11) [redacted], Pharmacokinetic study in the Cynomolgus Monkey, T-7485.1

T-7485

Final Report on the

A Pharmacokinetic Study of Perfluorobutanesulfonate in the Cynomolgus Monkey

To:

3M Corporation
P.O. Box 33327 • 55133-3327
3M Center, 224-IN-04
St. Paul, Minnesota 55144-1000

By:

P.E. Noker

Southern Research Institute
2000 Ninth Avenue South • 35205
P.O. Box 55305
Birmingham, Alabama 35255-5305

ABSTRACT

The pharmacokinetics and urinary excretion of perfluorobutanesulfonate were investigated in male and female cynomolgus monkeys. Three male and three female monkeys were administered a single IV bolus dose of 10 mg/kg of perfluorobutanesulfonate, potassium salt. At various times through 31 days after dosing, serum and urine (24-hour collections) samples were obtained and analyzed by HPLC/MS/MS for levels of intact perfluorobutanesulfonate. The lower limit of detection of the analytical method was 0.5 ng/mL. No sex-related differences in serum concentrations of perfluorobutanesulfonate were apparent among the six dosed monkeys. In individual animals, the concentration of perfluorobutanesulfonate in serum ranged from 19,628 to 61,740 ng/mL at 2 hours after dosing (earliest time point). By 48 hours after dosing, the serum concentration of perfluorobutanesulfonate ranged from 463 to 8,172 ng/mL in individual monkeys. Perfluorobutanesulfonate was not detectable in serum collected from any monkey on Day 31 post dose. The serum concentration versus time data were best fit to a three-compartment model. Mean serum half-life values for perfluorobutanesulfonate were 0.04, 0.55, and 4.0 days in male monkeys, and 0.06, 0.47, and 3.5 days in female monkeys. The mean AUC value calculated from serum concentrations of perfluorobutanesulfonate was 24,258 or 35,401 ng•day/mL in male or female monkeys, respectively. The total body clearance of perfluorobutanesulfonate was 511 mL/day/kg in male monkeys and 368 mL/day/kg in female monkeys. The volume of distribution at steady state (Vd_{ss}) was 254 or 255 mL/kg in male or female monkeys, respectively. For 5 of 6 monkeys, from 33.8 to 86.8% of the dose was recovered in urine within 24 hours after administration of perfluorobutanesulfonate. On Day 14, individual monkeys excreted less than 0.01% of the dose within a 24-hour interval. The results of this study indicated that the pharmacokinetics of perfluorobutanesulfonate were similar in male and female monkeys. Urinary excretion was a major route of elimination of the compound by monkeys.

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A Pharmacokinetic Study of Perfluorobutanesulfonate in the Cynomolgus Monkey

Patricia E. Noker 3/6/01

Patricia E. Noker, Ph.D., D.A.B.T. Date
Study Director
Supervisor, ADME and Pharmacokinetics

Reviewed by:

Ward R Richter 3/2/01

Ward R. Richter, D.V.M., M.S., D.A.C.V.P. Date
Director, Safety Assessment

We, the undersigned, were responsible for the conduct of the work and reporting of the results in the listed sections. We concur with the views relative to our body of work as expressed in the discussion and conclusions.

James D Johnson 3/6/01

James D. Johnson, M.S., M.B.A. Date
Manager, Bioanalytical Chemistry Group

Gregory S Gorman 3/6/01

Gregory S. Gorman, Ph.D. Date
Research Chemist

Tsu-Han Lin 3/6/01

Tsu-Han Lin, Ph.D. Date
Staff Pharmacologist

Good Laboratory Practices Disclaimer

This study described in this final report was not conducted in strict compliance with the U.S. Food and Drug Administration's (FDA) Good Laboratory Practice (GLP) regulations (21 CFR Part 58). Neither this report nor the study data were reviewed by the Southern Research Quality Assurance Unit (QAU). However, the study was conducted according to the protocol and the applicable Standard Operating Procedures (SOPs) of Southern Research. All study procedures, data recording, and reporting were performed in a manner consistent with the standard of GLPs.

