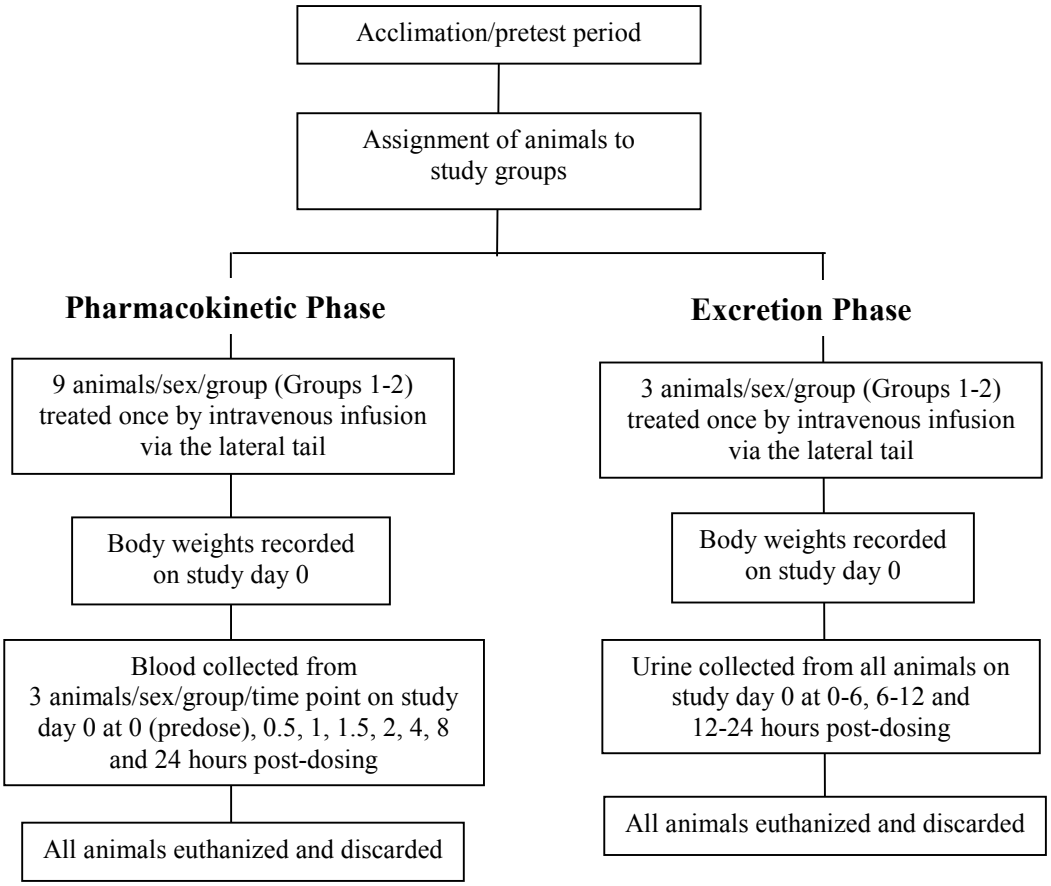
The following computer protocols were used for data collection during the study:

Computer Protocol	Type of Data Collected
WIL-534004A	Main study data (pharmacokinetic phase)
WIL-534004B	Main study data (excretion phase)
WIL-534004U	Unscheduled observations (pharmacokinetic phase)
WIL-534004V	Unscheduled observations (excretion phase)
WIL-534004P	Pretest data

2.2. KEY STUDY DATES

Date(s)	Event(s)
22 March 2005	Experimental starting date (animal receipt)
4 April 2005	Assignment to study groups
6 April 2005	Experimental start date (initiation of dose administration; study day 0)
7 April 2005	Scheduled necropsy (study day 1)
9 September 2005	Experimental termination (completion) date (date of last bioanalytical stability analysis)

3. STUDY DESIGN



4. EXPERIMENTAL PROCEDURES - MATERIALS AND METHODS

4.1. TEST ARTICLE AND VEHICLE

4.1.1. 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 PFHxA (approximately 2 g) was collected on 17 January 2005 and stored in the Archives of WIL Research Laboratories, LLC.

4.1.2. 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. 10410KC 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.

4.1.3. VEHICLE IDENTIFICATION

The vehicle used in preparation of the PFHxA and PFBS formulation groups was sterile water for injection, USP.

4.1.4. 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 septa. The test article formulations were prepared once as single formulations for each dosage level. The PFHxA formulation was divided into 2 aliquots (1 for a 2-day stability test, the other for dosing). All formulations were stored refrigerated. A small aliquot was removed from each group to measure the pH; the pH was 2.35 and 2.19 for the 10 mg/kg PFHxA and 10 mg/kg PFBS groups, respectively. The test article formulations were stirred continuously throughout the preparation procedures.

4.1.5. ADMINISTRATION

The test article formulations were administered by intravenous infusion via a lateral tail vein as a single dose. A constant dosage volume of 5 mL/kg was used. Individual doses were based on study day 0 body weights to provide the correct mg/kg dosage. The formulations were made the same day as dosing.

The following tables present the study group assignment:

Pharmacokinetic (Blood Collection) Groups (WIL-534004A)

Group Number	Test Article	Dosage Level (mg/kg/day)	Dose Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Animals	
					Males	Females
1	PFHxA	10	2	5	9	9
2	PFBS	10	2	5	9	9

Excretion (Urine Collection) Groups (WIL-534004B)

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

The selected route of administration for this study was intravenous infusion since this is an acceptable route of administration to assess systemic exposure. The animal model, the Crl:CD®(SD) rat, is recognized as appropriate for acute intravenous infusion studies and is a widely used strain for which significant historical control data are available.

4.1.6. SAMPLING AND ANALYSES

Homogeneity of the formulations was not assessed since the formulations were solutions. The PFBS test article formulations were determined to be stable for at least 2 days in a previous study (Kirkpatrick, 2005). Duplicate samples (1 mL each) for stability determinations were collected from the PFHxA dosing formulations and stored refrigerated for 2 days. Duplicate samples (1 mL each) for concentration analyses were collected from each group.

All analyses were conducted by the Analytical Chemistry Department, WIL Research Laboratories, LLC. The PFHxA formulations were found to be stable for at least 2 days. Both the PFHxA and PFBS formulations contained the amount of test article specified in the protocol. The methodology and results of these analyses are presented in Appendices B and C.

4.2. ANIMAL RECEIPT AND ACCLIMATION/PRETEST PERIOD

Thirty male and 30 female Crl:CD®(SD) rats were received in good health on 22 March 2005, from Charles River Laboratories, Inc., Raleigh, North Carolina. The animals were approximately 36 days old at receipt. Each animal was examined by a qualified technician on the day of receipt and weighed 2 days later. Each animal was uniquely identified by a metal eartag displaying the permanent identification number. All animals were housed for a 15-day acclimation/pretest period. During this period, each animal was observed twice daily for mortality and changes in general appearance or behavior.

Pretest data collection began on 24 March 2005. Individual body weights were recorded and detailed physical examinations were performed periodically during the pretest period. Food consumption data was also recorded for pretest animals prior to the initiation of dose administration. Pretest clinical observations are presented in Appendix D.

4.3. ANIMAL HOUSING

Upon arrival, all animals were housed 3 per cage by sex for 2 days. Thereafter, all animals were housed individually in clean, stainless steel, wire-mesh cages suspended above cage-board. Animals were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). 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

The basal diet used in this study, PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002 (meal), is a certified feed with appropriate analyses performed by the manufacturer and provided to WIL Research Laboratories, LLC. Reverse osmosis-treated (on-site) drinking water, delivered by an automatic watering system, and the basal diet were provided ad libitum throughout the study. Municipal water supplying the facility was 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 70.2°F to 71.0°F (21.2°C to 21.7°C) and mean daily relative humidity ranged from 40.8% to 48.6% during the study. Light timers were set to provide a 12-hour light (6 a.m. to 6 p.m.)/12-hour dark photoperiod. Air handling units were set to provide a minimum of 10 fresh air changes per hour.

4.6. ASSIGNMENT OF ANIMALS TO TREATMENT GROUPS

On 4 April 2005 (2 days prior to the initiation of dose administration), all available rats 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 selected for use in a computerized randomization procedure. A printout containing the animal numbers, corresponding body weights and individual group assignments was generated based on body weight stratification in a block design. The animals were then arranged into groups according to the printout. Individual body weights at randomization were within ± 20% of the mean for each sex. For the pharmacokinetic phase, each group consisted of 9 males and 9 females. For the excretion phase, each group consisted of 3 males and 3 females. The selected animals were approximately 7 weeks old at the initiation of dose administration; individual body weights ranged from 222 g to 254 g for males and from 166 g to 207 g for females in the pharmacokinetic phase, and from 228 g to 245 g for males and from 171 g to 209 g for females in the excretion phase.

5. PARAMETERS EVALUATED

5.1. CLINICAL OBSERVATIONS AND SURVIVAL (BOTH PHASES)

All animals were observed twice daily, once in the morning and once in the afternoon, for mortality and moribundity. Detailed physical examinations were conducted approximately weekly during the pretest period.

5.2. BODY WEIGHTS (BOTH PHASES)

Individual body weights were recorded during pretest, at randomization and on study day 0.

5.3. FOOD CONSUMPTION (BOTH PHASES)

Individual food consumption was recorded during the pretest period.

5.4. TOXICOKINETICS

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

5.4.1. PHARMACOKINETIC PROFILE (PHARMACOKINETIC PHASE)

Blood samples (approximately 0.5 mL each) for test article serum level determinations were collected at predose and 0.5, 1, 1.5, 2, 4, 8 and 24 hours after dose administration. Blood was collected via the retro-orbital sinus from isoflurane-anesthetized animals into tubes containing no anticoagulant. Samples were allowed to clot at room temperature, after which the samples were stored chilled until serum preparation. Serum was separated using a refrigerated centrifuge and frozen at approximately -20°C until transferred to the WIL Analytical Chemistry Department for analysis.

5.4.2. EXCRETION PROFILE (EXCRETION PHASE)

Urine was collected on wet ice over the following intervals: 0-6, 6-12 and 12-24 hours post-dosing. Animals were transferred into plastic metabolism cages for urine collection. 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.

5.5. STUDY TERMINATION (BOTH PHASES)

All animals were euthanized by carbon dioxide inhalation following the final blood or urine collection and discarded.

5.6. STATISTICAL METHODS

Statistical analyses were not conducted on this study.

5.7. 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 article, 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 (BOTH PHASES)

Individual Data: Tables 1, 2, 3, 4

All animals survived to study termination. There were no test article-related clinical observations. Urine containing red material was observed in the PFHxA and PFBS group males and females between 22 minutes to 1 hour and 29 minutes post-dosing in both phases of the study. This was most likely secondary to hemolysis caused by the intravenous injection procedure rather than to the test articles.

6.2. BODY WEIGHTS (BOTH PHASES)

Individual Data: Tables 5, 6

Body weights were collected for dose calculation purposes only.

6.3. TOXICOKINETICS (BOTH PHASES)

Summary Data: Appendices F, G, H

Individual Data: Appendices F, G

Serum and urine concentrations of PFHxA or PFBS were measured using a validated LC-MS/MS method. 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	52146	86539	1.0	0.116	0.175	2.1	84.0
Females	16538	12909	0.42	0.775	0.466	2.5	76.9
PFBS							
Males	68921	253837	2.1	0.0394	0.118	3.1	69.0
Females	30190	32197	0.64	0.311	0.288	2.4	71.6

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

Systemic exposures to PFBS were approximately 2.5- to 3-fold higher than exposures to PFHxA at equivalent dosages. This is partially due to a terminal half-life for PFBS that is longer than that for PFHxA. Exposure to both PFBS and PFHxA was approximately 7- to 8-fold higher for male rats than for female rats. This may be attributable to several related factors such as the shorter half-life and higher apparent clearance and volume of distribution in females than in males. The terminal half-life of PFHxA in serum was about 2.5-fold shorter for female rats than for male rats (0.42 hours compared to 1.0 hours). The terminal half-life of PFBS in serum was approximately 3-fold shorter for female rats than male rats (0.64 hours compared to 2.1 hours). Apparent clearance of PFHxA and PFBS from the serum was approximately 7- to 8-fold higher for female rats than for male rats; apparent volume of distribution for PFHxA and PFBS in the serum was approximately 2.5- to 3-fold higher for female rats than for male rats. Low apparent volumes of distribution for both male and female rats may reflect the rapid clearance and elimination rather than distribution outside of the vasculature.

Approximately 80% of the administered dose of PFHxA and approximately 70% of the administered dose of PFBS was recovered in the urine during 24 hours post-dosing regardless of gender. The half-life for urinary elimination of PFHxA and PFBS ranged from approximately 2 to 3 hours, regardless of gender.

7. CONCLUSIONS

In conclusion, PFHxA treatment resulted in lower systemic exposure in Crl:CD®(SD) rats than PFBS at equivalent dosages. PFHxA and PFBS in the serum was cleared more rapidly in the female rats than the male rats and the females had a wider volume of distribution than the male rats. There were no gender differences between PFHxA and PFBS for urinary elimination.

8. KEY STUDY PERSONNEL AND REPORT SUBMISSION

Report Submitted By:

Jeannie B. Kirkpatrick, MS — 17 Oct 2005
Staff Toxicologist — Date
Study Director

Report Prepared By:

Misty R. Lee, BA — 17 Oct 2005
Senior Study Analyst — Date

Report Reviewed By:

Christopher P. Chengelis, Ph.D., DABT — 17 Oct 2005
Director, Toxicology — Date

Jay G. Henson, B.S. — 17Oct05
Group Supervisor, Study Analysis and Reports — Date

Jozef J.W.M. Mertens, PhD, DABT — 17 Oct 05
Associate Director, General Toxicology — Date

Study Personnel:

Sally A. Keets, A.S.	Senior Operations Manager, Vivarium
Teresa D. Morris, B.S.	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)
06-Apr-2005	Animal Care and Equipment	08-Apr-2005	28-May-2005	K.Dobbs
09-May-2005	Study Records (Rx-1)	10-May-2005	29-Jun-2005	L.Goodrich
10-May-2005	Study Records (A-1 and A-2)	10-May-2005	29-Jun-2005	E.Crawford
10-May-2005	Study Records (I-1)	10-May-2005	29-Jun-2005	L.Goodrich
12-May-2005	Study Records (C-1)	12-May-2005	29-Jun-2005	L.Goodrich
30-May-2005	Draft AC Appendix-PFBS	31-May-2005	29-Jun-2005	E.Crawford
30-May-2005	Draft AC Appendix-PFHxA	31-May-2005	29-Jun-2005	E.Crawford
06-Sep-2005, 07-Sep-2005	Study Records (B-2 through B-7)	07-Sep-2005	17-Oct-2005	J.House
19-Sep-2005, 20-Sep-2005	Study Records (C-1 Supplemental, Toxicokinetic Data)	20-Sep-2005	17-Oct-2005	J.House
19-Sep-2005, 20-Sep-2005	Draft Report (without Analytical Chemistry, Bioanalytical or TK Appendices)	20-Sep-2005	17-Oct-2005	L.Goodrich
19-Sep-2005, 20-Sep-2005	Draft Report, Toxicokinetic Appendix	20-Sep-2005	17-Oct-2005	J.House
22-Sep-2005	Draft Report, PFBS Bioanalytical Appendix	22-Sep-2005	17-Oct-2005	J.House
22-Sep-2005	Draft Report, PFHxA Bioanalytical Appendix	22-Sep-2005	17-Oct-2005	J.House
22-Sep-2005	Study Records (B-8)	22-Sep-2005	17-Oct-2005	J.House
22-Sep-2005	Study Records (B-1)	22-Sep-2005	17-Oct-2005	J.House

This study was inspected in compliance with the United States EPA Good Laboratory Practice Standards (40 CFR Part 792), 18 September 1989; the OECD Principles of Good Laboratory Practice [C (97) 186/Final], 26 November 1997; 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) were 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 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:

Lori J. Goodrich, RQAP-GLP
Group Supervisor, Quality Assurance

17 Oct. 2005
Date

Report Released By:

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

17 Oct 2005
Date

10. REFERENCES

Kirkpatrick, J. A Pharmacokinetic (in Blood) and Excretion Study in Cynomolgus Monkeys (Study No. WIL-534002). WIL Research Laboratories, LLC, Ashland, OH, **2005.**

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 5.5** states that animals would weigh between 200 g and 350 g at initiation of dosing. Several females in the pharmacokinetic and excretion phases weighed between 166 g and 209 g.
- **Protocol Section 8** did not state for clinical observations to be performed. At approximately 20 minutes to 1.5 hours post-dosing, test article-related clinical findings were observed and recorded.

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

TABLES 1 - 6

TABLE 1 (PHARMACOKINETIC PHASE)

PROJECT NO.:WIL-534004A — PHARMACOKINETIC AND EXCRETION STUDY IN RATS — 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
78116	M	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78118	M	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78119	M	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78128	M	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78130	M	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78131	M	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78137	M	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78138	M	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78144	M	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78121	M	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1
78126	M	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1
78129	M	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1
78132	M	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1
78134	M	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1
78136	M	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1
78141	M	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1
78143	M	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1
78145	M	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1

A = CALCULATED TO THE NEAREST WHOLE WEEK USING THE MEAN AGE IN WEEKS AT INITIATION OF DOSING (7)

ANIMAL	SEX	GROUP	TYPE OF DEATH	AGE IN WEEKS A	DATE OF DEATH	DAYS ON STUDY
78148	F	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78156	F	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78150	F	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78151	F	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78152	F	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78159	F	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78166	F	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78167	F	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78173	F	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78146	F	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1
78155	F	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1
78157	F	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1
78158	F	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1
78161	F	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1
78163	F	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1
78165	F	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1
78174	F	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1
78175	F	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1

A = CALCULATED TO THE NEAREST WHOLE WEEK USING THE MEAN AGE IN WEEKS AT INITIATION OF DOSING (7)

TABLE 2 (EXCRETION PHASE)

PROJECT NO.:WIL-534004B — PHARMACOKINETIC AND EXCRETION STUDY IN RATS — 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
78123	M	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78124	M	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78125	M	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78133	M	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1
78135	M	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1
78139	M	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1

A = CALCULATED TO THE NEAREST WHOLE WEEK USING THE MEAN AGE IN WEEKS AT INITIATION OF DOSING (7)

ANIMAL	SEX	GROUP	TYPE OF DEATH	AGE IN WEEKS A	DATE OF DEATH	DAYS ON STUDY
78153	F	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78164	F	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78172	F	10 MG/KG PFHxA	SCHEDULED EUTHANASIA	7	07-APR-05	1
78160	F	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1
78169	F	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1
78170	F	10 MG/KG PFBS	SCHEDULED EUTHANASIA	7	07-APR-05	1

A = CALCULATED TO THE NEAREST WHOLE WEEK USING THE MEAN AGE IN WEEKS AT INITIATION OF DOSING (7)

TABLE 3 (UNSCHEDULED OBSERVATIONS - PHARMACOKINETIC PHASE)

PROJECT NO.:WIL-534004U — PHARMACOKINETIC AND EXCRETION STUDY IN RATS —

SPONSOR:AGC CHEMICAL — INDIVIDUAL CLINICAL OBSERVATIONS

TABLE RANGE: 04-06-05 TO 04-07-05

ANIMAL	SEX	GROUP	CATEGORY	DATE	TIME	GRADE	OBSERVATIONS
78132	M	10 MG/KG PFBS	EXCRETA	04-06-05	10:22	P	URINE CONTAINING RED MATERIAL
78141	M	10 MG/KG PFBS	EXCRETA	04-06-05	9:35	P	URINE CONTAINING RED MATERIAL
78151	F	10 MG/KG PFHxA	EXCRETA	04-06-05	10:10	P	URINE CONTAINING RED MATERIAL
78159	F	10 MG/KG PFHxA	EXCRETA	04-06-05	10:16	P	URINE CONTAINING RED MATERIAL
78163	F	10 MG/KG PFBS	EXCRETA	04-06-05	10:26	P	URINE CONTAINING RED MATERIAL

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

TABLE 4 (UNSCHEDULED OBSERVATIONS - EXCRETION PHASE)

PROJECT NO.:WIL-534004V — PHARMACOKINETIC AND EXCRETION STUDY IN RATS — PAGE 1

SPONSOR:AGC CHEMICAL — INDIVIDUAL CLINICAL OBSERVATIONS

TABLE RANGE: 04-06-05 TO 04-07-05

ANIMAL	SEX	GROUP	CATEGORY	DATE	TIME	GRADE	OBSERVATIONS
78124	M	10 MG/KG PFHxA	EXCRETA	04-06-05	9:25	P	URINE CONTAINING RED MATERIAL
78125	M	10 MG/KG PFHxA	EXCRETA	04-06-05	9:24	P	URINE CONTAINING RED MATERIAL
78153	F	10 MG/KG PFHxA	EXCRETA	04-06-05	9:07	P	URINE CONTAINING RED MATERIAL
78164	F	10 MG/KG PFHxA	EXCRETA	04-06-05	9:25	P	URINE CONTAINING RED MATERIAL
78172	F	10 MG/KG PFHxA	EXCRETA	04-06-05	9:24	P	URINE CONTAINING RED MATERIAL
78169	F	10 MG/KG PFBS	EXCRETA	04-06-05	9:25	P	URINE CONTAINING RED MATERIAL
78170	F	10 MG/KG PFBS	EXCRETA	04-06-05	9:25	P	URINE CONTAINING RED MATERIAL

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

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TABLE 5 (PHARMACOKINETIC PHASE)

PROJECT NO.:WIL-534004A — PHARMACOKINETIC AND EXCRETION STUDY IN RATS — PAGE 1

SPONSOR:AGC CHEMICAL — INDIVIDUAL BODY WEIGHTS [G]

MALE GROUP: 10 MG/KG PFHxA

DAY	-13	-8	-2	0
ANIMAL				
78116	111.	157.	219.	236.
78118	100.	149.	212.	228.
78119	114.	166.	222.	239.
78128	109.	153.	206.	224.
78130	108.	158.	217.	232.
78131	97.	150.	204.	223.
78137	115.	169.	231.	248.
78138	114.	171.	232.	253.
78144	112.	163.	227.	244.
MEAN	109.	160.	219.	236.
S.D.	6.4	8.1	10.2	10.6
N	9	9	9	9

MALE GROUP: 10 MG/KG PFBS

DAY	-13	-8	-2	0
ANIMAL				
78121	117.	169.	234.	254.
78126	113.	167.	228.	245.
78129	108.	153.	205.	222.
78132	116.	163.	221.	236.
78134	108.	157.	215.	229.
78136	110.	165.	223.	236.
78141	107.	156.	211.	229.
78143	116.	165.	218.	232.
78145	106.	156.	214.	226.
MEAN	111.	161.	219.	234.
S.D.	4.3	5.8	8.9	9.9
N	9	9	9	9

FEMALE GROUP: 10 MG/KG PFHxA

DAY	-13	-8	-2	0
ANIMAL				
78148	109.	146.	181.	193.
78156	92.	123.	159.	167.
78150	100.	141.	181.	191.
78151	100.	134.	165.	174.
78152	96.	134.	160.	174.
78159	104.	138.	175.	178.
78166	106.	151.	189.	199.
78167	107.	141.	168.	176.
78173	105.	136.	162.	166.
MEAN	102.	138.	171.	180.
S.D.	5.6	8.0	10.8	11.8
N	9	9	9	9

FEMALE GROUP: 10 MG/KG PFBS

DAY	-13	-8	-2	0
ANIMAL				
78146	96.	136.	167.	176.
78155	113.	151.	189.	193.
78157	102.	136.	160.	174.
78158	113.	153.	177.	207.
78161	97.	133.	168.	169.
78163	110.	149.	187.	200.
78165	102.	140.	169.	182.
78174	97.	132.	164.	168.
78175	109.	144.	175.	178.
MEAN	104.	142.	173.	183.
S.D.	7.0	8.0	10.0	13.9
N	9	9	9	9

TABLE 6 (EXCRETION PHASE)

PROJECT NO.:WIL-534004B | PHARMACOKINETIC AND EXCRETION STUDY IN RATS | PAGE 1

SPONSOR:AGC CHEMICAL | INDIVIDUAL BODY WEIGHTS [G]

MALE GROUP: 10 MG/KG PFHxA

DAY	-13	-8	-2	0
ANIMAL				
78123	109.	159.	214.	228.
78124	116.	166.	224.	242.
78125	112.	164.	226.	242.
MEAN	112.	163.	221.	237.
S.D.	3.5	3.6	6.4	8.1
N	3	3	3	3

TABLE 6 (EXCRETION PHASE)

PROJECT NO.:WIL-534004B | PHARMACOKINETIC AND EXCRETION STUDY IN RATS | PAGE 2

SPONSOR:AGC CHEMICAL | INDIVIDUAL BODY WEIGHTS [G]

MALE GROUP: 10 MG/KG PFBS

DAY	-13	-8	-2	0
ANIMAL				
78133	115.	168.	228.	242.
78135	111.	165.	224.	245.
78139	114.	160.	220.	238.
MEAN	113.	164.	224.	242.
S.D.	2.1	4.0	4.0	3.5
N	3	3	3	3

TABLE 6 (EXCRETION PHASE)

PROJECT NO.:WIL-534004B | PHARMACOKINETIC AND EXCRETION STUDY IN RATS | PAGE 3

SPONSOR:AGC CHEMICAL | INDIVIDUAL BODY WEIGHTS [G]

FEMALE GROUP: 10 MG/KG PFHxA

DAY	-13	-8	-2	0
ANIMAL				
78153	115.	159.	197.	209.
78164	116.	145.	175.	171.
78172	98.	135.	167.	175.
MEAN	110.	146.	180.	185.
S.D.	10.1	12.1	15.5	20.9
N	3	3	3	3

FEMALE GROUP: 10 MG/KG PFBS

DAY	-13	-8	-2	0
ANIMAL				
78160	121.	163.	187.	199.
78169	111.	153.	187.	185.
78170	99.	137.	173.	180.
MEAN	110.	151.	182.	188.
S.D.	11.0	13.1	8.1	9.8
N	3	3	3	3

APPENDIX A

Certificates 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

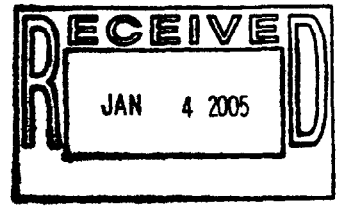
SIGMA-ALDRICH

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)

PHARMACOKINETIC (IN BLOOD) AND EXCRETION STUDY OF PERFLUOROHEXANOIC ACID AND NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

Analyses Of PFHxA Dosing Formulations

Analytical Chemistry Department

WIL Research Laboratories, LLC

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1. INTRODUCTION

This report provides a detailed description of an assay for the determination of perfluorohexanoic acid (PFHxA) in aqueous formulations using high performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS) in negative electrospray ionization (ESI-) mode. Assay specificity/selectivity, calibration reproducibility, accuracy, precision and ruggedness were assessed and validated in a previous study (Kirkpatrick, **Draft**). In the present study, PFHxA stability was assessed in formulations that were stored refrigerated for 2 days. The formulation used for dose administration was analyzed and the resulting concentration was within acceptance criteria (%RE within ± 10%).

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 5 µ 300A C18 cartridges or equivalent
Column Temperature:	35°C
Mobile Phase:	15% A: Deionized Water 85% B: Acetonitrile
Flow Rate:	1.0 mL/minute split to 0.3 mL/minute
Detector:	Mass spectrometer with conditions as described in Section 2.2.
Injection Volume:	5 µL
Retention Time:	Approximately 0.2 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:	3.00 kV
Cone:	45 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 313/269 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 deionized (DI) water. The solution was thoroughly mixed and vacuum degassed. The preparation was scaled as necessary.

2.4. PREPARATION OF THE STANDARD 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 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 QUALITY CONTROL STOCK SOLUTION

The quality control (QC) stock solution was prepared at 5000 µg PFHxA/mL by accurately weighing approximately 0.5000 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 was prepared fresh as needed.

2.6. PREPARATION OF CALIBRATION STANDARDS

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 a 50-mL polypropylene tube containing 1.0 mL of sterile water to yield a single QC sample at a concentration of 2.0 mg/mL. An appropriate volume of ACN was added to achieve a final volume of 40 mL. The QC sample was thoroughly mixed and a secondary dilution was performed with diluent in amber autosampler vials.

Level	Initial Concentration (mg/mL)	DI Water Volume (mL)	Stock Volume (mL)	ACN Volume (mL)	Total Volume (mL)	Diluted Concentration (µg/mL)
Blank	0.0	1.0	0.0	39.0	40	0.0
QC	2.0	1.0	0.4	38.6	40	50.0

Secondary Dilutions:

Level	Initial Concentration (mg/mL)	Primary Dilution Volume (mL)	Diluent Volume (mL)	Total Volume (mL)	Final Concentration (µg/mL)
Blank	0.0	0.300	0.700	1.0	0.0
QC	2.0	0.300	0.700	1.0	15.0

2.8. SAMPLE PROCESSING

Samples (1.0 mL) of the formulation 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 amber autosampler vials.

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 Dilution Volume (mL)	Diluent Volume (mL)	Total Volume (mL)	Final Concentration (ng/mL)
1	2.0	0.300	0.700	1.0	15.0

2.9. CALIBRATION AND QUANTITATION

Single injections were made of each standard and processed QC and formulation sample. A calibration curve was constructed for each set of analyses. 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

The validity of the assay procedure was established in a previous study (Kirkpatrick, **Draft**) through a careful study of the assay specificity/selectivity, calibration reproducibility, precision and accuracy, ruggedness and stability of PFHxA in processed samples. Under the described chromatographic conditions, the retention time of PFHxA was approximately 0.2 minutes. Figures 1, 2, 3 and 4 are typical chromatograms of a calibration sample, a processed QC sample, a processed formulation sample and a processed vehicle sample, respectively. The total analysis time required for each run was approximately 1.5 minutes. Formulations were prepared and evaluated for concentration verification. Two-day refrigerated stability was also assessed.

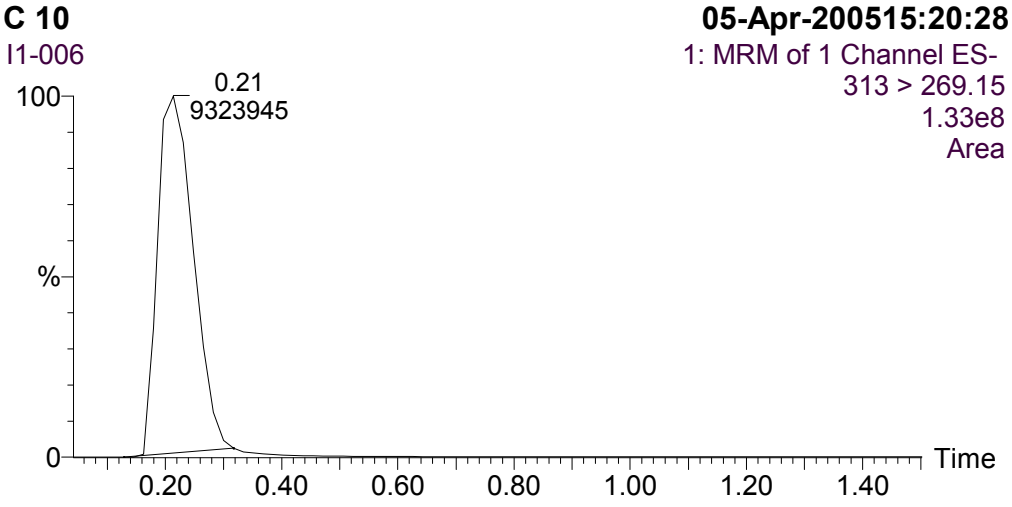

Figure 1: Representative Chromatogram Of A 10 µg/mL Calibration Sample

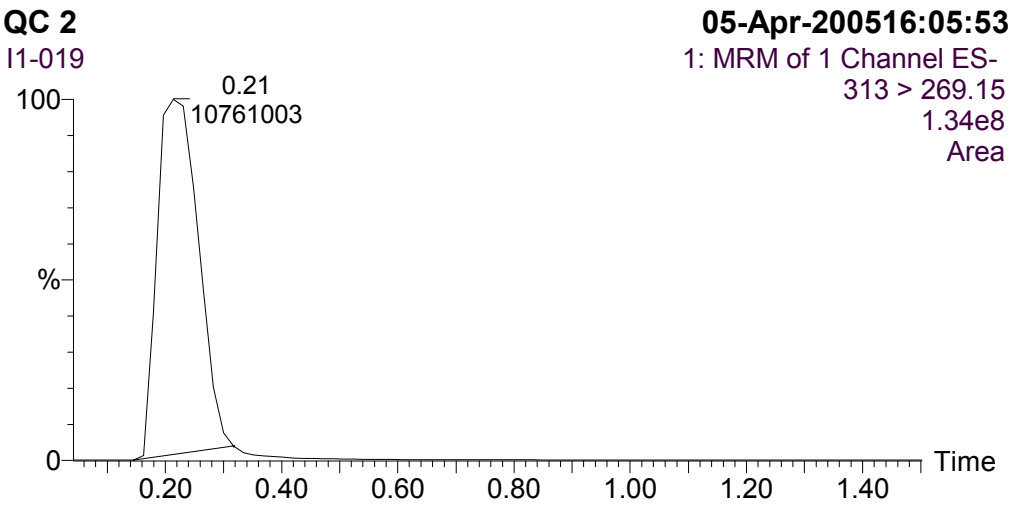

Figure 2: Representative Chromatogram Of A Processed 2.0 mg/mL QC Sample

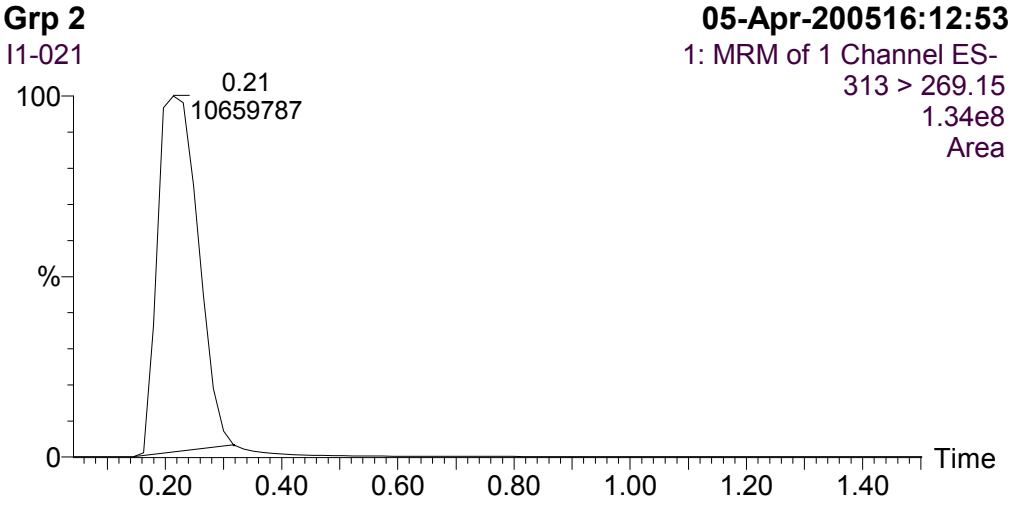

Figure 3: Representative Chromatogram Of A Processed 2 mg/mL Formulation Sample

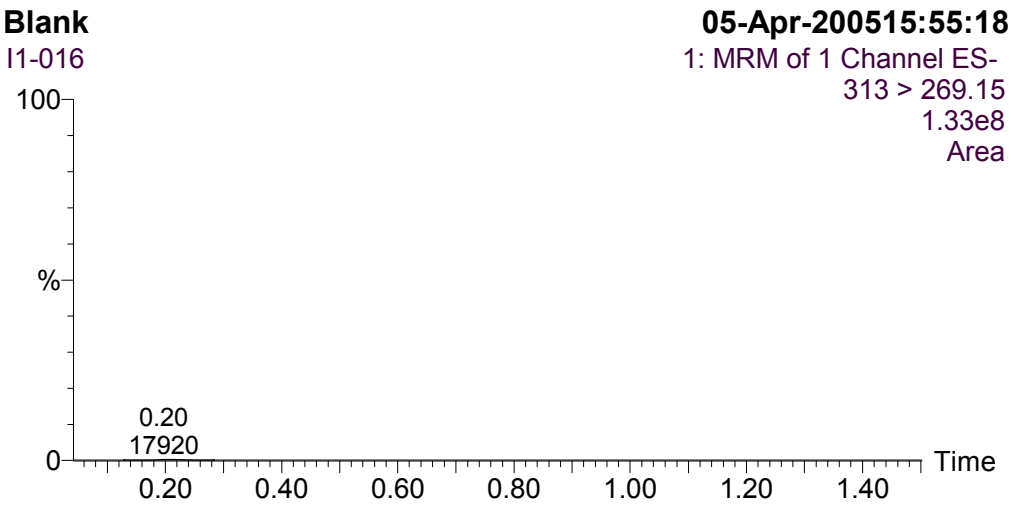

Figure 4: Representative Chromatogram Of A Processed Vehicle Sample

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 the vehicle revealed no significant peaks at or near the retention time for PFHxA (0.3 minutes).

3.2. CONCENTRATION ANALYSIS OF DOSING FORMULATIONS

The results of the concentration analysis of the dosing formulation are presented in Table 1, and the mean concentration (and % of target) value are summarized in the following table.

Formulation Date	Group 1 2 mg/mL (% of Target)
04 Apr 2005	2.07 (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 concentration was within 10% of the target dose concentration.

4. STABILITY

The dosing formulation prepared 04 Apr 2005 and analyzed on 04-05 Apr 2005, was refrigerated for 2 days and reanalyzed to assess test article stability in sterile water. The 2-day stability results for the formulation are presented in Table 2. The 2-day post-storage mean concentration was 98.4% of the time-zero value.

The test article was stable in the formulation following 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.

5. CONCLUSION

Assay specificity/selectivity, calibration reproducibility, precision, accuracy and ruggedness were assessed and validated in a previous study (Kirkpatrick, **Draft**). The formulation used for dosing was analyzed to confirm test article concentration and the results met all acceptance criteria (within 10% of the target concentration). Stability of the test article in dosing formulations stored refrigerated for 2 days was assessed and met acceptance criteria.

6. REFERENCES

Kirkpatrick, J.B. A Pharmacokinetic (In Blood) And Excretion Study In Cynomolgus Monkeys (Study No. WIL-534002). WIL Research Laboratories, LLC, Ashland, OH, **Draft.**

7. KEY STUDY PERSONNEL AND REPORT SUBMISSION

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TABLES 1 - 2

PHARMACOKINETIC (IN BLOOD) AND EXCRETION STUDY OF PERFLUOROHEXANOIC ACID AND NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

Table 1: Concentration Analysis of the 04Apr2005 PFHxA Formulations

(Analyzed 04-05Apr2005)

Group	Dose Conc (mg/mL)	Ref # (534004 -)	Run #	Analyzed Conc (mg/mL)	Percent of Target (%)	Mean Conc (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)
1	2	1 - 3	I1-0021	2.05	102	2.07	0.032	1.5	103
		1 - 4	I1-0022	2.09	105				

PHARMACOKINETIC (IN BLOOD) AND EXCRETION STUDY OF PERFLUOROHEXANOIC ACID AND NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

Table 2: 2-Day Refrigerated Stability Analysis of the 04Apr2005 PFHxA Formulations

(Analyzed 07Apr2005)

Group	Dose Conc (mg/mL)	Ref # (534004 -)	Run #	Analyzed Conc (mg/mL)	Percent of Target (%)	Mean Conc (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)	Percent of Time Zero (%)
1	2	10 - 3	I1-0061	2.09	105	2.04	0.076	3.7	102	98.4
		10 - 4	I1-0062	1.98	99.2					

	Group	(mg/mL)
Time Zero Concentration:	1	2.07

534004 PFHxA results.xls 2S2D
Printed: 06/13/05 7:57 AM

ATTACHMENT I

Supporting Data

Table A1: Calibration, Quality Control, and Formulations Samples from Sequence 534004a (PFHxA)

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534004.PRO\SampleDB\534004a
Last modified: Thu Apr 07 14:46:57 2005
Method: C:\MASSLYNX\534001.PRO\ACQUDB\534004 PFHXA
Last modified: Thu Apr 07 14:43:00 2005
Job Code:

Printed: Thu Apr 07 15:08:49 2005

Compound 1: PFHxA

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Analyzed Conc. (µg/mL)	% RE	Final Conc. (mg/mL)
I1-001		system suit	0.21	8268212	0	4.30		
I1-002		system suit	0.21	8694008	0	6.27		
I1-003		system suit	0.21	8805006	0	6.78		
I1-004		system suit	0.21	9259745	0	8.88		
I1-005		50:50 ACN\H2O	0.2	10657	0	0		
I1-006	4-2	C 10	0.21	9323945	0	9.18	-8.2	
I1-007	4-3	C 10	0.21	9065239	0	7.98	-20	
I1-008	4-4	C 10	0.21	9925855	0	12.0	20	
I1-009	4-5	C 17.5	0.21	11228247	0	18.0	2.7	
I1-010	4-6	C 17.5	0.21	11336447	0	18.5	5.5	
I1-011	4-7	C 17.5	0.21	11199726	0	17.8	1.9	
I1-012	4-8	C 25	0.21	12670822	0	24.6	-1.5	
I1-013	4-9	C 25	0.23	12668310	0	24.6	-1.5	
I1-014	4-10	C 25	0.21	12719402	0	24.9	-0.572	
I1-015		50:50 ACN\H2O	0.2	16852	0	0		
I1-016	5-6	Blank	0.2	17920	0	0		
I1-017	5-7	QC 2	0.21	10938690	133.3	2218	11	
I1-018	5-8	QC 2	0.21	10987923	133.3	2248	12	
I1-019	5-9	QC 2	0.21	10761003	133.3	2108	5.4	
I1-020		50:50 ACN\H2O	0.2	16268	0	0		
I1-021	1-3	Grp 1	0.21	10659787	133.3	2046	2.3	2.05
I1-022	1-4	Grp 1	0.21	10732950	133.3	2091	4.5	2.09
I1-023		50:50 ACN\H2O	0.2	15477	0	0		

Table A2: Calibration, Quality Control, and Formulations Samples from Sequence 534004c (PFHxA)

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534004.PRO\SampleDB\534004c
Last modified: Thu Apr 07 15:03:04 2005
Method: C:\MASSLYNX\534001.PRO\ACQUDB\534004 PFHXA
Last modified: Thu Apr 07 14:43:00 2005
Job Code:

Printed: Thu Apr 07 15:14:34 2005

Compound 1: PFHxA

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Analyzed Conc. (µg/mL)	% RE	Final Conc. (mg/mL)
I1-041		system suit	0.21	6335412	0	5.473		
I1-042		system suit	0.21	6958914	0	8.128		
I1-043		system suit	0.21	7302328	0	9.591		
I1-044		system suit	0.21	7342954	0	9.764		
I1-045		50:50 ACN\H2O	0.21	1627	0	0		
I1-046	12-2	C 10	0.21	7271952	0	9.46	-5.4	
I1-047	12-3	C 10	0.21	7195499	0	9.14	-8.6	
I1-048	12-4	C 10	0.21	7428600	0	10.1	1.3	
I1-049	12-5	C 17.5	0.21	9212966	0	17.7	1.3	
I1-050	12-6	C 17.5	0.21	9449611	0	18.7	7.1	
I1-051	12-7	C 17.5	0.21	9414555	0	18.6	6.2	
I1-052	12-8	C 25	0.21	10941516	0	25.1	0.35	
I1-053	12-9	C 25	0.21	10757472	0	24.3	-2.8	
I1-054	12-10	C 25	0.21	10764324	0	24.3	-2.7	
I1-055		50:50 ACN\H2O	0.21	1846	0	0		
I1-056	13-6	Blank	0.21	2973	0	0		
I1-057	13-7	QC 2	0.21	7586890	133.33	1440	-28	
I1-058	13-8	QC 2	0.21	6986155	133.33	1099	-45	
I1-059	13-9	QC 2	0.21	7592209	133.33	1443	-28	
I1-060		50:50 ACN\H2O	0.21	3109	0	0		
I1-061	10-3	Grp 1	0.21	8731909	133.33	2090	4.5	2.09
I1-062	10-4	Grp 1	0.21	8542991	133.33	1983	-0.84	1.98
I1-063		50:50 ACN\H2O	0.21	5663	0	0		
I1-064	13a-1	QC 2	0.21	8321058	133.33	1857	-7.1	
I1-065	13a-2	QC 2	0.21	8574002	133.33	2001	0.039	
I1-066	13a-3	QC 2	0.21	8663377	133.33	2052	2.6	

APPENDIX C

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

PHARMACOKINETIC (IN BLOOD) AND EXCRETION STUDY OF PERFLUOROHEXANOIC ACID AND NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

Analyses Of PFBS Dosing Formulations

Analytical Chemistry Department

WIL Research Laboratories, LLC

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1. INTRODUCTION

This report provides a detailed description of an assay for the determination of nonafluoro-1-butanesulfonic acid (PFBS) in aqueous formulations using high performance liquid chromatography mass spectrometry (HPLC/MS) in negative electrospray ionization (ESI-) mode. Assay specificity/selectivity, calibration reproducibility, accuracy, precision and ruggedness were assessed and validated in a previous study (Kirkpatrick, **Draft**). The formulation used for dose administration was analyzed and the resulting concentration was within acceptance criteria (%RE within ± 10%).

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 5 µ 300A C18 cartridges or equivalent
Column Temperature:	35°C
Mobile Phase:	15% A: Deionized water 85% 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 0.2 minutes for nonafluorobutane-1-sulfonic acid (PFBS)
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:	3.0 kV
Cone:	45 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:	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 deionized (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 quality control (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 Dilution 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 diluted 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 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)
2	2.0	1.0	39.0	40.0	50.0

Secondary Dilutions:

Group	Dose Concentration (mg/mL)	Primary Dilution Volume (mL)	Diluent Volume (mL)	Total Volume (mL)	Final Concentration (ng/mL)
2	2.0	0.100	9.90	10.0	500

2.9. CALIBRATION AND QUANTITATION

Single injections were made of each calibration and processed 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 and processed the retention time for PFBS was approximately 0.2 minutes. Figures 1, 2 and 3 are typical chromatograms of a calibration sample, a processed QC sample and a processed formulation sample, respectively. The total analysis time required for each run was approximately 1.5 minutes. Formulations were prepared and evaluated for concentration verification.