Patricia E. Noker, Ph.D., D.A.B.T. — Date
Study Director

3/6/01

Study Schedule and Personnel

Study Dates:

Study Initiation:	March 17, 2000
Day of Dosing:	April 10, 2000
Last Day of Sample Collection:	May 11, 2000
Study Completion:	TBD

Study Personnel:

Patricia E. Noker, Ph.D., D.A.B.T.	Study Director
James D. Johnson, M.S., M.B.A.	Manager, Bioanalytical Chemistry Group
Gregory S. Gorman, Ph.D.	Chemist
Tsu-Han Lin, Ph.D.	Pharmacokineticist
Darrell E. Hoskins, D.V.M., Ph.D., A.C.L.A.M. (Dipl.)	Veterinarian
Carolyn R. Oliver, B.S.	Preclinical Research Associate
LaJuana A. Durbin, B.S.	Supervisor, Large Animal Laboratory
Joan B. Belzer	Manager, Animal Facilities and Operations
Deborah S. Bailey, B.S.	Senior Supervisor, Animal Laboratory
Donna B. Hogan, B.S.	Senior Supervisor, Scientific Coordination

1.0 Introduction

The objectives of this study were to determine the concentration of perfluorobutanesulfonate in serum and to estimate urinary clearance at various times following administration of a single intravenous dose to monkeys. A copy of the protocol and any applicable amendments can be found in Appendix A.

2.0 Materials and Methods

2.1 Test System

The 3 male and 3 female monkeys designated for use in this study were selected from an in-house colony of monkeys that were housed at Southern Research Institute (Southern Research) prior to use on this study. These monkeys were purchased from Charles Rivers BRF, Inc. (Houston, TX). Individual animal identification was by chest tattoo. The cynomolgus monkey is an accepted species to support clinical studies of drugs used or intended for use in humans.

During the quarantine period, a complete physical examination including a fecal examination for internal parasites, complete blood count (CBC), body weight, and rectal temperature was performed on each of the monkeys. The following procedures were performed on the monkeys during quarantine: (1) Three tuberculin tests were administered to each animal at 2-week intervals. All tuberculin tests were administered intrapalpebrally. The three tuberculin tests were negative for all monkeys. (2) Blood was drawn for CBC and B virus titer. (3) Fecal cultures (screening for *Salmonella* and *Shigella*) were obtained, and fecal flotation tests were performed. (4) In general, primates were examined at least once weekly by an approved veterinarian and were observed (cage-side observations recorded by exception only) twice daily for abnormal clinical signs and mortality/moribundity. Housing, feed, water, and socialization procedures remained the same during the quarantine, holding, and study periods.

Certified, commercial, dry monkey chow #5048 (PMI Feeds, Inc., St. Louis, MO) was fed to the monkeys 2-3 times each day. The quantity of the daily ration was sufficient to meet nutritional requirements. In addition, the diet was supplemented with fresh fruit/treats several times each week. Tap water (Birmingham public water supply) was available to the monkeys *ad libitum* during the quarantine and study periods. The monkeys were housed individually in stainless steel cages during the quarantine and the study periods. From Day

0 to the end of the study, the monkeys were housed in a room that was maintained at a temperature of 68.0-70.3 °F and a relative humidity of 22.2-65.7%. The humidity was within the required range (30-70%) over 90% of the time during the study; excursions below the recommended humidity range were of short duration. Room lights were controlled by an automatic timer set to provide 12 hours of light (0600 to 1800 hours, CST) and 12 hours of dark per day. Cage size and animal care conformed to the guidelines of the *Guide for the Care and Use of Laboratory Animals*, 7th edition[1] and the U.S. Department of Agriculture through the Animal Welfare Act (Public Law 99-198) and to the applicable Standard Operating Procedures (SOPs) of Southern Research.

2.2 Test Article and Vehicle

Test Article: One bottle containing 9.58 grams of perfluorobutanesulfonate, potassium salt, was received from 3M Corporation (St. Paul, MN; T7485; expiration date not supplied; SRI Lot EO4/L-1) on March 17, 2000. The test article was stored at room temperature until used. Stability of the test article was the responsibility of the Sponsor.

Vehicle: The vehicle used for preparation of the dose formulation of perfluorobutanesulfonate was sterile saline, USP (Phoenix Pharmaceutical Company; St. Joseph, MO; Lot 8101069; expiration date October 2001). The vehicle was stored at room temperature and was considered to be stable when stored under these conditions.

Dose Formulation Preparation: For the single dose formulation of perfluorobutanesulfonate prepared to contain 5 mg/mL, the required amount of test article was weighed out in a volumetric flask. Sterile saline was added and the formulation was stirred until the test article was in solution. The formulation was stored refrigerated and used for dosing within 3 days after preparation; it was considered to be stable during this period.

Dose Formulation Analyses: Dose concentration and homogeneity analyses were not required to be performed.

2.3 Experimental Design

Group Assignment and Dose Procedure: As only one treatment group was used in this study, no formal randomization was required. On Day 0, each of the three male and three

female monkeys received a single intravenous (IV) dose of perfluorobutanesulfonate at 10 mg/kg by injection into a superficial arm or leg vein. Doses were based upon the Day 0 individual body weights. Doses were administered at a volume of 2 mL/kg.

Clinical Observations: All animals were checked twice daily for signs of mortality/moribundity. Each primate was examined shortly after dose administration for clinical signs of toxicity. Additional clinical observations (cage-side) were performed daily.

Body Weights: Each primate was weighed on Days 0, 4, 7, and 14.

Urine and Feces Collection: Urine and feces were collected for 24-hour intervals on the following days: prior to dose administration (Day -1; baseline), on Day 0 (0-24 hours postdose), and on Days 7 and 14. The volume of each urine sample was measured upon collection. Samples were stored frozen at -20 °C or below. Fecal samples will not be analyzed unless specifically requested by the Sponsor.

Serum Levels of Perfluorobutanesulfonate: Blood samples (approximately 2 mL) were collected from each primate at approximately 0 (predose) minutes; 2, 4, 8, 24, and 48 hours; and on Days 4, 7, 11, 14, and 31 postdose. Samples were collected into tubes without anticoagulant and were allowed to clot at room temperature. The blood samples were then centrifuged, and the serum separated and stored frozen (-20 °C or below) until analyzed.

Bioanalytical Method Development and Sample Analysis: A bioanalytical method was developed for the analysis of perfluorobutanesulfonate in serum and urine matrices. The method was validated for accuracy. This method was used for the analysis of serum and urine samples collected during the study (see Appendices B and C).

Data Analyses: Pharmacokinetic parameters were estimated from serum concentrations of unchanged perfluorobutanesulfonate using WinNonlin (Version 1.1; Scientific Consulting, Inc.; Apex, NC) The data were fit to a three compartment model. Mean values and standard deviations for each parameter were calculated using Microsoft® Excel software (Microsoft Corporation; Irvine, CA). The urinary excretion of perfluorobutanesulfonate at each collection interval was calculated and expressed as a percent of the administered dose. No other statistical analyses of the data were performed.

3.0 Results

3.1 Mortality

All of the monkeys in this study survived to the end of the study.

3.2 Clinical Observations

No adverse clinical signs were noted for any monkey during the course of this study.

3.3 Body Weights

Body weights are presented in Table 1. The body weight of each monkey remained essentially constant between Days 0 and 14.