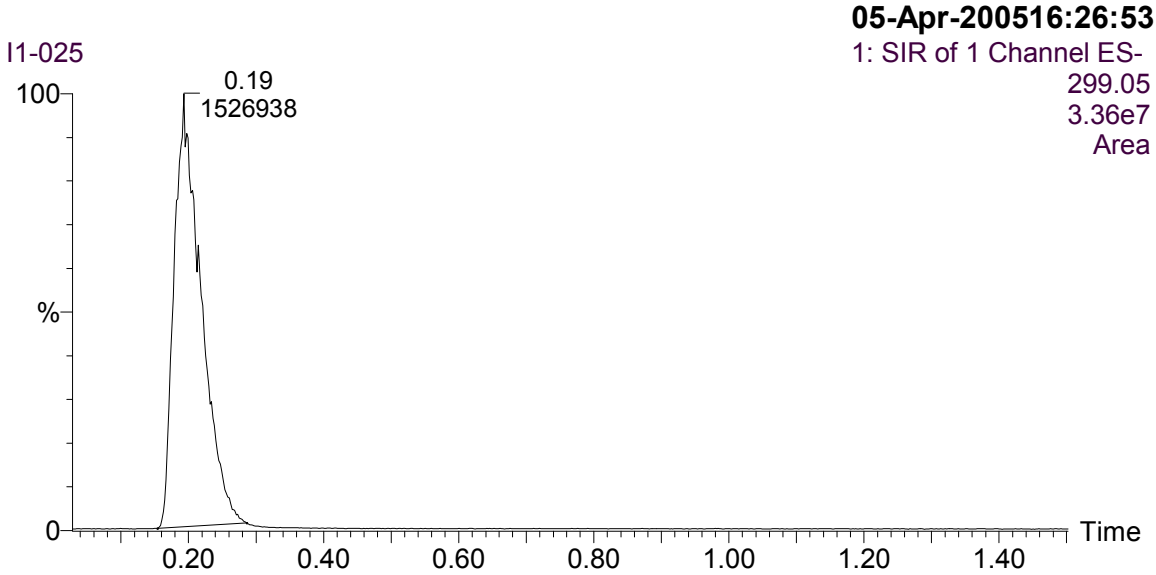

Figure 1: Representative Chromatogram Of A 200 µg PFBS/mL Calibration Sample

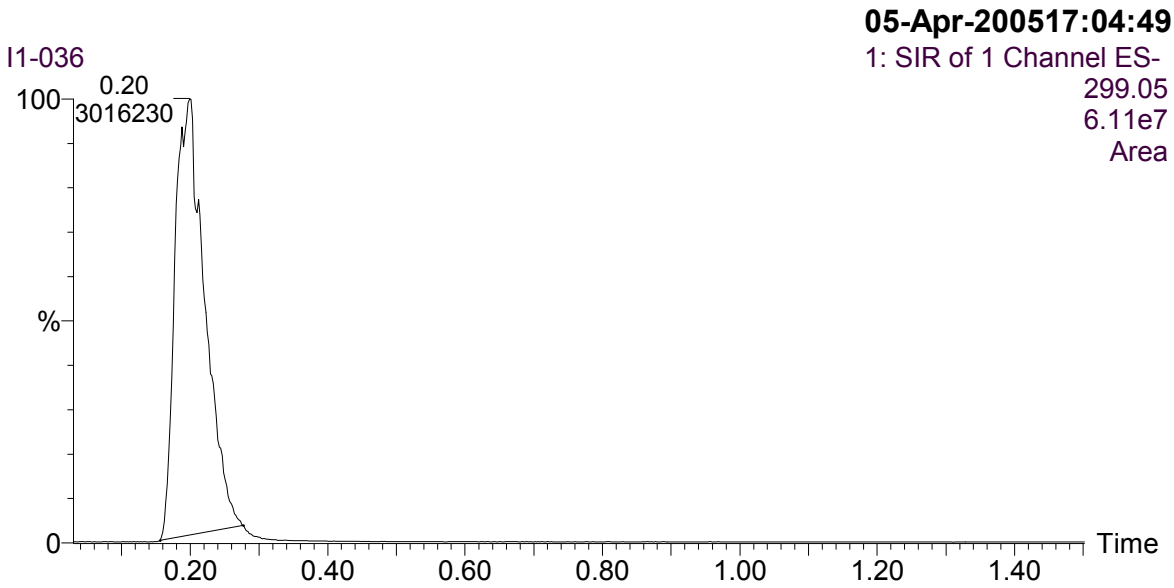

Figure 2: Representative Chromatogram Of A Processed 1.0 mg PFBS/mL QC Sample

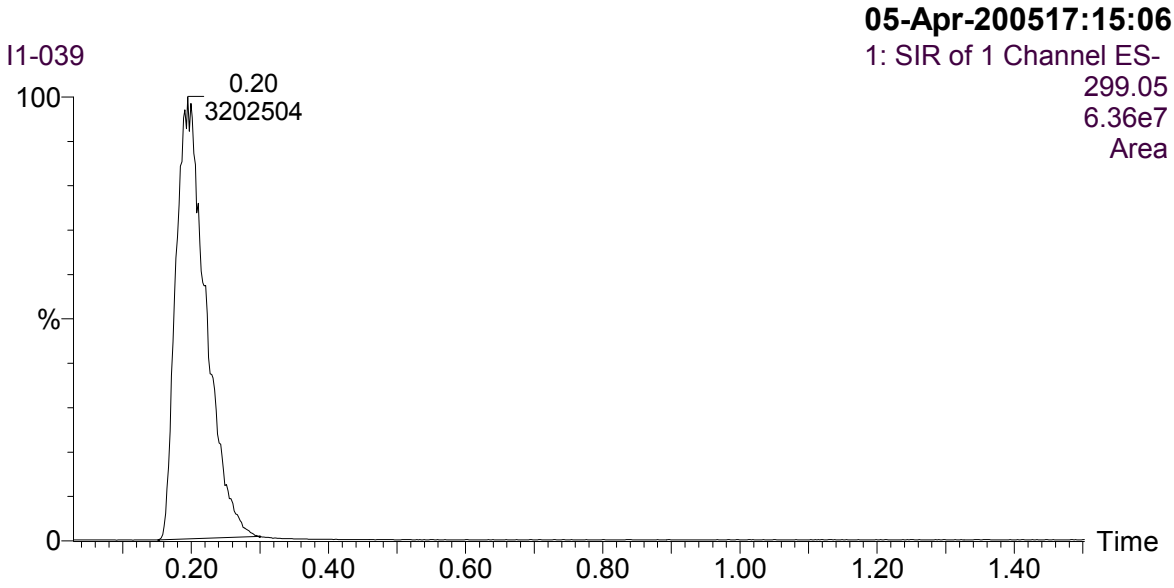

Figure 3: Representative Chromatogram Of A Processed 2 mg PFBS/mL Formulation Sample

3.1. SPECIFICITY/SELECTIVITY

Assay specificity/selectivity was demonstrated in a separate study (Kirkpatrick, **Draft**).

3.2. CONCENTRATION ANALYSIS OF DOSING FORMULATIONS

The results of the concentration analysis of the dosing formulation are presented in Table 1, and the mean concentration (and % of target) values are summarized in the following table.

Formulation Date	Group 2 2 mg/mL (% of Target)
04 Apr 2005	2.14 (107)

The analyzed formulation used for dose administration was 107% of target and, therefore, met the WIL SOP requirement for concentration acceptability, that is, the analyzed concentration was within 10% of the target dose concentration.

4. CONCLUSION

Assay specificity/selectivity, calibration reproducibility, precision, accuracy and ruggedness were assessed and validated in a previous study (Kirkpatrick, **Draft**). The formulation used for dosing was analyzed to confirm test article concentration and the results met all acceptance criteria (within 10% of the target concentration).

5. REFERENCES

Kirkpatrick, J.B. A Pharmacokinetic (In Blood) And Excretion Study In Cynomolgus Monkeys (Study No. WIL-534002). WIL Research Laboratories, LLC, Ashland, OH, **Draft.**

6. KEY STUDY PERSONNEL AND REPORT SUBMISSION

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TABLE 1

PHARMACOKINETIC (IN BLOOD) AND EXCRETION STUDY OF PERFLUOROHEXANOIC ACID AND NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

Table 1: Concentration Analysis Of The 04Apr2005 PFBS Formulations

(Analyzed 04-05Apr2005)

Group	Dose Conc (mg/mL)	Ref # (534004 -)	Run #	Analyzed Conc (mg/mL)	Percent of Target (%)	Mean Conc (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)
2	2	2 - 3	I1-0038	2.06	103	2.14	0.11	5.3	107
		2 - 4	I1-0039	2.22	111				

ATTACHMENT I

Supporting Data

Table A1: Calibration, Quality Control, and Formulations Samples from Sequence 534004b (PFBS)

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534004.PRO\SampleDB\534004b
Last modified: Tue Apr 05 13:22:10 2005
Method: C:\MASSLYNX\534001.PRO\ACQUDB\534004 PFBS
Last modified: Thu Apr 07 14:48:48 2005
Job Code:

Printed: Thu Apr 07 15:07:49 2005

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Analyzed Conc. (µg/mL)	% RE	Final Conc. (mg/mL)
I1-024	6-2	C200	0.2	1557885	0	192	-4.0	
I1-025	6-3	C200	0.19	1526938	0	185	-7.4	
I1-026	6-4	C200	0.2	1486496	0	176	-12	
I1-027	6-5	C400	0.19	2517481	0	404	0.95	
I1-028	6-6	C400	0.19	2560712	0	413	3.3	
I1-029	6-7	C400	0.19	2738694	0	453	13	
I1-030	6-8	C800	0.19	4154146	0	765	-4.4	
I1-031	6-9	C800	0.2	4363145	0	811	1.4	
I1-032	6-10	C800	0.19	4316585	0	801	0.097	
I1-033		50:50 acn/water	0.2	13716	0	0		
I1-034	7-7	QC 1	0.19	10843	2.02	0	-100	
I1-035	7-8	QC 1	0.2	3141653	2.02	1094	9.4	
I1-036	7-9	QC 1	0.2	3016230	2.02	1038	3.8	
I1-037		50:50 acn/water	0.19	14305	0	0		
I1-038	2-3	Grp 2	0.2	3020392	4	2059	3.0	2.06
I1-039	2-4	Grp 2	0.19	3202504	4	2220	11	2.22
I1-040		50:50 acn/water	0.2	2107824	0	313		

APPENDIX D

Pretest Clinical Observations

	----- M A L E -----
TABLE RANGE:	03-24-05 TO 04-05-05
GROUP:	1
NORMAL	
-NO SIGNIFICANT CLINICAL OBSERVATIONS	90/30

1- PRETEST

----- F E M A L E -----

TABLE RANGE: 03-24-05 TO 04-05-05
GROUP: 1

NORMAL
-NO SIGNIFICANT CLINICAL OBSERVATIONS 88/30

SPECIAL II
-SOFT PROTRUSION UMBILICAL AREA 2/ 1

1- PRETEST

APPENDIX E

Animal Room Environmental Conditions

PROJECT NO.:WIL- 534004 TEMPERATURE/HUMIDITY - DAILY SUMMARY REPORT BY STUDY
SPONSOR: AGC CHEMICAL

STUDY SPECIFICATIONS: 534004 DATE IN: 03/22/05 TIME IN: 7:00
DATE OUT: 04/07/05 TIME OUT: 16:00

ROOM SPECIFICATIONS: B ROOM 39 LOW TEMPERATURE °F: 66.0 HIGH TEMPERATURE °F: 76.0 LOW HUMIDITY: 30.0
SPECIES: RAT LOW TEMPERATURE °C: 18.9 HIGH TEMPERATURE °C: 24.4 HIGH HUMIDITY: 70.0

	TEMPERATURE		HUMIDITY
DATE	MEAN (°F)	MEAN (°C)	MEAN (%RH)
22-Mar-05	70.2	21.2	41.1
23-Mar-05	70.6	21.5	43.2
24-Mar-05	70.8	21.5	42.0
25-Mar-05	70.8	21.6	42.0
26-Mar-05	70.6	21.5	42.0
27-Mar-05	70.6	21.5	42.8
28-Mar-05	70.7	21.5	42.1
29-Mar-05	70.7	21.5	41.1
30-Mar-05	70.5	21.4	42.1
31-Mar-05	70.6	21.5	42.3
01-Apr-05	70.6	21.5	41.6
02-Apr-05	70.9	21.6	43.2
03-Apr-05	70.6	21.4	41.8
04-Apr-05	70.5	21.4	40.8
05-Apr-05	70.4	21.3	42.2
06-Apr-05	71.0	21.7	43.3
07-Apr-05	70.6	21.4	48.6

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
9/15/05 11:21

STUDY SPECIFICATIONS: 534004 DATE IN: 03/22/05 TIME IN: 7:00
DATE OUT: 04/07/05 TIME OUT: 16:00

ROOM SPECIFICATIONS: B ROOM 39 LOW TEMPERATURE °F: 66.0 HIGH TEMPERATURE °F: 76.0 LOW HUMIDITY: 30.0
SPECIES: RAT LOW TEMPERATURE °C: 18.9 HIGH TEMPERATURE °C: 24.4 HIGH HUMIDITY: 70.0

	TEMPERATURE		HUMIDITY
DATE	MEAN (°F)	MEAN (°C)	MEAN (%RH)

GRAND STATS	MEAN	MIN	MAX
TEMPERATURE °F	70.6	70.2	71.0
TEMPERATURE °C	21.5	21.2	21.7
HUMIDITY (%RH)	42.5	40.8	48.6
N DAYS	17		

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 39		
SPECIES:	RAT		
LOW TEMPERATURE:	66.0	DATE IN:	03/22/05
HIGH TEMPERATURE:	76.0	TIME IN:	7:00
LOW HUMIDITY:	30.0	DATE OUT:	04/07/05
HIGH HUMIDITY:	70.0	TIME OUT:	16:00

ROOM B ROOM 39 SUMMARY

	TEMPERATURE	HUMIDITY
MEAN	70.7	42.4
MIN	69.3	36.0
MAX	72.3	70.0
SD	0.85	3.34
N SAMPLES	392	392
FIRST DAY	03/22/05	
LAST DAY	04/07/05	
N DAYS	17	

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

STUDY 534004 SUMMARY

MEAN	70.7	42.4
MIN	69.3	36.0
MAX	72.3	70.0
SD	0.85	3.34
N SAMPLES	392	392
FIRST DAY	03/22/05	
LAST DAY	04/07/05	
N DAYS	17	

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]

PHARMACOKINETIC (IN BLOOD) AND EXCRETION STUDY OF PERFLUOROHEXANOIC ACID AND NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

Analysis Of PFHxA In Serum And Urine

Analytical Chemistry Department

WIL Research Laboratories, LLC

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1. INTRODUCTION

This report provides a detailed description 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 rat serum and urine. Method sensitivity, specificity/selectivity, calibration reproducibility, accuracy and precision were assessed and validated in a separate study (Kirkpatrick, 2005).

This report details the analytical results from the determination of PFHxA in rat serum and urine samples. Analysis of serum samples resulted in levels ranging from less than the lower limit of quantitation (LLOQ, 30 ng/mL) to 40,117 ng PFHxA/mL. Analysis of urine samples resulted in levels ranging from 2482 to 294,713 ng PFHxA/mL.

2. BLANK MATRIX IDENTIFICATION

Blank rat 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
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 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.

3.5. PREPARATION OF CALIBRATION SAMPLES

An aliquot of the primary stock solution was diluted with ACN to yield a secondary stock solution at 25 µg 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 Section 3.7. (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 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 were prepared at concentrations of 30, 300 and 5000 ng PFHxA/mL 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 100,000 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 Section 3.7. (Sample Processing).

3.7. SAMPLE PROCESSING

Aliquots (0.1 mL) of the experimental samples were transferred to 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.

3.8. 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

Method sensitivity, specificity/selectivity, calibration reproducibility, accuracy and precision were assessed and validated in a separate study (Kirkpatrick, 2005). 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 28 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 28).

Figures 29 through 46 illustrate typical chromatograms for a processed solvent blank (Figure 29), a processed urine blank (Figure 30), processed urine calibration samples (Figures 31 through 36), processed urine QC samples (Figures 37 through 40) and experimental urine samples (Figures 41 through 46).

4.2. ANALYSIS OF EXPERIMENTAL SAMPLES

Rat serum and urine samples were analyzed for PFHxA and the results are summarized in Tables 1 and 2, respectively. In addition to the experimental (unknown) samples, each set of analyses consisted of at least duplicate calibration samples, 1 solvent blank, 1 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 rat serum and urine was validated in a separate study (Kirkpatrick, 2005). Analysis of serum samples resulted in levels ranging from less than the lower limit of quantitation (LLOQ, 30 ng/mL) to 40,117 ng PFHxA/mL. Analysis of urine samples resulted in levels ranging from 2482 to 294,713 ng PFHxA/mL.

6. REFERENCES

Kirkpatrick, J.B. A Combined 28-Day Repeated Dose Oral Toxicity Study With The Reproduction/Developmental Toxicity Screening Test Of Perfluorohexanoic Acid And 1H, 1H, 2H, 2H-Tridecafluoro-1-Octanol In Rats, With Recovery (Study No. WIL-534001). WIL Research Laboratories, LLC, Ashland, OH, **2005.**

7. KEY STUDY PERSONNEL AND REPORT SUBMISSION

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Staff Toxicologist
Study Director

17 Oct 2005
Date

Report Prepared By:

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17 October 2005
Date

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Date

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TABLES 1-2

PHARMACOKINETIC (IN BLOOD) AND EXCRETION
STUDY OF PERFLUOROHEXANOIC ACID AND
NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

Table 1: Day 0 Rat Serum Experimental Sample PFHxA Concentrations

Run #	Ref #	Animal #	Sex	Timepoint (hrs)	PFHxA ng/mL
I1-0736	63-1	78116	M	0	< LLOQ
I1-0737	63-2	78118	M	0	< LLOQ
I1-0738	63-3	78119	M	0	< LLOQ
I1-0739	63-4	78148	F	0	< LLOQ
I1-0740	63-5	78149	F	0	< LLOQ
I1-0741	63-6	78150	F	0	< LLOQ
I1-0706	62-1	78128	M	0.5	39355
I1-0707	62-2	78130	M	0.5	40117
I1-0708	62-3	78131	M	0.5	33082
I1-0709	62-4	78151	F	0.5	9109
I1-0710	62-5	78152	F	0.5	11495
I1-0711	62-6	78159	F	0.5	17820
I1-0712	62-7	78137	M	1	30337
I1-0713	62-8	78138	M	1	27638
I1-0714	62-9	78144	M	1	30143
I1-0715	62-10	78166	F	1	2842
I1-0797	68-1	78167	F	1	1298
I1-0798	68-2	78173	F	1	1904
I1-0718	62-13	78116	M	1.5	19045
I1-0719	62-14	78118	M	1.5	17737
I1-0720	62-15	78119	M	1.5	15118
I1-0799	68-3	78148	F	1.5	746
I1-0801	68-5	78150	F	1.5	1161
I1-0800	68-4	78156	F	1.5	1073
I1-0724	62-19	78128	M	2	11117
I1-0725	62-20	78130	M	2	18042
I1-0726	62-21	78131	M	2	8783
I1-0808	68-6	78151	F	2	272
I1-0809	68-7	78152	F	2	506
I1-0810	68-8	78159	F	2	1124

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

PHARMACOKINETIC (IN BLOOD) AND EXCRETION
STUDY OF PERFLUOROHEXANOIC ACID AND
NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

Table 1: Day 0 Rat Serum Experimental Sample PFHxA Concentrations

Run #	Ref #	Animal #	Sex	Timepoint (hrs)	PFHxA ng/mL
I1-0811	68-9	78137	M	4	8069
I1-0743	63-8	78138	M	4	1829
I1-0744	63-9	78144	M	4	3200
I1-0745	63-10	78166	F	4	39.9
I1-0746	63-11	78167	F	4	33.6
I1-0747	63-12	78173	F	4	< LLOQ
I1-0748	63-13	78116	M	8	217
I1-0749	63-14	78118	M	8	381
I1-0750	63-15	78119	M	8	152
I1-0751	63-16	78148	F	8	< LLOQ
I1-0752	63-17	78156	F	8	< LLOQ
I1-0753	63-18	78150	F	8	< LLOQ
I1-0754	63-19	78128	M	24	< LLOQ
I1-0755	63-20	78130	M	24	< LLOQ
I1-0756	63-21	78131	M	24	< LLOQ
I1-0757	63-22	78151	F	24	< LLOQ
I1-0758	63-23	78152	F	24	< LLOQ
I1-0759	63-24	78159	F	24	< LLOQ

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

PHARMACOKINETIC (IN BLOOD) AND EXCRETION
STUDY OF PERFLUOROHEXANOIC ACID AND
NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

Table 2: Day 0 Rat Urine Experimental Sample PFHxA Concentrations

Run #	Ref #	Animal #	Sex	Timept. (hrs)	PFHxA ng/mL	Urine Vol (mL)	Total PFHxA (µg)
I1-0304	23-1	78123	M	0-6	285217	6	1711
I1-0305	23-2	78124	M	0-6	254763	6	1529
I1-0306	23-3	78125	M	0-6	294713	7	2063
I1-0307	23-4	78153	F	0-6	206246	7	1444
I1-0308	23-5	78164	F	0-6	293267	4	1173
I1-0309	23-6	78172	F	0-6	171766	7	1202
I1-0310	23-7	78123	M	6-12	29035	4	116
I1-0311	23-8	78124	M	6-12	45736	7	320
I1-0312	23-9	78125	M	6-12	29181	4	117
I1-0313	23-10	78153	F	6-12	14876	6	89.3
I1-0314	23-11	78164	F	6-12	25628	4	103
I1-0315	23-12	78172	F	6-12	24415	4	97.7
I1-0874a	76-1	78123	M	12-24	2482	15	37.2
I1-0875a	76-2	78124	M	12-24	3718	13	48.3
I1-0885a	76-3	78125	M	12-24	2912	11	32.0
I1-0319	23-16	78153	F	12-24	3904	21	82.0
I1-0886a	76-4	78164	F	12-24	3479	11	38.3
I1-0321	23-18	78172	F	12-24	4625	9	41.6

FIGURES 1-46

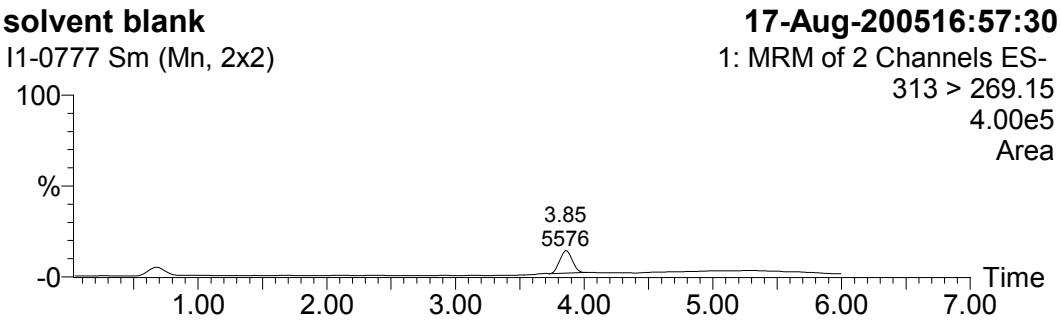

Figure 1: Representative Chromatogram Of A Processed Solvent Blank (Serum Assay)

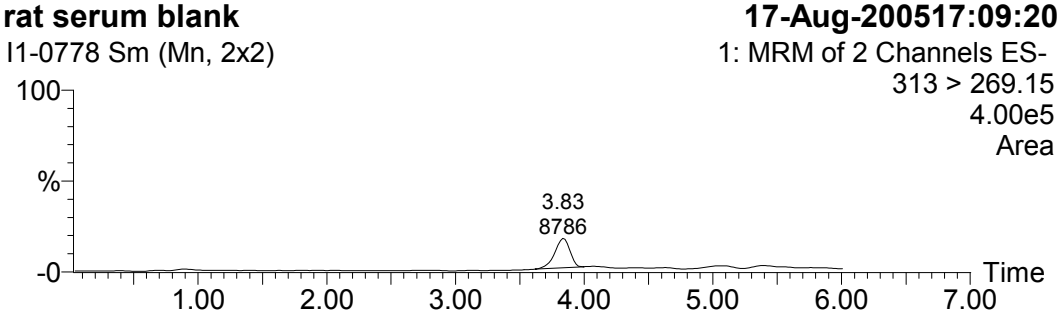

Figure 2: Representative Chromatogram Of A Processed Serum Blank

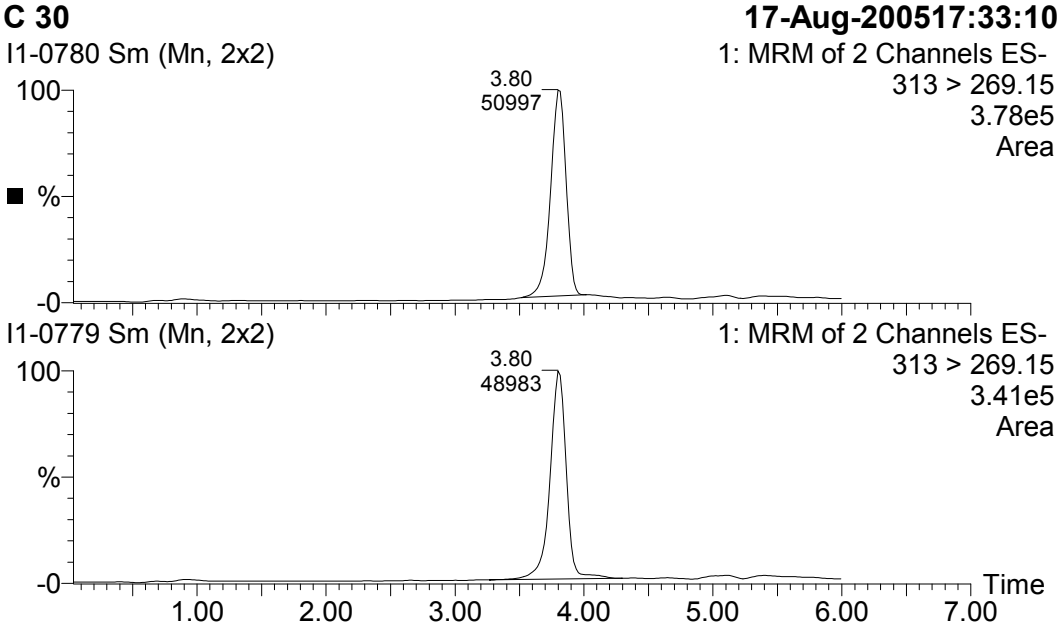

Figure 3: Representative Chromatogram Of 30 ng/mL Serum Calibration Samples

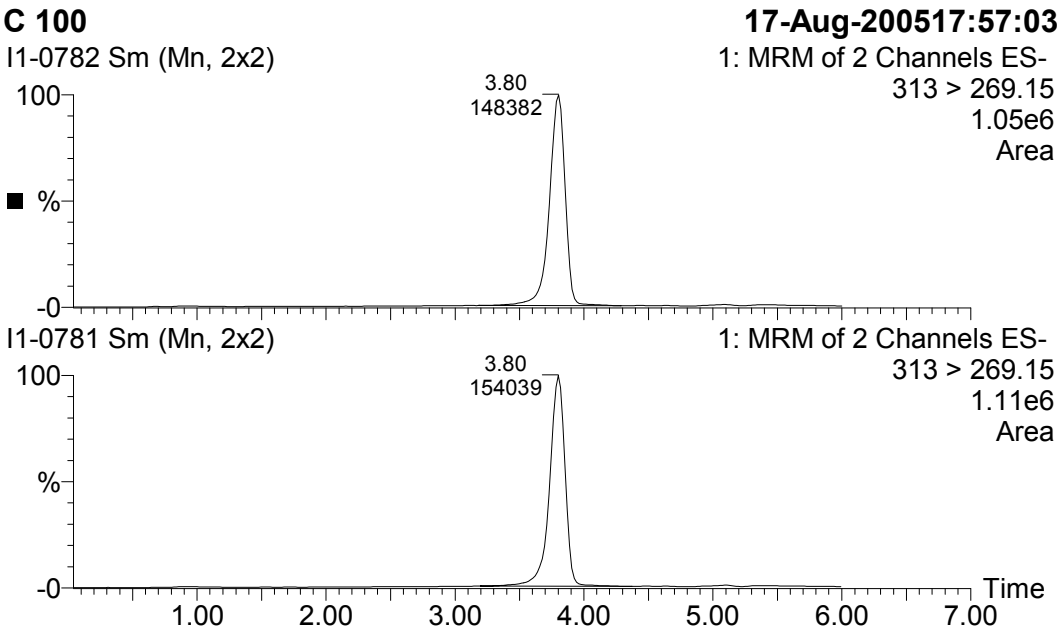

Figure 4: Representative Chromatogram Of 100 ng/mL Serum Calibration Samples

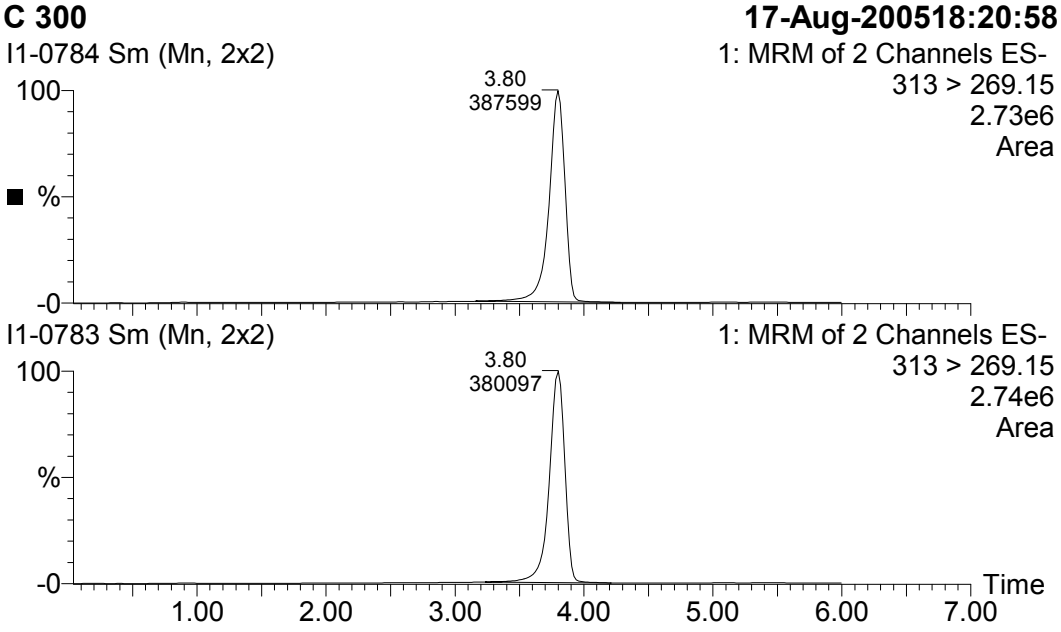

Figure 5: Representative Chromatogram Of 300 ng/mL Serum Calibration Samples

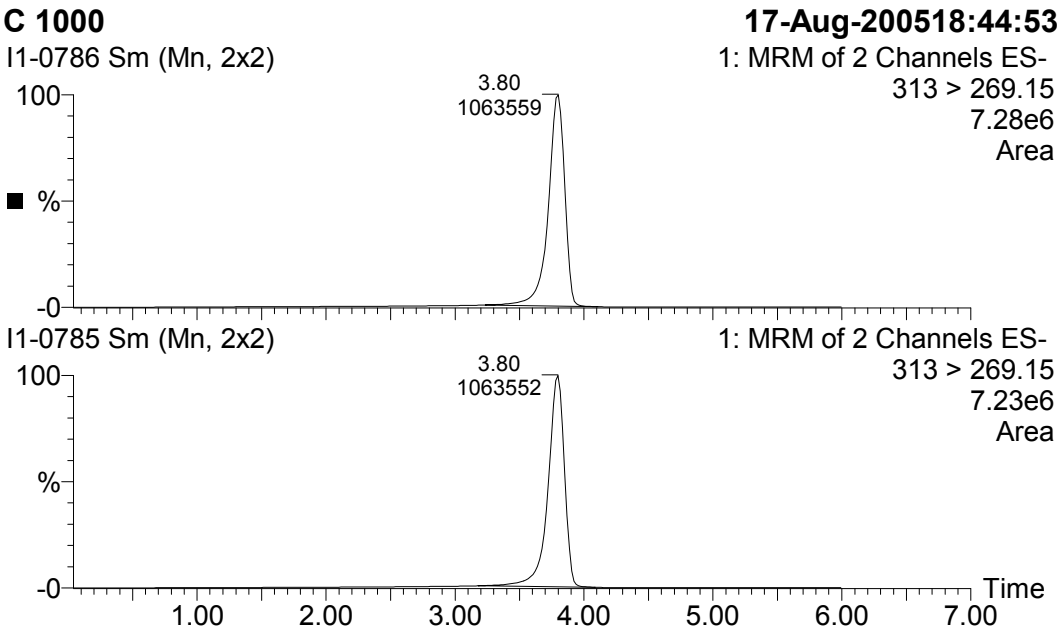

Figure 6: Representative Chromatogram Of 1000 ng/mL Serum Calibration Samples

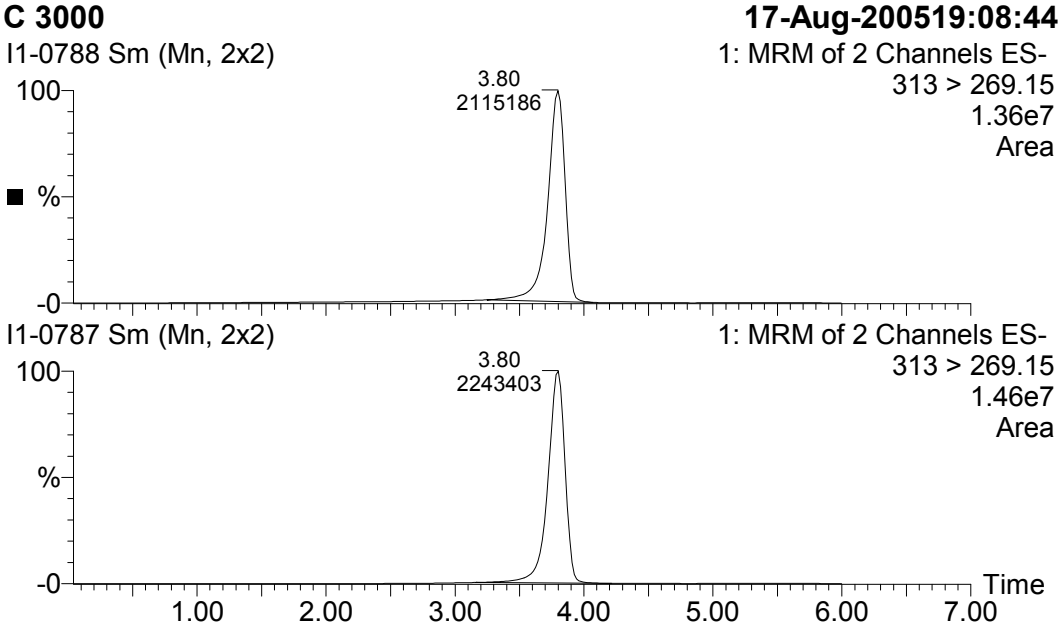

Figure 7: Representative Chromatogram Of 3000 ng/mL Serum Calibration Samples

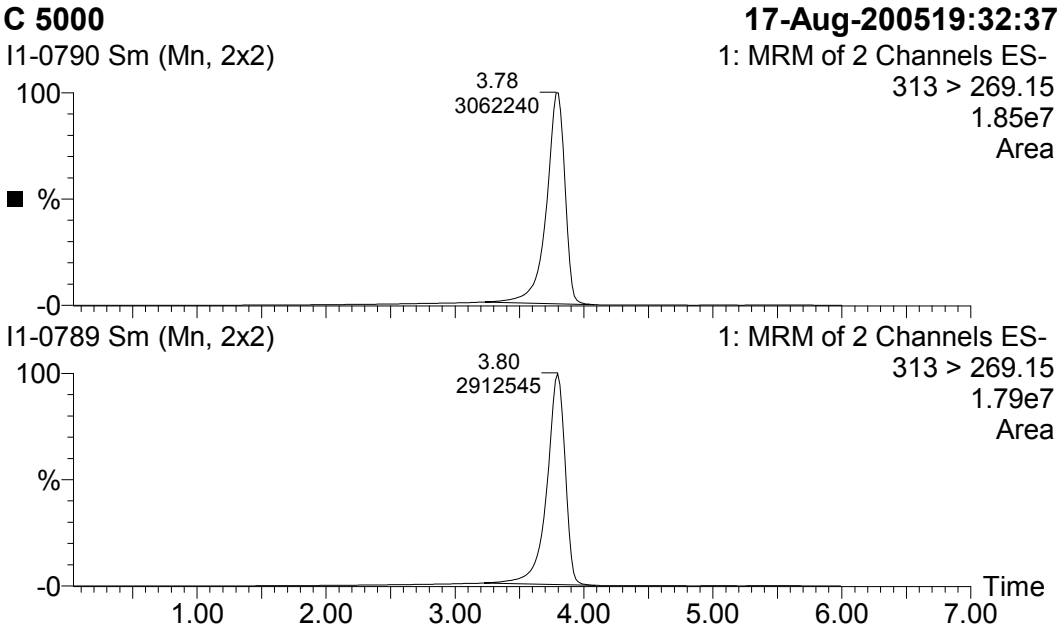

Figure 8: Representative Chromatogram Of 5000 ng/mL Serum Calibration Samples

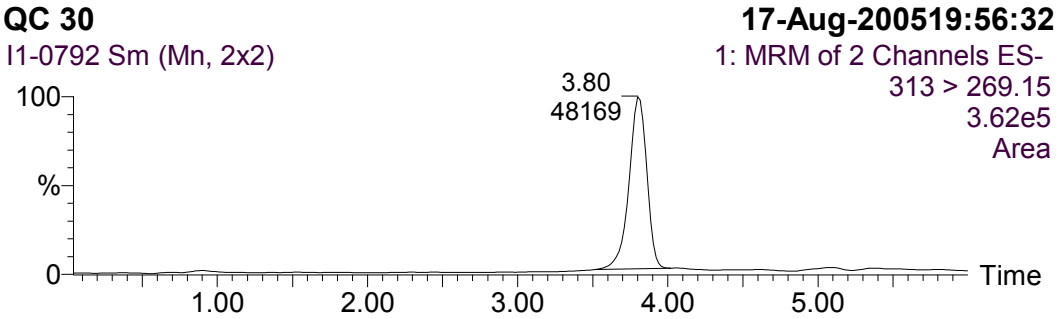

Figure 9: Representative Chromatogram Of A 30 ng/mL Serum Quality Control Sample

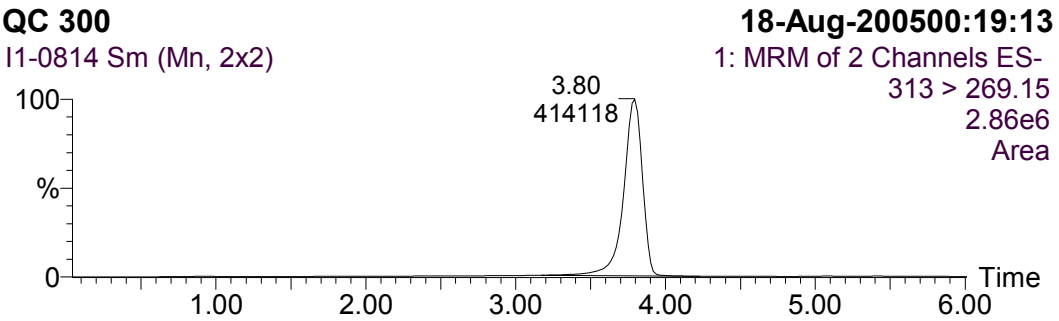

Figure 10: Representative Chromatogram Of A 300 ng/mL Serum Quality Control Sample

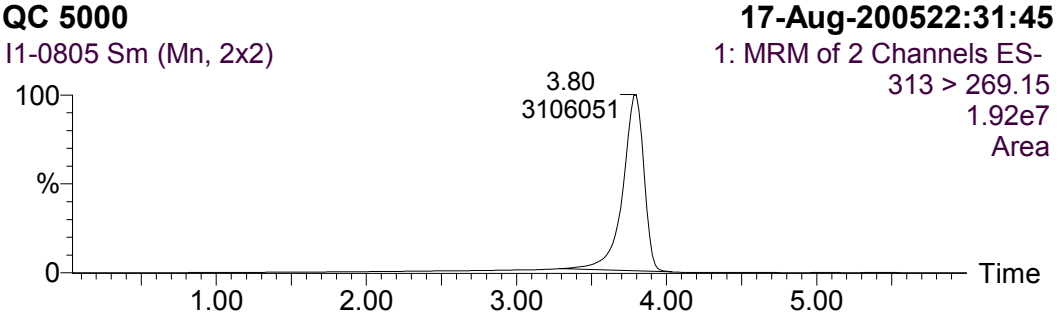

Figure 11: Representative Chromatogram Of A 5000 ng/mL Serum Quality Control Sample

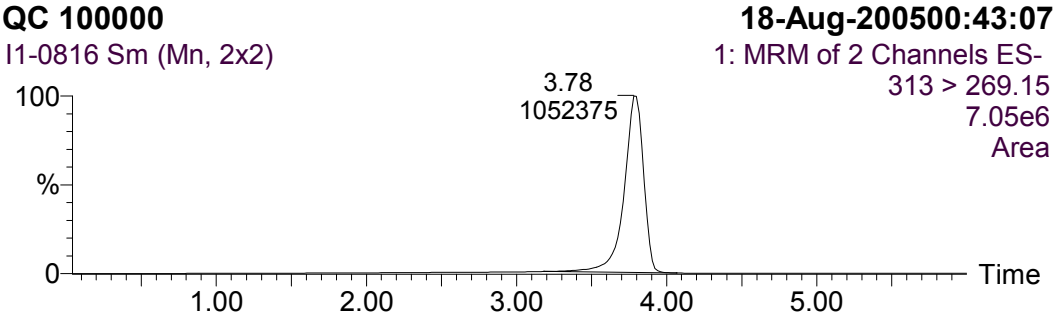

Figure 12: Representative Chromatogram Of A Dilutional 100,000 ng/mL Serum Quality Control Sample

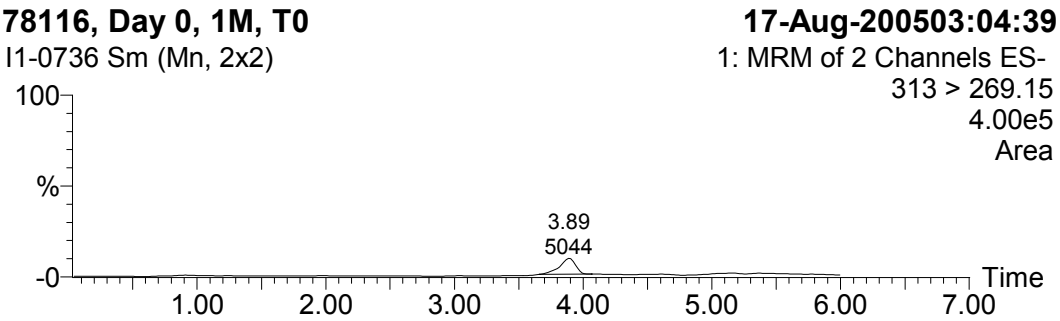

Figure 13: Chromatogram Of Animal No. 78116, Group 1 Male, Pre-Dose Serum Sample

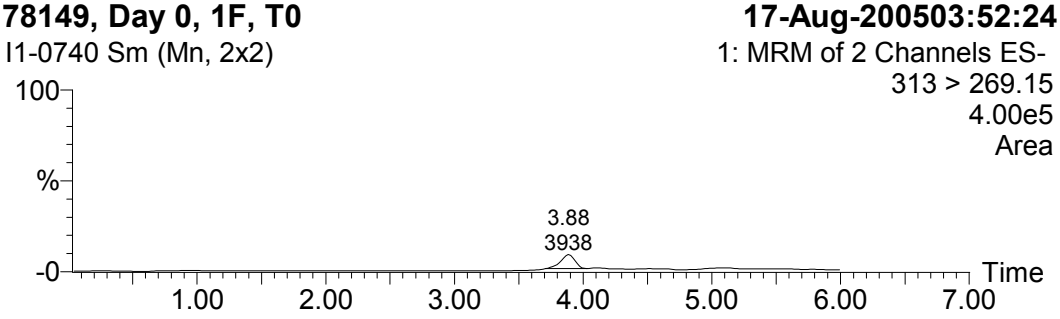

Figure 14: Chromatogram Of Animal No. 78149, Group 1 Female, Pre-Dose Serum Sample

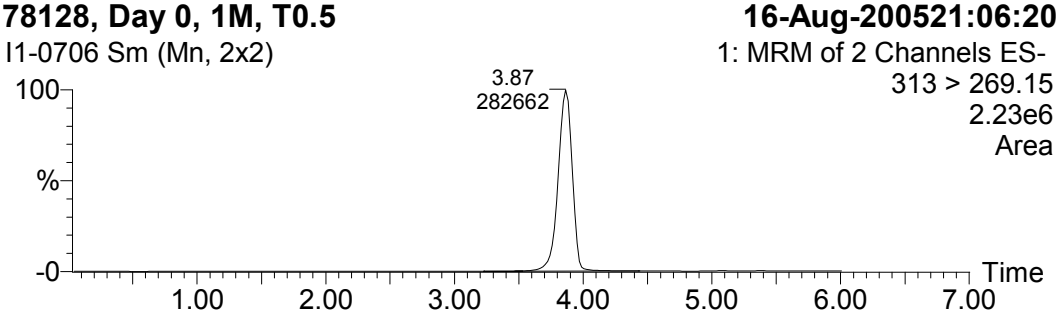

Figure 15: Chromatogram Of Animal No. 78128, Group 1 Male, 0.5 Hours Post-Dose Serum Sample

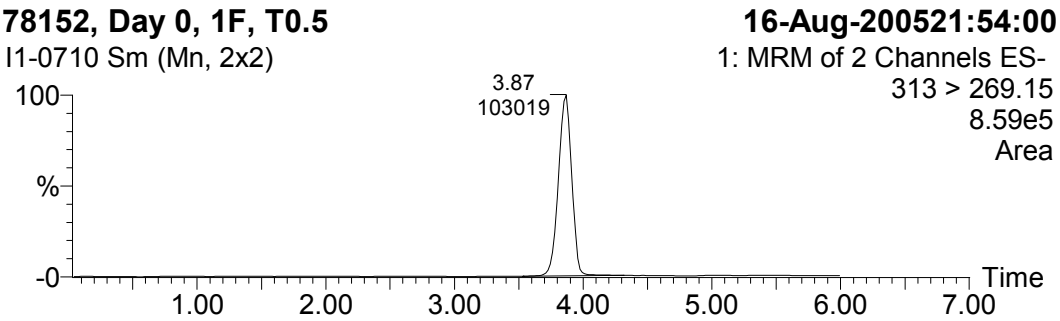

Figure 16: Chromatogram Of Animal No. 78152, Group 1 Female, 0.5 Hours Post-Dose Serum Sample

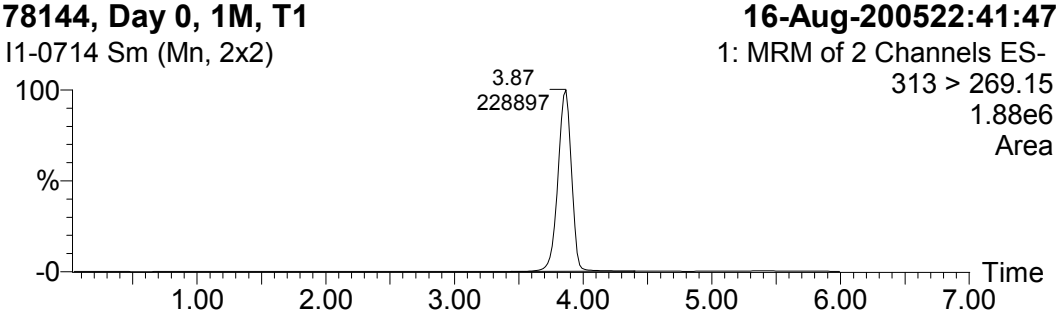

Figure 17: Chromatogram Of Animal No. 78144, Group 1, Male 1 Hour Post-Dose Serum Sample

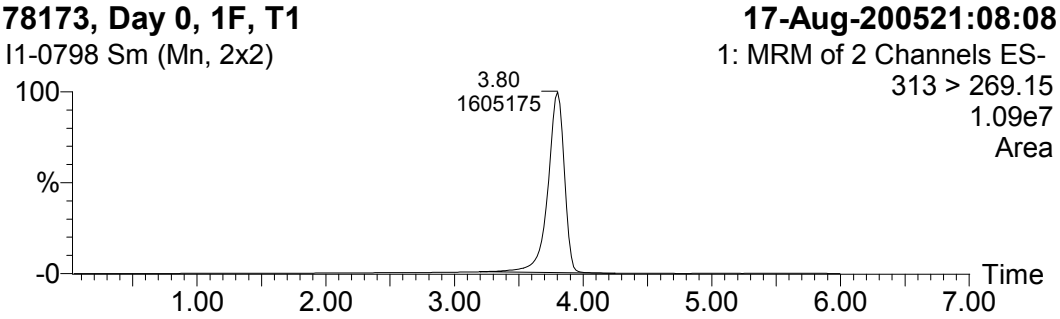

Figure 18: Chromatogram Of Animal No. 78173, Group 1 Female, 1 Hour Post-Dose Serum Sample

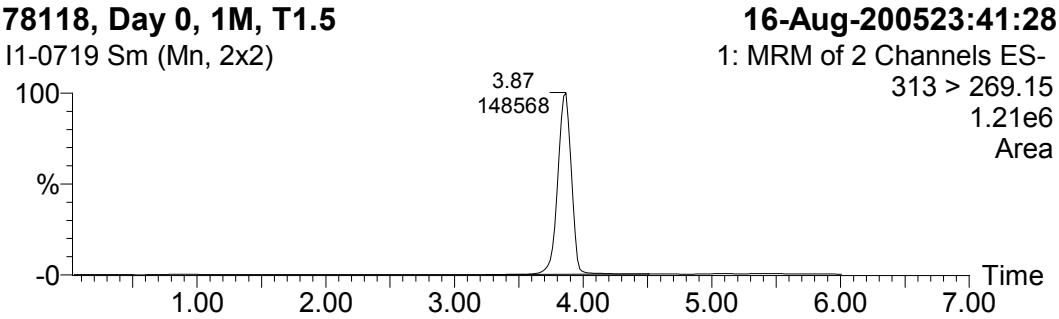

Figure 19: Chromatogram Of Animal No. 78118, Group 1 Male, 1.5 Hours Post-Dose Serum Sample

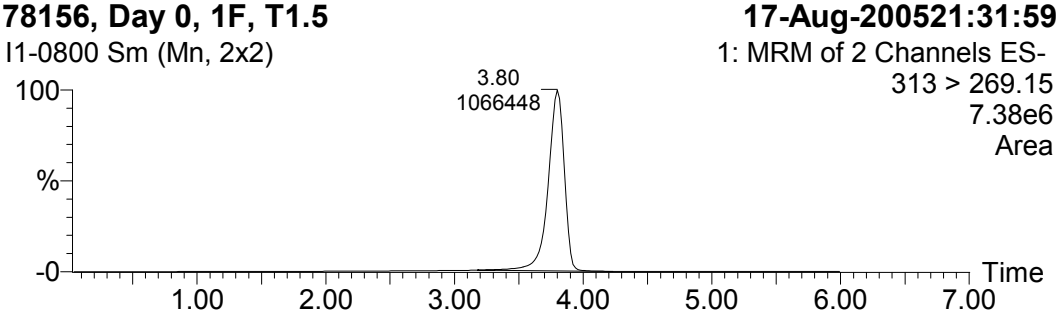

Figure 20: Chromatogram Of Animal No. 78156, Group 1 Female, 1.5 Hours Post-Dose Serum Sample

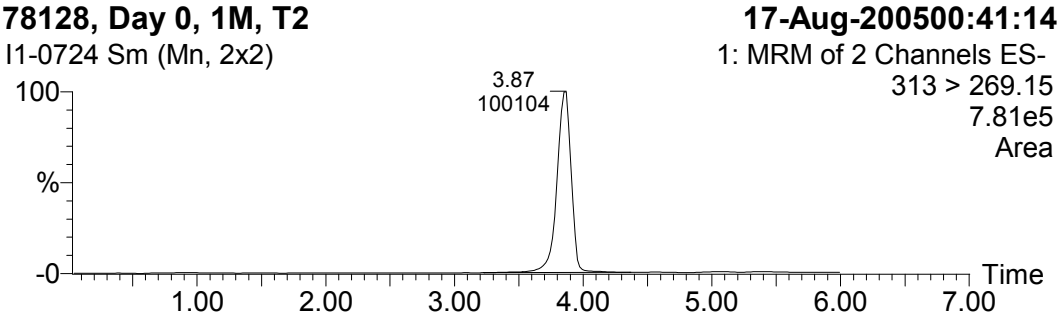

Figure 21: Chromatogram Of Animal No. 78128, Group 1 Male, 2 Hours Post-Dose Serum Sample

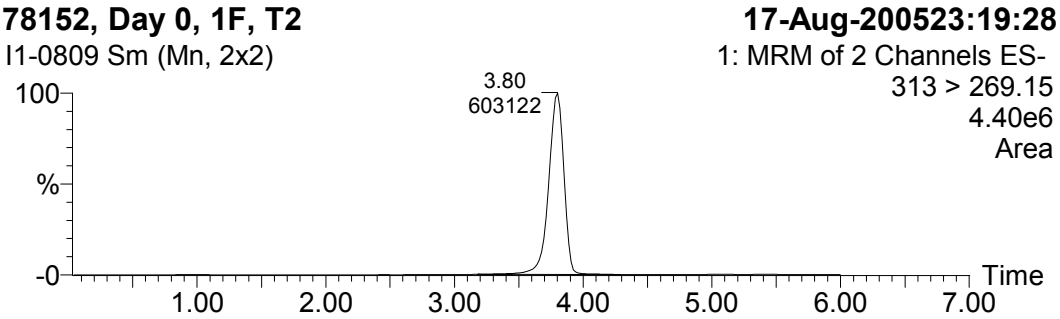

Figure 22: Chromatogram Of Animal No. 78152, Group 1 Female, 2 Hours Post-Dose Serum Sample

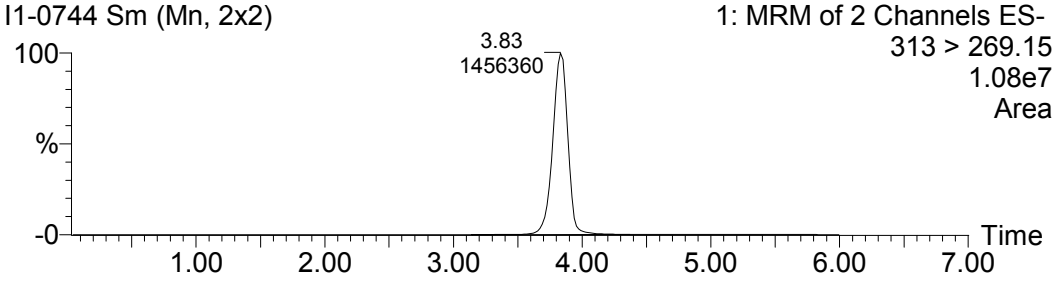

Figure 23: Chromatogram Of Animal No. 78144, Group 1 Male, 4 Hours Post-Dose Serum Sample

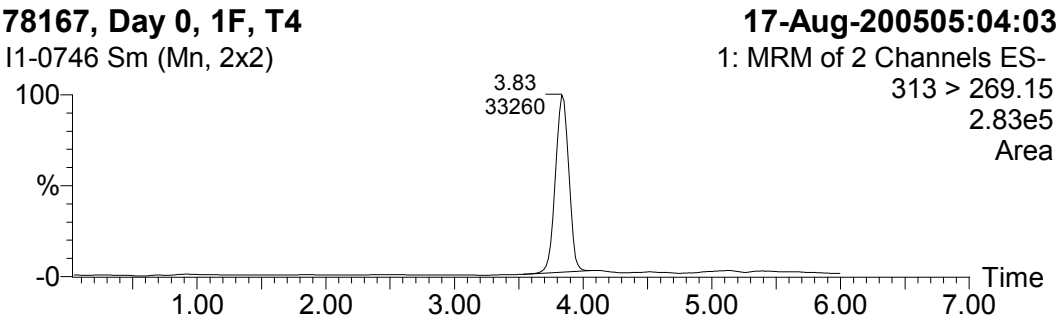

Figure 24: Chromatogram Of Animal No. 78167, Group 1 Female, 4 Hours Post-Dose Serum Sample

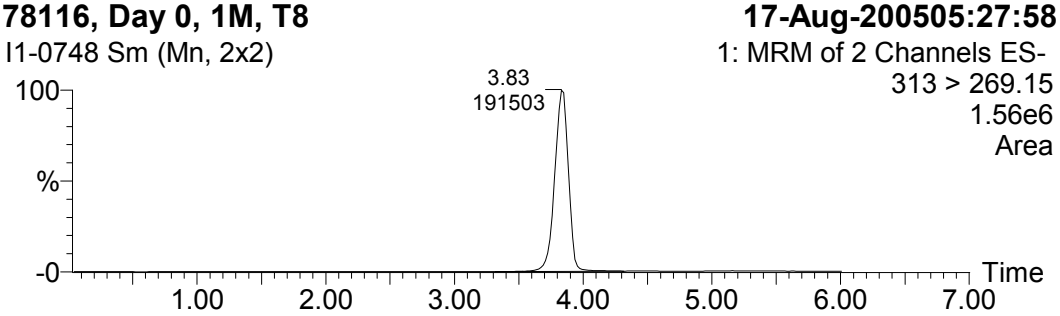

Figure 25: Chromatogram Of Animal No. 78116, Group 1 Male, 8 Hours Post-Dose Serum Sample

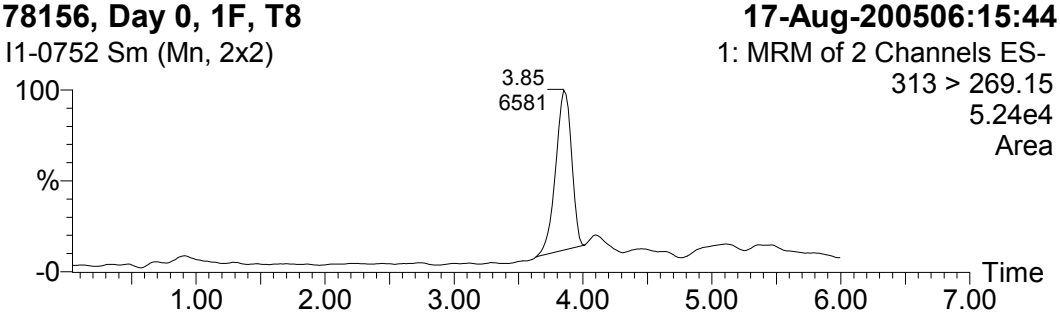

Figure 26: Chromatogram Of Animal No. 78156, Group 1 Female, 8 Hours Post-Dose Serum Sample

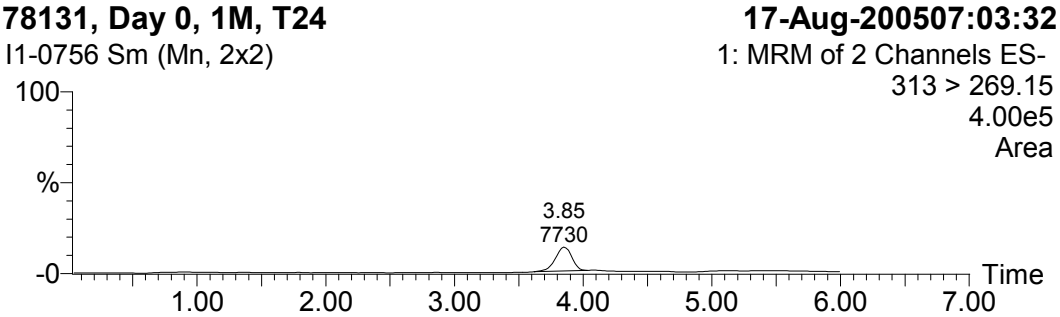

Figure 27: Chromatogram Of Animal No. 78131, Group 1 Male, 24 Hours Post-Dose Serum Sample

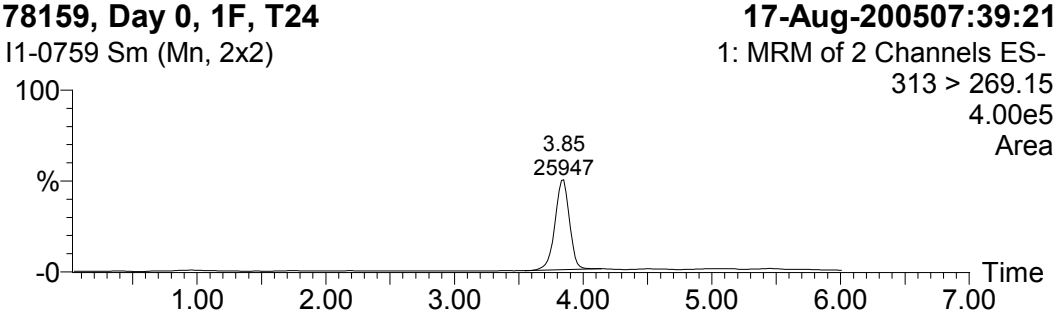

Figure 28: Chromatogram Of Animal No. 78159, Group 1 Female, 24 Hours Post-Dose Serum Sample

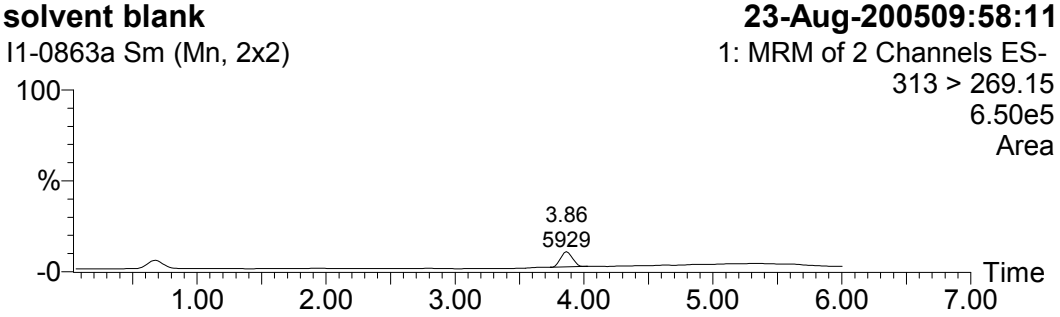

Figure 29: Representative Chromatogram Of A Processed Solvent Blank (Urine Assay)

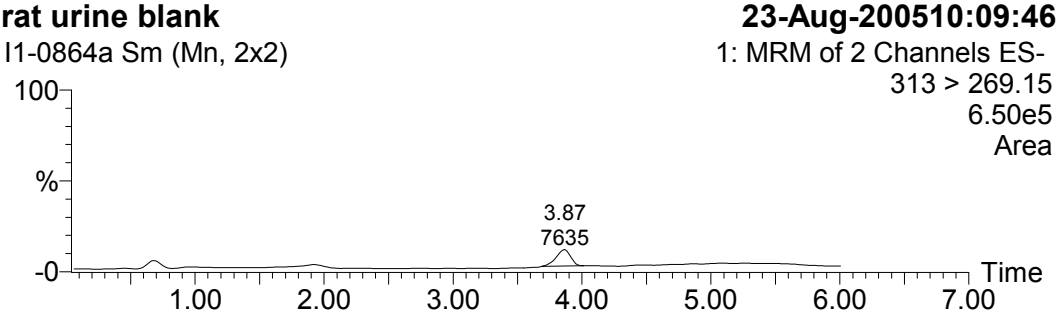

Figure 30: Representative Chromatogram Of A Processed Urine Blank

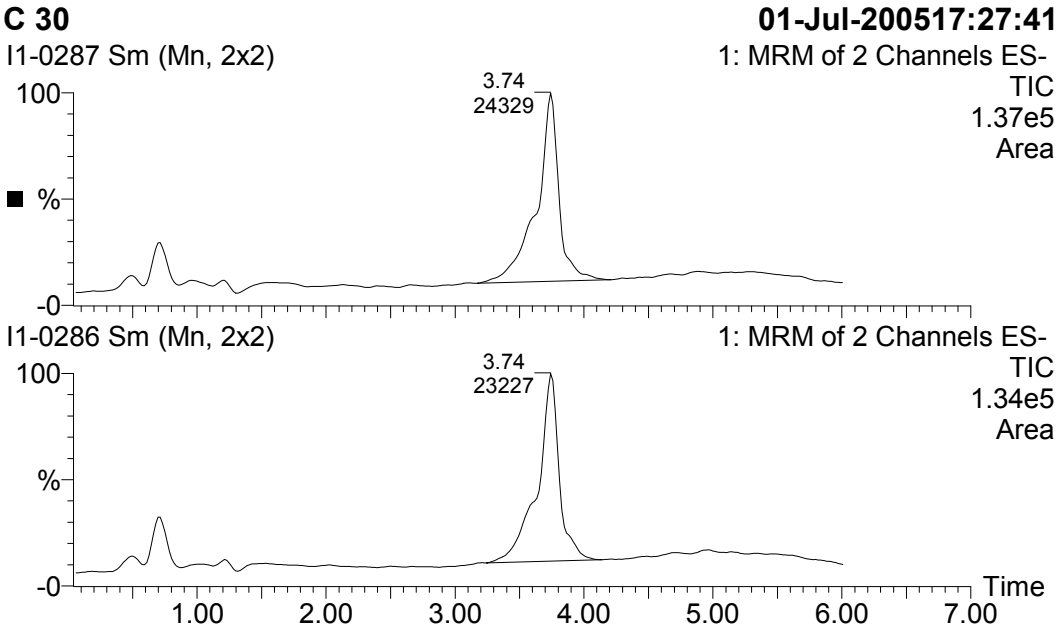

Figure 31: Representative Chromatogram Of 30 ng/mL Urine Calibration Samples

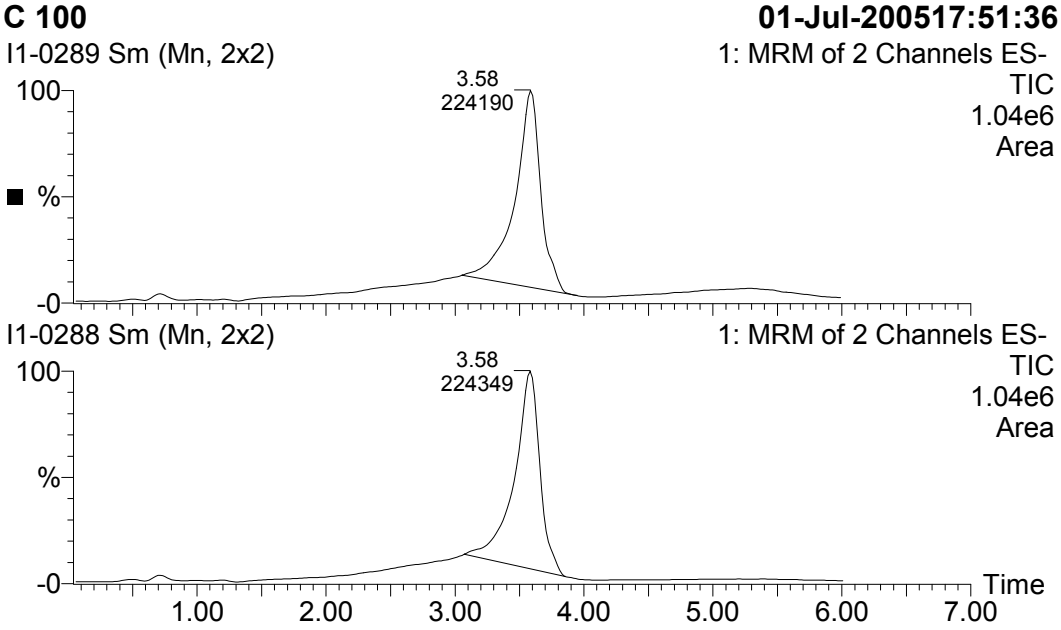

Figure 32: Representative Chromatogram Of 100 ng/mL Urine Calibration Samples

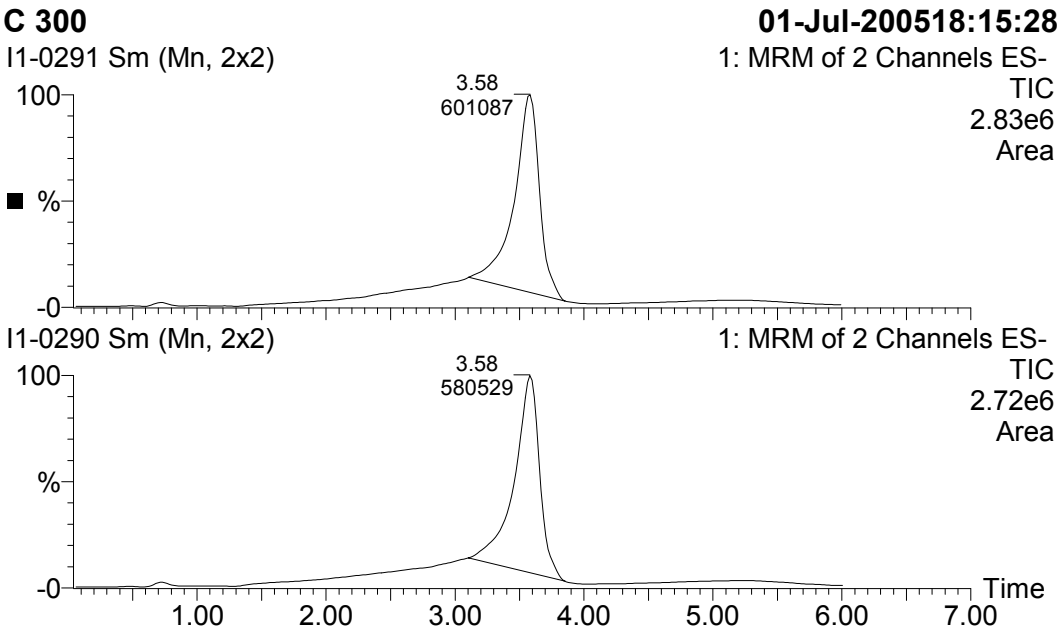

Figure 33: Representative Chromatogram Of 300 ng/mL Urine Calibration Samples

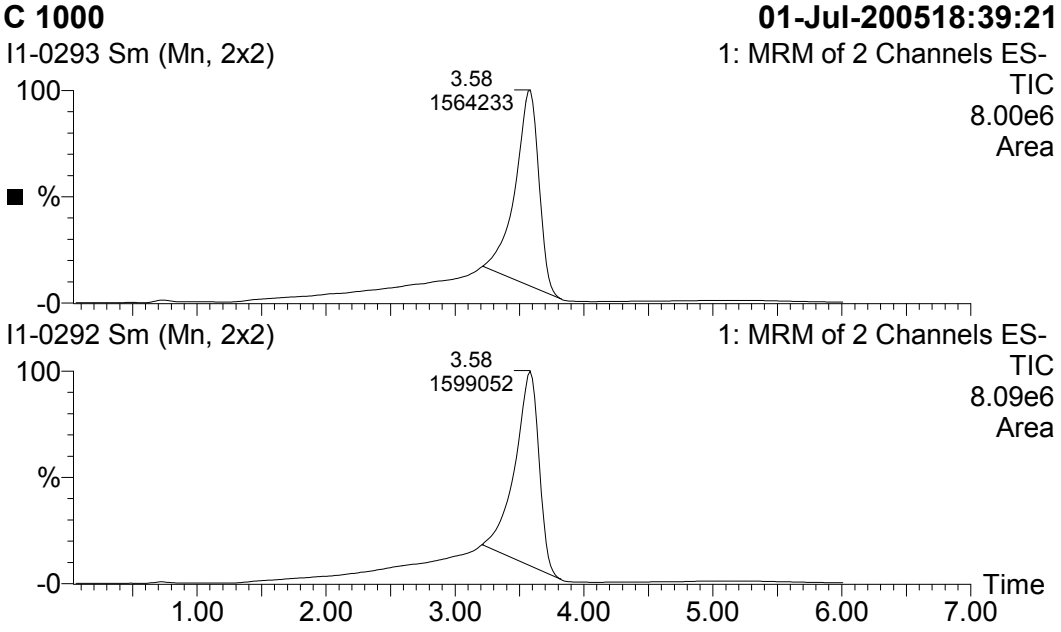

Figure 34: Representative Chromatogram Of 1000 ng/mL Urine Calibration Samples

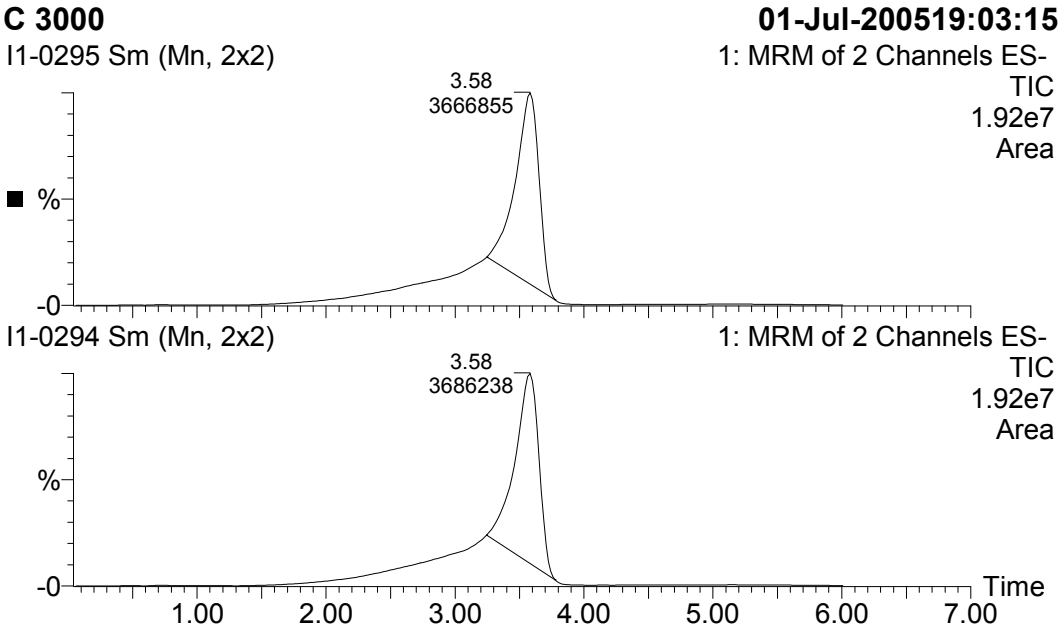

Figure 35: Representative Chromatogram Of 3000 ng/mL Urine Calibration Samples

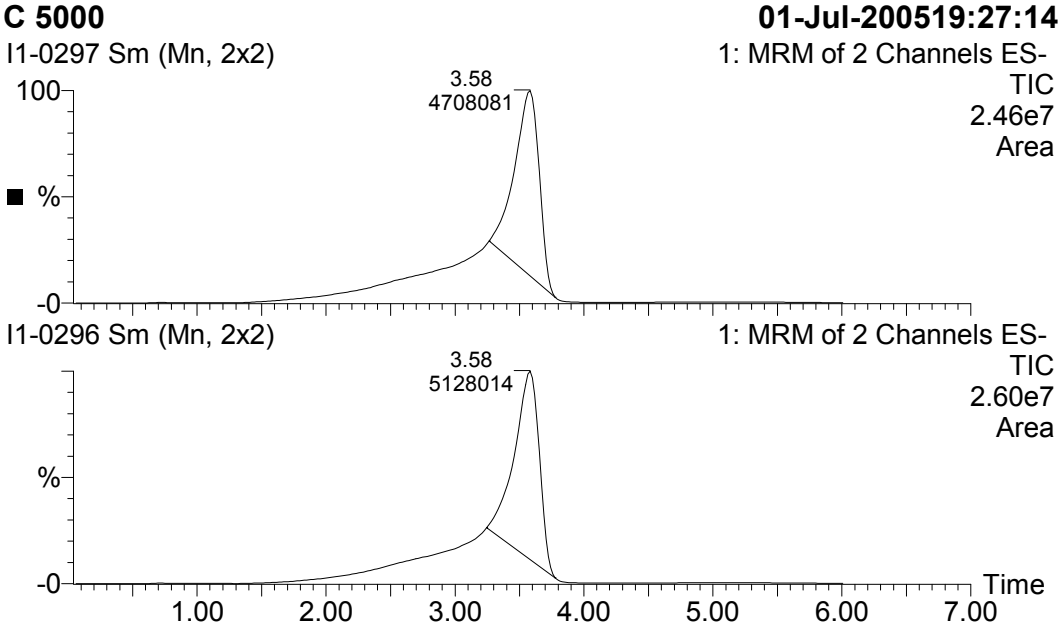

Figure 36: Representative Chromatogram Of 5000 ng/mL Urine Calibration Samples

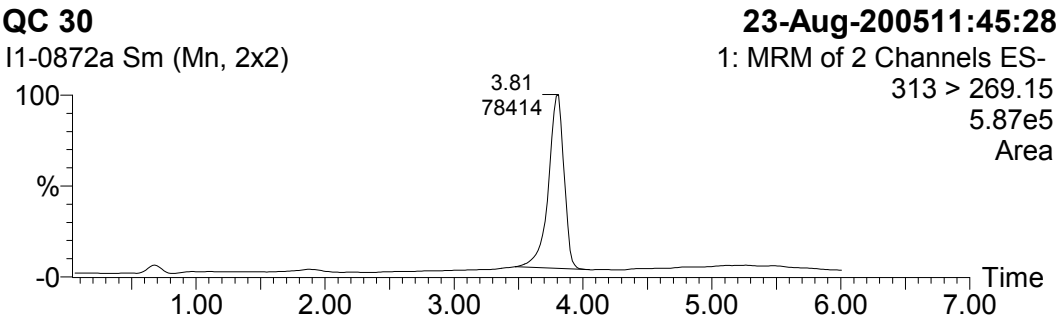

Figure 37: Representative Chromatogram Of A 30 ng/mL Urine Quality Control Sample

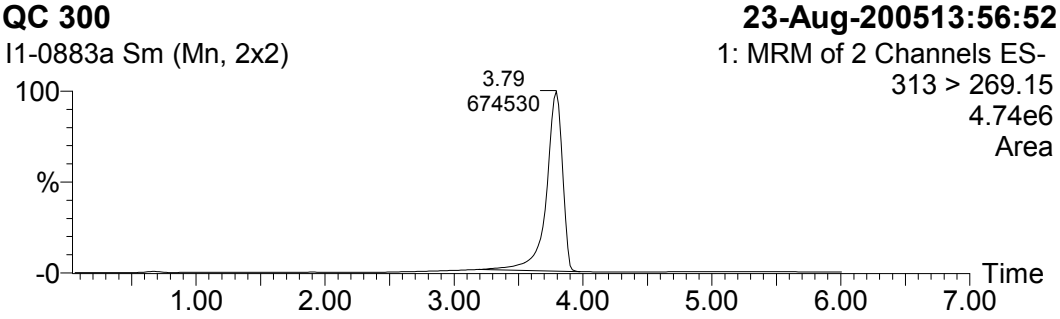

Figure 38: Representative Chromatogram Of A 300 ng/mL Urine Quality Control Sample

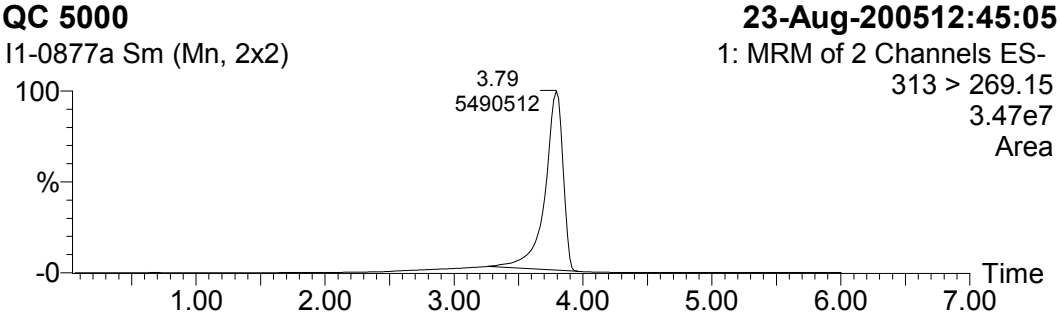

Figure 39: Representative Chromatogram Of A 5000 ng/mL Urine Quality Control Sample

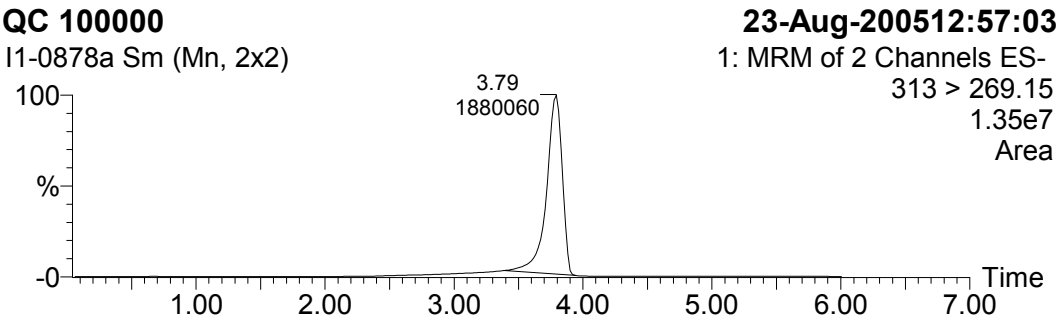

Figure 40: Representative Chromatogram Of A Dilutional 100,000 ng/mL Urine Quality Control Sample

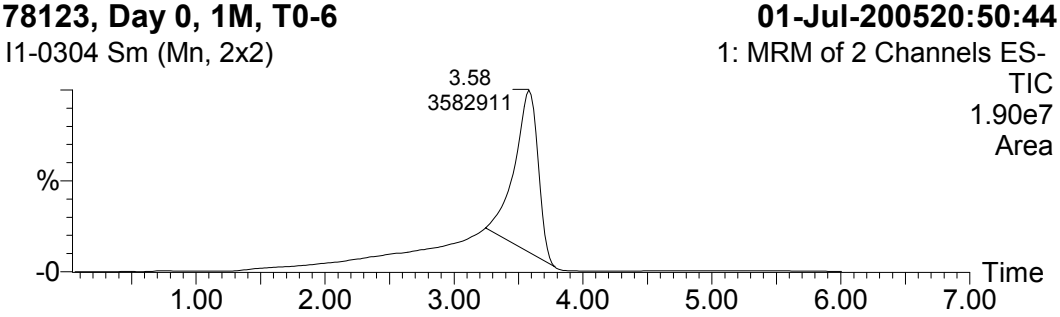

Figure 41: Chromatogram Of Animal No. 78123, Group 1 Male, 0 To 6 Hours Post-Dose Urine Sample

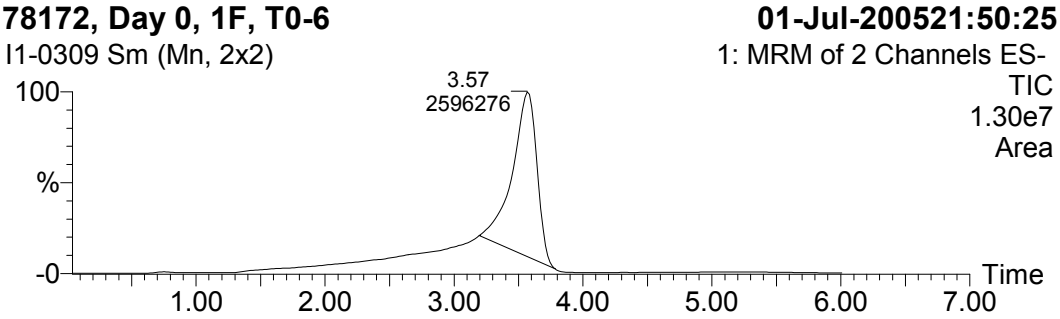

Figure 42: Chromatogram Of Animal No. 78172, Group 1 Female, 0 To 6 Hours Post-Dose Urine Sample

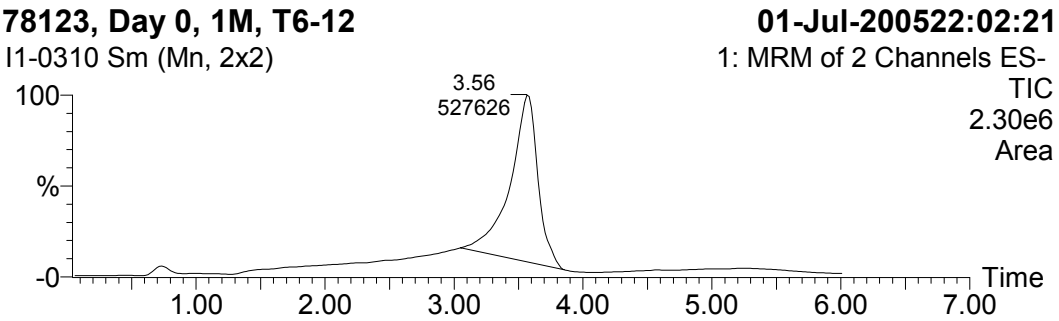

Figure 43: Chromatogram Of Animal No. 78123, Group 1, Male, 6 To 12 Hours Post-Dose Urine Sample

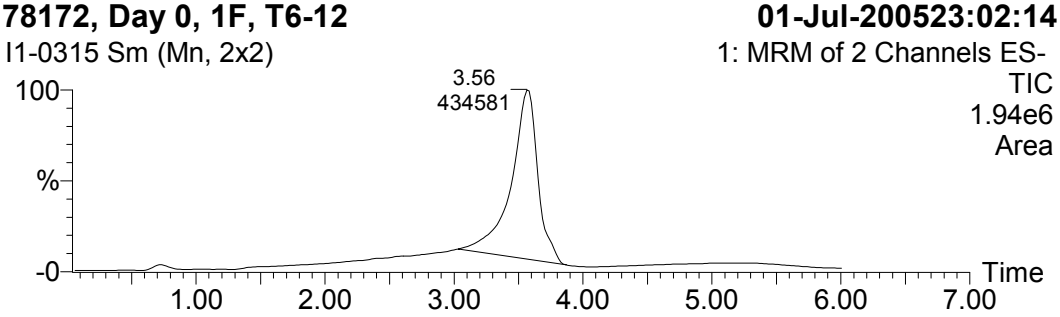

Figure 44: Chromatogram Of Animal No. 78172, Group 1 Female, 6 To 12 Hours Post-Dose Urine Sample

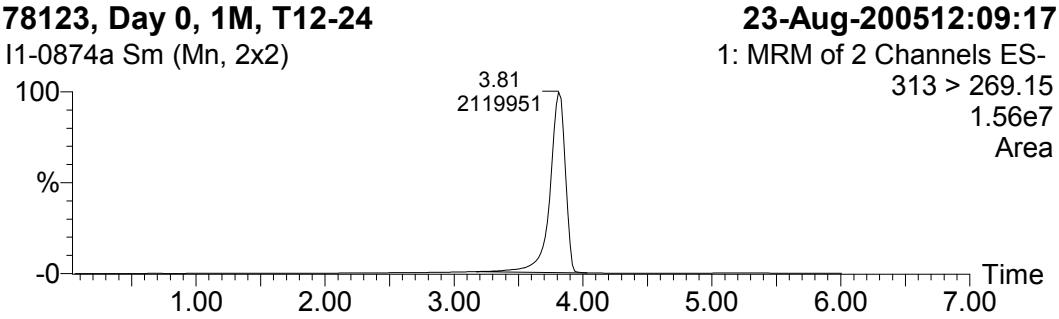

Figure 45: Chromatogram Of Animal No. 78123, Group 1 Male, 12 To 24 Hours Post-Dose Urine Sample

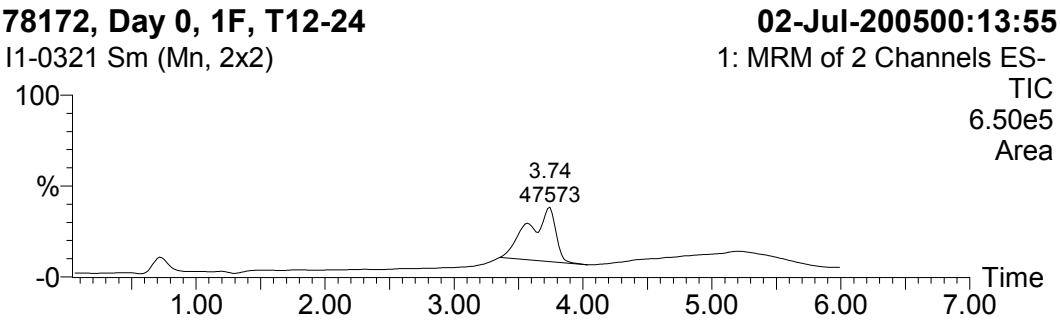

Figure 46: Chromatogram Of Animal No. 78172, Group 1 Female, 12 To 24 Hours Post-Dose Urine Sample

ATTACHMENT I

Supporting Data

Table A1: Calibration, QC And Experimental Samples For Sequence 534004(PFHxA)ARU

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534004.PRO\SampleDB\534004(PFHxA)ARU
Last modified: Fri Jul 01 16:07:13 2005
Method: C:\MASSLYNX\534004.PRO\MethDB\534001bioPFHXA
Last modified: Tue Jul 05 08:05:10 2005
Job Code:

Printed: Thu Sep 01 15:29:15 2005

Compound 1: PFHxA

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0279	21-3	system suitability	3.76	27772	0	32.7	
I1-0280	21-3	system suitability	3.74	25723.2	0	31.2	
I1-0281	21-3	system suitability	3.74	25002	0	30.7	
I1-0282	21-3	system suitability	3.74	25991.7	0	31.4	
I1-0283		50:50 ACN\H2O	3.74	30541.1	0	34.8	
I1-0284	21-1	solvent blank	3.76	20987.5	0	27.5	
I1-0285	21-2	rat urine blank	3.74	17554	0	24.7	
I1-0286	21-3	C 30	3.74	23226.8	0	29.3	-2.3
I1-0287	21-4	C 30	3.74	24328.7	0	30.2	0.53
I1-0288	21-5	C 100	3.58	224348.9	0	141	41
I1-0289	21-6	C 100	3.59	224189.6	0	141	41
I1-0290	21-7	C 300	3.58	580528.5	0	317	5.7
I1-0291	21-8	C 300	3.58	601086.9	0	327	9.1
I1-0292	21-9	C 1000	3.58	1599052.1	0	912	-8.8
I1-0293	21-10	C 1000	3.59	1564232.6	0	888	-11
I1-0294	21-11	C 3000	3.58	3686238.3	0	2998	-0.080
I1-0295	21-12	C 3000	3.58	3666855	0	2970	-1.0
I1-0296	21-13	C 5000	3.58	5128013.5	0	5892	18
I1-0297	21-14	C 5000	3.58	4708081	0	4839	-3.2
I1-0298		50:50 ACN\H2O	3.76	51525.1	0	48.7	
I1-0299	22-1	QC 30	3.74	19334.5	0	26.2	-13
I1-0300	22-4	QC 300	3.58	518083.2	0	286	-4.8
I1-0301	22-7	QC 5000	3.56	4824335.5	0	5108	2.2
I1-0302	22-10	QC 100000	3.58	1448843.6	100	81217	-19
I1-0303		50:50 ACN\H2O	3.74	50243.9	0	47.9	
I1-0304	23-1	78123, Day 0, 1M, T0-6	3.58	3582911	100	285217	
I1-0305	23-2	78124, Day 0, 1M, T0-6	3.58	3351957.5	100	254763	
I1-0306	23-3	78125, Day 0, 1M, T0-6	3.58	3650836.5	100	294713	
I1-0307	23-4	78153, Day 0, 1F, T0-6	3.58	2936010	100	206246	
I1-0308	23-5	78164, Day 0, 1F, T0-6	3.58	3640608.5	100	293267	

Compound 1: PFHxA

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0309	23-6	78172, Day 0, 1F, T0-6	3.57	2596276	100	171766	
I1-0310	23-7	78123, Day 0, 1M, T6-12	3.57	527625.8	100	29035	
I1-0311	23-8	78124, Day 0, 1M, T6-12	3.56	849323.4	100	45736	
I1-0312	23-9	78125, Day 0, 1M, T6-12	3.57	530545.9	100	29181	
I1-0313	23-10	78153, Day 0, 1F, T6-12	3.57	240288.7	100	14876	
I1-0314	23-11	78164, Day 0, 1F, T6-12	3.56	459130.7	100	25628	
I1-0315	23-12	78172, Day 0, 1F, T6-12	3.56	434581.1	100	24415	
I1-0316	23-13	78123, Day 0, 1M, T12-24	3.74	21962	100	2832	
I1-0317	23-14	78124, Day 0, 1M, T12-24	3.74	22122.6	100	2844	
I1-0318	23-15	78125, Day 0, 1M, T12-24	3.74	18478.9	100	2549	
I1-0319	23-16	78153, Day 0, 1F, T12-24	3.74	36663	100	3904	
I1-0320	23-17	78164, Day 0, 1F, T12-24	3.74	20771.8	100	2737	
I1-0321	23-18	78172, Day 0, 1F, T12-24	3.74	47572.6	100	4625	
I1-0322		50:50 ACN\H2O	3.74	41237.1	0	42.1	
I1-0323	22-2	QC 30	3.74	26348.3	0	31.7	5.6
I1-0324	22-5	QC 300	3.56	485530.5	0	269	-10
I1-0325	22-8	QC 5000	3.57	4509062.5	0	4411	-12
I1-0326	22-11	QC 100000	3.56	1669396.9	100	96040	-4.0
I1-0327		50:50 ACN\H2O	3.74	40432.2	0	41.6	
I1-0328	24-1	300 ng/mL LTS	3.62	562600.4	0	308	
I1-0329	24-2	300 ng/mL LTS	3.62	521176.6	0	287	
I1-0330	24-3	300 ng/mL LTS	3.62	499488.6	0	276	
I1-0331	24-4	5000 ng/mL LTS	3.6	4801559.5	0	5054	
I1-0332	24-5	5000 ng/mL LTS	3.6	4444009	0	4280	
I1-0333	24-6	5000 ng/mL LTS	3.6	4941887	0	5397	
I1-0334	24-7	300 ng/mL FTS1	3.62	558850.5	0	306	
I1-0335	24-8	300 ng/mL FTS1	3.6	646155.9	0	350	
I1-0336	24-9	300 ng/mL FTS1	3.6	612750.5	0	333	
I1-0337	24-10	300 ng/mL FTS2	3.62	562430.7	0	308	
I1-0338	24-11	300 ng/mL FTS2	3.62	546988.8	0	300	
I1-0339	24-12	300 ng/mL FTS2	3.6	591496.2	0	323	
I1-0340	24-13	300 ng/mL FTS3	3.62	605638.5	0	330	
I1-0341	24-14	300 ng/mL FTS3	3.62	583491.6	0	318	
I1-0342	24-15	300 ng/mL FTS3	3.62	522999.4	0	288	
I1-0343		50:50 ACN\H2O	3.74	41068	0	42.0	
I1-0344	22-3	QC 30	3.72	17193.2	0	24.4	-19
I1-0345	22-6	QC 300	3.55	426187	0	240	-20
I1-0346	22-9	QC 5000	3.55	4098436	0	3643	-27
I1-0347	22-12	QC 100000	3.55	1151153.6	100	62791	-37
I1-0348		50:50 ACN\H2O	3.74	39582.4	0	41.0	

Table A2: Calibration, QC And Experimental Samples For Sequence 534004(PFHxA)HRS1

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534004.PRO\SampleDB\534004(PFHxA)HRS1
Last modified: Wed Aug 17 11:38:25 2005
Method: C:\MASSLYNX\534004.PRO\MethDB\534004bioPFHXAa
Last modified: Wed Aug 17 09:23:10 2005
Job Code:

Printed: Tue Sep 06 15:41:00 2005

Compound 1: PFHxA

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0681a	60-3	system suitability	3.9	24546.9	0	23.6	
I1-0682a	60-3	system suitability	3.9	30834	0	30.0	
I1-0683a	60-3	system suitability	3.9	31358.8	0	30.5	
I1-0684a	60-3	system suitability	3.89	33369	0	32.6	
I1-0685		50:50 ACN\H2O	3.94	3800.2	0	3.70	
I1-0686	60-1	solvent blank	3.92	5134.8	0	4.92	
I1-0687	60-2	rat serum blank	3.92	4906.9	0	4.72	
I1-0688	60-3	C 30	3.88	28805.5	0	27.9	-7.0
I1-0689	60-4	C 30	3.88	30974.6	0	30.1	0.43
I1-0690	60-5	C 100	3.87	96311.8	0	106	6.3
I1-0691	60-6	C 100	3.88	96688	0	107	6.8
I1-0692	60-7	C 300	3.87	241532.3	0	322	7.5
I1-0693	60-8	C 300	3.87	227790.8	0	300	-0.13
I1-0694	60-9	C 1000	3.87	537736.3	0	920	-8.0
I1-0695	60-10	C 1000	3.87	513216.3	0	863	-14
I1-0696	60-11	C 3000	3.87	1218340.5	0	2975	-0.84
I1-0697	60-12	C 3000	3.87	1269883	0	3168	5.6
I1-0698	60-13	C 5000	3.87	1808443.1	0	5499	10
I1-0699	60-14	C 5000	3.87	1665807.1	0	4825	-3.5
I1-0700		50:50 ACN\H2O	3.92	3831.4	0	3.73	
I1-0701	61-1	QC 30	3.87	30297.2	0	29.4	-1.9
I1-0702	61-4	QC 300	3.86	218198.2	0	284	-5.4
I1-0703	61-7	QC 5000	3.87	1533584.5	0	4237	-15
I1-0704	61-10	QC 100000	3.87	668067.9	100	124174	24
I1-0705		50:50 ACN\H2O	3.92	5061.2	0	4.86	
I1-0706	62-1	78128, Day 0, 1M, T0.5	3.87	282662.1	100	39355	
I1-0707	62-2	78130, Day 0, 1M, T0.5	3.87	286937.6	100	40117	
I1-0708	62-3	78131, Day 0, 1M, T0.5	3.87	246502.4	100	33082	
I1-0709	62-4	78151, Day 0, 1F, T0.5	3.85	84252.3	100	9109	
I1-0710	62-5	78152, Day 0, 1F, T0.5	3.87	103018.5	100	11495	

Compound 1: PFHxA

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0711	62-6	78159, Day 0, 1F, T0.5	3.87	149139.9	100	17820	
I1-0712	62-7	78137, Day 0, 1M, T1	3.87	230072.2	100	30337	
I1-0713	62-8	78138, Day 0, 1M, T1	3.87	213516.8	100	27638	
I1-0714	62-9	78144, Day 0, 1M, T1	3.87	228896.6	100	30143	
I1-0715	62-10	78166, Day 0, 1F, T1	3.87	29323	100	2842	
I1-0716	62-11	78167, Day 0, 1F, T1	3.87	17650.9	100	1672	
I1-0717	62-12	78173, Day 0, 1F, T1	3.87	21211.6	100	2022	
I1-0718	62-13	78116, Day 0, 1M, T1.5	3.87	157591.3	100	19045	
I1-0719	62-14	78118, Day 0, 1M, T1.5	3.87	148567.8	100	17737	
I1-0720	62-15	78119, Day 0, 1M, T1.5	3.87	129994.4	100	15118	
I1-0721	62-16	78148, Day 0, 1F, T1.5	3.87	12163.5	100	1146	
I1-0722	62-17	78149, Day 0, 1F, T1.5	3.87	14116.4	100	1331	
I1-0723	62-18	78150, Day 0, 1F, T1.5	3.87	16223.9	100	1534	
I1-0724	62-19	78128, Day 0, 1M, T2	3.87	100104	100	11117	
I1-0725	62-20	78130, Day 0, 1M, T2	3.87	150684.1	100	18042	
I1-0726	62-21	78131, Day 0, 1M, T2	3.87	81620.2	100	8783	
I1-0727	62-22	78151, Day 0, 1F, T2	3.87	7572.2	100	717	
I1-0728	62-23	78152, Day 0, 1F, T2	3.87	9172.4	100	865	
I1-0729	62-24	78159, Day 0, 1F, T2	3.87	14099.4	100	1330	
I1-0730		50:50 ACN\H2O	3.9	3866.5	0	3.76	
I1-0731	61-2	QC 30	3.85	28513.1	0	27.6	-8.0
I1-0732	61-5	QC 300	3.85	226467.8	0	297	-0.86
I1-0733	61-8	QC 5000	3.85	1559751.1	0	4350	-13
I1-0734	61-11	QC 100000	3.85	589348.4	100	104310	4.3
I1-0735		50:50 ACN\H2O	3.9	3667.2	0	3.58	
I1-0736	63-1	78116, Day 0, 1M, T0	3.89	5044.2	0	4.84	
I1-0737	63-2	78118, Day 0, 1M, T0	3.89	3938.9	0	3.83	
I1-0738	63-3	78119, Day 0, 1M, T0	3.89	3603	0	3.52	
I1-0739	63-4	78148, Day 0, 1F, T0	3.89	5269.5	0	5.05	
I1-0740	63-5	78149, Day 0, 1F, T0	3.88	3938.1	0	3.83	
I1-0741	63-6	78150, Day 0, 1F, T0	3.88	5076.1	0	4.87	
I1-0742	63-7	78137, Day 0, 1M, T4	3.83	2178986.3	0	7444	
I1-0743	63-8	78138, Day 0, 1M, T4	3.85	1002499.6	0	2224	
I1-0744	63-9	78144, Day 0, 1M, T4	3.83	1456360.1	0	3910	
I1-0745	63-10	78166, Day 0, 1F, T4	3.85	39571.2	0	39.2	
I1-0746	63-11	78167, Day 0, 1F, T4	3.84	33259.8	0	32.5	
I1-0747	63-12	78173, Day 0, 1F, T4	3.85	21099.3	0	20.1	
I1-0748	63-13	78116, Day 0, 1M, T8	3.83	191502.8	0	242	
I1-0749	63-14	78118, Day 0, 1M, T8	3.83	306948.4	0	437	
I1-0750	63-15	78119, Day 0, 1M, T8	3.83	140166.4	0	165	
I1-0751	63-16	78148, Day 0, 1F, T8	3.84	19929.1	0	19.0	
I1-0752	63-17	78156, Day 0, 1F, T8	3.85	6580.6	0	6.25	

Compound 1: PFHxA

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0753	63-18	78150, Day 0, 1F, T8	3.87	6279	0	5.97	
I1-0754	63-19	78128, Day 0, 1M, T24	3.85	8648.2	0	8.16	
I1-0755	63-20	78130, Day 0, 1M, T24	3.85	15109.2	0	14.3	
I1-0756	63-21	78131, Day 0, 1M, T24	3.85	7729.7	0	7.31	
I1-0757	63-22	78151, Day 0, 1F, T24	3.87	6429.5	0	6.11	
I1-0758	63-23	78152, Day 0, 1F, T24	3.87	7081.5	0	6.71	
I1-0759	63-24	78159, Day 0, 1F, T24	3.85	25947.5	0	25.0	
I1-0760		50:50 ACN\H2O	3.89	3568.8	0	3.49	
I1-0761	61-3	QC 30	3.83	35960.6	0	35.4	18
I1-0762	61-6	QC 300	3.83	267714.1	0	367	22
I1-0763	61-9	QC 5000	3.83	2012771.4	0	6537	31
I1-0764	61-12	QC 100000	3.83	646810.2	100	118686	19
I1-0765		50:50 ACN\H2O	3.88	3498	0	3.43	
I1-0766	*60-3	C 30	3.85	29845.6	0	29.0	-3.5
I1-0767	*60-5	C 100	3.82	95093.8	0	105	4.7
I1-0768	*60-7	C 300	3.82	248522.3	0	334	11
I1-0769	*60-9	C 1000	3.82	669993.6	0	1247	25
I1-0770	*60-11	C 3000	3.82	1377010.3	0	3586	20
I1-0771	*60-13	C 5000	3.82	1929449.1	0	6103	22

* Not included in regression equation due to drift in instrument reponse.

Table A3: Calibration, QC And Experimental Samples For Sequence 534004(PFHxA)HRS2

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534004.PRO\SampleDB\534004(PFHxA)HRS2
Last modified: Wed Aug 17 11:37:06 2005
Method: C:\MASSLYNX\534004.PRO\MethDB\534004bioPFHXAa
Last modified: Wed Aug 17 09:23:10 2005
Job Code:

Printed: Mon Aug 29 15:34:55 2005

Compound 1: PFHxA

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0681a	60-3	system suitability	3.9	24546.9	0	25.0	
I1-0682a	60-3	system suitability	3.9	30834	0	31.2	
I1-0683a	60-3	system suitability	3.9	31358.8	0	31.7	
I1-0684a	60-3	system suitability	3.89	33369	0	33.7	
I1-0685		50:50 ACN\H2O	3.94	3800.2	0	4.62	
I1-0686	60-1	solvent blank	3.92	5134.8	0	5.98	
I1-0687	60-2	rat serum blank	3.92	4906.9	0	5.75	
I1-0688	*60-3	C 30	3.88	28805.5	0	29.2	-2.8
I1-0689	*60-4	C 30	3.88	30974.6	0	31.3	4.3
I1-0690	*60-5	C 100	3.87	96311.8	0	101	0.57
I1-0691	*60-6	C 100	3.88	96688	0	101	0.99
I1-0692	*60-7	C 300	3.87	241532.3	0	285	-5.0
I1-0693	*60-8	C 300	3.87	227790.8	0	266	-11
I1-0694	*60-9	C 1000	3.87	537736.3	0	775	-23
I1-0695	*60-10	C 1000	3.87	513216.3	0	729	-27
I1-0696	*60-11	C 3000	3.87	1218340.5	0	2438	-19
I1-0697	*60-12	C 3000	3.87	1269883	0	2595	-14
I1-0698	*60-13	C 5000	3.87	1808443.1	0	4509	-9.8
I1-0699	*60-14	C 5000	3.87	1665807.1	0	3952	-21
I1-0700		50:50 ACN\H2O	3.92	3831.4	0	4.65	
I1-0701	61-1	QC 30	3.87	30297.2	0	30.6	2.1
I1-0702	61-4	QC 300	3.86	218198.2	0	253	-16
I1-0703	61-7	QC 5000	3.87	1533584.5	0	3468	-31
I1-0704	61-10	QC 100000	3.87	668067.9	100	103571	3.6
I1-0705		50:50 ACN\H2O	3.92	5061.2	0	5.905	
I1-0706	62-1	78128, Day 0, 1M, T0.5	3.87	282662.1	100	34423	
I1-0707	62-2	78130, Day 0, 1M, T0.5	3.87	286937.6	100	35055	
I1-0708	62-3	78131, Day 0, 1M, T0.5	3.87	246502.4	100	29201	

* Not included in regression equation due to drift in instrument reponse.

Compound 1: PFHxA

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0709	62-4	78151, Day 0, 1F, T0.5	3.85	84252.3	100	8710	
I1-0710	62-5	78152, Day 0, 1F, T0.5	3.87	103018.5	100	10819	
I1-0711	62-6	78159, Day 0, 1F, T0.5	3.87	149139.9	100	16303	
I1-0712	62-7	78137, Day 0, 1M, T1	3.87	230072.2	100	26904	
I1-0713	62-8	78138, Day 0, 1M, T1	3.87	213516.8	100	24638	
I1-0714	62-9	78144, Day 0, 1M, T1	3.87	228896.6	100	26741	
I1-0715	62-10	78166, Day 0, 1F, T1	3.87	29323	100	2966	
I1-0716	62-11	78167, Day 0, 1F, T1	3.87	17650.9	100	1823	
I1-0717	62-12	78173, Day 0, 1F, T1	3.87	21211.6	100	2170	
I1-0718	62-13	78116, Day 0, 1M, T1.5	3.87	157591.3	100	17353	
I1-0719	62-14	78118, Day 0, 1M, T1.5	3.87	148567.8	100	16233	
I1-0720	62-15	78119, Day 0, 1M, T1.5	3.87	129994.4	100	13976	
I1-0721	62-16	78148, Day 0, 1F, T1.5	3.87	12163.5	100	1290	
I1-0722	62-17	78156, Day 0, 1F, T1.5	3.87	14116.4	100	1480	
I1-0723	62-18	78150, Day 0, 1F, T1.5	3.87	16223.9	100	1684	
I1-0724	62-19	78128, Day 0, 1M, T2	3.87	100104	100	10487	
I1-0725	62-20	78130, Day 0, 1M, T2	3.87	150684.1	100	16494	
I1-0726	62-21	78131, Day 0, 1M, T2	3.87	81620.2	100	8420	
I1-0727	62-22	78151, Day 0, 1F, T2	3.87	7572.2	100	841	
I1-0728	62-23	78152, Day 0, 1F, T2	3.87	9172.4	100	998	
I1-0729	62-24	78159, Day 0, 1F, T2	3.87	14099.4	100	1478	
I1-0730		50:50 ACN\H2O	3.9	3866.5	0	4.69	
I1-0731	61-2	QC 30	3.85	28513.1	0	28.9	-3.8
I1-0732	61-5	QC 300	3.85	226467.8	0	264	-12
I1-0733	61-8	QC 5000	3.85	1559751.1	0	3561	-29
I1-0734	61-11	QC 100000	3.85	589348.4	100	87493	-13
I1-0735		50:50 ACN\H2O	3.9	3667.2	0	4.48	
I1-0736	63-1	78116, Day 0, 1M, T0	3.89	5044.2	0	5.89	
I1-0737	63-2	78118, Day 0, 1M, T0	3.89	3938.9	0	4.76	
I1-0738	63-3	78119, Day 0, 1M, T0	3.89	3603	0	4.41	
I1-0739	63-4	78148, Day 0, 1F, T0	3.89	5269.5	0	6.12	
I1-0740	63-5	78149, Day 0, 1F, T0	3.88	3938.1	0	4.76	
I1-0741	63-6	78150, Day 0, 1F, T0	3.88	5076.1	0	5.92	
I1-0742	63-7	78137, Day 0, 1M, T4	3.83	2178986.3	0	6135	
I1-0743	63-8	78138, Day 0, 1M, T4	3.85	1002499.6	0	1829	
I1-0744	63-9	78144, Day 0, 1M, T4	3.83	1456360.1	0	3200	
I1-0745	63-10	78166, Day 0, 1F, T4	3.85	39571.2	0	39.9	
I1-0746	63-11	78167, Day 0, 1F, T4	3.84	33259.8	0	33.6	
I1-0747	63-12	78173, Day 0, 1F, T4	3.85	21099.3	0	21.6	
I1-0748	63-13	78116, Day 0, 1M, T8	3.83	191502.8	0	217	
I1-0749	63-14	78118, Day 0, 1M, T8	3.83	306948.4	0	381	
I1-0750	63-15	78119, Day 0, 1M, T8	3.83	140166.4	0	152	
I1-0751	63-16	78148, Day 0, 1F, T8	3.84	19929.1	0	20.4	
I1-0752	63-17	78156, Day 0, 1F, T8	3.85	6580.6	0	7.43	

Compound 1: PFHxA

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0753	63-18	78150, Day 0, 1F, T8	3.87	6279	0	7.13	
I1-0754	63-19	78128, Day 0, 1M, T24	3.85	8648.2	0	9.47	
I1-0755	63-20	78130, Day 0, 1M, T24	3.85	15109.2	0	15.8	
I1-0756	63-21	78131, Day 0, 1M, T24	3.85	7729.7	0	8.57	
I1-0757	63-22	78151, Day 0, 1F, T24	3.87	6429.5	0	7.28	
I1-0758	63-23	78152, Day 0, 1F, T24	3.87	7081.5	0	7.93	
I1-0759	63-24	78159, Day 0, 1F, T24	3.85	25947.5	0	26.3	
I1-0760		50:50 ACN\H2O	3.89	3568.8	0	4.38	
I1-0761	61-3	QC 30	3.83	35960.6	0	36.3	21
I1-0762	61-6	QC 300	3.83	267714.1	0	322	7.5
I1-0763	61-9	QC 5000	3.83	2012771.4	0	5373	7.5
I1-0764	61-12	QC 100000	3.83	646810.2	100	99132	-0.87
I1-0765		50:50 ACN\H2O	3.88	3498	0	4.30	
I1-0766	60-3	C 30	3.85	29845.6	0	30.2	0.59
I1-0767	60-5	C 100	3.82	95093.8	0	99.2	-0.81
I1-0768	60-7	C 300	3.82	248522.3	0	295	-1.7
I1-0769	60-9	C 1000	3.82	669993.6	0	1040	4.0
I1-0770	60-11	C 3000	3.82	1377010.3	0	2935	-2.2
I1-0771	60-13	C 5000	3.82	1929449.1	0	5011	0.22

Table A4: Calibration, QC And Experimental Samples For Sequence 534004(PFHxA)IRS

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534004.PRO\SampleDB\534004(PFHxA)IRS
Last modified: Wed Aug 17 15:41:25 2005
Method: C:\MASSLYNX\534004.PRO\MethDB\534004bioPFHXAa
Last modified: Wed Aug 17 09:23:10 2005
Job Code:

Printed: Mon Aug 29 08:54:08 2005

Compound 1: PFHxA

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0772	66-3	system suitability	3.8	35175.9	0	21.4	
I1-0773	66-3	system suitability	3.76	31701.1	0	19.3	
I1-0774	66-3	system suitability	3.78	41408.5	0	25.2	
I1-0775	66-3	system suitability	3.8	44587.6	0	27.1	
I1-0776		50:50 ACN\H2O	3.85	5770	0	3.95	
I1-0777	66-1	solvent blank	3.85	5576.3	0	3.83	
I1-0778	66-2	rat serum blank	3.83	8786	0	5.75	
I1-0779	66-3	C 30	3.8	48982.7	0	29.8	-0.67
I1-0780	66-4	C 30	3.8	50996.6	0	31.0	3.5
I1-0781	66-5	C 100	3.8	154038.7	0	100	0.36
I1-0782	66-6	C 100	3.8	148382.5	0	96.3	-3.7
I1-0783	66-7	C 300	3.8	380097	0	286	-4.8
I1-0784	66-8	C 300	3.8	387598.9	0	292	-2.5
I1-0785	66-9	C 1000	3.8	1063551.5	0	1069	6.9
I1-0786	66-10	C 1000	3.8	1063558.6	0	1069	6.9
I1-0787	66-11	C 3000	3.8	2243402.5	0	3128	4.3
I1-0788	66-12	C 3000	3.8	2115186	0	2861	-4.6
I1-0789	66-13	C 5000	3.8	2912545.3	0	4695	-6.1
I1-0790	66-14	C 5000	3.78	3062239.8	0	5087	1.7
I1-0791		50:50 ACN\H2O	3.85	6388.6	0	4.321	
I1-0792	67-1	QC 30	3.8	48169	0	29.3	-2.3
I1-0793	67-4	QC 300	3.8	397552.5	0	302	0.54
I1-0794	67-7	QC 5000	3.8	2813232.3	0	4444	-11
I1-0795	67-10	QC 100000	3.8	1085357.1	100	109882	9.9
I1-0796		50:50 ACN\H2O	3.85	7247.1	0	4.836	
I1-0797	68-1	78167, Day 0, 1F, T1	3.8	1224857.4	0	1298	
I1-0798	68-2	78173, Day 0, 1F, T1	3.8	1605175.4	0	1904	
I1-0799	68-3	78148, Day 0, 1F, T1.5	3.8	813368.4	0	746	
I1-0800	68-4	78156, Day 0, 1F, T1.5	3.8	1066448.4	0	1073	
I1-0801	68-5	78150, Day 0, 1F, T1.5	3.8	1129524.6	0	1161	

Compound 1: PFHxA

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0802		50:50 ACN\H2O	3.85	6242.7	0	4.23	
I1-0803	67-2	QC 30	3.8	50507.4	0	30.7	2.5
I1-0804	67-5	QC 300	3.8	380245.7	0	286	-4.7
I1-0805	67-8	QC 5000	3.8	3106050.5	0	5204	4.1
I1-0806	67-11	QC 100000	3.8	1053575.3	100	105513	5.5
I1-0807		50:50 ACN\H2O	3.85	6597.7	0	4.45	
I1-0808	68-6	78151, Day 0, 1F, T2	3.8	364920	0	272	
I1-0809	68-7	78152, Day 0, 1F, T2	3.8	603122.3	0	506	
I1-0810	68-8	78159, Day 0, 1F, T2	3.8	1103745.1	0	1124	
I1-0811	68-9	78137, Day 0, 1M, T4	3.8	503515.4	20	8069	
I1-0812		50:50 ACN\H2O	3.85	6880.4	0	4.62	
I1-0813	67-3	QC 30	3.79	49533.3	0	30.1	0.46
I1-0814	67-6	QC 300	3.8	414118.2	0	317	5.7
I1-0815	67-9	QC 5000	3.8	3011233.8	0	4952	-0.97
I1-0816	67-12	QC 100000	3.78	1052375.1	100	105350	5.4
I1-0817		50:50 ACN\H2O	3.84	6338.2	0	4.29	

Table A5: Calibration, QC And Experimental Samples For Sequence 534004(PFHxA)KRU1

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534004.PRO\SampleDB\534004(PFHxA)kRU1
Last modified: Tue Aug 23 09:12:28 2005
Method: C:\MASSLYNX\534004.PRO\MethDB\534004bioPFHXAa
Last modified: Wed Aug 17 09:23:10 2005
Job Code:

Printed: Mon Aug 29 16:10:15 2005

Compound 1: PFHxA

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0859a	74-3	system suitability	3.74	48338.8	0	16.8	
I1-0860a	74-3	system suitability	3.78	60464.1	0	20.9	
I1-0861a	74-3	system suitability	3.78	68305.6	0	23.6	
I1-0862a		50:50 ACN\H2O	3.85	7381.1	0	2.92	
I1-0863a	74-1	solvent blank	3.86	5928.6	0	2.41	
I1-0864a	74-2	rat urine blank	3.87	7634.9	0	3.01	
I1-0865a	74-3	C 30	3.81	84852.2	0	29.4	-1.9
I1-0866a	74-5	C 100	3.81	276465.4	0	103	3.2
I1-0867a	74-7	C 300	3.81	645485.3	0	274	-8.7
I1-0868a	74-9	C 1000	3.79	1984180.3	0	1135	13
I1-0869a	74-11	C 3000	3.79	4161641	0	3223	7.4
I1-0870a	74-13	C 5000	3.79	5998092	0	5619	12
I1-0871a		50:50 ACN\H2O	3.86	7941.8	0	3.12	
I1-0872a	75-1	QC 30	3.81	78414	0	27.2	-9.5
I1-0873a	75-4	QC 300	3.81	773158.6	0	341	14
I1-0874a	76-1	78123, Day 0, 1M, T12-24	3.81	2119950.5	2	2482	
I1-0875a	76-2	78124, Day 0, 1M, T12-24	3.81	2841547.5	2	3718	
I1-0876a	75-7	QC 5000	3.79	5294771.5	0	4632	-7.4
I1-0877a	75-8	QC 5000	3.79	5490512	0	4898	-2.0
I1-0878a	75-11	QC 100000	3.8	1880060.4	100	105581	5.6
I1-0879a	75-10	QC 100000	3.8	2055425.5	100	119015	19
I1-0880a		50:50 ACN\H2O	3.87	7781.1	0	3.06	
I1-0881a	75-3	QC 30	3.79	90381.2	0	31.4	4.6
I1-0882a	75-2	QC 30	3.79	96763.4	0	33.7	12
I1-0883a	75-5	QC 300	3.79	674530.1	0	289	-3.7
I1-0884a	75-6	QC 300	3.79	725978.6	0	316	5.2
I1-0885a	76-3	78125, Day 0, 1M, T12-24	3.81	2383085	2	2912	
I1-0886a	76-4	78164, Day 0, 1F, T12-24	3.81	2709608.3	2	3479	
I1-0887a	75-9	QC 5000	3.79	4949990	0	4180	-16
I1-0888a	75-12	QC 100000	3.79	2176244	100	128591	29

Compound 1: PFHxA

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0889a		50:50 ACN\H2O	3.87	7552.8	0	2.98	
I1-0890a	74-4	C 30	3.79	89412.7	0	31.0	3.5
I1-0891a	74-6	C 100	3.79	260320.8	0	96.5	-3.5
I1-0892a	74-8	C 300	3.79	700477.4	0	302	0.75
I1-0893a	74-10	C 1000	3.79	1799562.1	0	996	-0.4
I1-0894a	74-12	C 3000	3.79	3694655	0	2706	-9.8
I1-0895a	74-14	C 5000	3.79	5105064.5	0	4381	-12

APPENDIX G

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

PHARMACOKINETIC (IN BLOOD) AND EXCRETION STUDY OF PERFLUOROHEXANOIC ACID AND NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

Analysis Of PFBS In Serum And Urine Samples

Analytical Chemistry Department

WIL Research Laboratories, LLC

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1. INTRODUCTION

This report provides a detailed description and cross-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 rat serum and urine. The method was developed in a separate study (Kirkpatrick, 2005) for determination of PFBS in monkey serum and urine, where method sensitivity, specificity/selectivity, calibration reproducibility, accuracy and precision were assessed and validated. In the present study, the method was cross-validated for the determination of PFBS in rat serum and 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 (minimum of 4 hours) room temperature storage and in samples after the freeze-thaw process was evaluated.

This report also details the analytical results from the determination of PFBS in rat serum and urine samples. Analysis of serum samples resulted in levels ranging from less than the lower limit of quantitation (LLOQ, 30 ng/mL) to 70,466 ng PFBS/mL. Analysis of urine samples resulted in levels ranging from 2,769 to 545,125 ng PFBS/mL.

2. BLANK MATRIX IDENTIFICATION

Blank rat 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 (serum or urine) 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 100,000 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 to 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 cross-validated for the determination of PFBS in rat serum and urine where a single validation session was conducted with each matrix. Method sensitivity, specificity/selectivity, calibration reproducibility, accuracy and precision were assessed and validated. The results of the cross-validations are summarized in Tables 1 and 2 (calibration samples) and 3 and 4 (QC samples). Stability of PFBS in processed samples, in samples during frozen storage (-20°C), in samples after short-term (minimum of

4 hours) 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 27 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 27).

Figures 28 through 44 illustrate typical chromatograms for a processed solvent blank (Figure 28), a processed urine blank (Figure 29), processed urine calibration samples (Figures 30 through 34), processed urine QC samples (Figures 35 through 38) and experimental urine samples (Figures 39 through 44).

4.2. SENSITIVITY

According to WIL SOP, the 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 rat serum and urine. The rat 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:	1.4	2.9
Urine:	1.9	4.8

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. 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 intra-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-session statistics for PFBS calibration samples used during the single cross-validation session for rat 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-session statistics for the calibration samples are summarized in the following table.

	%RE Range	%RSD Range
Serum:	-1.8 to 2.9	0.92 to 2.9
Urine:	-2.7 to 2.8	4.3 to 6.0

Based on the results in Tables 1 and 2, the reproducibility of the calibration data was acceptable for the determination of PFBS concentration in rat 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 ≤ 15%, except at the lowest concentration level where ≤ 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 ≤ 20% was acceptable.

The back-calculated concentration values and the associated intra-session statistics for the PFBS QC samples used during the single cross-validation session for rat 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-session statistics for the QC samples are summarized in the following table.

	%RE Range	%RSD Range
Serum:	11 to 15	1.9 to 2.9
Urine:	-13 to 1.0	3.9 to 8.6

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 rat serum and urine.

4.6. STABILITY

Stability of PFBS in processed samples, in samples during frozen storage (-20°C), in samples after short-term (minimum of 4 hours) room temperature storage and in samples after the freeze-thaw process was evaluated. According to WIL standard operating procedures (SOP), stability is demonstrated 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 rat 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 PFBS concentrations.

The mean measured concentrations in serum samples after 6 days of refrigerated storage ranged from 81.3% to 88.7% of the corresponding time-zero concentrations (Table 5). Since post-storage concentrations of PFBS did not meet the specified criteria, samples should be analyzed as soon as possible after sample processing along with standards and QC samples processed at the same time.

The mean measured concentrations in urine samples after 1 day of refrigerated storage ranged from 90.3% to 97.0% of the corresponding time-zero concentrations (Table 6). Since the post-storage concentrations met the specified criteria, PFBS in processed rat urine samples was considered to be stable through 1 day of refrigerated storage.

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

Frozen (-20°C) storage stability of PFBS in rat serum and urine was assessed. Chemical degradation usually occurs at a rate described by a monoexponential decay function: $C_t = C_0 \times e^{(kt)}$ where C_0 is the analyte concentration at t = 0, C_t is the analyte concentration after storage time = t days and k is the degradation constant. Tables 7

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

	Serum	Urine
100 ng/mL:	$-4.54e^{-04}$	$2.79e^{-03}$
750 ng/mL:	$-1.03e^{-03}$	$1.20e^{-03}$

For urine samples, degradation constants were positive, indicating no detectable loss during the storage of the samples. Based on the largest (most negative) degradation constant for serum (-0.00103), a 10% decrease in PFBS concentration would occur in 102 days.

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 a minimum of 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 4.5 hours of room temperature storage were 105% and 97.4% of the corresponding time-zero concentrations (Table 9). Since post-storage concentrations of PFBS met the specified criteria, PFBS in rat serum samples was considered to be stable after 4.5 hours of room temperature storage.

The mean measured concentrations in urine samples after 4 hours of room temperature storage were 120% and 98.1% of the corresponding time-zero concentrations (Table 10). Since post-storage concentrations of PFBS met the specified criteria, PFBS in rat 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 rat 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 95.0% to 105% of the corresponding time-zero concentrations (Table 11). Since concentrations of PFBS after freezing and thawing met the specified criteria, PFBS in rat 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 91.5% to 114% of the corresponding time-zero concentrations (Table 12) with the exception of the second freeze-thaw cycle at 100 ng/mL (87.4%). Since concentrations of PFBS after the third freeze-thaw cycle met the specified criteria, PFBS in rat urine samples was considered to be stable through 3 freeze-thaw cycles.

4.7. ANALYSIS OF EXPERIMENTAL SAMPLES

Rat 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 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 stated criteria, all the PFBS analyses were acceptable.

5. CONCLUSION

An HPLC/MS/MS ESI method for the determination of PFBS in rat serum and urine was cross-validated. Stability of PFBS in processed samples, in samples during frozen storage (-20°C), in samples after short-term (minimum of 4 hours) room temperature storage and in samples after the freeze-thaw process was evaluated. Analysis of serum samples resulted in levels ranging from less than the LLOQ (30 ng/mL) to 70,466 ng PFBS/mL. Analysis of urine samples resulted in levels ranging from 2,769 to 545,125 ng PFBS/mL.

6. REFERENCES

Kirkpatrick, J.B. A Pharmacokinetic (In Blood) And Excretion Study In Cynomolgus Monkeys (Study No. WIL-534002). WIL Research Laboratories, LLC, Ashland, OH, **2005.**

7. KEY STUDY PERSONNEL AND REPORT SUBMISSION

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17 Oct 2005
Date

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TABLES 1-14

PHARMACOKINETIC (IN BLOOD) AND EXCRETION
STUDY OF PERFLUOROHEXANOIC ACID AND
NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

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

Theo. Conc. (ng/mL)	**30**	**60**	**100**	**300**	**1000**
Set 1 (29 Jun 2005), 534004(PFBS)BRS, analyst SMH					
Samp 1	30.0	58.6	101	314	1001
Samp 2	29.9	59.1	96.9	301	1002
Samp 3	31.4	59.7	96.6	311	969
Intra-session Statistics					
n	3	3	3	3	3
Mean	30.4	59.1	98.2	309	991
SD	0.87	0.54	2.6	6.8	18.5
RSD	2.9	0.92	2.7	2.2	1.9
%RE	1.4	-1.4	-1.8	2.9	-0.94

PHARMACOKINETIC (IN BLOOD) AND EXCRETION
STUDY OF PERFLUOROHEXANOIC ACID AND
NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

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

Theo. Conc. (ng/mL)	**30**	**60**	**100**	**300**	**1000**
Set 1 (29 Jun 2005), 534004(PFBS)BRU, analyst SMH					
Samp 1	30.7	60.8	104	293	1010
Samp 2	29.1	59.9	100	304	1021
Samp 3	32.0	54.5	93.4	329	943
Intra-session Statistics					
n	3	3	3	3	3
Mean	30.6	58.4	99.4	308	991
SD	1.5	3.4	5.5	19	43
RSD	4.8	5.8	5.5	6.0	4.3
%RE	1.9	-2.7	-0.62	2.8	-0.86

PHARMACOKINETIC (IN BLOOD) AND EXCRETION
STUDY OF PERFLUOROHEXANOIC ACID AND
NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

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

Theo. Conc. (ng/mL)	**30**	**100**	**750**
Set 1 (29 Jun 2005), 534004(PFBS)BRS, analyst SMH			
Samp 1	32.5	115	837
Samp 2	33.8	109	879
Samp 3	33.3	112	882
Intra-session Statistics			
n	3	3	3
Mean	33.2	112	866
SD	0.62	3.2	25
RSD	1.9	2.9	2.9
%RE	11	12	15

PHARMACOKINETIC (IN BLOOD) AND EXCRETION
STUDY OF PERFLUOROHEXANOIC ACID AND
NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

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

Theo. Conc. (ng/mL)	**30**	**100**	**750**
Set 1 (29 Jun 2005), 534004(PFBS)BRU, analyst SMH			
Samp 1	24.7	93.8	730
Samp 2	27.9	89.4	755
Samp 3	26.0	79.2	789
Intra-set Statistics			
n	3	3	3
Mean	26.2	87.5	758
SD	1.6	7.5	29
RSD	6.2	8.6	3.9
%RE	-13	-13	1.0

534004 PFBS bio results.xls Summary II (PFBS)RU
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PHARMACOKINETIC (IN BLOOD) AND EXCRETION
STUDY OF PERFLUOROHEXANOIC ACID AND
NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

Table 5: 6-Day Refrigerated PFBS Stability Analysis Of Processed Rat Serum Samples

Time Point	Date Analyzed	Theo. Conc (ng/mL)	Ref # (534004 -)	Run #	Conc.	Mean Conc.	Percent of Time Zero (%)
Quality Control Samples							
T = 0	27 Aug 2005	30	94 - 1	I1-1033	29.9	29.3	---
			94 - 2	I1-1053	29.2		
			94 - 3	I1-1073	28.8		
6-Day	02 Sep 2005		94 - 1	I1-1272	27.2	25.7	87.7
			94 - 2	I1-1273	25.0		
			94 - 3	I1-1274	24.9		
T = 0	27 Aug 2005	100	94 - 4	I1-1034	97.0	98.3	---
			94 - 5	I1-1054	96.2		
			94 - 6	I1-1074	102		
6-Day	02 Sep 2005		94 - 4	I1-1278	75.2	79.9	81.3
			94 - 5	I1-1279	72.3		
			94 - 6	I1-1280	92.4		
T = 0	27 Aug 2005	750	94 - 7	I1-1035	790	748	---
			94 - 8	I1-1055	727		
			94 - 9	I1-1075	729		
6-Day	02 Sep 2005		94 - 7	I1-1284	730	664	88.7
			94 - 8	I1-1285	701		
			94 - 9	I1-1286	560		

534004 PFBS bio results.xls PSS(PFBS) RS
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PHARMACOKINETIC (IN BLOOD) AND EXCRETION
STUDY OF PERFLUOROHEXANOIC ACID AND
NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

Table 6: 1-Day Refrigerated PFBS Stability Analysis Of Processed Rat Urine Samples

Time Point	Date Analyzed	Theo. Conc (ng/mL)	Ref # (534004 -)	Run #	Conc.	Mean Conc.	Percent of Time Zero (%)
Quality Control Samples							
T = 0	30 Jun 2005	30	12 - 1	I1-0198	24.7	26.2	---
			12 - 2	I1-0199	27.9		
			12 - 3	I1-0200	26.0		
1-Day	01 Jul 2005		12 - 1	I1-0264	24.8	23.7	90.3
			12 - 2	I1-0265	23.8		
			12 - 3	I1-0266	22.3		
T = 0	30 Jun 2005	100	12 - 4	I1-0201	93.8	87.5	---
			12 - 5	I1-0202	89.4		
			12 - 6	I1-0203	79.2		
1-Day	01 Jul 2005		12 - 4	I1-0267	79.2	81.0	92.6
			12 - 5	I1-0268	81.1		
			12 - 6	I1-0269	82.9		
T = 0	30 Jun 2005	750	12 - 7	I1-0204	730	758	---
			12 - 8	I1-0205	755		
			12 - 9	I1-0206	789		
1-Day	01 Jul 2005		12 - 7	I1-0270	725	735	97.0
			12 - 8	I1-0271	718		
			12 - 9	I1-0272	763		

534004 PFBS bio results.xls PSS(PFBS) RU
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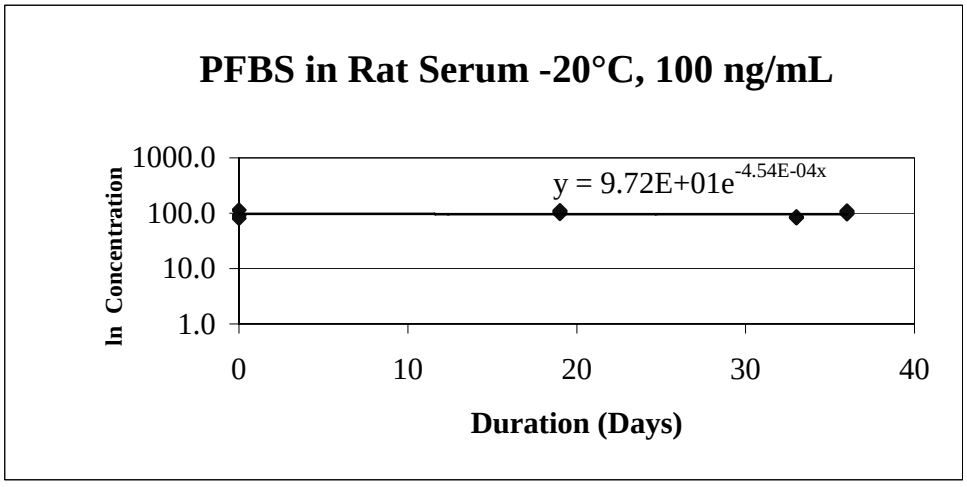
PHARMACOKINETIC (IN BLOOD) AND EXCRETION
STUDY OF PERFLUOROHEXANOIC ACID AND
NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

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

Theo. Conc. (ng/mL)	Storage Duration (Days)	Run# (534004-)	Ref. # (534004-)	Assay Conc. (ng/mL)	Mean Conc. (ng/mL)	Mean % of Target	k
100	0	I1-0446	30-4	80.9	95.6	95.6	-4.54E-04
	0	I1-0476	30-5	91.3			
	0	I1-0506	30-6	115			
	19	I1-0669	56-1	98.1	105	105	
	19	I1-0670	56-2	105			
	19	I1-0671	56-3	111			
	33	I1-0948c	78-1	83.5	84.3	84.3	
	33	I1-0949c	78-2	86.4			
	33	I1-0950c	78-3	83.0			
	36	I1-1037	95-1	97.0	103	103	
	36	I1-1038	95-2	110.5			
	36	I1-1039	95-3	102.2			



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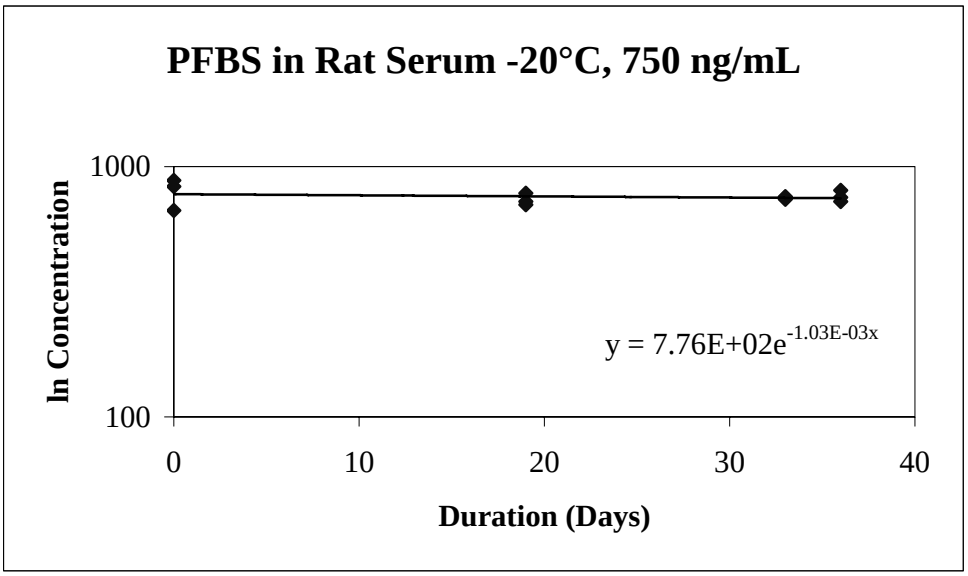
PHARMACOKINETIC (IN BLOOD) AND EXCRETION
STUDY OF PERFLUOROHEXANOIC ACID AND
NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

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

Theo. Conc. (ng/mL)	Storage Duration (Days)	Run# (534004-)	Ref. # (534004-)	Assay Conc. (ng/mL)	Mean Conc. (ng/mL)	Mean % of Target	k
750	0	I1-0447	30-7	668	794	106	-1.03E-03
	0	I1-0477	30-8	834			
	0	I1-0507	30-9	880			
	19	I1-0672	56-4	784	737	98.3	
	19	I1-0673	56-5	724			
	19	I1-0674	56-6	704			
	33	I1-0951c	78-4	758	751	100	
	33	I1-0952c	78-5	740			
	33	I1-0953c	78-6	756			
	36	I1-1057	95-4	754	761	101	
	36	I1-1058	95-5	726			
	36	I1-1059	95-6	804			



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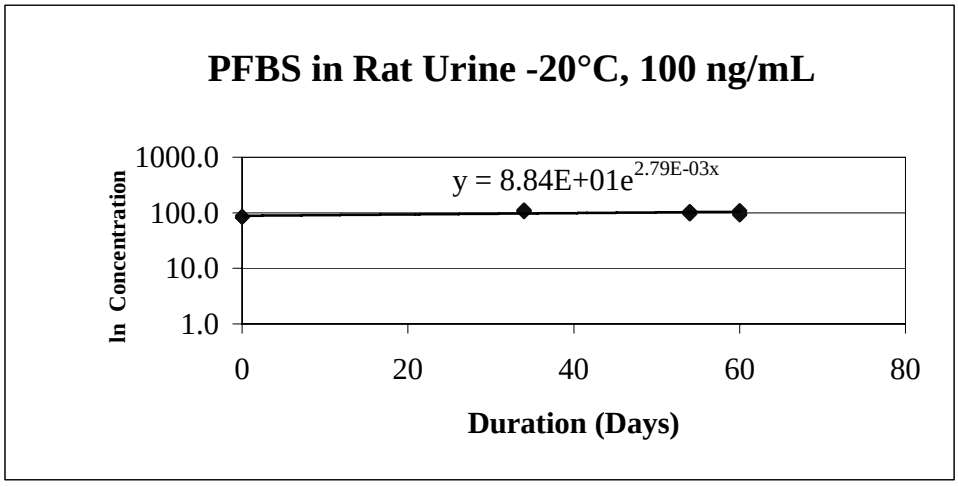
PHARMACOKINETIC (IN BLOOD) AND EXCRETION
STUDY OF PERFLUOROHEXANOIC ACID AND
NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

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

Theo. Conc. (ng/mL)	Storage Duration (Days)	Run# (534004-)	Ref. # (534004-)	Assay Conc. (ng/mL)	Mean Conc. (ng/mL)	Mean % of Target	k
100	0	I1-0235	14-4	86.2	84.5	84.5	2.79E-03
	0	I1-0253	14-5	83.8			
	0	I1-0275	14-6	83.5			
	34	I1-0595	49-1	104	108	108	
	34	I1-0596	49-2	112			
	34	I1-0597	49-3	108			
	54	I1-0998b	83-1	95.7	101	101	
	54	I1-0999b	83-2	104.9			
	54	I1-1000b	83-3	103.0			
	60	I1-1178	102-1	92.5	99.9	99.9	
	60	I1-1179	102-2	98.6			
	60	I1-1180	102-3	108.7			



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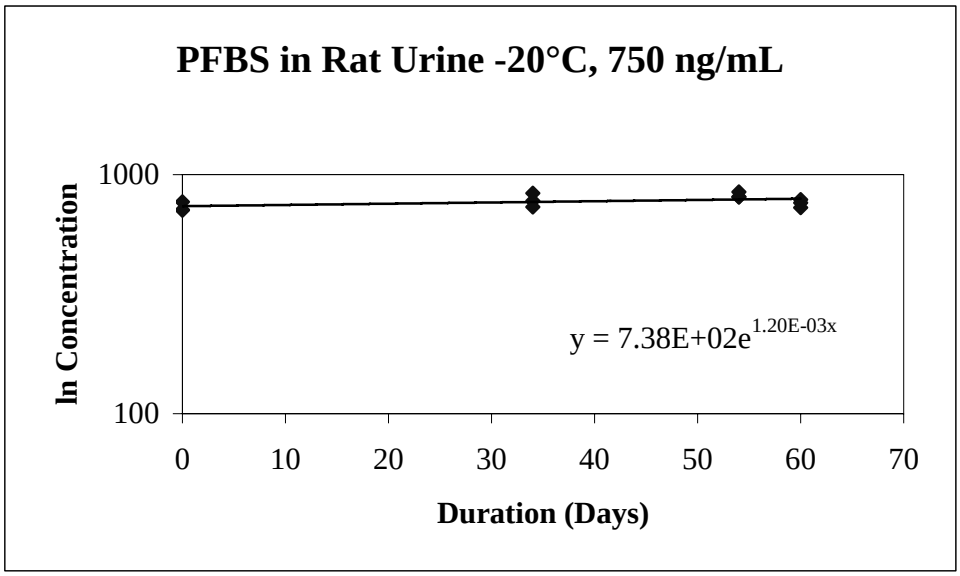
PHARMACOKINETIC (IN BLOOD) AND EXCRETION
STUDY OF PERFLUOROHEXANOIC ACID AND
NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

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

Theo. Conc. (ng/mL)	Storage Duration (Days)	Run# (534004-)	Ref. # (534004-)	Assay Conc. (ng/mL)	Mean Conc. (ng/mL)	Mean % of Target	k
750	0	I1-0236	14-7	710	731	97.5	1.20E-03
	0	I1-0254	14-8	712			
	0	I1-0276	14-9	771			
	34	I1-0598	49-4	732	781	104	
	34	I1-0599	49-5	835			
	34	I1-0600	49-6	776			
	54	I1-1001b	83-4	805	820	109	
	54	I1-1002b	83-5	846			
	54	I1-1003b	83-6	807			
	60	I1-1181	102-4	787	759	101	
	60	I1-1182	102-5	728			
	60	I1-1183	102-6	763			



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PHARMACOKINETIC (IN BLOOD) AND EXCRETION
STUDY OF PERFLUOROHEXANOIC ACID AND
NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

Table 9: 4.5-Hour Room Temperature Stability Of PFBS In Rat Serum

Theo. Conc. (ng/mL)	Time hr (hr)	Run # (534004-)	Analyzed Conc (ng/mL)	% Target (%)	Mean Conc (ng/mL)	RSD (%)	Mean % Target (%)	% of Time Zero (%)
100	0	I1-0446	80.9	80.9	95.6	18	95.6	---
		I1-0476	91.3	91.3				
		I1-0506	115	115				
	4.5	I1-1040	105	105	100	5.0	100	105
		I1-1041	99.5	99.5				
		I1-1042	95.6	95.6				
750	0	I1-0447	668	89.1	794	14	106	---
		I1-0477	834	111				
		I1-0507	880	117				
	4.5	I1-1060	762	102	774	1.9	103	97.4
		I1-1061	790	105				
		I1-1062	769	103				

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PHARMACOKINETIC (IN BLOOD) AND EXCRETION
STUDY OF PERFLUOROHEXANOIC ACID AND
NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

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

Theo. Conc. (ng/mL)	Time hr (hr)	Run # (534004-)	Analyzed Conc (ng/mL)	% Target (%)	Mean Conc (ng/mL)	RSD (%)	Mean % Target (%)	% of Time Zero (%)
100	0	I1-0235	86.2	86.2	84.5	1.784	84.5	---
		I1-0253	83.8	83.8				
		I1-0275	83.5	83.5				
	4	I1-0606	100	100	101	8.3	101	120
		I1-0607	110	110				
		I1-0608	93.1	93.1				
750	0	I1-0236	710	94.7	731	4.7	97.5	---
		I1-0254	712	95.0				
		I1-0276	771	103				
	4	I1-0609	690	92.0	717	9.8	95.6	98.1
		I1-0610	797	106.2				
		I1-0611	663	88.4				

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PHARMACOKINETIC (IN BLOOD) AND EXCRETION
STUDY OF PERFLUOROHEXANOIC ACID AND
NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

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

Theo. Conc. (ng/mL)	# of Cycles	Run # (534004-)	Analyzed Conc (ng/mL)	% Target (%)	Mean Conc (ng/mL)	RSD (%)	Mean % Target (%)	% of Time Zero (%)
Rat Serum								
100	0	I1-0446	80.9	80.9	95.6	18	95.6	---
		I1-0476	91.3	91.3				
		I1-0506	115	115				
	1	I1-1043	103	103	97.8	4.5	97.8	102
		I1-1044	95.2	95.2				
		I1-1045	95.3	95.3				
	2	I1-1046	98.5	98.5	101	2.1	101	105
		I1-1047	101	101				
		I1-1048	103	103				
	3	I1-1049	100	100	98.5	1.8	98.5	103
		I1-1050	96.6	96.6				
		I1-1051	98.6	98.6				
750	0	I1-0447	668	89.1	794	14	106	---
		I1-0477	834	111				
		I1-0507	880	117				
	1	I1-1063	742	99.0	791	6.0	105	99.6
		I1-1064	837	112				
		I1-1065	794	106				
	2	I1-1066	791	105	785	5.8	105	98.9
		I1-1067	828	110				
		I1-1068	738	98.3				
	3	I1-1069	800	107	754	5.3	100.5	95.0
		I1-1070	736	98.1				
		I1-1071	727	96.9				

534004 PFBS bio results.xls FT (PFBS) RS
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PHARMACOKINETIC (IN BLOOD) AND EXCRETION
STUDY OF PERFLUOROHEXANOIC ACID AND
NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

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

Theo. Conc. (ng/mL)	# of Cycles	Run # (534004-)	Analyzed Conc (ng/mL)	% Target (%)	Mean Conc (ng/mL)	RSD (%)	Mean % Target (%)	% of Time Zero (%)
Rat Urine								
100	0	I1-0592	102	102	97.5	5.3	97.5	---
		I1-0603	99.1	99.1				
		I1-0614	91.8	91.8				
	1	I1-1352	81.2	81.2	94.9	16.2	94.9	97.3
		I1-1353	112	112				
		I1-1354	92.0	92.0				
	2	I1-1355	80.2	80.2	85.3	7.4	85.3	87.4
		I1-1356	83.2	83.2				
		I1-1357	92.4	92.4				
	3	I1-1358	91.4	91.4	89.2	3.9	89.2	91.5
		I1-1359	85.2	85.2				
		I1-1360	91.1	91.1				
750	0	I1-0593	745	99.3	767	2.5	102	---
		I1-0604	774	103				
		I1-0615	781	104				
	1	I1-1364	880	117	875	12	117	114
		I1-1365	982	131				
		I1-1366	764	102				
	2	I1-1367	933	124	833	11	111	109
		I1-1368	769	103				
		I1-1369	795	106				
	3	I1-1370	820	109	771	5.5	103	101
		I1-1371	746	99.4				
		I1-1372	749	99.8				

534004 PFBS bio results.xls FT (PFBS) RU
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PHARMACOKINETIC (IN BLOOD) AND EXCRETION
STUDY OF PERFLUOROHEXANOIC ACID AND
NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

Table 13: Day 0 Rat Serum Experimental Sample PFBS Concentrations

Run #	Ref #	Animal #	Sex	Timepoint (hrs)	PFBS ng/mL
I1-0450	34-1	78121	M	0	< LLOQ
I1-0451	34-2	78126	M	0	< LLOQ
I1-0452	34-3	78129	M	0	< LLOQ
I1-0453	34-4	78146	F	0	< LLOQ
I1-0454	34-5	78155	F	0	< LLOQ
I1-0455	34-6	78157	F	0	< LLOQ
I1-0456	31-1	78132	M	0.5	70466
I1-0457	31-2	78134	M	0.5	48916
I1-0458	31-3	78136	M	0.5	57418
I1-0459	31-4	78158	F	0.5	24796
I1-0460	31-5	78161	F	0.5	21875
I1-0461	31-6	78163	F	0.5	25414
I1-0462	31-7	78141	M	1	47082
I1-0463	31-8	78143	M	1	56092
I1-0464	31-9	78145	M	1	45263
I1-0465	31-10	78165	F	1	9621
I1-0466	31-11	78174	F	1	8869
I1-0467	31-12	78175	F	1	7218
I1-0468	32-1	78121	M	1.5	39153
I1-0469	32-2	78126	M	1.5	33474
I1-0470	32-3	78129	M	1.5	31249
I1-0645	55-1	78146	F	1.5	5342
I1-0646	55-2	78155	F	1.5	4681
I1-0647	55-3	78157	F	1.5	3346
I1-0480	32-7	78132	M	2	37057
I1-0481	32-8	78134	M	2	19728
I1-0482	32-9	78136	M	2	34107
I1-0928c	77a-1	78158	F	2	3267
I1-0649	55-5	78161	F	2	3384
I1-0650	55-6	78163	F	2	4923

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

PHARMACOKINETIC (IN BLOOD) AND EXCRETION
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Table 13: Day 0 Rat Serum Experimental Sample PFBS Concentrations

Run #	Ref #	Animal #	Sex	Timepoint (hrs)	PFBS ng/mL
I1-0486	33-1	78141	M	4	44574
I1-0487	33-2	78143	M	4	20529
I1-0488	33-3	78145	M	4	18535
I1-0929c	77a-2	78165	F	4	512
I1-0930c	77a-3	78174	F	4	507
I1-0931c	77a-4	78175	F	4	282
I1-0492	33-7	78121	M	8	4937
I1-0493	33-8	78126	M	8	4494
I1-0494	33-9	78129	M	8	3569
I1-0932c	77a-5	78146	F	8	< LLOQ
I1-0933c	77a-6	78155	F	8	< LLOQ
I1-0934c	77a-7	78157	F	8	< LLOQ
I1-0935c	77a-8	78132	M	24	66.1
I1-0936c	77a-9	78134	M	24	< LLOQ
I1-0937c	77a-10	78136	M	24	< LLOQ
I1-0944c	77a-11	78158	F	24	< LLOQ
I1-0945c	77a-12	78161	F	24	< LLOQ
I1-0946c	77a-13	78163	F	24	< LLOQ

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

PHARMACOKINETIC (IN BLOOD) AND EXCRETION
STUDY OF PERFLUOROHEXANOIC ACID AND
NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

Table 14: Day 0 Rat Urine Experimental Sample PFBS Concentrations

Run #	Ref #	Animal #	Sex	Timept. (hrs)	PFBS ng/mL	Urine Vol (mL)	Total PFBS (µg)
I1-0544	42-1	78133	M	0-6	148632	5	743
I1-0545	42-2	78135	M	0-6	244468	5	1222
I1-0546	42-3	78139	M	0-6	203697	6	1222
I1-0242	15-4	78160	F	0-6	545125	3	1635
I1-0547	42-4	78169	F	0-6	184499	5	922
I1-0548	42-5	78170	F	0-6	187637	5	938
I1-0245	16-1	78133	M	6-12	132013	6	792
I1-0246	16-2	78135	M	6-12	36298	9	327
I1-0247	16-3	78139	M	6-12	35919	10	359
I1-0248	16-4	78160	F	6-12	40937	4	164
I1-0549	43-1	78169	F	6-12	25053	5	125
I1-0556	43-2	78170	F	6-12	18446	7	129
I1-0257	17-1	78133	M	12-24	17164	8	137
I1-1238	111-1	78135	M	12-24	5631	14	78.8
I1-1174	104-1	78139	M	12-24	9291	14	130
I1-1175	104-2	78160	F	12-24	5683	9	51.1
I1-1176	104-3	78169	F	12-24	7514	6	45.1
I1-1177	104-4	78170	F	12-24	2769	11	30.5

FIGURES 1-44

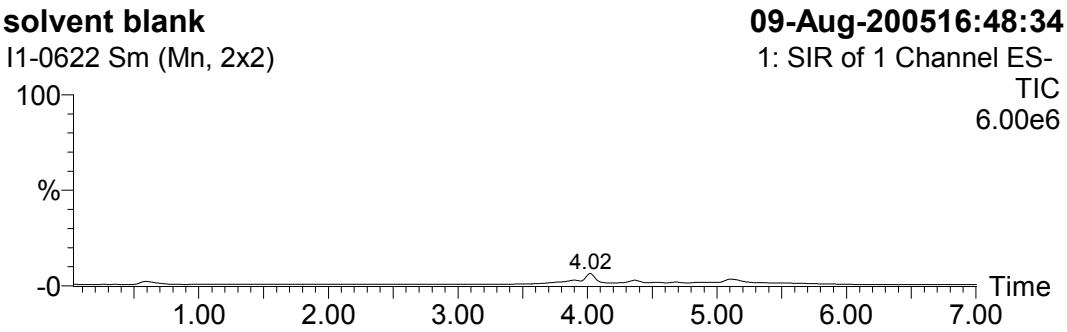

Figure 1: Representative Chromatogram Of A Processed Solvent Blank (Serum Assay)

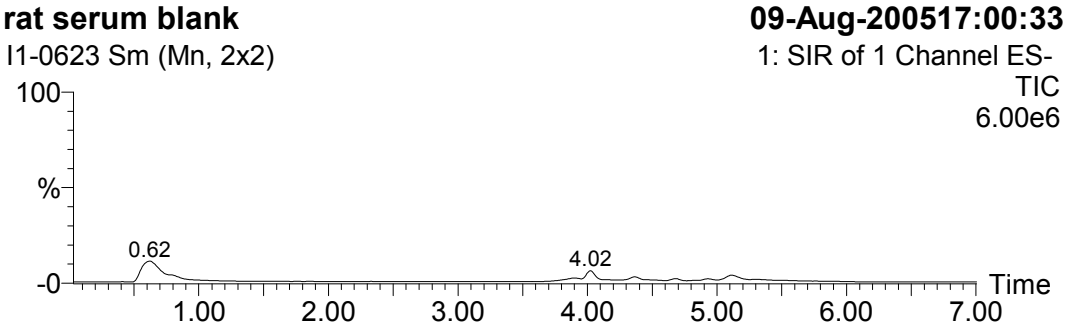

Figure 2: Representative Chromatogram Of A Processed Blank Serum Sample

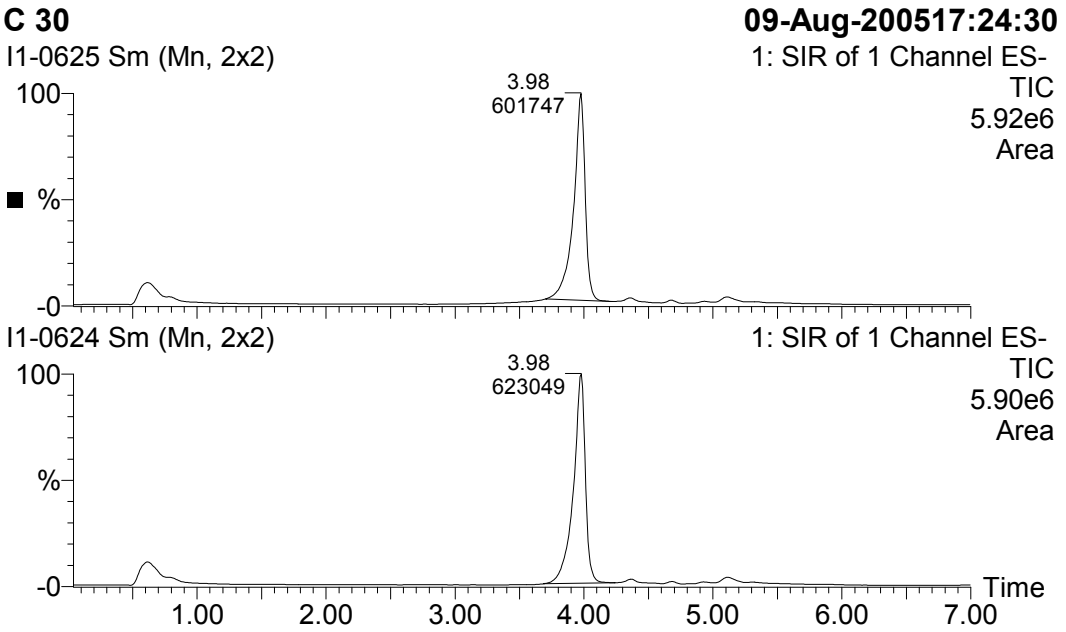

Figure 3: Representative Chromatograms Of 30 ng/mL Serum Calibration Samples

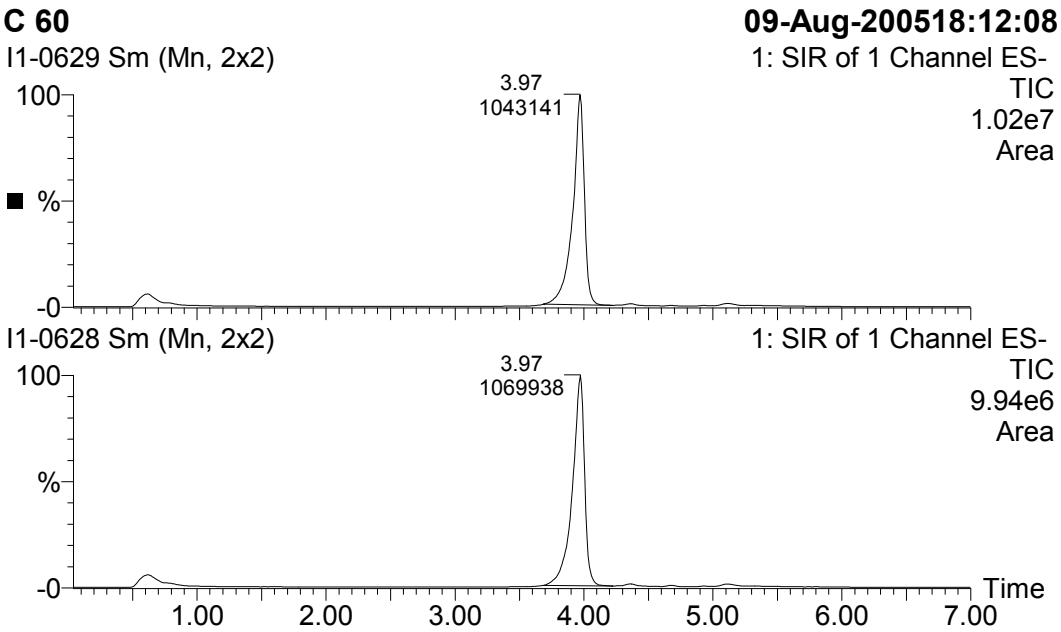

Figure 4: Representative Chromatograms Of 60 ng/mL Serum Calibration Samples

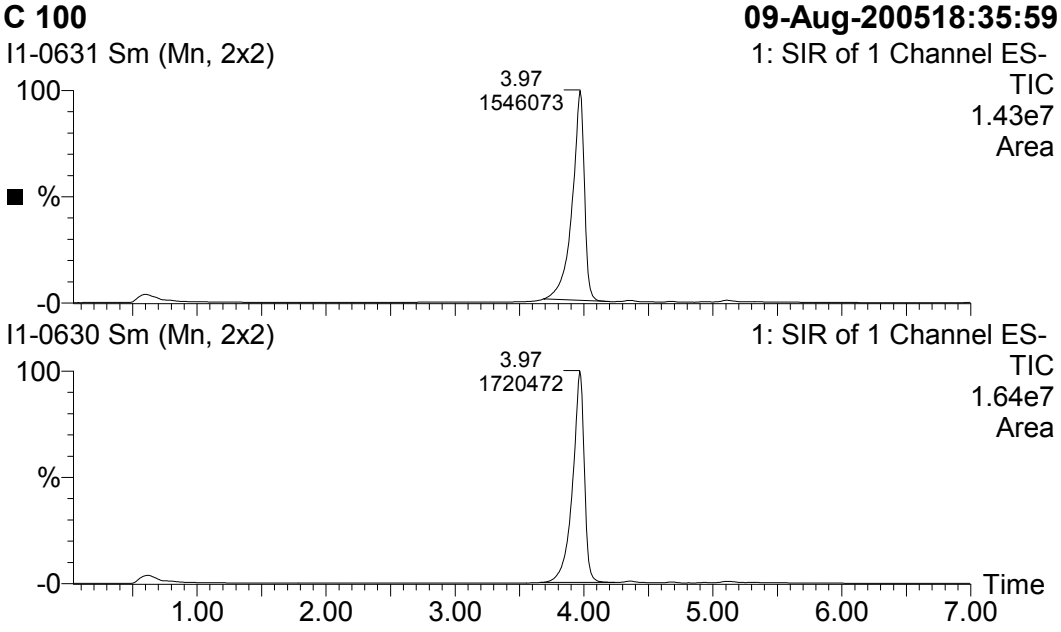

Figure 5: Representative Chromatograms Of 100 ng/mL Serum Calibration Samples

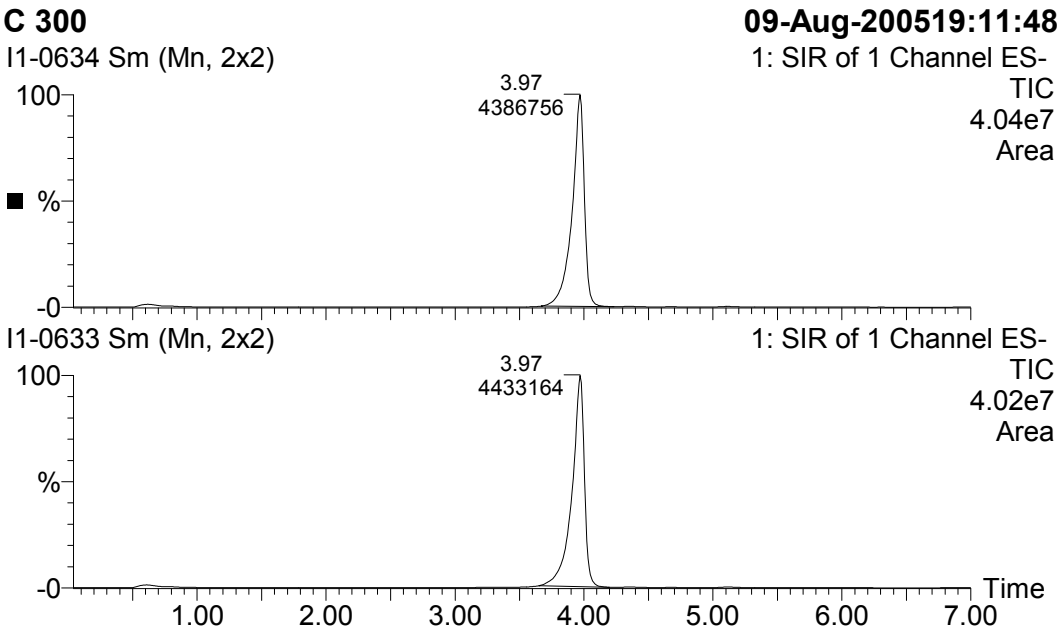

Figure 6: Representative Chromatograms Of 300 ng/mL Serum Calibration Samples

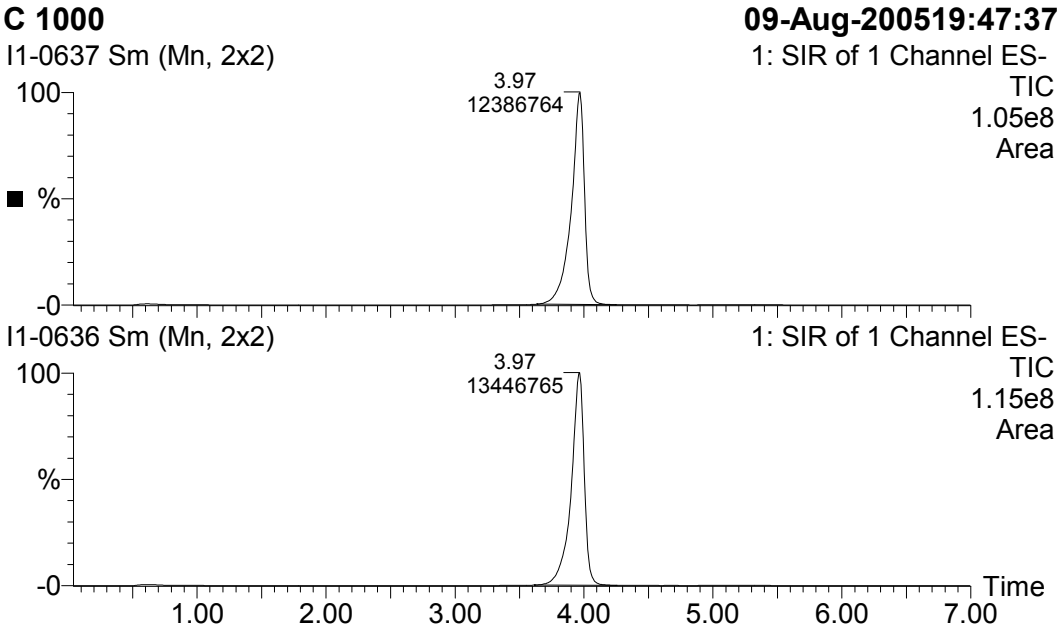

Figure 7: Representative Chromatograms Of 1000 ng/mL Serum Calibration Samples

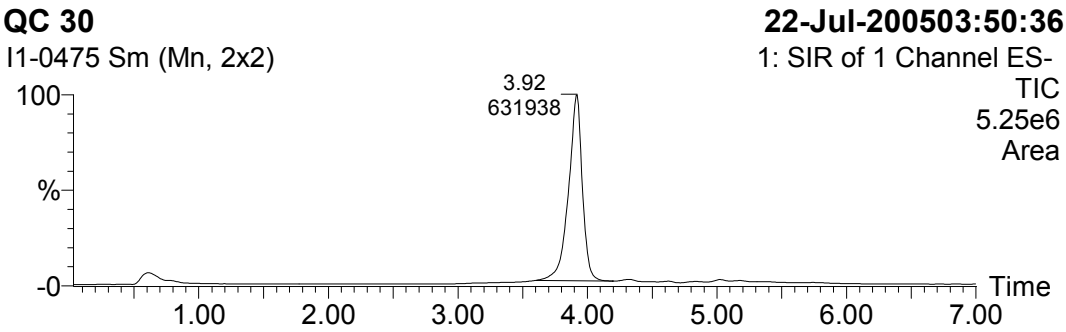

Figure 8: Representative Chromatogram Of A 30 ng/mL Serum QC Sample

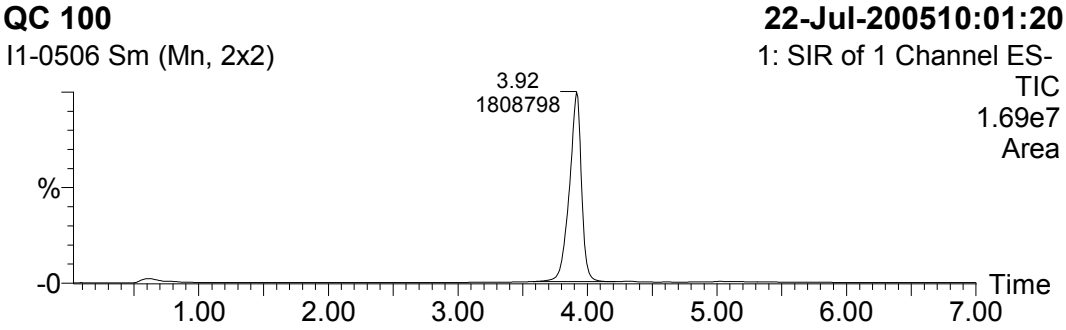

Figure 9: Representative Chromatogram Of A 100 ng/mL Serum QC Sample

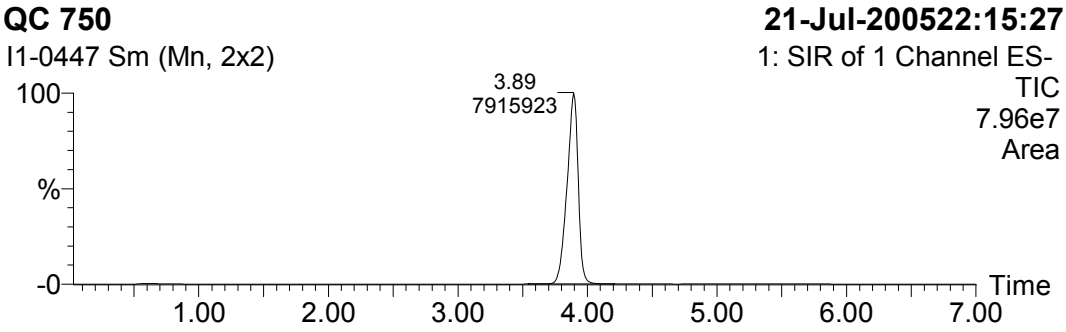

Figure 10: Representative Chromatogram Of A 750 ng/mL Serum QC Sample

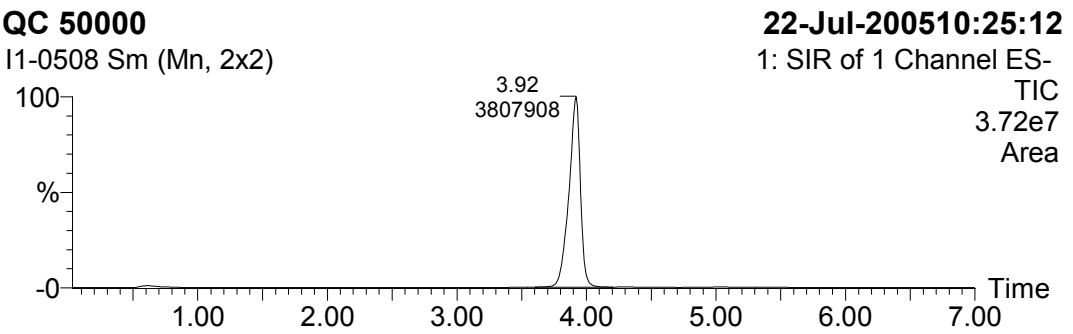

Figure 11: Representative Chromatogram Of A Dilutional 50,000 ng/mL Serum QC Sample

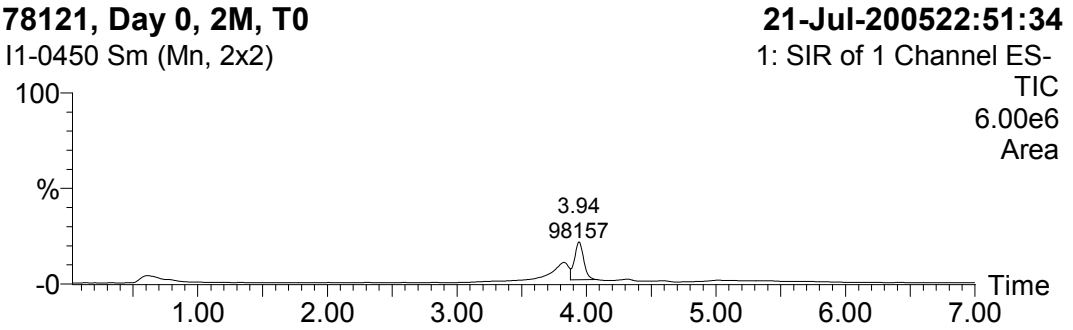

Figure 12: Chromatogram Of Animal No. 78121, Group T2, Male, Pre-Dose Serum Sample

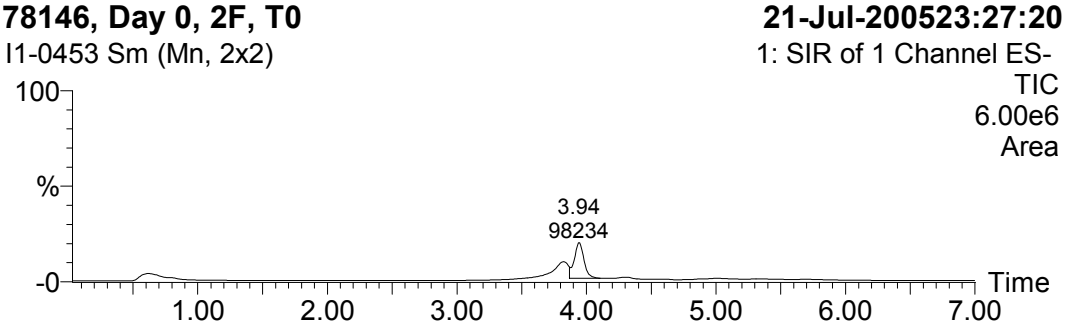

Figure 13: Chromatogram Of Animal No. 78146, Group T2, Female, Pre-Dose Serum Sample

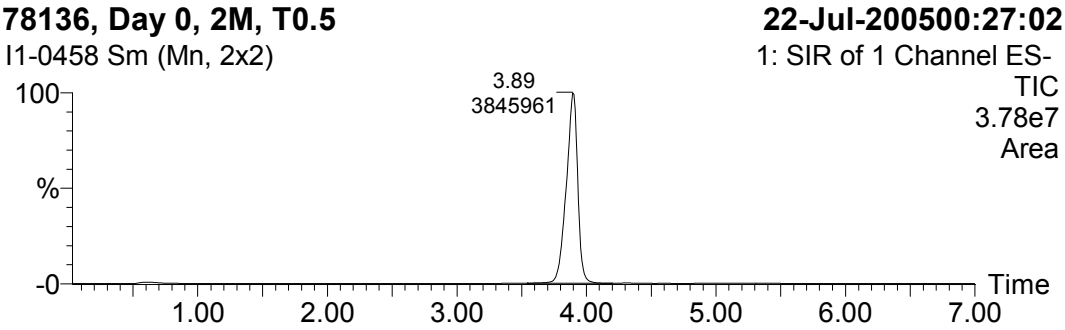

Figure 14: Chromatogram Of Animal No. 78136, Group T2, Male, 0.5 Hour Post-Dose Serum Sample

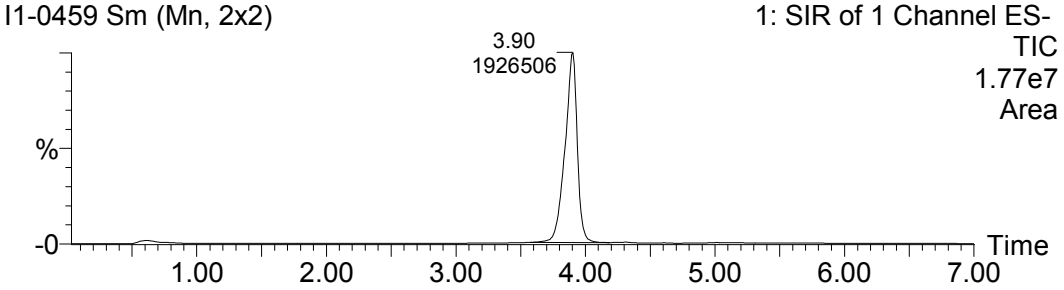

Figure 15: Chromatogram Of Animal No. 78158, Group T2, Female, 0.5 Hour Post-Dose Serum Sample

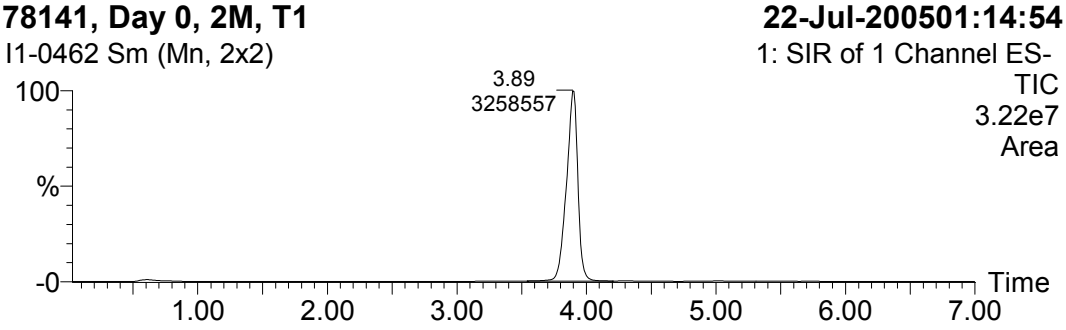

Figure 16: Chromatogram Of Animal No. 78141, Group T2, Male, 1 Hour Post-Dose Serum Sample

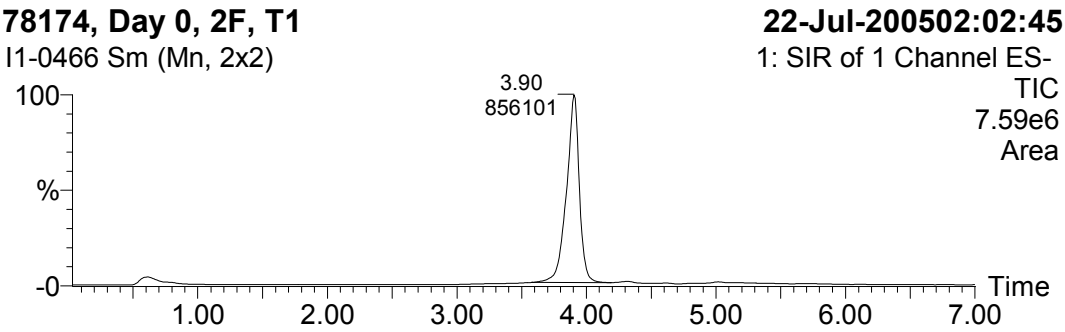

Figure 17: Chromatogram Of Animal No. 78174, Group T2, Female, 1 Hour Post-Dose Serum Sample

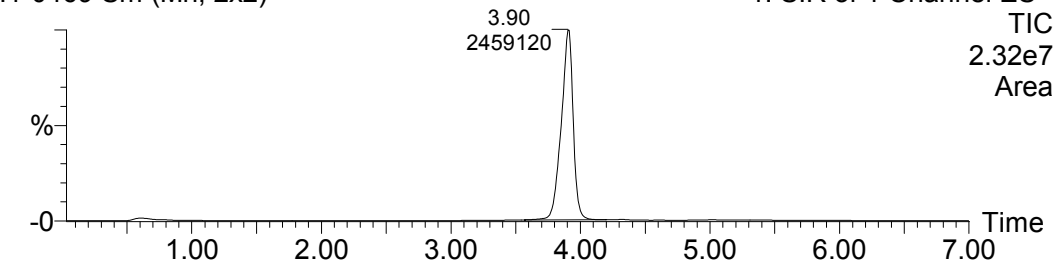

Figure 18: Chromatogram Of Animal No. 78126, Group T2, Male, 1.5 Hour Post-Dose Serum Sample

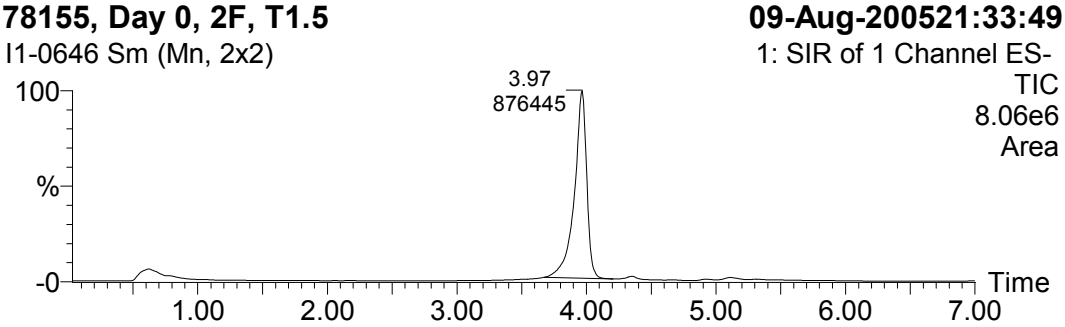

Figure 19: Chromatogram Of Animal No. 78155, Group T2, Female, 1.5 Hour Post-Dose Serum Sample

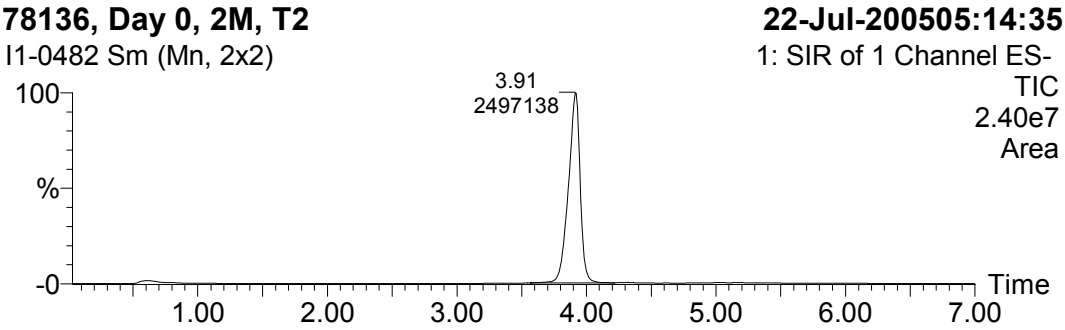

Figure 20: Chromatogram Of Animal No. 78136, Group T2, Male, 2 Hour Post-Dose Serum Sample

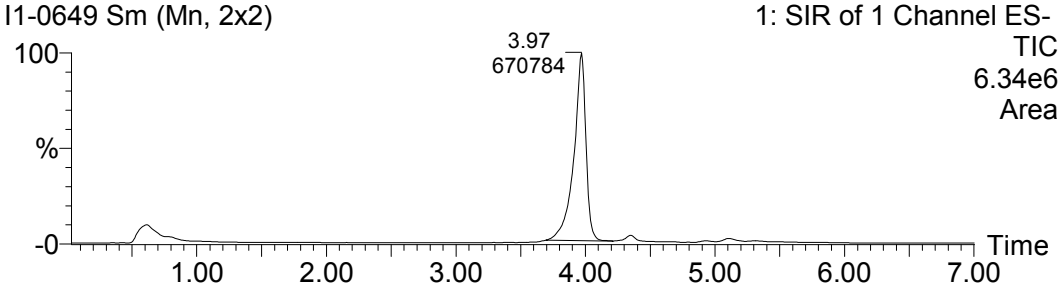

Figure 21: Chromatogram Of Animal No. 78161, Group T2, Female, 2 Hour Post-Dose Serum Sample

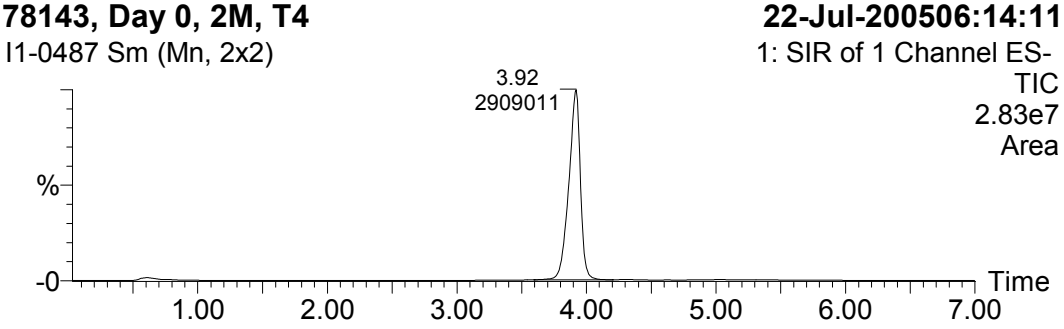

Figure 22: Chromatogram Of Animal No. 78143, Group T2, Male, 4 Hour Post-Dose Serum Sample

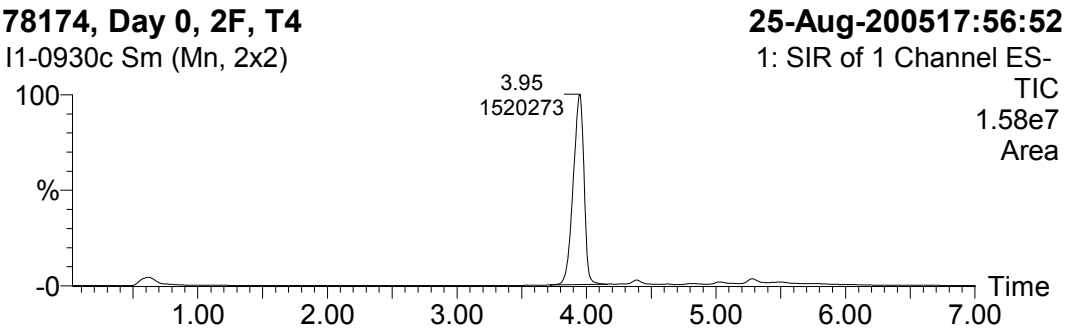

Figure 23: Chromatogram Of Animal No. 78174, Group T2, Female, 4 Hour Post-Dose Serum Sample

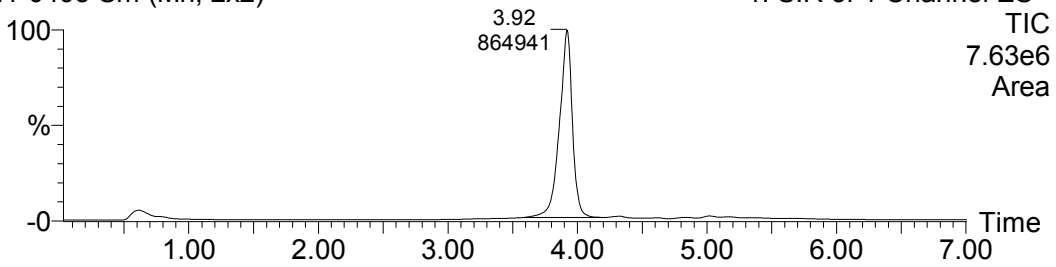

Figure 24: Chromatogram Of Animal No. 78126, Group T2, Male, 8 Hour Post-Dose Serum Sample

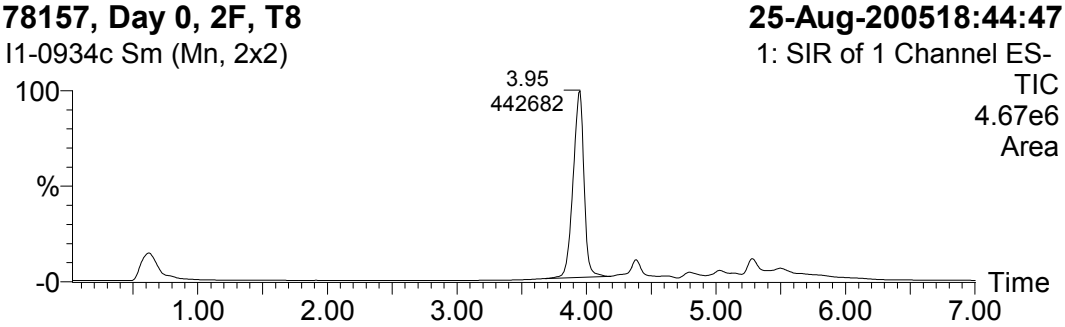

Figure 25: Chromatogram Of Animal No. 78157, Group T2, Female, 8 Hour Post-Dose Serum Sample

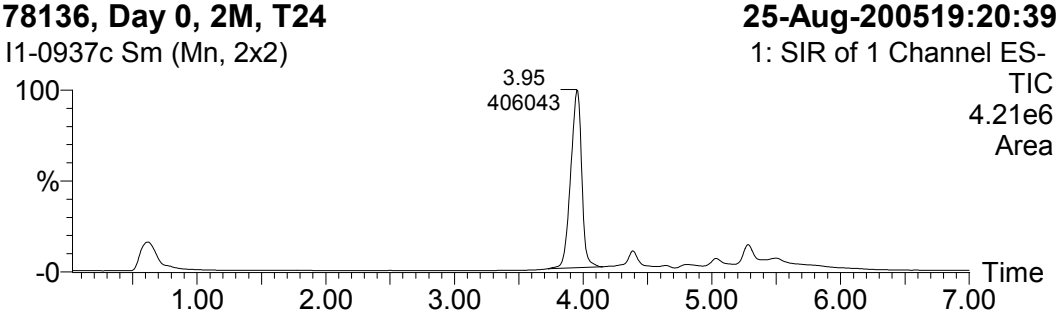

Figure 26: Chromatogram Of Animal No. 78136, Group T2, Male, 24 Hour Post-Dose Serum Sample

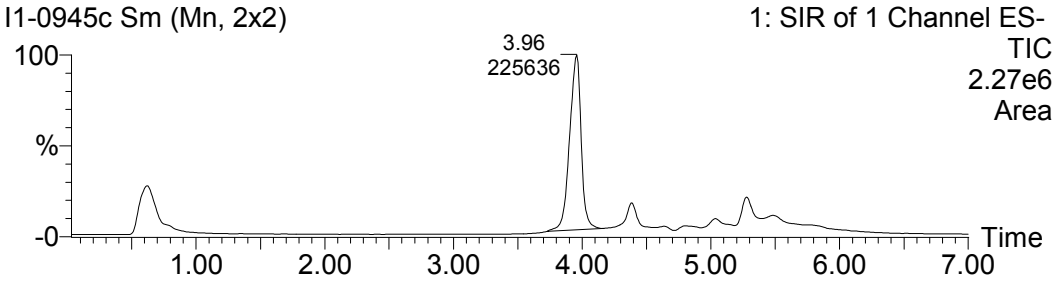

Figure 27: Chromatogram Of Animal No. 78161, Group T2, Female, 24 Hour Post-Dose Serum Sample

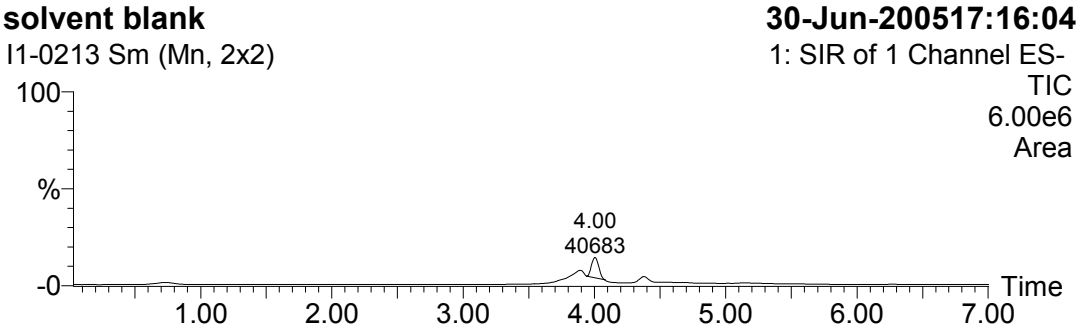

Figure 28: Representative Chromatogram Of A Processed Solvent Blank (Urine Assay)

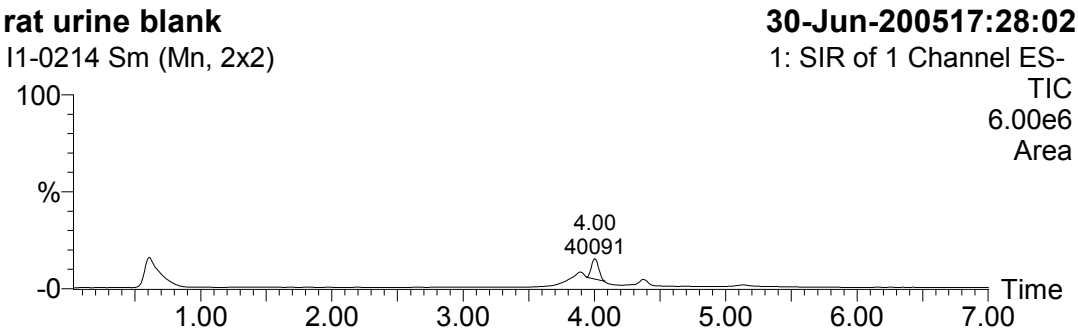

Figure 29: Representative Chromatogram Of A Processed Blank Urine Sample

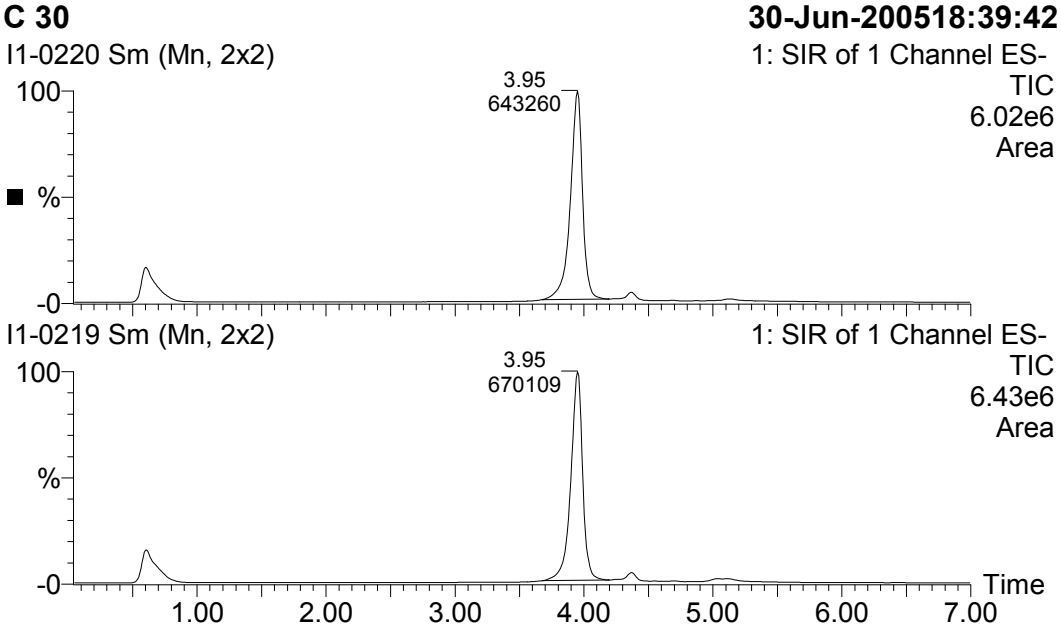

Figure 30: Representative Chromatograms Of 30 ng/mL Urine Calibration Samples

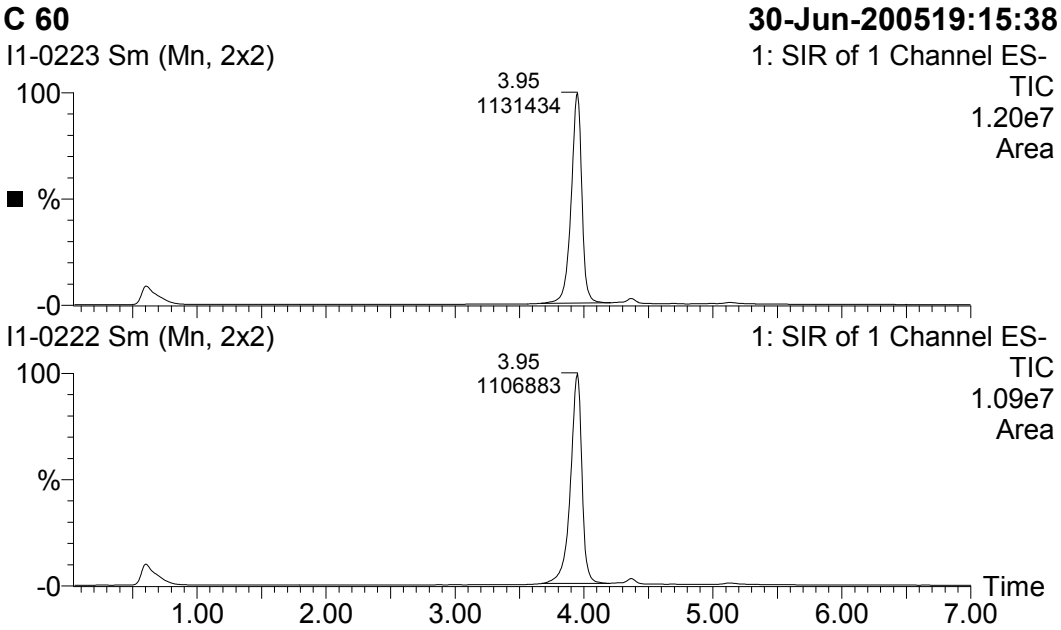

Figure 31: Representative Chromatograms Of 60 ng/mL Urine Calibration Samples

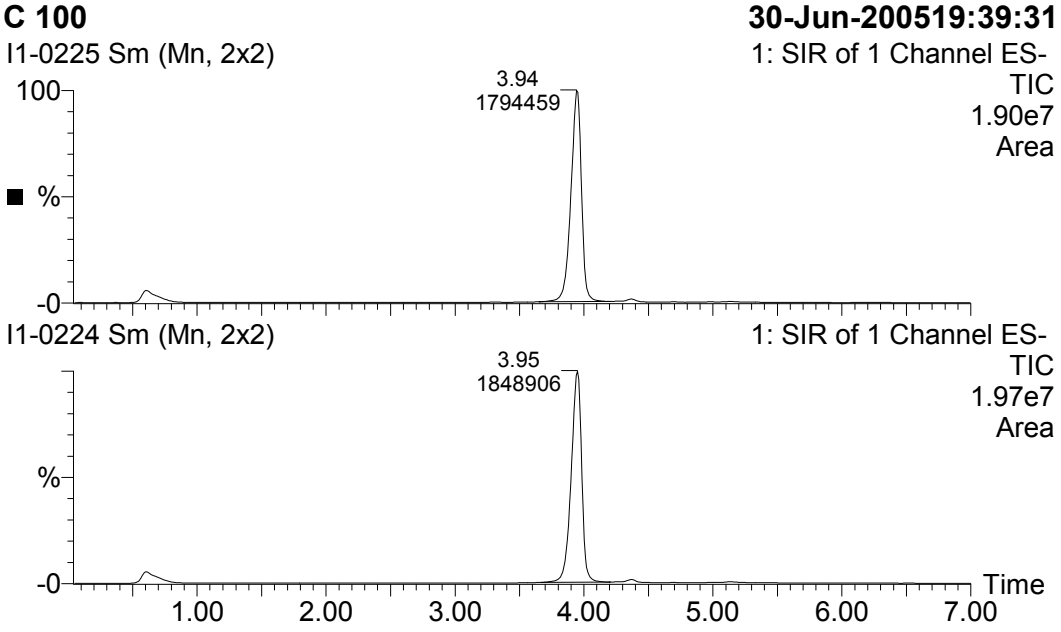

Figure 32: Representative Chromatograms Of 100 ng/mL Urine Calibration Samples

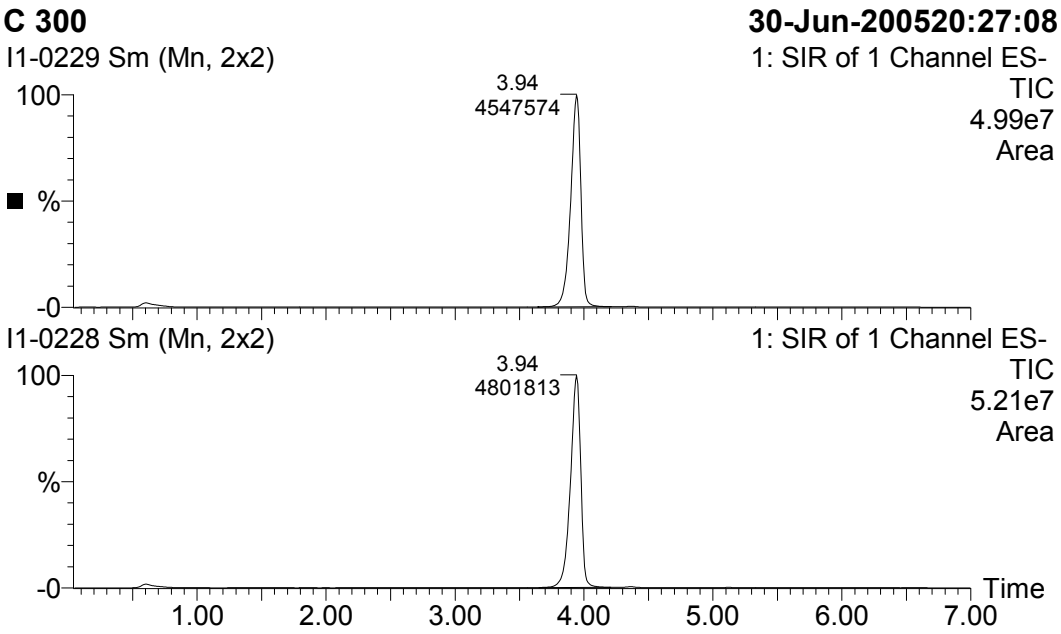

Figure 33: Representative Chromatograms Of 300 ng/mL Urine Calibration Samples

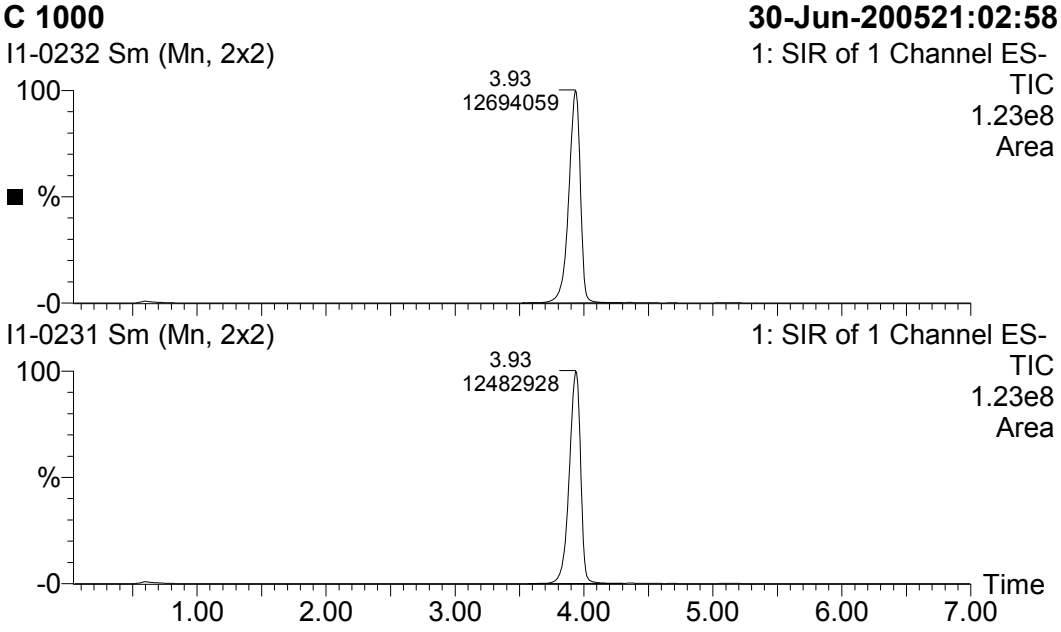

Figure 34: Representative Chromatograms Of 1000 ng/mL Urine Calibration Samples

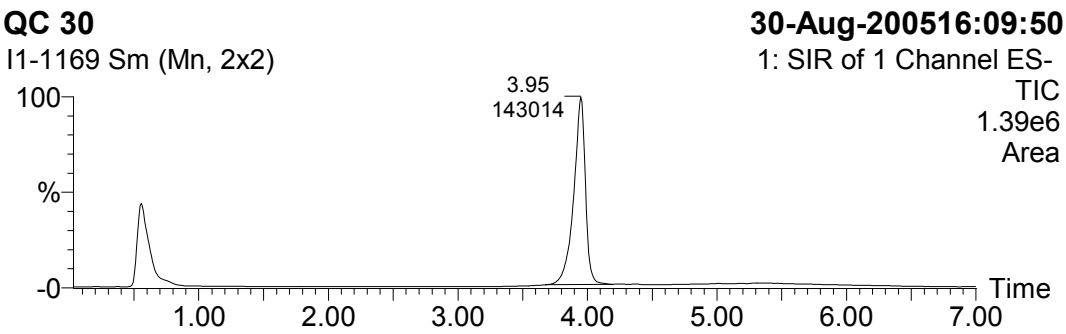

Figure 35: Representative Chromatogram Of A 30 ng/mL Urine QC Sample

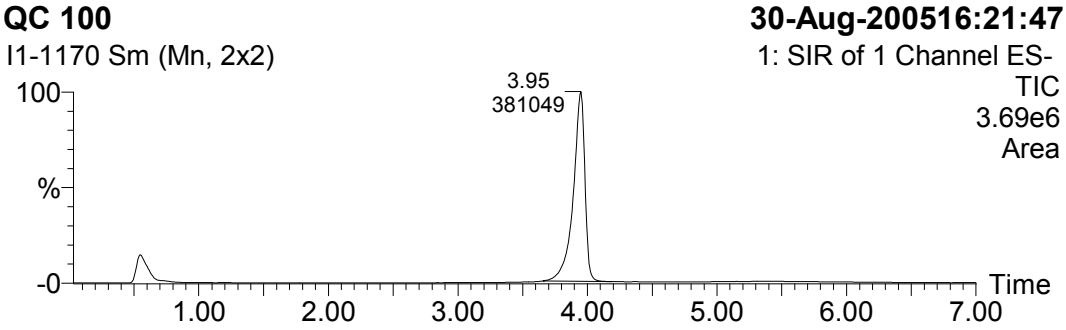

Figure 36: Representative Chromatogram Of A 100 ng/mL Urine QC Sample

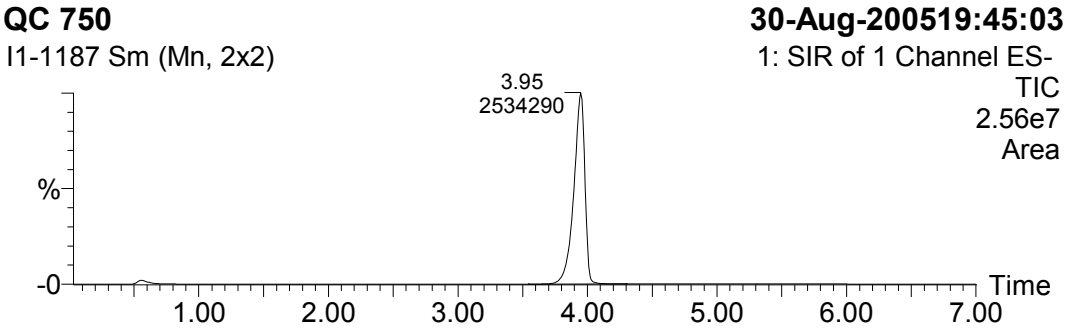

Figure 37: Representative Chromatogram Of A 750 ng/mL Urine QC Sample

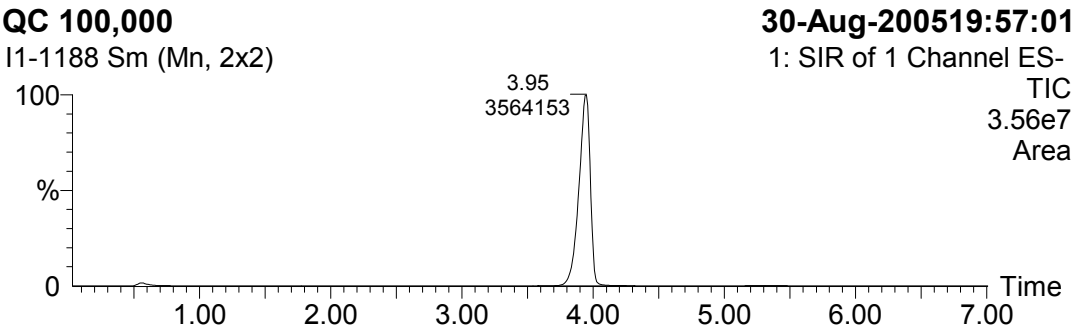

Figure 38: Representative Chromatogram Of A Dilutional 100,000 ng/mL Urine QC Sample

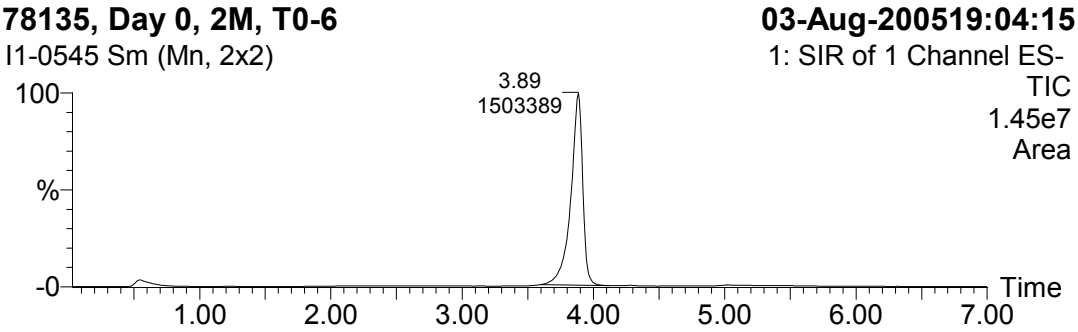

Figure 39: Chromatogram Of Animal No. 78135, Group T2, Male, 0 To 6 Hours Post-Dose Urine Sample

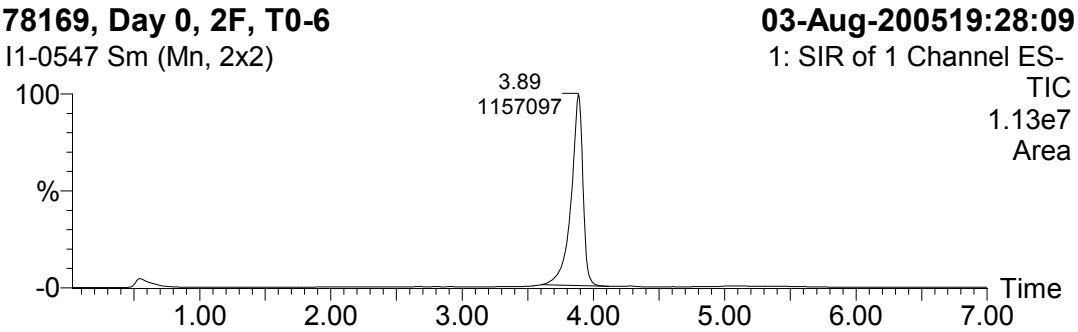

Figure 40: Chromatogram Of Animal No. 78169, Group T2, Female, 0 To 6 Hours Post-Dose Urine Sample

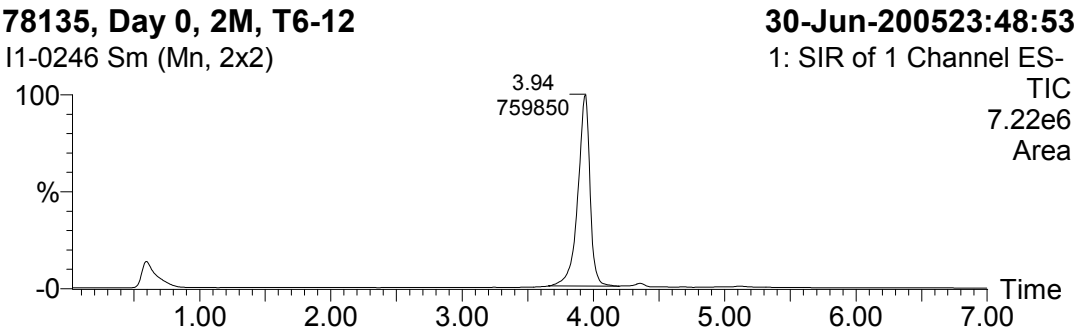

Figure 41: Chromatogram Of Animal No. 78135, Group T2, Male, 6 To 12 Hours Post-Dose Urine Sample

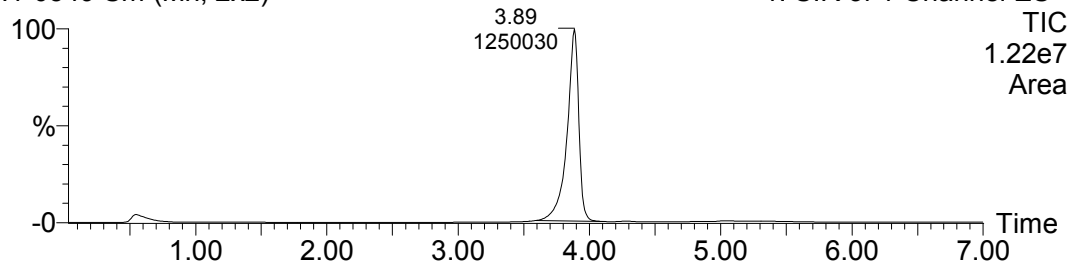

Figure 42: Chromatogram Of Animal No. 78169, Group T2, Female, 6 To 12 Hours Post-Dose Urine Sample

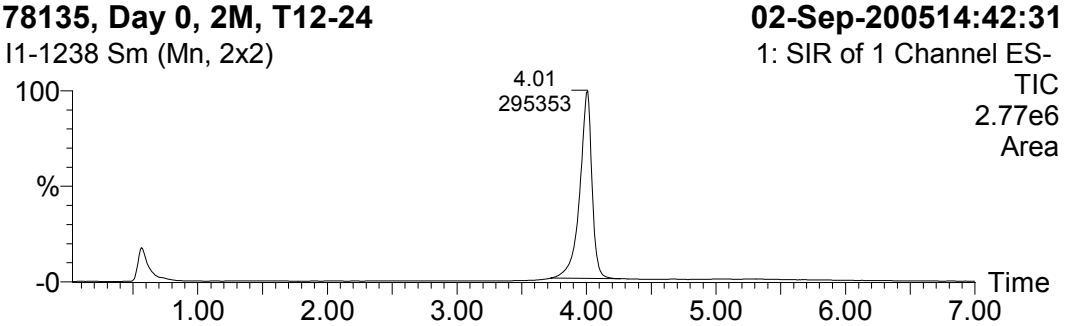

Figure 43: Chromatogram Of Animal No. 78135, Group T2, Male, 12 To 24 Hours Post-Dose Urine Sample

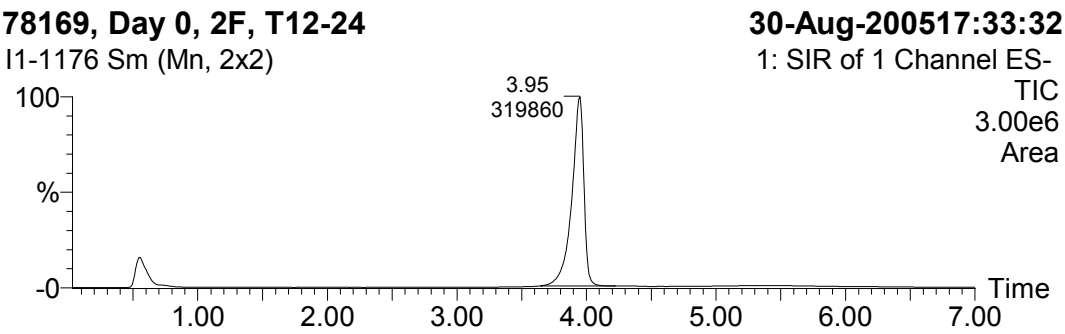

Figure 44: Chromatogram Of Animal No. 78169, Group T2, Female, 12 To 24 Hours Post-Dose Urine Sample

ATTACHMENT I

Supporting Data

Table A1: Calibration and Quality Control Samples For Sequence 534004(PFBS)BRS

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534004.PRO\SampleDB\534004(PFBS)BRS
Last modified: Thu Jun 30 07:53:22 2005
Method: C:\MASSLYNX\534004.PRO\MethDB\534004 PFBSbio2
Last modified: Thu Jun 30 08:21:18 2005
Job Code:

Printed: Thu Jun 30 08:28:50 2005

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0136	9-6	sys suit	3.99	102779	0	6.201	
I1-0137	9-6	sys suit			0		
I1-0138	9-6	sys suit			0		
I1-0139		ACN			0		
I1-0140		ACN			0		
I1-0141	9-1	solvent blank	3.98	53891	0	3.10	
I1-0142	9-2	rat serum blank	3.97	49202	0	2.81	
I1-0143	9-3	C 10	3.93	198740	0	12.6	26
I1-0144	9-4	C 10	3.94	193545	0	12.3	23
I1-0145	9-5	C 10	3.94	197723	0	12.6	26
I1-0146	9-6	C 30	3.93	441673	0	30.0	-0.070
I1-0147	9-7	C 30	3.93	440068	0	29.9	-0.46
I1-0148	9-8	C 30	3.93	461286	0	31.4	4.8
I1-0149	9-9	C 60	3.93	819431	0	58.6	-2.3
I1-0150	9-10	C 60	3.92	825888	0	59.1	-1.5
I1-0151	9-11	C 60	3.92	833338	0	59.7	-0.50
I1-0152	9-12	C 100	3.92	1354727	0	101	1.3
I1-0153	9-13	C 100	3.93	1300632	0	96.9	-3.1
I1-0154	9-14	C 100	3.93	1297197	0	96.6	-3.4
I1-0155	9-15	C 300	3.93	3824036	0	314	4.6
I1-0156	9-16	C 300	3.93	3679665	0	301	0.32
I1-0157	9-17	C 300	3.92	3793757	0	311	3.7
I1-0158	9-18	C 1000	3.92	11029299	0	1001	0.07
I1-0159	9-19	C 1000	3.92	11041268	0	1002	0.19
I1-0160	9-20	C 1000	3.92	10712528	0	969	-3.1
I1-0161		ACN	3.96	101577	0	6.12	
I1-0162	10-1	QC 30	3.92	476237	0	32.5	8.4
I1-0163	10-2	QC 30	3.92	492808	0	33.8	13
I1-0164	10-3	QC 30	3.93	486586	0	33.3	11
I1-0165	10-4	QC 100	3.93	1526107	0	115	15

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0166	10-5	QC 100	3.92	1447830	0	109	8.9
I1-0167	10-6	QC 100	3.93	1480595	0	112	12
I1-0168	10-7	QC 750	3.92	9366469	0	837	12
I1-0169	10-8	QC 750	3.92	9799687	0	879	17
I1-0170	10-9	QC 750	3.93	9828484	0	882	18
I1-0171		ACN	3.97	105565	0	6.38	

Table A2: Calibration and Quality Control Samples For Sequence 534004(PFBS)BRU

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534004.PRO\SampleDB\534004(PFBS)BRU
Last modified: Thu Jun 30 07:58:52 2005
Method: C:\MASSLYNX\534004.PRO\MethDB\534004 PFBSbio2
Last modified: Thu Jun 30 08:21:18 2005
Job Code:

Printed: Thu Jun 30 08:30:45 2005

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0172	11-6	sys suit	3.98	124698	0	4.88	
I1-0173	11-6	sys suit	3.98	114041	0	4.38	
I1-0174	11-6	sys suit	3.97	116012	0	4.48	
I1-0175		ACN	3.97	138650	0	5.55	
I1-0176		ACN	3.97	119600	0	4.64	
I1-0177	11-1	solvent blank	3.98	62482	0	2.11	
I1-0178	11-2	rat urine blank	3.98	66279	0	2.27	
I1-0179	11-3	C 10	3.93	271724	0	12.4	24
I1-0180	11-4	C 10	3.93	280276	0	12.9	29
I1-0181	11-5	C 10	3.93	267690	0	12.2	22
I1-0182	11-6	C 30	3.93	587072	0	30.7	2.3
I1-0183	11-7	C 30	3.93	560550	0	29.1	-3.1
I1-0184	11-8	C 30	3.93	608620	0	32.0	6.7
I1-0185	11-9	C 60	3.93	1057709	0	60.8	1.3
I1-0186	11-10	C 60	3.93	1044349	0	59.9	-0.16
I1-0187	11-11	C 60	3.92	962362	0	54.5	-9.2
I1-0188	11-12	C 100	3.92	1690795	0	104	4.3
I1-0189	11-13	C 100	3.93	1636181	0	100	0.43
I1-0190	11-14	C 100	3.92	1536256	0	93.4	-6.6
I1-0191	11-15	C 300	3.92	4192805	0	293	-2.5
I1-0192	11-16	C 300	3.93	4333412	0	304	1.2
I1-0193	11-17	C 300	3.92	4651505	0	329	9.6
I1-0194	11-18	C 1000	3.93	12728408	0	1010	1.0
I1-0195	11-19	C 1000	3.92	12853872	0	1021	2.1
I1-0196	11-20	C 1000	3.92	11956274	0	943	-5.7
I1-0197		ACN	3.96	112671	0	4.32	
I1-0198	12-1	QC 30	3.92	487852	0	24.7	-18
I1-0199	12-2	QC 30	3.92	541743	0	27.9	-6.9
I1-0200	12-3	QC 30	3.93	508959	0	26.0	-13
I1-0201	12-4	QC 100	3.92	1541731	0	93.8	-6.2

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0202	12-5	QC 100	3.92	1478655	0	89.4	-11
I1-0203	12-6	QC 100	3.91	1330291	0	79.2	-21
I1-0204	12-7	QC 750	3.92	9494874	0	730	-2.7
I1-0205	12-8	QC 750	3.93	9783209	0	755	0.64
I1-0206	12-9	QC 750	3.93	10178886	0	789	5.2
I1-0207		ACN	3.97	123654	0	4.83	

Table A3: Calibration, QC And Experimental Samples For Sequence 534004(PFBS)CRU

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534004.PRO\SampleDB\534004(PFBS)CRU
Last modified: Fri Jul 01 07:56:47 2005
Method: C:\MASSLYNX\534004.PRO\MethDB\534004 PFBSbio2
Last modified: Thu Jun 30 08:21:18 2005
Job Code:

Printed: Tue Jul 05 09:09:55 2005

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0208	13-6	sys suit	3.97	560306		25.4	
I1-0209	13-6	sys suit	3.95	637029	0	29.5	
I1-0210	13-6	sys suit	3.96	624076	0	28.8	
I1-0211		ACN	3.99	120940	0	4.25	
I1-0212		ACN	3.99	126120	0	4.46	
I1-0213	13-1	solvent blank	4	40683	0	1.20	
I1-0214	13-2	rat urine blank	4	40091	0	1.18	
I1-0215	13-3	C 10	3.96	293077	0	11.9	19
I1-0216	13-4	C 10	3.96	311445	0	12.8	28
I1-0217	13-5	C 10	3.95	302817	0	12.4	24
I1-0218	13-6	C 30	3.95	677794	0	31.8	5.8
I1-0219	13-7	C 30	3.95	670109	0	31.3	4.4
I1-0220	13-8	C 30	3.95	643260	0	29.9	-0.44
I1-0221	13-9	C 60	3.95	1095636	0	55.7	-7.1
I1-0222	13-10	C 60	3.95	1106883	0	56.4	-6.0
I1-0223	13-11	C 60	3.95	1131434	0	57.9	-3.6
I1-0224	13-12	C 100	3.95	1848906	0	103	3.0
I1-0225	13-13	C 100	3.94	1794459	0	99.4	-0.59
I1-0226	13-14	C 100	3.95	1770353	0	97.8	-2.2
I1-0227	13-15	C 300	3.94	4906458	0	324	8.2
I1-0228	13-16	C 300	3.94	4801813	0	316	5.4
I1-0229	13-17	C 300	3.94	4547574	0	297	-1.1
I1-0230	13-18	C 1000	3.94	12556968	0	983	-1.7
I1-0231	13-19	C 1000	3.93	12482928	0	976	-2.4
I1-0232	13-20	C 1000	3.93	12694059	0	996	-0.42
I1-0233		ACN	3.98	121587	0	4.27	
I1-0234	14-1	QC 30	3.94	594218	0	27.2	-9.3
I1-0235	14-4	QC 100	3.93	1589916	0	86.2	-14
I1-0236	14-7	QC 750	3.93	9532238	0	710	-5.3
I1-0237	14-10	QC 100000	3.94	1645082	1000	89767	-10

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0238		ACN	3.99	120683	0	4.24	
I1-0239	15-1	78133, Day 0, 2M, T0-6	3.94	462471	10000	203053	
I1-0240	15-2	78135, Day 0, 2M, T0-6	3.93	545708	10000	246404	
I1-0241	15-3	78139, Day 0, 2M, T0-6	3.93	531544	10000	238941	
I1-0242	15-4	78160, Day 0, 2F, T0-6	3.94	1075224	10000	545125	
I1-0243	15-5	78169, Day 0, 2F, T0-6	3.93	509230	10000	227254	
I1-0244	15-6	78170, Day 0, 2F, T0-6	3.93	518307	10000	231998	
I1-0245	16-1	78133, Day 0, 2M, T6-12	3.94	2284654	1000	132013	
I1-0246	16-2	78135, Day 0, 2M, T6-12	3.94	759850	1000	36298	
I1-0247	16-3	78139, Day 0, 2M, T6-12	3.93	753084	1000	35919	
I1-0248	16-4	78160, Day 0, 2F, T6-12	3.94	842052	1000	40937	
I1-0249	16-5	78169, Day 0, 2F, T6-12	3.93	612795	1000	28220	
I1-0250	16-6	78170, Day 0, 2F, T6-12	3.93	429871	1000	18643	
I1-0251		ACN	3.98	115909	0	4.04	
I1-0252	14-2	QC 30	3.93	566430	0	25.7	-14
I1-0253	14-5	QC 100	3.93	1551864	0	83.8	-16
I1-0254	14-8	QC 750	3.93	9558222	0	712	-5.0
I1-0255	14-11	QC 100000	3.93	1583669	1000	85847	-14
I1-0256		ACN	3.98	114357	0	3.98	
I1-0257	17-1	78133, Day 0, 2M, T12-24	3.94	724486	500	17164	
I1-0258	17-2	78135, Day 0, 2M, T12-24	3.94	296183	500	6033	
I1-0259	17-3	78139, Day 0, 2M, T12-24	3.93	427085	500	9251	
I1-0260	17-4	78160, Day 0, 2F, T12-24	3.94	255361	500	5074	
I1-0261	17-5	78169, Day 0, 2F, T12-24	3.93	372830	500	7893	
I1-0262	17-6	78170, Day 0, 2F, T12-24	3.95	166136	500	3074	
I1-0263		ACN	3.97	114756	0	4.00	
I1-0264	12-1	QC 30	3.93	549231	0	24.8	-17
I1-0265	12-2	QC 30	3.93	529477	0	23.8	-21
I1-0266	12-3	QC 30	3.93	502012	0	22.3	-26
I1-0267	12-4	QC 100	3.92	1477908	0	79.2	-21
I1-0268	12-5	QC 100	3.93	1508226	0	81.1	-19
I1-0269	12-6	QC 100	3.93	1536589	0	82.9	-17
I1-0270	12-7	QC 750	3.92	9698305	0	725	-3.4
I1-0271	12-8	QC 750	3.92	9619913	0	718	-4.3
I1-0272	12-9	QC 750	3.93	10132192	0	763	1.7
I1-0273		ACN	3.98	110115	0	3.81	
I1-0274	14-3	QC 30	3.93	590996	0	27.0	-9.8
I1-0275	14-6	QC 100	3.93	1546304	0	83.5	-17
I1-0276	14-9	QC 750	3.93	10217634	0	771	2.8
I1-0277	14-12	QC 100000	3.93	1749630	1000	96500	-3.5
I1-0278		ACN	3.98	114543	0	3.99	

Table A4: Calibration, QC And Experimental Samples For Sequence 534004(PFBS)DRS

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534004.PRO\SampleDB\534004(PFBS)DRS
Last modified: Fri Jul 22 12:16:11 2005
Method: C:\MASSLYNX\534004.PRO\MethDB\534004 PFBSbio2
Last modified: Thu Jun 30 08:21:18 2005
Job Code:

Printed: Fri Jul 22 13:39:07 2005

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0419	29-6	sys suit	3.89	480569	0	20.7	
I1-0420	29-6	sys suit	3.88	459359	0	19.4	
I1-0421	29-6	sys suit	3.89	524401	0	23.2	
I1-0422		ACN	3.94	69006	0	1.24	
I1-0423		ACN	3.93	67964	0	1.21	
I1-0424	29-1	solvent blank	3.93	58187	0	0.948	
I1-0425	29-2	rat serum blank	3.93	53489	0	0.829	
I1-0426	*29-3	C 10	3.9	276946	0	9.69	-3.1
I1-0427	*29-4	C 10	3.9	263190	0	9.02	-9.8
I1-0428	*29-5	C 10	3.9	269659	0	9.33	-6.7
I1-0429	*29-6	C 30	3.89	451289	0	19.0	-37
I1-0430	*29-7	C 30	3.89	462045	0	19.6	-35
I1-0431	*29-8	C 30	3.89	451271	0	19.0	-37
I1-0432	*29-9	C 60	3.89	791618	0	40.0	-33
I1-0433	*29-10	C 60	3.89	765293	0	38.3	-36
I1-0434	*29-11	C 60	3.89	749217	0	37.3	-38
I1-0435	*29-12	C 100	3.89	1144574	0	64.4	-36
I1-0436	*29-13	C 100	3.89	1249528	0	72.1	-28
I1-0437	*29-14	C 100	3.89	1181567	0	67.1	-33
I1-0438	*29-15	C 300	3.89	3174924	0	228	-24
I1-0439	*29-16	C 300	3.89	3295421	0	239	-20
I1-0440	*29-17	C 300	3.89	3482713	0	255	-15
I1-0441	*29-18	C 1000	3.89	9961395	0	869	-13
I1-0442	*29-19	C 1000	3.89	9628591	0	836	-16
I1-0443	*29-20	C 1000	3.89	9609324	0	834	-17
I1-0444		ACN	3.94	61588	0	1.04	
I1-0445	30-1	QC 30	3.89	537650	0	24.0	-20
I1-0446	30-4	QC 100	3.89	1368552	0	80.9	-19

* Not included in regression equation due to drift in instrument reponse.

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0447	30-7	QC 750	3.89	7915923	0	668	-11
I1-0448	30-10	QC 50000	3.89	3360859	200	48864	-2.3
I1-0449		ACN	3.94	64770	0	1.12	
I1-0450	34-1	78121, Day 0, 2M, T0	3.94	98157	0	2.14	
I1-0451	34-2	78126, Day 0, 2M, T0	3.94	97226	0	2.11	
I1-0452	34-3	78129, Day 0, 2M, T0	3.9	352998	0	13.6	
I1-0453	34-4	78146, Day 0, 2F, T0	3.94	98234	0	2.14	
I1-0454	34-5	78155, Day 0, 2F, T0	3.95	102435	0	2.28	
I1-0455	34-6	78157, Day 0, 2F, T0	3.95	99559	0	2.18	
I1-0456	31-1	78132, Day 0, 2M, T0.5	3.89	4570168	200	70466	
I1-0457	31-2	78134, Day 0, 2M, T0.5	3.89	3363853	200	48916	
I1-0458	31-3	78136, Day 0, 2M, T0.5	3.89	3845961	200	57418	
I1-0459	31-4	78158, Day 0, 2F, T0.5	3.9	1926506	200	24796	
I1-0460	31-5	78161, Day 0, 2F, T0.5	3.9	1741502	200	21875	
I1-0461	31-6	78163, Day 0, 2F, T0.5	3.9	1965222	200	25414	
I1-0462	31-7	78141, Day 0, 2M, T1	3.89	3258557	200	47082	
I1-0463	31-8	78143, Day 0, 2M, T1	3.9	3771372	200	56092	
I1-0464	31-9	78145, Day 0, 2M, T1	3.9	3153651	200	45263	
I1-0465	31-10	78165, Day 0, 2F, T1	3.9	911562	200	9621	
I1-0466	31-11	78174, Day 0, 2F, T1	3.9	856101	200	8869	
I1-0467	31-12	78175, Day 0, 2F, T1	3.9	731078	200	7218	
I1-0468	32-1	78121, Day 0, 2M, T1.5	3.9	2797111	200	39153	
I1-0469	32-2	78126, Day 0, 2M, T1.5	3.9	2459120	200	33474	
I1-0470	32-3	78129, Day 0, 2M, T1.5	3.9	2324647	200	31249	
I1-0471	32-4	78146, Day 0, 2F, T1.5	3.91	601841	200	5584	
I1-0472	32-5	78155, Day 0, 2F, T1.5	3.91	465418	200	3957	
I1-0473	32-6	78157, Day 0, 2F, T1.5	3.91	466397	200	3968	
I1-0474		ACN	3.96	74715	0	1.41	
I1-0475	30-2	QC 30	3.92	631938	0	29.8	-0.72
I1-0476	30-5	QC 100	3.91	1507825	0	91.3	-8.7
I1-0477	30-8	QC 750	3.91	9603575	0	834	11
I1-0478	30-11	QC 50000	3.91	4270391	200	65028	30
I1-0479		ACN	3.96	85294	0	1.72	
I1-0480	32-7	78132, Day 0, 2M, T2	3.92	2673176	200	37057	
I1-0481	32-8	78134, Day 0, 2M, T2	3.92	1603140	200	19728	
I1-0482	32-9	78136, Day 0, 2M, T2	3.91	2497138	200	34107	
I1-0483	32-10	78158, Day 0, 2F, T2	3.93	417290	200	3413	
I1-0484	32-11	78161, Day 0, 2F, T2	3.92	473035	200	4045	
I1-0485	32-12	78163, Day 0, 2F, T2	3.92	581583	200	5335	
I1-0486	33-1	78141, Day 0, 2M, T4	3.92	5582267	100	44574	
I1-0487	33-2	78143, Day 0, 2M, T4	3.92	2909011	100	20529	
I1-0488	33-3	78145, Day 0, 2M, T4	3.92	2673911	100	18535	

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0489	33-4	78165, Day 0, 2F, T4	3.96	271788	100	944	
I1-0490	33-5	78174, Day 0, 2F, T4	3.95	285553	100	1011	
I1-0491	33-6	78175, Day 0, 2F, T4	3.97	225392	100	725	
I1-0492	33-7	78121, Day 0, 2M, T8	3.92	930098	100	4937	
I1-0493	33-8	78126, Day 0, 2M, T8	3.92	864941	100	4494	
I1-0494	33-9	78129, Day 0, 2M, T8	3.92	724910	100	3569	
I1-0495	33-10	78146, Day 0, 2F, T8	3.96	194421	100	587	
I1-0496	33-11	78155, Day 0, 2F, T8	3.96	196254	100	595	
I1-0497	33-12	78157, Day 0, 2F, T8	3.96	172824	100	495	
I1-0498	33-13	78132, Day 0, 2M, T24	3.97	180886	100	529	
I1-0499	33-14	78134, Day 0, 2M, T24	3.97	170851	100	487	
I1-0500	33-15	78136, Day 0, 2M, T24	3.96	188688	100	562	
I1-0501	33-16	78158, Day 0, 2F, T24	3.96	187310	100	556	
I1-0502	33-17	78161, Day 0, 2F, T24	3.96	145014	100	383	
I1-0503	33-18	78163, Day 0, 2F, T24	3.96	172864	100	495	
I1-0504		ACN	3.96	76209	0	1.45	
I1-0505	30-3	QC 30	3.93	652230	0	31.1	3.5
I1-0506	30-6	QC 100	3.92	1808798	0	115	15
I1-0507	30-9	QC 750	3.91	10071502	0	880	17
I1-0508	30-12	QC 50000	3.92	3807908	200	56741	13
I1-0509		ACN	3.96	73548	0	1.37	
I1-0510	29-6	C 30	3.92	628661	0	29.6	-1.4
I1-0511	29-9	C 60	3.92	1107272	0	61.8	3.0
I1-0512	29-12	C 100	3.92	1609809	0	99.2	-0.85
I1-0513	29-15	C 300	3.92	3953953	0	297	-1.1
I1-0514	29-18	C 1000	3.92	11317152	0	1004	0.44
I1-0515		ACN	3.96	70025	0	1.27	

Table A5: Calibration, QC And Experimental Samples For Sequence 534004(PFBS)EFRU

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534004.PRO\SampleDB\534004(PFBS)EFRU
Last modified: Thu Aug 04 09:37:12 2005
Method: C:\MASSLYNX\534004.PRO\MethDB\534004 PFBSbio2
Last modified: Thu Jun 30 08:21:18 2005
Job Code:

Printed: Wed Aug 31 13:04:55 2005

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0538		ACN			0		
I1-0539	41-1	QC 30	3.89	327350.6	0	24.5	-18
I1-0540	41-4	QC 100	3.89	892601.2	0	69.9	-30
I1-0541	41-7	QC 750	3.89	5627732	0	524	-30
I1-0542	41-10	QC 100000	3.89	1066664.4	1000	84562	-15
I1-0543		ACN			0		
I1-0544	42-1	78133, Day 0, 2M, T0-6	3.89	944944.9	2000	148632	
I1-0545	42-2	78135, Day 0, 2M, T0-6	3.89	1503388.9	2000	244468	
I1-0546	42-3	78139, Day 0, 2M, T0-6	3.89	1269016.5	2000	203697	
I1-0547	42-4	78169, Day 0, 2F, T0-6	3.89	1157096.9	2000	184499	
I1-0548	42-5	78170, Day 0, 2F, T0-6	3.89	1175468.4	2000	187637	
I1-0549	43-1	78169, Day 0, 2F, T6-12	3.89	1250030.3	250	25053	
I1-0550		ACN			250		
I1-0551	41-2	QC 30	3.89	349727.6	0	26.2	-13
I1-0552	41-5	QC 100	3.89	1075810.5	0	85.3	-15
I1-0553	41-8	QC 750	3.89	5986001.5	0	562	-25
I1-0554	41-11	QC 100000	3.89	1139703.3	1000	90766	-9.2
I1-0555		ACN			250		
I1-0556	43-2	78170, Day 0, 2F, T6-12	3.89	938606	250	18446	
I1-0557	44-1	78135, Day 0, 2M, T12-24	3.89	192253	500	7103	
I1-0558	44-2	78139, Day 0, 2M, T12-24	3.89	256581	500	9533	
I1-0559	44-3	78160, Day 0, 2F, T12-24	3.89	166091	500	6124	
I1-0560	44-4	78169, Day 0, 2F, T12-24	3.89	224077	500	8301	
I1-0561	44-5	78170, Day 0, 2F, T12-24	3.89	109244	500	4019	
I1-0562		ACN			0		
I1-0563	41-3	QC 30	3.89	382816.1	0	28.8	-4.0
I1-0564	41-6	QC 100	3.89	1096566.1	0	87.1	-13
I1-0565	41-9	QC 750	3.89	6589280.5	0	626	-16
I1-0566	41-12	QC 100000	3.89	1220056.9	1000	97638	-2.4
I1-0567		ACN			0		

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0568	47-6	sys suit	3.89	383780.6	0	28.9	
I1-0569	47-6	sys suit	3.89	385371.9	0	29.0	
I1-0570	47-6	sys suit	3.89	366215.2	0	27.5	
I1-0571		ACN			0		
I1-0572		ACN			0		
I1-0573	47-1	solvent blank			0		
I1-0574	47-2	rat urine blank			0		
I1-0575	47-6	C 30	3.89	402941.6	0	30.4	1.2
I1-0576	47-7	C 30	3.89	411574.5	0	31.0	3.4
I1-0577	47-8	C 30	3.89	385936.4	0	29.0	-3.2
I1-0578	47-9	C 60	3.89	773673.6	0	60.1	0.19
I1-0579	47-10	C 60	3.89	782668.1	0	60.9	1.4
I1-0580	47-11	C 60	3.89	757359.1	0	58.8	-2.1
I1-0581	47-12	C 100	3.89	1230227.3	0	98.5	-1.5
I1-0582	47-13	C 100	3.89	1231755	0	98.6	-1.4
I1-0583	47-14	C 100	3.89	1241730.8	0	99.5	-0.50
I1-0584	47-15	C 300	3.89	3429344	0	301	0.42
I1-0585	47-16	C 300	3.89	3361263.5	0	295	-1.8
I1-0586	47-17	C 300	3.89	3580905	0	316	5.4
I1-0587	47-18	C 1000	3.89	10240793	0	1037	3.7
I1-0588	47-19	C 1000	3.89	9368214	0	936	-6.4
I1-0589	47-20	C 1000	3.89	10079478	0	1018	1.8
I1-0590		ACN			0		
I1-0591	48-1	QC 30	3.89	465415.2	0	35.3	18
I1-0592	48-4	QC 100	3.89	1268369.9	0	102	1.8
I1-0593	48-7	QC 750	3.89	7673273	0	745	-0.69
I1-0594		ACN			0		
I1-0595	49-1	LT STB 100 ng/mL	3.89	1299290.9	0	104	
I1-0596	49-2	LT STB 100 ng/mL	3.89	1388285.4	0	112	
I1-0597	49-3	LT STB 100 ng/mL	3.89	1335671.5	0	108	
I1-0598	49-4	LT STB 750 ng/mL	3.89	7560180	0	732	
I1-0599	49-5	LT STB 750 ng/mL	3.89	8483920	0	835	
I1-0600	49-6	LT STB 750 ng/mL	3.89	7952784.5	0	776	
I1-0601		ACN			0		
I1-0602	48-2	QC 30	3.89	444669.8	0	33.6	12
I1-0603	48-5	QC 100	3.89	1236854.1	0	99.1	-0.92
I1-0604	48-8	QC 750	3.89	7938433	0	774	3.2
I1-0605		ACN			0		
I1-0606	50-1	4 Hr STB 100 ng/mL	3.89	1253103.6	0	100	
I1-0607	50-2	4 Hr STB 100 ng/mL	3.89	1361884.4	0	110	
I1-0608	50-3	4 Hr STB 100 ng/mL	3.89	1167386.8	0	93.1	
I1-0609	50-4	4 Hr STB 750 ng/mL	3.89	7176171.5	0	690	
I1-0610	50-5	4 Hr STB 750 ng/mL	3.89	8139935	0	797	
I1-0611	50-6	4 Hr STB 750 ng/mL	3.89	6929929	0	663	
I1-0612		ACN			0		
I1-0613	48-3	QC 30	3.89	400959.6	0	30.2	0.68
I1-0614	48-6	QC 100	3.89	1151354.3	0	91.8	-8.2
I1-0615	48-9	QC 750	3.89	7995891.5	0	781	4.1
I1-0616		ACN			0		

Table A6: Calibration, QC And Experimental Samples For Sequence 534004(PFBS)GRS

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534004.PRO\SampleDB\534004(PFBS)GRS
Last modified: Wed Aug 10 08:14:51 2005
Method: C:\MASSLYNX\534004.PRO\MethDB\534004 PFBSbio2
Last modified: Thu Jun 30 08:21:18 2005
Job Code:

Printed: Wed Aug 31 12:45:19 2005

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0617	53-6	sys suit	4	709045.1	0	36.2	
I1-0618	53-6	sys suit	3.99	699037	0	35.6	
I1-0619	53-6	sys suit	3.98	730440.3	0	37.5	
I1-0620		ACN			0		
I1-0621		ACN			0		
I1-0622	53-1	solvent blank			0		
I1-0623	53-2	rat serum blank			0		
I1-0624	53-6	C 30	3.98	623049.2	0	30.9	3.1
I1-0625	53-7	C 30	3.98	601747.3	0	29.6	-1.3
I1-0626	53-8	C 30	3.97	611990.9	0	30.2	0.81
I1-0627	53-9	C 60	3.97	1095403.4	0	61.2	1.9
I1-0628	53-10	C 60	3.97	1069937.9	0	59.5	-0.89
I1-0629	53-11	C 60	3.97	1043140.9	0	57.7	-3.8
I1-0630	53-12	C 100	3.97	1720471.6	0	104	4.2
I1-0631	53-13	C 100	3.97	1546073	0	91.9	-8.1
I1-0632	53-14	C 100	3.97	1684817.3	0	102	1.7
I1-0633	53-15	C 300	3.97	4433164	0	307	2.4
I1-0634	53-16	C 300	3.97	4386755.5	0	304	1.2
I1-0635	53-17	C 300	3.97	4369883.5	0	302	0.80
I1-0636	53-18	C 1000	3.97	13446765	0	1035	3.5
I1-0637	53-19	C 1000	3.97	12386764	0	948	-5.2
I1-0638	53-20	C 1000	3.96	13088100	0	1005	0.52
I1-0639		ACN			0		
I1-0640	54-1	QC 30	3.97	528065.2	0	25.2	-16
I1-0641	54-4	QC 100	3.97	1598775.6	0	95.6	-4.4
I1-0642	54-7	QC 750	3.97	9622811	0.0	722	-3.8
I1-0643	54-10	QC 50000	3.97	4492608.5	200.0	62385	25
I1-0644		ACN			0		
I1-0645	55-1	78146, Day 0, 2F, T1.5	3.97	978166.4	100	5342	
I1-0646	55-2	78155, Day 0, 2F, T1.5	3.97	876444.9	100	4681	

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0647	55-3	78157, Day 0, 2F, T1.5	3.96	664605.5	100	3346	
I1-0648	55-4	78158, Day 0, 2F, T2	3.97	514261.1	100	2440	
I1-0649	55-5	78161, Day 0, 2F, T2	3.97	670784.4	100	3384	
I1-0650	55-6	78163, Day 0, 2F, T2	3.97	913921.3	100	4923	
I1-0651	55-7	78165, Day 0, 2F, T4	3.97	430405.6	20	391	
I1-0652	55-8	78174, Day 0, 2F, T4	3.97	446437.7	20	409	
I1-0653	55-9	78175, Day 0, 2F, T4	3.97	226214.9	20	173	
I1-0654	55-10	78146, Day 0, 2F, T8	3.99	69497	10	17.7	
I1-0655	55-11	78155, Day 0, 2F, T8	3.97	93799.1	10	26.8	
I1-0656	55-12	78157, Day 0, 2F, T8	3.98	75328.4	10	19.8	
I1-0657		ACN			0		
I1-0658	54-2	QC 30	3.97	646056.8	0	32.3	7.7
I1-0659	54-5	QC 100	3.97	1872342	0.0	115	15
I1-0660	54-8	QC 750	3.97	11504621	0	875	17
I1-0661	54-11	QC 50000	3.97	5046499.5	200	71028	42
I1-0662		ACN			0		
I1-0663	55-13	78132, Day 0, 2M, T24	3.97	234426.6	10	90.3	
I1-0664	55-14	78134, Day 0, 2M, T24	3.98	72076.6	10	18.6	
I1-0665	55-15	78136, Day 0, 2M, T24	3.97	137032	10	44.6	
I1-0666	55-16	78158, Day 0, 2F, T24			10		
I1-0667	55-17	78161, Day 0, 2F, T24			10		
I1-0668	55-18	78163, Day 0, 2F, T24			10		
I1-0669	56-1	100 ng/mL LTS	3.96	1633592.4	0	98.1	
I1-0670	56-2	100 ng/mL LTS	3.96	1732657.6	0	105	
I1-0671	56-3	100 ng/mL LTS	3.96	1813291.1	0	111	
I1-0672	56-4	750 ng/mL LTS	3.96	10385427	0	784	
I1-0673	56-5	750 ng/mL LTS	3.96	9656694	0	724	
I1-0674	56-6	750 ng/mL LTS	3.96	9400169	0	704	
I1-0675		ACN			0		
I1-0676	54-3	QC 30	3.96	503584.1	0	23.8	-21
I1-0677	54-6	QC 100	3.96	1440034.1	0	84.6	-15
I1-0678	54-9	QC 750	3.96	8821272	0	657	-12
I1-0679	54-12	QC 50000	3.96	4149696.3	200	57075	14
I1-0680		ACN			0		

Table A7: Calibration, QC And Experimental Samples For Sequence 534004(PFBS)LRS2

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534004.PRO\SampleDB\534004(PFBS)LRS2
Last modified: Thu Aug 25 12:00:51 2005
Method: C:\MASSLYNX\534004.PRO\MethDB\534004 PFBSbio3
Last modified: Thu Aug 25 08:03:03 2005
Job Code:

Printed: Tue Aug 30 11:27:29 2005

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0900c	86-6	sys suit	3.96	903819.9	0	30.8	
I1-0901c	86-6	sys suit	3.94	881122.9	0	30.1	
I1-0902c	86-6	sys suit	3.94	900167.4	0	30.7	
I1-0903d		ACN			0		
I1-0904d		ACN			0		
I1-0905c	86-1	solvent blank	3.95	94006.7	0	4.84	
I1-0906d	86-2	rat serum blank	3.95	87535.5	0	4.60	
I1-0907d	86-6	C 30	3.94	880826.9	0	30.1	0.17
I1-0908c	86-7	C 30	3.95	912748.8	0	31.1	3.5
I1-0909c	86-8	C 30	3.94	911414.1	0	31.0	3.4
I1-0910c	86-9	C 60	3.95	1757173.9	0	58.6	-2.3
I1-0911c	86-10	C 60	3.94	1750668	0	58.4	-2.7
I1-0912c	86-11	C 60	3.94	1728055.9	0	57.6	-3.9
I1-0913c	86-12	C 100	3.94	2808380.8	0	96.4	-3.7
I1-0914c	86-13	C 100	3.94	2833445	0	97.3	-2.7
I1-0915c	86-14	C 100	3.94	2835459.5	0	97.4	-2.6
I1-0916c	86-15	C 300	3.94	7710179.5	0	335	12
I1-0917c	86-16	C 300	3.95	7491331	0	322	7.4
I1-0918c	86-17	C 300	3.95	7196226.5	0	305	1.6
I1-0919c	86-18	C 1000	3.94	15053997	0	967	-3.3
I1-0920c	86-19	C 1000	3.94	15067719	0	969	-3.2
I1-0921c	86-20	C 1000	3.94	15134263	0	976	-2.4
I1-0922c		ACN			0		
I1-0923c	87-1	QC 30	3.95	865222.9	0	29.6	-1.5
I1-0924c	87-4	QC 100	3.95	2578661	0	87.8	-12
I1-0925c	87-7	QC 750	3.94	14024275	0	852	14
I1-0926c	87-10	QC 50000	3.95	6969920	200	58367	17
I1-0927c		ACN			0		
I1-0928c	77a-1	78158, Day 0, 2F, T2	3.95	4433015	20	3267	
I1-0929c	77a-2	78165, Day 0, 2F, T4	3.95	1535958.8	10	512	

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0930c	77a-3	78174, Day 0, 2F, T4	3.95	1520273.1	10	507	
I1-0931c	77a-4	78175, Day 0, 2F, T4	3.95	820482.5	10	282	
I1-0932c	77a-5	78146, Day 0, 2F, T8	3.95	584711.9	0	20.8	
I1-0933c	77a-6	78155, Day 0, 2F, T8	3.95	555636.1	0	19.9	
I1-0934c	77a-7	78157, Day 0, 2F, T8	3.95	442681.6	2	32.7	
I1-0935c	77a-8	78132, Day 0, 2M, T24	3.95	1973858.9	0	66.1	
I1-0936c	77a-9	78134, Day 0, 2M, T24	3.95	695077.1	0	24.2	
I1-0937c	77a-10	78136, Day 0, 2M, T24	3.95	406042.9	5	76.1	
I1-0938c		ACN			0		
I1-0939c	87-2	QC 30	3.95	879045	0	30.0	-0.010
I1-0940c	87-5	QC 100	3.95	2591551.3	0	88.2	-12
I1-0941c	87-8	QC 750	3.94	13439108	0	791	5.5
I1-0942c	87-11	QC 50000	3.95	7543950	200	65084	30
I1-0943c		ACN			0		
I1-0944c	77a-11	78158, Day 0, 2F, T24	3.96	406321.4	0	15.2	
I1-0945c	77a-12	78161, Day 0, 2F, T24	3.96	225635.8	0	9.42	
I1-0946c	77a-13	78163, Day 0, 2F, T24	3.96	235442.8	5	48.7	
I1-0947c		ACN			0		
I1-0948c	78-1	100 ng/mL LTS	3.95	2461677.8	0	83.5	
I1-0949c	78-2	100 ng/mL LTS	3.95	2542041.5	0	86.4	
I1-0950c	78-3	100 ng/mL LTS	3.96	2449791	0	83.0	
I1-0951c	78-4	750 ng/mL LTS	3.95	13107769	0	758	
I1-0952c	78-5	750 ng/mL LTS	3.95	12919866	0	740	
I1-0953c	78-6	750 ng/mL LTS	3.95	13080980	0	756	
I1-0954c		ACN			0		
I1-0955c	87-3	QC 30	3.96	830794.9	0	28.5	-5.1
I1-0956c	87-6	QC 100	3.96	2541837	0	86.4	-14
I1-0957c	87-9	QC 750	3.96	13046191	0	752	0.29
I1-0958c	87-12	QC 50000	3.96	6862502	200	57149	14
I1-0959c		ACN			0		

Table A8: Calibration, QC And Experimental Samples For Sequence 534004(PFBS)MRU2

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534004.PRO\SampleDB\534004(PFBS)MRU2
Last modified: Mon Aug 29 13:56:51 2005
Method: C:\MASSLYNX\534004.PRO\MethDB\534004 PFBSbio3
Last modified: Thu Aug 25 08:03:03 2005
Job Code:

Printed: Tue Aug 30 11:13:45 2005

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0960c	89-6	sys suit	3.92	497387.3	0	30.3	-100
I1-0961b	89-6	sys suit	3.9	347424.5	0	20.7	
I1-0962b	89-6	sys suit	3.91	265216.4	0	15.7	
I1-0963b		ACN			0		
I1-0964b		ACN			0		
I1-0965b	89-1	solvent blank			0		
I1-0966b	89-2	rat urine blank			0		
I1-0967b	89-6	C 30	3.95	495477	0	30.2	0.54
I1-0968b	89-7	C 30	3.94	489771	0	29.8	-0.69
I1-0969b	89-8	C 30	3.95	482920	0	29.4	-2.2
I1-0970b	89-9	C 60	3.95	938322.8	0	59.9	-0.18
I1-0971b	89-10	C 60	3.95	972966.8	0	62.3	3.8
I1-0972b	89-11	C 60	3.95	938811.4	0	59.9	-0.13
I1-0973b	89-12	C 100	3.94	1468781.6	0	97.9	-2.1
I1-0974b	89-13	C 100	3.95	1508223.6	0	101	0.84
I1-0975b	89-14	C 100	3.95	1534433.6	0	103	2.8
I1-0976b	89-15	C 300	3.95	3789341	0	286	-4.8
I1-0977b	89-16	C 300	3.95	3791162.3	0	286	-4.7
I1-0978b	89-17	C 300	3.94	4150752.8	0	317	5.8
I1-0979b	89-18	C 1000	3.94	10586793	0	960	-4.0
I1-0980b	89-19	C 1000	3.94	10852608	0	990	-1.0
I1-0981b	89-20	C 1000	3.94	11558009	0	1068	6.8
I1-0982b		ACN			0		
I1-0983b	90-1	QC 30	3.95	484656.6	0	29.5	-1.8
I1-0984b	90-4	QC 100	3.95	1567021.8	0	105	5.2
I1-0985b	90-7	QC 750	3.94	9226839	0	814	8.5
I1-0986b	90-10	QC 100000	3.94	3850958.5	100	29102	191
I1-0987b		ACN			0		
I1-0988b	82-1	78139, Day 0, 2M, T12-24	3.94	1435418.4	100	9547	
I1-0989b	82-2	78160, Day 0, 2F, T12-24	3.94	799464.6	100	5034	

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-0990b	82-3	78169, Day 0, 2F, T12-25	3.94	1405085.3	100	9324	
I1-0991b	82-4	78170, Day 0, 2F, T12-24	3.94	506371	100	3087	
I1-0992b		ACN			0		
I1-0993b	90-2	QC 30	3.94	996292.1	0	63.9	113
I1-0994b	90-5	QC 100	3.94	1550858.6	0	104	4.0
I1-0995b	90-8	QC 750	3.94	8935368	0	783	4.4
I1-0996b	90-11	QC 100000	3.94	4336491.5	100	33386	234
I1-0997b		ACN			0		
I1-0998b	83-1	100 ng/mL LTS	3.95	1439223.1	0	95.7	
I1-0999b	83-2	100 ng/mL LTS	3.94	1563206.6	0	105	
I1-1000b	83-3	100 ng/mL LTS	3.94	1537459	0	103	
I1-1001b	83-4	750 ng/mL LTS	3.94	9142205	0	805	
I1-1002b	83-5	750 ng/mL LTS	3.94	9532922	0	846	
I1-1003b	83-6	750 ng/mL LTS	3.94	9165519	0	807	
I1-1004b		ACN			0		
I1-1005b	90-3	QC 30	3.94	532344.9	0	32.6	8.5
I1-1006b	90-6	QC 100	3.94	1486866	0	99.3	-0.74
I1-1007b	90-9	QC 750	3.94	9071021	0	797	6.3
I1-1008b	90-12	QC 100000	3.94	4026889.5	100	30642	206
I1-1009b		ACN			0		

Table A9: Calibration and Quality Control Samples For Sequence 534004(PFBS)NRS

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534004.PRO\SampleDB\534004(PFBS)NRS
Last modified: Mon Aug 29 08:15:23 2005
Method: C:\MASSLYNX\534004.PRO\MethDB\534004 PFBSbio3
Last modified: Thu Aug 25 08:03:03 2005
Job Code:

Printed: Tue Aug 30 11:14:56 2005

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-1010	93-6	sys suit	3.94	388330.8	0	28.9	
I1-1011	93-6	sys suit	3.94	403119.9	0	30.0	
I1-1012	93-6	sys suit	3.94	390317.8	0	29.1	
I1-1013		ACN			0		
I1-1014		ACN			0		
I1-1015	93-1	solvent blank			0		
I1-1016	93-2	rat serum blank			0		
I1-1017	93-6	C 30	3.94	386337.8	0	28.8	-4.0
I1-1018	93-7	C 30	3.94	385064.5	0	28.7	-4.3
I1-1019	93-8	C 30	3.94	407629.8	0	30.3	1.0
I1-1020	93-9	C 60	3.94	863504.5	0	62.9	4.9
I1-1021	93-10	C 60	3.95	841158.9	0	61.3	2.2
I1-1022	93-11	C 60	3.95	900472.6	0	65.6	9.4
I1-1023	93-12	C 100	3.94	1337725.3	0	98.2	-1.8
I1-1024	93-13	C 100	3.94	1324077.9	0	97.2	-2.8
I1-1025	93-14	C 100	3.94	1380274.3	0	101	1.5
I1-1026	93-15	C 300	3.95	3596832.8	0	286	-4.6
I1-1027	93-16	C 300	3.95	3735679.3	0	299	-0.42
I1-1028	93-17	C 300	3.94	3620306.8	0	288	-3.9
I1-1029	93-18	C 1000	3.94	10281967	0	1005	0.47
I1-1030	93-19	C 1000	3.94	10621455	0	1047	4.7
I1-1031	93-20	C 1000	3.94	10164882	0	990	-0.98
I1-1032		ACN			0		
I1-1033	94-1	QC 30	3.94	401401.6	0	29.9	-0.46
I1-1034	94-4	QC 100	3.94	1321357.8	0	97.0	-3.0
I1-1035	94-7	QC 750	3.94	8486595	0	790	5.3
I1-1036		ACN			0		
I1-1037	95-1	100 ng/mL LTS	3.94	1321993.6	0	97.0	
I1-1038	95-2	100 ng/mL LTS	3.94	1497441.5	0	110	
I1-1039	95-3	100 ng/mL LTS	3.94	1390483	0	102	

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-1040	96-1	100 ng/mL 4 Hr STB	3.94	1432659.4	0	105	
I1-1041	96-2	100 ng/mL 4 Hr STB	3.94	1354086.6	0	99.5	
I1-1042	96-3	100 ng/mL 4 Hr STB	3.94	1303546.9	0	95.6	
I1-1043	97-1	100 ng/mL FTS 1	3.94	1399420.1	0	103	
I1-1044	97-2	100 ng/mL FTS 1	3.94	1298019.9	0	95.2	
I1-1045	97-3	100 ng/mL FTS 1	3.93	1299493.9	0	95.3	
I1-1046	97-4	100 ng/mL FTS 2	3.93	1341691.8	0	98.5	
I1-1047	97-5	100 ng/mL FTS 2	3.94	1370744.4	0	101	
I1-1048	97-6	100 ng/mL FTS 2	3.94	1398391.4	0	103	
I1-1049	97-7	100 ng/mL FTS 3	3.93	1363808.8	0	100	
I1-1050	97-8	100 ng/mL FTS 3	3.93	1316609.6	0	96.6	
I1-1051	97-9	100 ng/mL FTS 3	3.93	1342886.8	0	98.6	
I1-1052		ACN			0		
I1-1053	94-2	QC 30	3.94	392532.9	0	29.2	-2.5
I1-1054	94-5	QC 100	3.94	1311358.6	0	96.2	-3.8
I1-1055	94-8	QC 750	3.94	7931918.5	0	727	-3.1
I1-1056		ACN			0		
I1-1057	95-4	750 ng/mL LTS	3.94	8171297	0	754	
I1-1058	95-5	750 ng/mL LTS	3.93	7929461.5	0	726	
I1-1059	95-6	750 ng/mL LTS	3.94	8605016	0	804	
I1-1060	96-4	750 ng/mL 4 Hr STB	3.94	8239614	0	762	
I1-1061	96-5	750 ng/mL 4 Hr STB	3.94	8487599	0	790	
I1-1062	96-6	750 ng/mL 4 Hr STB	3.94	8304574.5	0	769	
I1-1063	97-10	750 ng/mL FTS 1	3.94	8069739	0	742	
I1-1064	97-11	750 ng/mL FTS 1	3.94	8888741	0	837	
I1-1065	97-12	750 ng/mL FTS 1	3.94	8518067	0	794	
I1-1066	97-13	750 ng/mL FTS 2	3.94	8493804	0	791	
I1-1067	97-14	750 ng/mL FTS 2	3.94	8812191	0	828	
I1-1068	97-15	750 ng/mL FTS 2	3.94	8028083	0	738	
I1-1069	97-16	750 ng/mL FTS 3	3.94	8571724	0	800	
I1-1070	97-17	750 ng/mL FTS 3	3.94	8013141	0	736	
I1-1071	97-18	750 ng/mL FTS 3	3.94	7930766	0	727	
I1-1072		ACN			0		
I1-1073	94-3	QC 30	3.94	386752.2	0	28.8	-3.9
I1-1074	94-6	QC 100	3.94	1381742	0	102	1.6
I1-1075	94-9	QC 750	3.94	7951439	0	729	-2.8
I1-1076		ACN			0		

Table A10: Calibration, QC And Experimental Samples For Sequence 534004(PFBS)ORU1

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534004.PRO\SampleDB\534004(PFBS)ORU1
Last modified: Tue Aug 30 15:39:28 2005
Method: C:\MASSLYNX\534004.PRO\MethDB\534004 PFBSbio3
Last modified: Thu Aug 25 08:03:03 2005
Job Code:

Printed: Wed Aug 31 08:14:12 2005

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-1150		ACN			0		
I1-1151	107-1	solvent blank			0		
I1-1152	107-2	rat urine blank			0		
I1-1153	107-6	C 30	3.95	149316.8	0	30.1	0.48
I1-1154	107-7	C 30	3.95	147870.1	0	29.8	-0.73
I1-1155	107-8	C 30	3.95	155210.2	0	31.6	5.4
I1-1156	107-9	C 60	3.95	261174.9	0	59.3	-1.2
I1-1157	107-10	C 60	3.95	258191.7	0	58.5	-2.5
I1-1158	107-11	C 60	3.95	235179.1	0	52.3	-13
I1-1159	107-12	C 100	3.95	455934.8	0	113	13
I1-1160	107-13	C 100	3.95	405387.2	0	98.7	-1.3
I1-1161	107-14	C 100	3.95	427098.2	0	105	4.7
I1-1162	107-15	C 300	3.95	1180765.6	0	318	6.1
I1-1163	107-16	C 300	3.95	1032160.1	0	276	-8.0
I1-1164	107-17	C 300	3.95	1108149.6	0	298	-0.82
I1-1165	107-18	C 1000	3.95	3834089.5	0	1053	5.3
I1-1166	107-19	C 1000	3.95	3488228	0	959	-4.1
I1-1167	107-20	C 1000	3.95	3615000.3	0	993	-0.65
I1-1168		ACN			0		
I1-1169	101-1	QC 30	3.95	143014.1	0	28.6	-4.8
I1-1170	101-4	QC 100	3.95	381048.5	0	91.9	-8.1
I1-1171	101-7	QC 750	3.95	2827216.8	0	779	3.8
I1-1172	101-10	QC 100000	3.95	4711359	100	128654	29
I1-1173		ACN			0		
I1-1174	104-1	78139, Day 0, 2M, T12-24	3.95	384569	100	9291	
I1-1175	104-2	78160, Day 0, 2F, T12-24	3.95	252049.1	100	5683	
I1-1176	104-3	78169, Day 0, 2F, T12-24	3.95	319859.6	100	7514	
I1-1177	104-4	78170, Day 0, 2F, T12-24	3.95	139484.9	100	2769	
I1-1178	102-1	100 ng/mL LTS	3.94	383199.6	0	92.5	
I1-1179	102-2	100 ng/mL LTS	3.95	405192.7	0	98.6	

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-1180	102-3	100 ng/mL LTS	3.95	441317.2	0	109	
I1-1181	102-4	750 ng/mL LTS	3.95	2856125.5	0	787	
I1-1182	102-5	750 ng/mL LTS	3.94	2643309.8	0	728	
I1-1183	102-6	750 ng/mL LTS	3.94	2771368.5	0	763	
I1-1184		ACN			0		
I1-1185	101-2	QC 30	3.95	137129.2	0	27.1	-9.6
I1-1186	101-5	QC 100	3.95	345673.6	0	82.2	-18
I1-1187	101-8	QC 750	3.95	2534290.3	0	698	-6.9
I1-1188	101-11	QC 100000	3.95	3564153	100	97975	-2.0
I1-1189		ACN			0		
I1-1190	103-1	100 ng/mL FTS 1	3.95	425046.2	0	104	
I1-1191	103-2	100 ng/mL FTS 1	3.94	388203.2	0	93.9	
I1-1192	103-3	100 ng/mL FTS 1	3.95	410258.9	0	100	
I1-1193	103-4	100 ng/mL FTS 2	3.94	371775.7	0	89.4	
I1-1194	103-5	100 ng/mL FTS 2	3.94	351671.8	0	83.9	
I1-1195	103-6	100 ng/mL FTS 2	3.94	340144.8	0	80.7	
I1-1196	103-7	100 ng/mL FTS 3	3.94	304079.1	0	70.8	
I1-1197	103-8	100 ng/mL FTS 3	3.94	297491.9	0	69.1	
I1-1198	103-9	100 ng/mL FTS 3	3.94	335314.8	0	79.4	
I1-1199	103-10	750 ng/mL FTS 1	3.94	1941573.9	0	533	
I1-1200	103-11	750 ng/mL FTS 1	3.94	2204628.5	0	607	
I1-1201	103-12	750 ng/mL FTS 1	3.94	2098824.5	0	577	
I1-1202	103-13	750 ng/mL FTS 2	3.94	2196924.5	0	604	
I1-1203	103-14	750 ng/mL FTS 2	3.94	2231110.3	0	614	
I1-1204	103-15	750 ng/mL FTS 2	3.94	1860720.1	0	510	
I1-1205	103-16	750 ng/mL FTS 3	3.94	2196247.5	0	604	
I1-1206	103-17	750 ng/mL FTS 3	3.94	2143200.5	0	589	
I1-1207	103-18	750 ng/mL FTS 3	3.94	2323018.5	0	639	
I1-1208		ACN			0		
I1-1209	101-3	QC 30	3.94	139132	0	27.6	-8.0
I1-1210	101-6	QC 100	3.94	302648.2	0	70.5	-30
I1-1211	101-9	QC 750	3.93	2127525	0	585	-22
I1-1212	101-12	QC 100000	3.94	3287830.8	100	90478	-9.5
I1-1213		ACN			0		

Table A11: Calibration, QC And Experimental Samples For Sequence 534004(PFBS)PRU

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534004.PRO\SampleDB\534004(PFBS)PRU
Last modified: Fri Sep 02 16:52:10 2005
Method: C:\MASSLYNX\534004.PRO\MethDB\534004 PFBSbio3
Last modified: Thu Aug 25 08:03:03 2005
Job Code:

Printed: Tue Sep 06 08:20:41 2005

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-1214	109-5	sys suit	4.07	184013.7	0	29.7	
I1-1215	109-5	sys suit	4.07	218221.4	0	37.5	
I1-1216	109-5	sys suit	4.06	204655.7	0	34.4	
I1-1217		ACN	4.1	32894.7	0	2.06	
I1-1218	109-1	solvent blank	4.09	30445.1	0	1.79	
I1-1219	109-2	rat urine blank	4.09	37911.4	0	2.63	
I1-1220	*109-5	C 30	4.05	238312.5	0	42.3	40.9
I1-1221	*109-6	C 30	4.05	220723.4	0	38.1	27.0
I1-1222	*109-7	C 60	4.05	383432.3	0	79.2	32.0
I1-1223	*109-8	C 60	4.05	424759.6	0	90.3	50.5
I1-1224	*109-9	C 100	4.04	647098.8	0	153	53.0
I1-1225	*109-10	C 100	4.04	619193.1	0	145	44.9
I1-1226	*109-11	C 300	4.03	1281313.4	0	346	15.5
I1-1227	*109-12	C 300	4.03	1473881	0	407	35.8
I1-1228	*109-13	C 1000	4.03	4451630.5	0	1387	38.7
I1-1229	*109-14	C 1000	4.02	4034802.5	0	1248	24.8
I1-1230		ACN	4.06	35499.2	0	2.35	
I1-1231	110-1	QC 30	4.03	246072.2	0	44.1	47
I1-1232	110-2	QC 30	4.02	239747.6	0	42.6	42
I1-1233	110-4	QC 100	4.02	684526.3	0	164	64
I1-1234	110-5	QC 100	4.01	441362.1	0	94.8	-5.2
I1-1235	110-7	QC 750	4.01	2826757.8	0	848	13
I1-1236	110-10	QC 100000	4	3375756.5	100	102936	2.9
I1-1237		ACN			0		
I1-1238	111-1	78135, Day 0, 2M, T12-24	4.01	295352.7	100	5631	
I1-1239		ACN			0		
I1-1240	110-3	QC 30	4	172983.9	0	27.2	-9.3
I1-1241	110-6	QC 100	4	457131.6	0	99.1	-0.87

* Not included in regression equation due to drift in instrument reponse.

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-1243	109-9	C 100	4	437816.8	0	93.8	-6.2
I1-1244	109-11	C 300	4	1157141.9	0	308	2.5
I1-1242	110-8	QC 750	4	2808013.5	0	842	12
I1-1245	110-9	QC 750	4	2592910.5	0	771	2.8
I1-1246	110-11	QC 100000	3.99	3755288.5	100	115523	16
I1-1247	110-12	QC 100000	3.99	3728302.5	100	114627	15
I1-1248	109-13	C 1000	3.99	3271311.8	0	995	-0.52
I1-1249	109-5	C 30	4	184131.9	0	29.7	-1.0
I1-1250	109-7	C 60	4	322932.2	0	63.3	5.6
I1-1251		ACN			0		

Table A12: Calibration and Quality Control Samples For Sequence 534004(PFBS)QRS

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534004.PRO\SampleDB\534004(PFBS)QRS
Last modified: Fri Sep 02 16:36:12 2005
Method: C:\MASSLYNX\534004.PRO\MethDB\534004 PFBSbio3
Last modified: Thu Aug 25 08:03:03 2005
Job Code:

Printed: Tue Sep 06 08:33:09 2005

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-1252	113-5	sys suit	4	200260.3	0	34.1	
I1-1253	113-5	sys suit	4	173495.1	0	28.5	
I1-1254	113-5	sys suit	4	172148.6	0	28.2	
I1-1255		ACN			0		
I1-1256	113-1	solvent blank			0		
I1-1257	113-2	rat serum blank			0		
I1-1258	113-5	C 30	4	195361.3	0	33.1	10
I1-1259	113-6	C 30	3.99	170540.5	0	27.9	-7.0
I1-1260	113-7	C 60	3.99	309116.8	0	58.4	-2.6
I1-1261	113-8	C 60	3.99	303233.3	0	57.1	-4.9
I1-1262	113-9	C 100	3.98	867729.9	0	211	111
I1-1263	113-10	C 100	3.98	499773.6	0	106	6.1
I1-1264	113-11	C 300	3.98	1115400.6	0	288	-4.1
I1-1265	113-12	C 300	3.98	1187769.3	0	311	3.7
I1-1266	113-13	C 1000	3.98	3092176	0	1023	2.3
I1-1267	113-14	C 1000	3.98	2979016.3	0	976	-2.4
I1-1268		ACN			0		
I1-1269	114-1	QC 30	3.98	189090.5	0	31.7	5.8
I1-1270	114-2	QC 30	3.98	181630.3	0	30.2	0.62
I1-1271	114-3	QC 30	3.98	174089.5	0	28.6	-4.5
I1-1272	94-1	QC 30	3.98	167028.8	0	27.2	-9.3
I1-1273	94-2	QC 30	3.98	156163.9	0	25.0	-17
I1-1274	94-3	QC 30	3.98	155670.1	0	24.9	-17
I1-1275	114-4	QC 100	3.97	401112.7	0	80.8	-19
I1-1276	114-5	QC 100	3.97	482076.2	0	101	1.5
I1-1277	114-6	QC 100	3.97	412570.1	0	83.6	-16
I1-1278	94-4	QC 100	3.97	378670.7	0	75.2	-25
I1-1279	94-5	QC 100	3.98	366759.9	0	72.3	-28
I1-1280	94-6	QC 100	3.97	447016.9	0	92.4	-7.6
I1-1281	114-7	QC 750	3.97	2491581.3	0	782	4.2

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-1282	114-8	QC 750	3.97	2292516.5	0	705	-6.0
I1-1283	114-9	QC 750	3.97	2080994.5	0	625	-17
I1-1284	94-7	QC 750	3.97	2358406	0	730	-2.7
I1-1285	94-8	QC 750	3.97	2283035.5	0	701	-6.5
I1-1286	94-9	QC 750	3.97	1906261	0	560	-25
I1-1287		ACN			0		

Table A13: Calibration and Quality Control Samples For Sequence 534004(PFBS)RRU3a

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534004.PRO\SampleDB\534004(PFBS)RRU3a
Last modified: Fri Sep 09 08:29:15 2005
Method: C:\MASSLYNX\534004.PRO\MethDB\534004 PFBSbio3
Last modified: Thu Aug 25 08:03:03 2005
Job Code:

Printed: Fri Sep 09 09:05:06 2005

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-1337	116-5	sys suit	3.93	258673.5	0	44.2	
I1-1338	116-5	sys suit	3.91	218785.4	0	37.9	
I1-1339	116-5	sys suit	3.92	250686.4	0	42.9	
I1-1340					0		
I1-1341	116-1				0		
I1-1342	116-2				0		
I1-1343	116-5	C 30	3.93	171285.1	0	30.3	0.93
I1-1344	116-7	C 60	3.94	371958.6	0	62.1	3.5
I1-1345	116-9	C 100	3.93	554236.6	0	91.0	-9.1
I1-1346	116-11	C 300	3.94	1958282.5	0	323	7.8
I1-1347	116-13	C 1000	3.94	5424581.5	0	977	-2.3
I1-1348					0		
I1-1349	117-1	QC 30	3.94	204598.8	0	35.6	19
I1-1350	117-4	QC 100	3.93	543419.9	0	89.2	-11
I1-1351	117-7	QC 750	3.93	3548690	0	610	-19
I1-1352	118-1	100 ng/mL FTS 1	3.93	492782.4	0	81.2	
I1-1353	118-2	100 ng/mL FTS 1	3.94	683637.1	0	112	
I1-1354	118-3	100 ng/mL FTS 1	3.93	560672.3	0	92.0	
I1-1355	118-4	100 ng/mL FTS 2	3.93	486633.7	0	80.2	
I1-1356	118-5	100 ng/mL FTS 2	3.93	505598.4	0	83.2	
I1-1357	118-6	100 ng/mL FTS 2	3.93	563404.9	0	92.4	
I1-1358	118-7	100 ng/mL FTS 3	3.93	557186.7	0	91.4	
I1-1359	118-8	100 ng/mL FTS 3	3.93	518047.4	0	85.2	
I1-1360	118-9	100 ng/mL FTS 3	3.93	554873.9	0	91.1	
I1-1361	117-2	QC 30	3.93	130501.2	0	23.7	-21
I1-1362	117-5	QC 100	3.94	558833.5	0	91.7	-8.3
I1-1363	117-8	QC 750	3.94	3726731.3	0	644	-14

Table A14: Calibration and Quality Control Samples For Sequence 534004(PFBS)RRU3b

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\534004.PRO\SampleDB\534004(PFBS)RRU3b
Last modified: Fri Sep 09 08:37:32 2005
Method: C:\MASSLYNX\534004.PRO\MethDB\534004 PFBSbio3
Last modified: Thu Aug 25 08:03:03 2005
Job Code:

Printed: Fri Sep 09 09:02:51 2005

Compound 1: PFBS

Run #	Ref. # (534004-)	Sample Text	RT	Area	Mult.	Conc. (ng/mL)	% RE
I1-1361	117-2	QC 30	3.93	130501.2	0	33.8	13
I1-1362	117-5	QC 100	3.94	558833.5	0	136	36
I1-1363	117-8	QC 750	3.94	3726731.3	0	909	21
I1-1364	118-10	750 ng/mL FTS 1	3.94	3609075.5	0	880	
I1-1365	118-11	750 ng/mL FTS 1	3.94	4013859.5	0	982	
I1-1366	118-12	750 ng/mL FTS 1	3.94	3146625	0	764	
I1-1367	118-13	750 ng/mL FTS 2	3.94	3823120.5	0	933	
I1-1368	118-14	750 ng/mL FTS 2	3.94	3168622.8	0	769	
I1-1369	118-15	750 ng/mL FTS 2	3.94	3271947	0	795	
I1-1370	118-16	750 ng/mL FTS 3	3.94	3371375	0	820	
I1-1371	118-17	750 ng/mL FTS 3	3.94	3073515	0	746	
I1-1372	118-18	750 ng/mL FTS 3	3.94	3085492.3	0	749	
I1-1373					0		
I1-1374	117-3	QC 30	3.94	118535.5	0	30.9	2.9
I1-1375	116-6	C 30	3.94	116238.9	0	30.3	0.99
I1-1376	116-8	C 60	3.94	256784.8	0	64.0	6.7
I1-1377	116-10	C 100	3.94	349611.2	0	86.1	-14
I1-1378	117-6	QC 100	3.94	456292.9	0	111	11
I1-1379	116-12	C 300	3.94	1392718.8	0	334	11
I1-1380	117-9	QC 750	3.94	3013797	0	731	-2.6
I1-1381	116-14	C 1000	3.94	3962145.3	0	969	-3.1

APPENDIX H

Toxicokinetic Report (WIL Research Laboratories, LLC)

STUDY TITLE

PHARMACOKINETIC (IN BLOOD) AND EXCRETION STUDY OF PERFLUOROHEXANOIC ACID AND NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

REPORT TITLE

PHARMACOKINETICS OF PERFLUOROHEXANOIC ACID AND NONAFLUORO-1-BUTANESULFONIC ACID IN SERUM AND URINE FOLLOWING A SINGLE INTRAVENOUS DOSE TO RATS

REPORT DATE

17 October 2005

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1.0 SUMMARY

For the pharmacokinetic phase, two groups of nine male and nine female Crl:CD®(SD) rats received a single intravenous (bolus) injection of perfluorohexanoic acid (PFHxA) or nonafluoro-1-butanesulfonic acid (PFBS). The dosage level for both test articles was 10 mg/kg. Blood samples were collected from three animals/sex/group at 0 (prior to dosing), 0.5, 1, 1.5, 2, 4, 8, and 24 hours following each dose.

For the urine excretion phase, two groups of three male and three female Crl:CD®(SD) rats received a single intravenous (bolus) injection of PFHxA or PFBS. The dosage level for both test articles was 10 mg/kg. Urine was collected from each animal over the following intervals: 0-6, 6-12, and 12-24 hours 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 a regression analysis of the measured values. The mean concentrations in serum and mean amounts excreted in urine were used for pharmacokinetic analysis.

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

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	52146	86539	1.0	0.116	0.175	2.1	84.0
Females	16538	12909	0.42	0.775	0.466	2.5	76.9
PFBS							
Males	68921	253837	2.1	0.0394	0.118	3.1	69.0
Females	30190	32197	0.64	0.311	0.288	2.4	71.6

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

Systemic exposures to PFBS were approximately 2.5- to 3-fold higher than exposures to PFHxA at equivalent dosages. This is partially due to a terminal half-life for PFBS that is longer than that for PFHxA. Exposure to both PFBS and PFHxA was approximately 7- to 8-fold higher for male rats than for female rats. This may be attributable to several related factors such as the shorter half-life and higher apparent clearance and volume of distribution in females than in males. The terminal half-life of PFHxA in serum was approximately 2.5-fold shorter for female rats than for male rats. The terminal half-life of PFBS in serum was approximately 3-fold shorter for female rats than for male rats. Apparent clearance of PFHxA and PFBS from the serum was approximately 7- to 8-fold higher for female rats than for male rats; apparent volume of distribution for PFHxA and PFBS in the serum was approximately 2.5- to 3-fold higher for female rats than for male

rats. Low apparent volumes of distribution for both male and female rats may reflect the rapid clearance and elimination rather than distribution outside of the vasculature.

Approximately 80% of the administered dose of PFHxA and approximately 70% of the administered dose of PFBS was recovered in the urine during 24 hours post-dosing regardless of gender. The half-life for urinary elimination of PFHxA and PFBS ranged from approximately 2 to 3 hours, regardless of gender.

2.0 INTRODUCTION

For the pharmacokinetic phase, two groups of nine male and nine female Crl:CD®(SD) rats received a single intravenous (bolus) injection of perfluorohexanoic acid (PFHxA) or nonafluoro-1-butanesulfonic acid (PFBS). The dosage level for both test articles was 10 mg/kg. Blood samples (approximately 0.5 mL) were collected from three animals/sex/group at 0 (prior to dosing), 0.5, 1, 1.5, 2, 4, 8, and 24 hours following each dose. Blood samples were collected via a retro-orbital sinus while the animal was under isoflurane anesthesia into tubes containing no anticoagulant. Serum was separated using a refrigerated centrifuge. Samples were stored at approximately -20°C until analysis.

For the urine excretion phase, two groups of three male and three female Crl:CD®(SD) rats received a single intravenous (bolus) injection of PFHxA or PFBS. The dosage

level for both test articles was 10 mg/kg. Urine was collected from each animal over the following intervals: 0-6, 6-12, and 12-24 hours post-dosing. Urine samples were maintained on wet ice during collection. Samples were stored at approximately -20°C until analysis.

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 based on a regression analysis of the measured values. The mean concentrations in serum and mean 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 for PFHxA and PFBS, respectively. The lower limit of quantitation (LLOQ) was 30 ng/mL for both test articles in both matrices.

3.3 Toxicokinetic Evaluation & Statistical Analysis

All toxicokinetic parameters were calculated from the mean 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 measured values.
$AUC_{0\text{-}24}$	The area under the serum concentration vs. time curve from 0 to 24 h post-dosing. The values were calculated by linear trapezoidal summation using the equation: $$AUC_{0\text{-}24} = \Sigma\ (0.5 \bullet (y_1 + y_2) \bullet \Delta\ t)$$ where y_1 and y_2 are successive serum concentrations and Δt is the sampling interval, in hours, between y_1 and y_2.
$AUC_{0\text{-}\infty}$	The estimate of the area under the serum concentration vs. time curve from 0 h to infinity. The values were calculated using the formula: $$AUC_{0\text{-}\infty} = AUC_{0\text{-}24} + (\text{serum concentration at 24 h}/K_{el})$$ where $AUC_{0\text{-}24}$ 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 0.5 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: $Cl = \text{Dosage}/ AUC_{0-\infty}$ 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 * Mean ARE at 0 h/(Mean BW * Dosage) where ARE is defined previously and BW is the mean body weight used to calculate the administered dose. Mean body weights were obtained from Table 6 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. The estimation of C_0 may be inaccurate because the first sampling point was 0.5 h post-dosing.

4.0 RESULTS AND DISCUSSION

4.1 Serum Concentration Data

Concentrations of PFHxA and PFBS in individual serum samples can be found in the tables of the bioanalytical reports (Appendices F and G, respectively).

4.1.1 PFHxA in Male Rats

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

Table 1. Mean ± SD Concentrations of PFHxA in Serum of Male Rats following Intravenous Administration of 10 mg PFHxA/kg

Hours Post-Dosing	Mean (ng/mL)	SD
0*	52146	N/A
0.5	37518	3861
1	29372	1505
1.5	17300	2000
2	12648	4815
4	4366	3279
8	250	118
24	0.00	0.00

*Estimated value. N/A = not applicable.

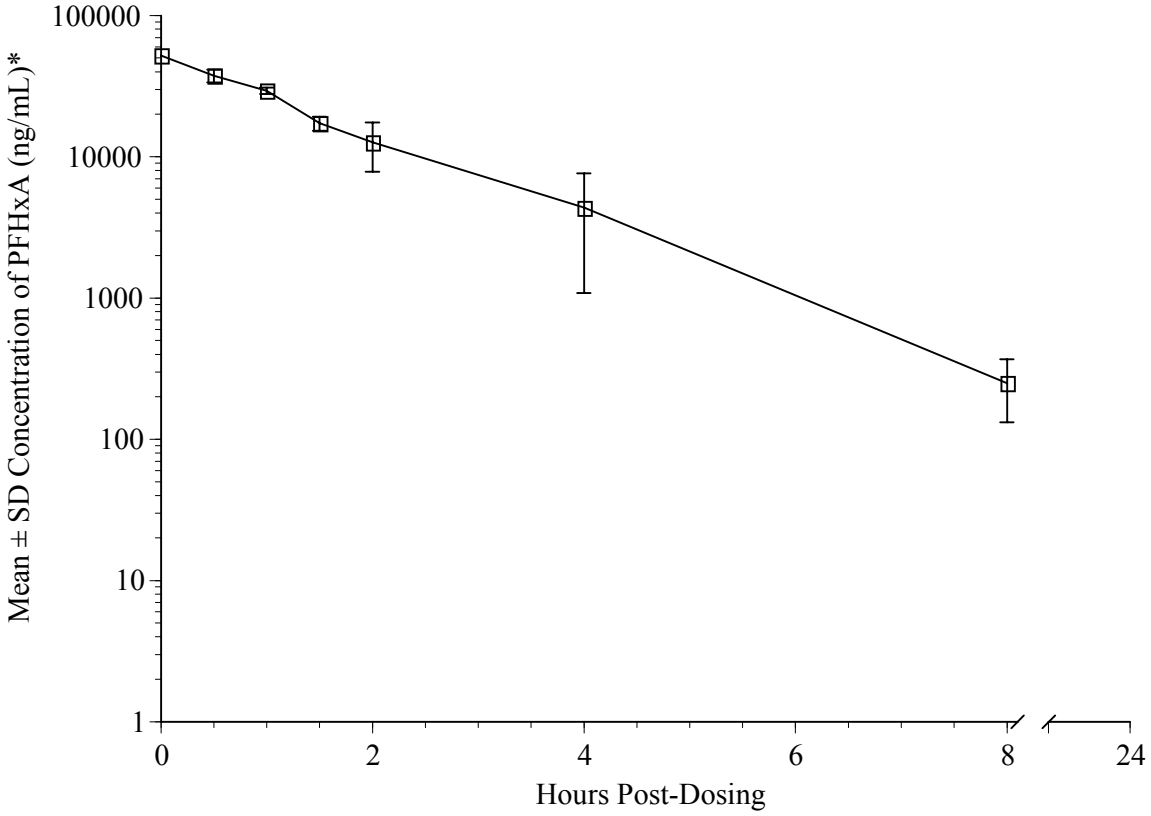

Figure 1. Mean Concentrations of PFHxA in Serum of Male Rats following Intravenous Administration of 10 mg PFHxA/kg

*Concentration at 0 h estimated.

The concentrations of PFHxA in serum of male rats appeared to decrease in a log-linear manner from the first sampling time (0.5 h post-dosing) through 8 hours post-dosing. The concentration of PFHxA was below the LLOQ (30 ng/mL) by 24 hours post-dosing.

4.1.2 PFHxA in Female Rats

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

Table 2. Mean ± SD Concentrations of PFHxA in Serum of Female Rats following Intravenous Administration of 10 mg PFHxA/kg

Hours Post-Dosing	Mean (ng/mL)	SD
0*	16538	N/A
0.5	12808	4501
1	2015	778
1.5	993	218
2	634	440
4	24.5	21.4
8	0.00	0.00
24	0.00	0.00

*Estimated value. N/A = not applicable.

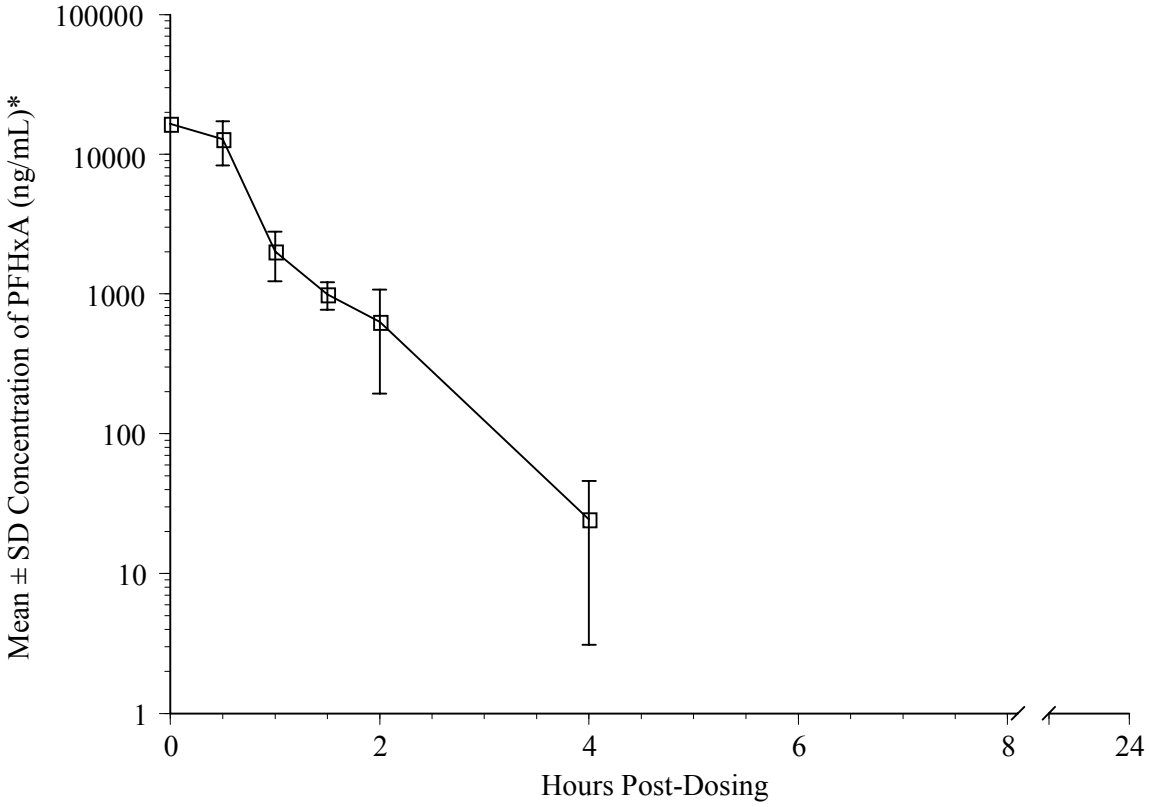

Figure 2. Mean Concentrations of PFHxA in Serum of Female Rats following Intravenous Administration of 10 mg PFHxA/kg

*Concentration at 0 h estimated.

The concentrations of PFHxA in serum of female rats appeared to decrease in a log-linear manner from the first sampling time (0.5 h post-dosing) through 4 hours post-dosing. The concentration of PFHxA was below the LLOQ (30 ng/mL) by 8 hours post-dosing.

4.1.3 PFBS in Male Rats

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

Table 3. Mean ± SD Concentrations of PFBS in Serum of Male Rats following Intravenous Administration of 10 mg PFBS/kg

Hours Post-Dosing	Mean (ng/mL)	SD
0*	68921	N/A
0.5	58933	10854
1	49479	5799
1.5	34626	4076
2	30297	9272
4	27879	14492
8	4334	698
24	22.0	38.1

*Estimated value. N/A = not applicable.

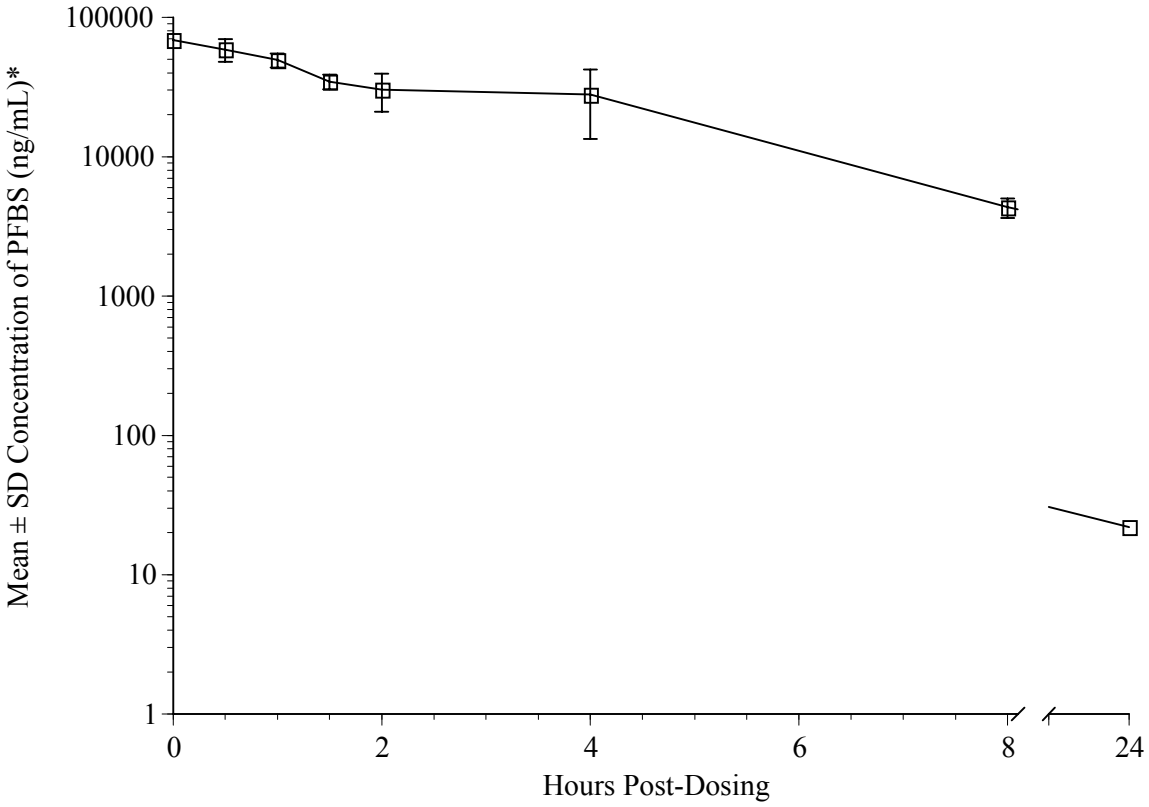

Figure 3. Mean Concentrations of PFBS in Serum of Male Rats following Intravenous Administration of 10 mg PFBS/kg

*Concentration at 0 h estimated.

The concentrations of PFBS in serum of male rats appeared to decrease in a near log-linear manner from the first sampling time (0.5 h post-dosing) through 24 hours post-dosing, however, only one of the three animals sampled at 24 hours post-dosing had a concentration of PFBS that was above the LLOQ (30 ng/mL).

4.1.4 PFBS in Female Rats

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

Table 4. Mean ± SD Concentrations of PFBS in Serum of Female Rats following Intravenous Administration of 10 mg PFBS/kg

Hours Post-Dosing	Mean (ng/mL)	SD
0*	30190	N/A
0.5	24028	1890
1	8569	1229
1.5	4456	1017
2	3858	924
4	433	132
8	0.00	0.00
24	0.00	0.00

*Estimated value. N/A = not applicable.

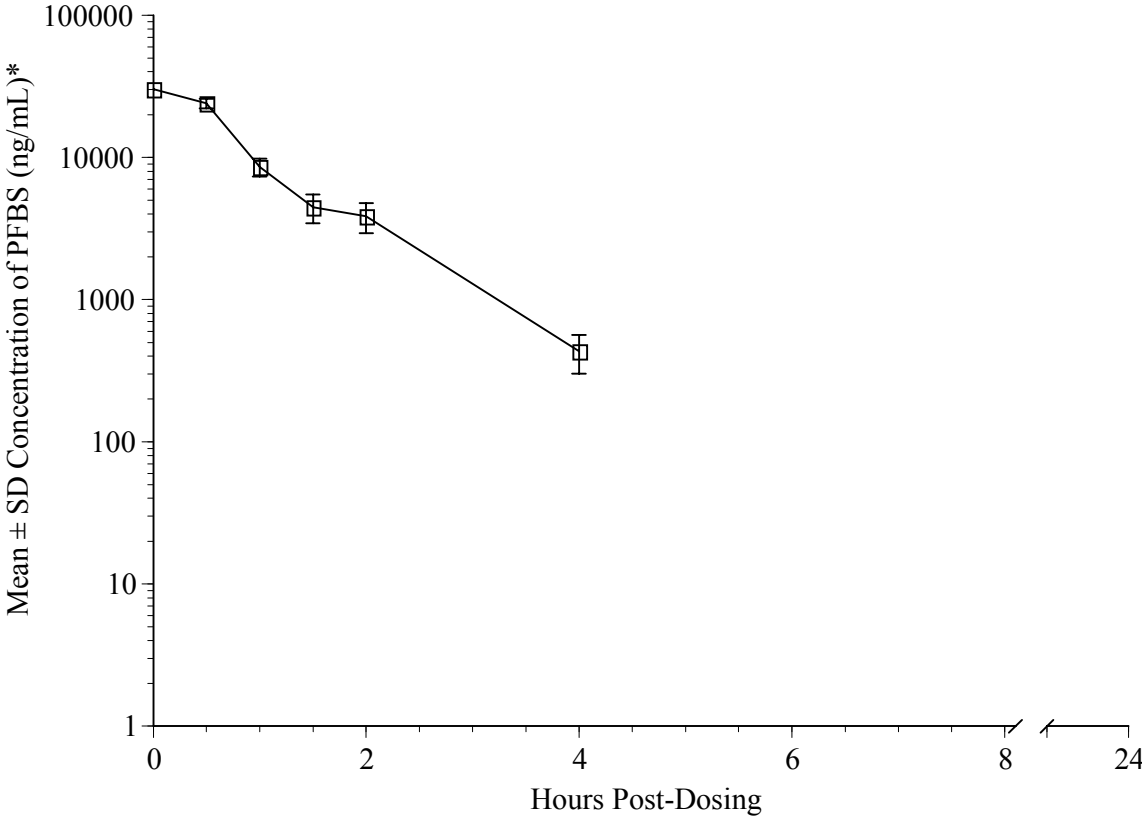

Figure 4. Mean Concentrations of PFBS in Serum of Female Rats following Intravenous Administration of 10 mg PFBS/kg

*Concentration at 0 h estimated.

The concentrations of PFBS in serum of female rats appeared to decrease in a log-linear manner from the first sampling time (0.5 h post-dosing) through 4 hours post-dosing. The concentration of PFBS was below the LLOQ (30 ng/mL) by 8 hours post-dosing.

4.2 Serum Pharmacokinetics

4.2.1 PFHxA Pharmacokinetics

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

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

	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	52146	86539	0.661	1.0	0.116	0.175
Females	16538	12909	1.66	0.42	0.775	0.466

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

The pharmacokinetic parameters for PFHxA differed between the genders. Systemic exposure ($AUC_{0-\infty}$) for male rats was almost 7-fold higher than for female rats. This may be attributable to several related factors as follows. The terminal half-life of PFHxA in serum was about 2.5-fold shorter for female rats than for male rats. Apparent clearance of PFHxA from the serum and the apparent volume of distribution were approximately 7-fold higher and 3-fold higher, respectively, for female rats than for male rats. Low apparent volumes of distribution for both male and female rats may reflect the rapid clearance and elimination rather than distribution outside of the vasculature.

4.2.2 PFBS Pharmacokinetics

The pharmacokinetic parameters for PFBS in serum of male and female rats are presented in Table 6.

Table 6. PFBS Serum Pharmacokinetic Parameters following Intravenous Administration of 10 mg PFBS/kg to Male and Female Rats

	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	68921	253837	0.335	2.1	0.0394	0.118
Females	30190	32197	1.08	0.64	0.311	0.288

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

The pharmacokinetic parameters for PFBS differed between the genders. Systemic exposure ($AUC_{0-\infty}$) for male rats was almost 8-fold higher than for female rats. This may be attributable to several related factors as follows. The terminal half-life of PFBS in serum was about 3-fold shorter for female rats than for male rats. Apparent clearance of PFBS from the serum and the apparent volume of distribution were approximately 8-fold higher and 2.5-fold higher, respectively, for female rats than for male rats. Low apparent volumes of distribution for both male and female rats may reflect the rapid clearance and elimination rather than distribution 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 reports (Appendices F and G, respectively).

4.3.1 PFHxA Urinary Elimination in Male Rats

Mean amounts of PFHxA eliminated in urine and urinary pharmacokinetic parameters following a single intravenous dosage of 10 mg PFHxA/kg to male rats are presented in Table 7, with the amount remaining to be eliminated illustrated in Figure 5.

Table 7. Mean ± SD Amounts of PFHxA in Urine of Male Rats following Intravenous Administration of 10 mg PFHxA/kg

Hours Post-Dosing	Mean (µg)	SD
0-6	1768	272
6-12	184	118
12-24	39.2	8.33
Total Eliminated as a % of Dose	84.0	N/A
Elimination Rate Constant (1/h)*	0.327	N/A
Half-life (h)*	2.1	N/A

*Based on 0-12 h. N/A = not applicable.

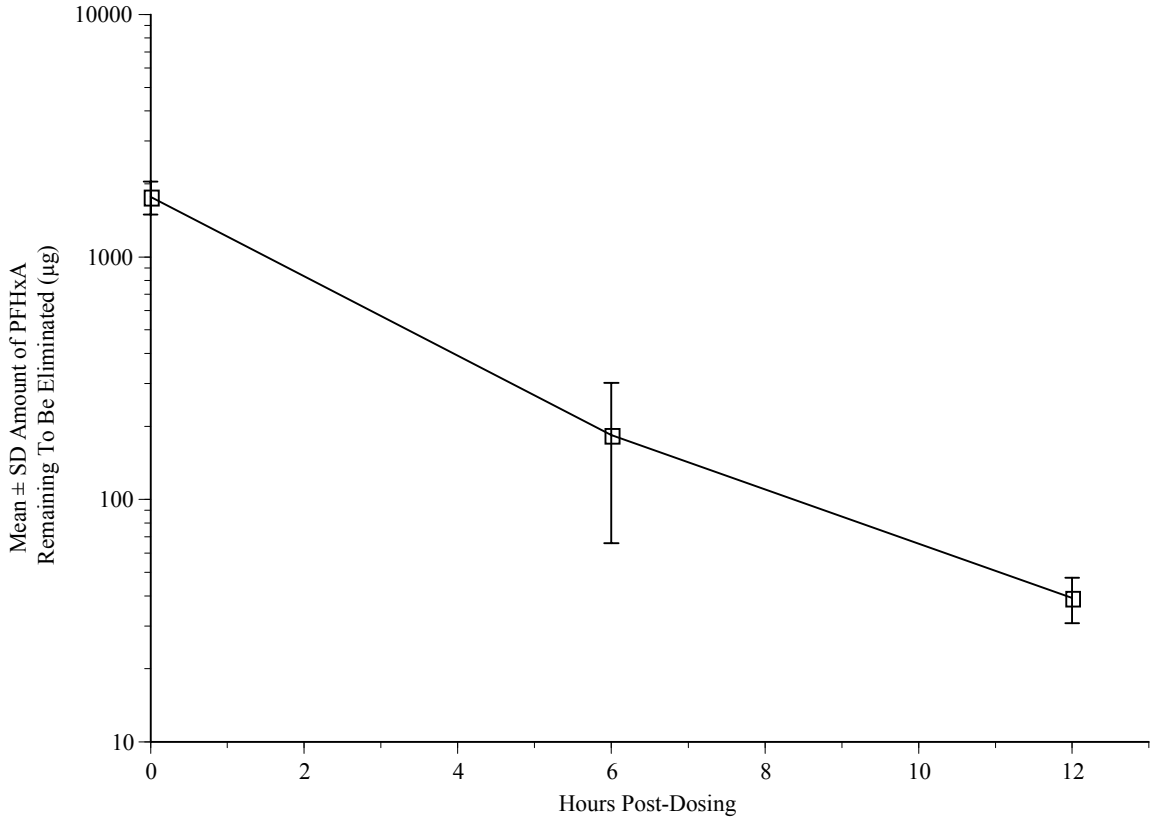

Figure 5. Mean Amounts of PFHxA Remaining To Be Eliminated in Urine of Male Rats following Intravenous Administration of 10 mg PFHxA/kg

The amount of PFHxA recovered in the urine of male rats accounted for 84% of the administered dose. The elimination of PFHxA in the urine of male rats appeared to be monoexponential with a calculated half-life of 2.1 h.

4.3.2 PFHxA Urinary Elimination in Female Rats

Mean amounts of PFHxA eliminated in urine and urinary pharmacokinetic parameters following a single intravenous dosage of 10 mg PFHxA/kg to female rats are presented in Table 8, with the amount remaining to be eliminated illustrated in Figure 6.

Table 8. Mean ± SD Amounts of PFHxA in Urine of Female Rats following Intravenous Administration of 10 mg PFHxA/kg

Hours Post-Dosing	Mean (µg)	SD
0-6	1273	149
6-12	96.5	6.71
12-24	54.0	24.3
Total Eliminated as a % of Dose	76.9	N/A
Elimination Rate Constant (1/h)*	0.273	N/A
Half-life (h)*	2.5	N/A

*Based on 0-12 h. N/A = not applicable.

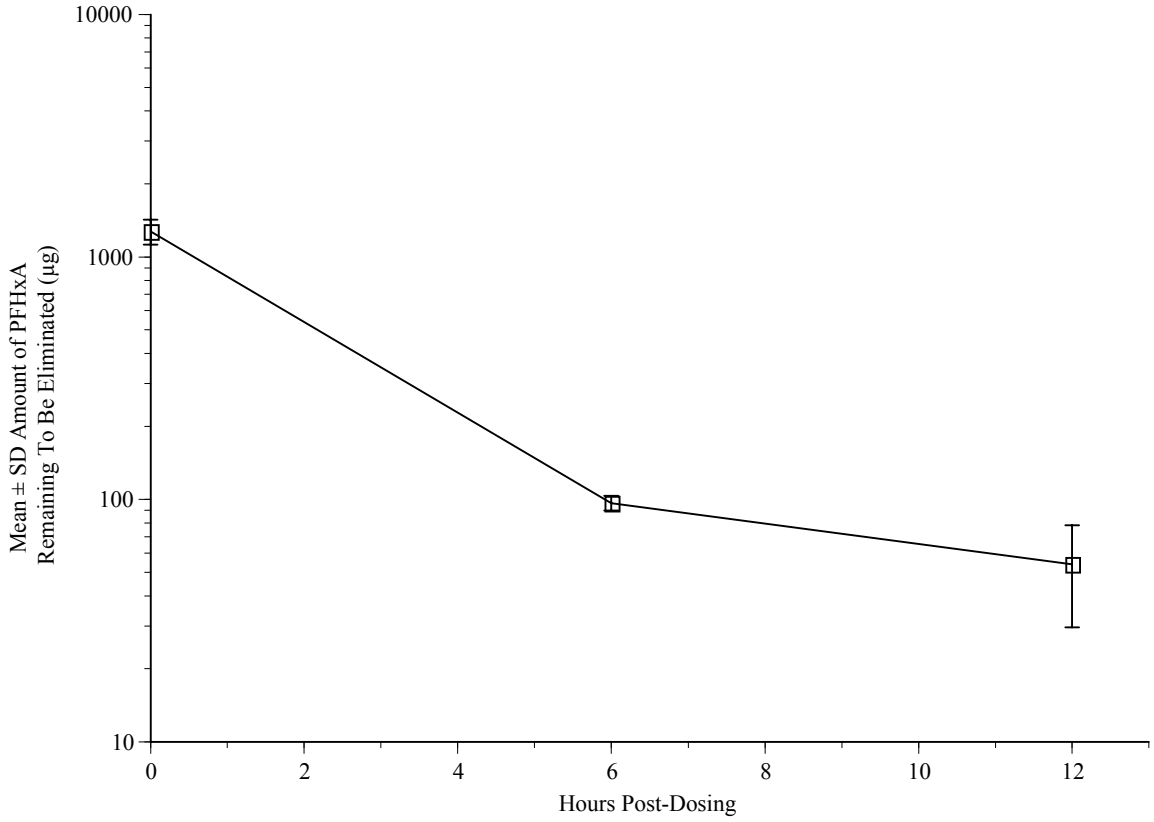

Figure 6. Mean Amounts of PFHxA Remaining To Be Eliminated in Urine of Female Rats following Intravenous Administration of 10 mg PFHxA/kg

The amount of PFHxA recovered in the urine of female rats accounted for approximately 77% of the administered dose. The elimination of PFHxA in the urine of female rats appeared to be monoexponential with a calculated half-life of 2.5 h.

4.3.3 PFBS Urinary Elimination in Male Rats

Mean amounts of PFBS eliminated in urine and urinary pharmacokinetic parameters following a single intravenous dosage of 10 mg PFBS/kg to male rats are presented in Table 9, with the amount remaining to be eliminated illustrated in Figure 7.

Table 9. Mean ± SD Amounts of PFBS in Urine of Male Rats following Intravenous Administration of 10 mg PFBS/kg

Hours Post-Dosing	Mean (µg)	SD
0-6	1063	277
6-12	493	260
12-24	115	31.9
Total Eliminated as a % of Dose	69.0	N/A
Elimination Rate Constant (1/h)*	0.223	N/A
Half-life (h)*	3.1	N/A

*Based on 0-12 h. N/A = not applicable.

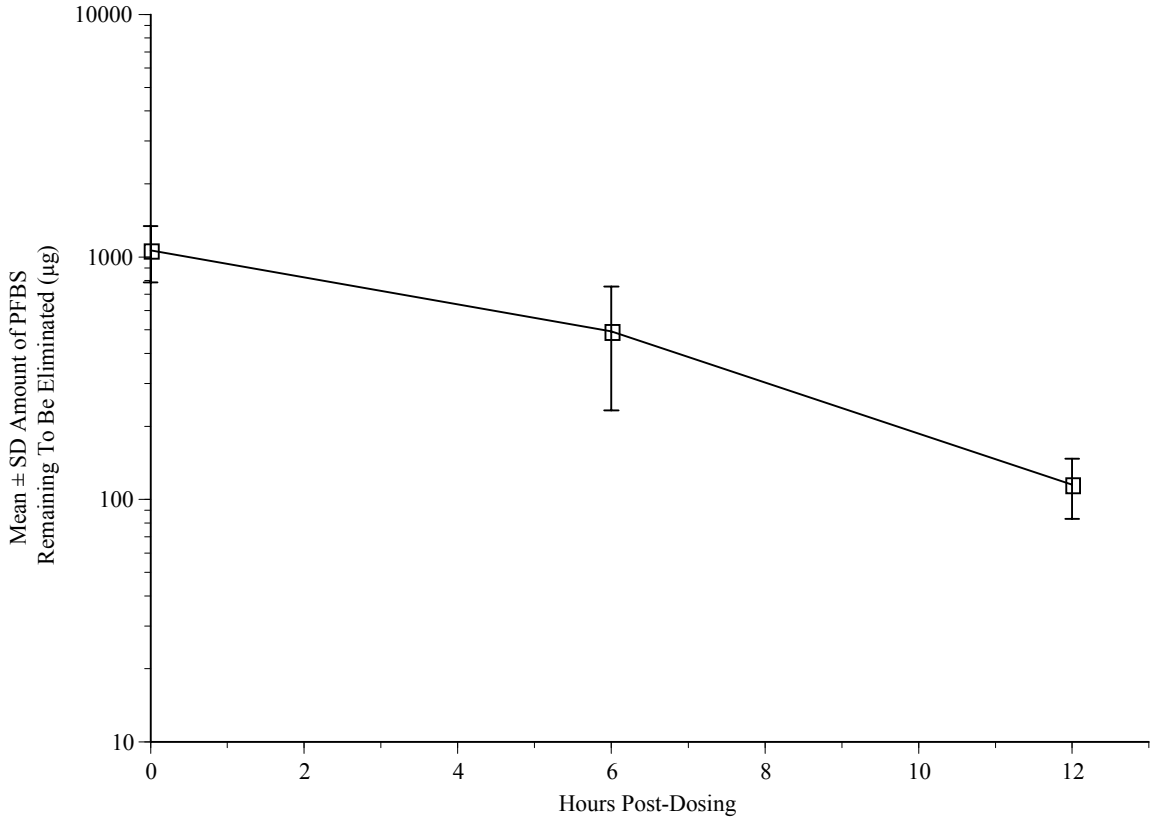

Figure 7. Mean Amounts of PFBS Remaining To Be Eliminated in Urine of Male Rats following Intravenous Administration of 10 mg PFBS/kg

The amount of PFBS recovered in the urine of male rats accounted for approximately 70% of the administered dose. The elimination of PFBS in the urine of male rats appeared to be monoexponential with a calculated half-life of 3.1 h.

4.3.4 PFBS Urinary Elimination in Female Rats

Mean amounts of PFBS eliminated in urine and urinary pharmacokinetic parameters following a single intravenous dosage of 10 mg PFBS/kg to female rats are presented in Table 10, with the amount remaining to be eliminated illustrated in Figure 8.

Table 10. Mean ± SD Amounts of PFBS in Urine of Female Rats following Intravenous Administration of 10 mg PFBS/kg

Hours Post-Dosing	Mean (µg)	SD
0-6	1165	407
6-12	139	21.2
12-24	42.2	10.6
Total Eliminated as a % of Dose	71.6	N/A
Elimination Rate Constant (1/h)*	0.289	N/A
Half-life (h)*	2.4	N/A

*Based on 0-12 h. N/A = not applicable.

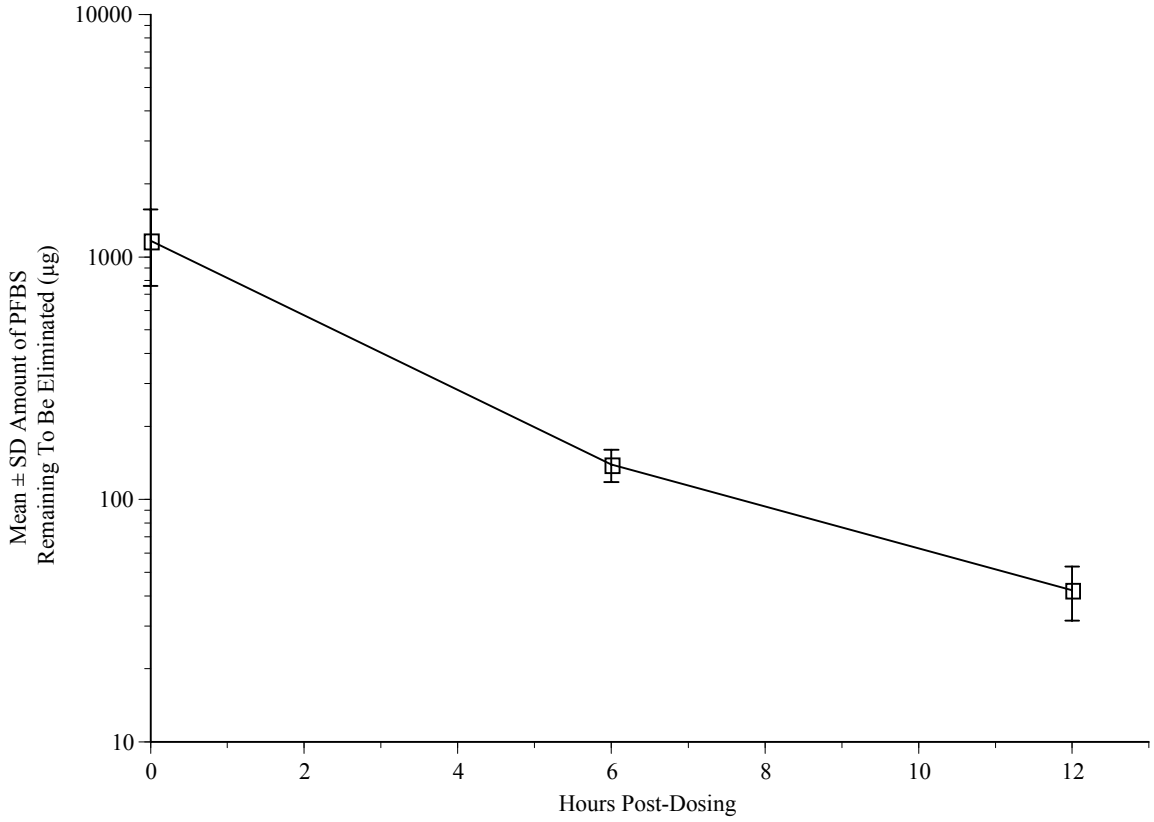

Figure 8. Mean Amounts of PFBS Remaining To Be Eliminated in Urine of Female Rats following Intravenous Administration of 10 mg PFBS/kg

The amount of PFBS recovered in the urine of female rats accounted for approximately 72% of the administered dose. The elimination of PFBS in the urine of female rats appeared to be monoexponential with a calculated half-life of 2.4 h.

5.0 CONCLUSIONS

Systemic exposures to PFBS were approximately 2.5- to 3-fold higher than exposures to PFHxA at equivalent dosages. This is partially due to a terminal half-life for PFBS that is longer than that for PFHxA. Exposure to both PFBS and PFHxA was approximately 7- to 8-fold higher for male rats than for female rats. This may be attributable to several related factors such as the shorter half-life and higher apparent clearance and volume of distribution in females than in males. The terminal half-life of PFHxA in serum was approximately 2.5-fold shorter for female rats than for male rats. The terminal half-life of PFBS in serum was approximately 3-fold shorter for female rats than for male rats. Apparent clearance of PFHxA and PFBS from the serum was approximately 7- to 8-fold higher for female rats than for male rats; apparent volume of distribution for PFHxA and PFBS in the serum was approximately 2.5- to 3-fold higher for female rats than for male rats. Low apparent volumes of distribution for both male and female rats may reflect the rapid clearance and elimination rather than distribution outside of the vasculature.

Approximately 80% of the administered dose of PFHxA and approximately 70% of the administered dose of PFBS was recovered in the urine during 24 hours post-dosing regardless of gender. The half-life for urinary elimination of PFHxA and PFBS ranged from approximately 2 to 3 hours, regardless of gender.

6.0 RESPONSIBLE PERSONNEL

Report Prepared By:

for 17 Oct 2005

Daniel W. Sved, Ph.D.
Director, Metabolism and Analytical Chemistry

Date

Report Reviewed By:

Jeannie B. Kirkpatrick 17 Oct 2005

Jeannie B. Kirkpatrick, M.S.
Staff Toxicologist;
Study Director

Date

APPENDIX I

Study Protocol

Study Number: WIL-534004

PROTOCOL AMENDMENT I

Sponsor:AGC Chemical

A. Title of Study:

Pharmacokinetic (in Blood) and Excretion Study of Perfluorohexanoic Acid and Nonafluoro-1-Butanesulfonic Acid in Rats.

B. Protocol Modification:

1) **3 STUDY SCHEDULE:**

Proposed Experimental Termination (Completion) Date:	September 9, 2005
Proposed Audited Draft Report Date:	September 30, 2005

2) **4.2.8 Personnel Safety:**

In accordance with the study director notification dated April 6, 2005, nitrile gloves are to be worn when handling the test article or dosing formulations.

3) **7.5.2 Homogeneity and Stability of Test Article Formulations:**

In accordance with the study director notification dated April 6, 2005, two-day stability evaluations will be performed for the PFHxA formulation.

8.5 Toxicokinetics for Elimination:

In accordance with the study director notification dated April 5, 2005, the following changes were made:

4)	Target Blood Volumes	Tubes for blood collection will not be chilled prior to collection of blood samples.
5)	Serum Preparation	The centrifuge temperature will be set at 4°C.

C. Reasons for Protocol Modifications:

1) Proposed experimental completion and audited draft report dates are added.

2) Addition of personnel safety precautions.

3) Addition of two-day stability evaluation for the PFHxA formulation to provide information which was inadvertently not collected in WIL-534002.

4) Clarification that sampling tubes will not be pre-chilled.

5) Correction of sample type and centrifuge temperature.

Approved By:

AGC Chemical

Motoki Shinohara
Sponsor Representative

4/10/2005
Date

Prepared By:

WIL Research Laboratories, LLC

Jeannie B. Kirkpatrick, MS
Study Director

11 October 2005
Date

Christopher P. Chengelis, PhD, DABT
Director, Toxicology

11 Oct 2005
Date

PROTOCOL

PHARMACOKINETIC (IN BLOOD) AND EXCRETION STUDY OF PERFLUOROHEXANOIC ACID AND NONAFLUORO-1-BUTANESULFONIC ACID IN RATS

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 articles in rats.

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:

Joseph F. Holson, Ph.D.
President, Director

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

Ronald E. Wilson, B.S.
Director, Informational Systems

Jay G. Henson, B.S.
Group Manager, Study Analysis and Reports

Philip L. Stetson, M.D., Ph.D.
Associate Director, Analytical Chemistry

Carol A. Kopp, B.S., L.A.T.
Manager, Gross Pathology and
Developmental Toxicology Laboratory

Heather L. Osborn, B.S., RQAP-GLP
Manager, Quality Assurance

Daniel W. Sved, Ph.D.
Director, Metabolism and Analytical Chemistry

Susan C. Haley, B.S.
Manager, Clinical Pathology

Lisa T. Snyder, D.V.M.
Clinical Veterinarian

3 STUDY SCHEDULE:

Proposed Experimental Starting Date (Animal Receipt):	March 22, 2005
Proposed Experimental Start Date:	April 6, 2005
Proposed Euthanasia Date:	April 7, 2005
Proposed Experimental Termination (Completion) Date:	To be added by protocol amendment
Proposed Audited Draft Report Date:	To be determined.

4 TEST ARTICLE DATA:

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:

The test article is considered to be stable under the storage conditions provided by 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 Data:

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. A material safety data sheet (MSDS) should accompany the test article upon arrival at the laboratory.

4.1.9 Vehicle:

Sterile water for injection.

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:

Sterile water for injection.

5 TEST SYSTEM:

5.1 Species:

Rat

5.2 Strain:

Sprague-Dawley Crl:CD®(SD)

5.3 Source:

Charles River Laboratories, Inc.
(Facility to be documented in the study records)

5.4 Number on Study:

Thirty (30) animals of each sex will be ordered. Twenty-four males and 24 females will be placed on study. Animals not utilized on study will be deemed as part of the stock colony or euthanized by CO_2 inhalation and discarded.

This number of animals is considered to be the minimum required for meaningful interpretation of the data and fulfillment of agency requirements.

5.5 Approximate Age and Weight:

Animals will be approximately 5 to 7 weeks of age when received and 7 to 9 weeks of age at initiation of dosing. Body weight will be approximately 200 to 350 grams at initiation of dosing.

5.6 Identification System:

The animals will be uniquely identified by a metal eartag displaying the animal number. Individual cage cards will be affixed to each cage and will display the animal number, group number, study number, dosage level and sex of the animal.

5.7 Justification for Selection:

This species and strain of animal is recognized to be appropriate for subchronic toxicity studies. The Sprague-Dawley rat will be used because it is a widely used strain for which significant control data are available. The number of animals is the minimum required to yield scientifically meaningful and statistically significant data.

6 SPECIFIC MAINTENANCE SCHEDULE:

6.1 Animal Housing:

Animals will be housed in a study-dedicated, environmentally controlled room three per cage by sex in clean, suspended, wire-mesh cages for approximately two to four days following receipt. If the number of animals received does not allow for all animals to be housed three per cage by sex, then some animals will be pair-housed by sex. Thereafter, all animals will be housed individually. The cages will be elevated above cage-board or other suitable material, which will be changed at least three times each week. 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 ± 5°F (22 ± 3°C) 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. Temporary adjustments to the light/dark cycles may be made 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-purified water will be available *ad libitum*. Filters servicing the automatic watering system are changed regularly according to WIL Standard Operating Procedures. The municipal water supplying the laboratory is analyzed according to WIL Standard Operating Procedures on a routine basis to assure that contaminants are not present in concentrations that would be expected to affect the outcome of the study.

6.4 Basal Diet:

PMI Nutrition International, LLC Certified Rodent LabDiet® 5002 (meal) will be offered *ad libitum* during the study. Periodic analyses of the certified feed are performed by the manufacturer to ensure that heavy metals and pesticides are not present at concentrations that would be expected to affect the outcome of the study. Results of the analyses are provided to WIL Research Laboratories, LLC by the manufacturer. Feeders will be changed and sanitized once per week.

7 EXPERIMENTAL DESIGN:

7.1 Animal Receipt and Quarantine:

Each animal will be inspected by a qualified technician upon receipt. Animals judged to be in good health will be placed immediately in acclimation for at least seven days. All animals will be weighed and assigned a permanent animal number. During the acclimation period, each animal will be observed twice daily for changes in general appearance and behavior. There will be a pretreatment week (as part of the acclimation period) during which body weights and food consumption will be recorded and general health will be monitored, but the rats will not be dosed. All animals will receive a detailed physical examination approximately one week prior to initiation of dosing and at the time of animal selection for randomization.

7.2 Randomization:

At the conclusion of the acclimation period, animals judged to be suitable for testing will be assigned to groups at random, based on body weight stratification into a block design, using a computer program. A printout containing the animal numbers and individual group assignments will be generated. Animals will then be arranged into the groups according to the printout. Body weights at randomization will be within ± 20% of the mean for each sex.

7.3 Route and Rationale of Test Article Administration:

The route of administration will be intravenous since this is an acceptable route of administration to assess systemic exposure.

7.4 Organization of Test Groups, Dosage Levels and Treatment Regimen:

7.4.1 Organization of Test Groups:

The dosage levels will be determined from the results of previous studies and will be provided by the Sponsor Representative after consultation with the WIL Study Director. The following diagram presents the study group arrangement.

Pharmacokinetic (Blood Collection) Groups:

Group Number	Treatment	Dosage Level (mg/kg)	Dosage Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Animals	
					Males	Females
1	PFHxA	10	2	5	9	9
2	PFBS	10	2	5	9	9

Excretion (Urine Collection) Groups:

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

Data for pharmacokinetic groups and excretion groups will be collected in separate computer protocols.

7.4.2 Vehicle:

Sterile water for injection will be used as the vehicle.

7.4.3 Treatment Regimen:

Animals will be appropriately restrained and administered dosing solutions by a slow bolus intravenous injection (sterile needle and syringe) via a lateral tail vein. A constant volume of 5 mL/kg will be used. The treatment period will be 1 day. Day 0 will be the day of dosing.

7.5 Preparation and Analysis of Test Article Preparations:

7.5.1 Test Article Preparation:

The test articles will be prepared for dosing as weight-to-volume mixtures in a 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 Homogeneity and Stability of Test Article Formulations:

Homogeneity assessments will not be performed, as the formulations are solutions. Analyses to demonstrate the stability of the test article formulations were conducted previously as part of study WIL-534002.

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 article concentration.

The Analytical Chemistry report will be appended to the final report for this study.

8 EXPERIMENTAL OBSERVATIONS:

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 euthanized to ensure that tissues will not be lost due to autolysis.

8.2 Detailed Physical Examination:

All animals will receive a detailed physical examination at least once during the pre-treatment period. Animals without signs will be noted individually.

8.3 Individual Body Weights:

Individual body weights will be recorded during acclimation, at pretest initiation, at randomization and on Day 0.

8.4 Individual Food Consumption:

Individual food consumption will be recorded during the pretreatment period only.

8.5 Toxicokinetics for Elimination:

Blood samples will be obtained from the blood collection groups for determination of concentration of the PFHxA and PFBS test article in serum at the time points outlined in the following table:

Intervals	1 | Day 0
Time points post-dosing	• Prior to dosing and approximately 0.5, 1, 1.5, 2, 4, 8 and 24 hours after dosing • Clock times of collection recorded
Number of Animals	• 3 animals/sex bled per time point • Each animal sampled no more than three times in a 24 hour period
Sample Collection	Retro-orbital sinus under isoflurane (inhalation) anesthesia.
Target Blood Volumes	• 0.5 mL/time point • collect into chilled sampling tubes
Anticoagulant	None.
Sample Handling	Samples will be allowed to clot at room temperature, after which they will be kept chilled (ice water bath, as appropriate) after collection and during processing.
Plasma Preparation	Beckman 6R centrifuge 2400-2700 rpm at -4ºC
Aliquots	Recovery all serum possible and place in Nunc© plastic vials.
Label information	Study number, dose group, animal number, sample type, date of collection, time of collection.
Storage	• Approximately -20ºC until analysis • Time placed in freezer recorded

Moribund animals will be euthanized by CO_2 inhalation. Animals found dead or euthanized *in extremis* after start of dosing will be examined to determine possible cause of death and discarded. Following the final blood collection, all animals will be euthanized by carbon dioxide inhalation and discarded.

Urine collection animals will be transferred into plastic metabolism cages for urine collection following dosing. Urine will be collected on wet ice over the following intervals: 0-6, 6-12 and 12-24 hours post-dosing. The volume of each urine sample will be recorded, after which the urine samples will be frozen with minimal delay in a freezer set to maintain temperature of approximately -20°C until preparation for analysis. Following the final urine collection, all animals used for urine collection will be euthanized by carbon dioxide inhalation and discarded.

Serum and urine samples will be analyzed for PFHxA or PFBS, as appropriate, 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 test articles following a single dose of the test material.

9 STATISTICAL METHODS:

No statistical test will be performed.

10 QUALITY ASSURANCE:

The study will be audited by the WIL Quality Assurance Unit while in progress to assure compliance with the study protocol and protocol amendments, WIL Standard Operating Procedures and the appropriate provisions of the U.S. EPA TSCA and FIFRA Good Laboratory Practice Standards published in the Federal Register (40 CFR Part 792 and 40 CFR Part 160) and the OECD Good Laboratory Practice Regulations [C(97)186 Final]. The raw data and draft report will be audited by the WIL Quality Assurance Unit prior to submission to the Sponsor Representative to assure that the final report accurately describes the conduct and the findings of the study.

This study 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 as described in Section 12 in the Archives at WIL Research Laboratories, LLC.

12 WORK PRODUCT:

The Sponsor will have title to all documentation records, raw data, slides, specimens and other work product generated during the performance of the study. All work product, including raw paper data, pertinent electronic storage media and specimens, will be retained for a period of 10 years following issuance of the final report in the Archives at WIL Research Laboratories, LLC. Thereafter, WIL Research Laboratories, LLC 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 EPA specifications.

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 ANIMAL WELFARE ACT COMPLIANCE:

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act (AWA) regulations (9 CFR Parts 1, 2 and 3). 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 designed 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.

15 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 protocol amendment. All alterations of the protocol and reasons for the modification(s) will be signed by the Study Director and the Sponsor Representative.

16 PROTOCOL APPROVAL:

Sponsor approval received via email on March 29, 2005.

AGC Chemical

Motoki Shinohara — 4/8/2005
Motoki Shinohara, Sponsor Representative — Date

WIL Research Laboratories, LLC

Jeannie B. Kirkpatrick — 4/1/2005
Jeannie B. Kirkpatrick, M.S., Study Director — Date

Christopher P. Chengelis — 14 April 05
Christopher P. Chengelis, Ph.D., D.A.B.T., Director, Toxicology — Date