3.4 Serum and Urine Concentrations of [Perfluorobutanesulfonate]

Prior to the initiation of the in-life aspects of the study, an HPLC/MS/MS method was developed and validated for the analysis of [perfluorobutanesulfonate] in monkey serum. Details of the method validation procedure and specifications of the method are provided in Appendix B. Details of the analytical method are provided in Appendix C. This method was also applied to the analysis of [perfluorobutanesulfonate] in monkey urine.

Serum concentrations of [perfluorobutanesulfonate] in three male and three female monkeys at various times through Day 31 after administration of a single IV dose of the compound are presented in Table 2. No sex-related differences in serum concentrations of [perfluorobutanesulfonate] were apparent. Among the six dosed monkeys, the concentration of [perfluorobutanesulfonate] in serum ranged from 19,628 to 61,740 ng/mL at 2 hours after dosing (earliest time point). By 48 hours after dosing, the serum concentration of [perfluorobutanesulfonate] ranged from 463 to 8,172 ng/mL in individual monkeys. [Perfluorobutanesulfonate] was not detectable in serum collected from any monkey on Day 31. For the analyses conducted on the Day 31 serum samples, the standard curve was extended to include a [perfluorobutanesulfonate] concentration of 0.5 ng/mL; thus, the serum concentration of [perfluorobutanesulfonate] was below this level in all monkeys on Day 31.

Pharmacokinetic data calculated from serum concentrations of [perfluorobutanesulfonate] in individual monkeys are presented in Table 3 and are plotted in Figures 1-6. The serum concentration versus time data were fitted to a three-compartment model. Mean serum half-

life values for perfluorobutanesulfonate in the male monkeys were 0.04, 0.55, and 4.0 days for the α-, β-, and γ-phases, respectively; the corresponding values in the female monkeys were 0.06, 0.47, and 3.5 days. The mean AUC value calculated from serum concentrations of perfluorobutanesulfonate was 24,258 or 35,401 ng•day/mL in male or female monkeys, respectively. The total body clearance of perfluorobutanesulfonate was 511 mL/day/kg in male monkeys and 368 mL/day/kg in female monkeys. The volume of distribution at steady state (Vd_{ss}) was 254 or 255 mL/kg in male or female monkeys, respectively.

The amount of perfluorobutanesulfonate eliminated in urine by individual monkeys at various times after dosing is presented in Table 4. For 5/6 monkeys, from 33.8% to 86.8% of the dose was recovered in urine within 24 hours after administration of perfluorobutanesulfonate. For the remaining monkey, less than 1% of the dose was recovered in urine during this same time interval. In that the serum concentrations of perfluorobutanesulfonate indicated this animal had been properly dosed, loss or spillage of urine excreted by this animal most likely occurred during the collection interval. With time after dosing, the rate of urinary excretion of perfluorobutanesulfonate, expressed as percent of dose per day, decreased. On Day 14, individual monkeys excreted less than 0.01% of the dose within a 24-hour interval.

4.0 Discussion

In male and female monkeys administered a single IV dose of 10 mg/kg of perfluorobutanesulfonate, no sex-related differences in serum concentrations of the compound were apparent. Serum concentrations of perfluorobutanesulfonate versus time were best fit to a three-compartment pharmacokinetic model. Mean half-life values for perfluorobutanesulfonate in serum were 0.04, 0.55, and 4.0 days in male monkeys and 0.06, 0.47, and 3.5 days in female monkeys. The small value calculated for the steady state of volume of distribution of perfluorobutanesulfonate in individual monkeys indicated the compound was not distributed to total body water. Urinary excretion was a major route of elimination of perfluorobutanesulfonate in that as much as 86.8% of the dose was eliminated as unchanged parent compound within the first 24 hours after dosing.

5.0 Conclusions

No sex-related differences in serum concentrations and urinary excretion of perfluorobutanesulfonate were apparent among male and female monkeys administered a single IV dose of 10 mg/kg. The mean terminal serum half-life of perfluorobutanesulfonate in male and female monkeys was 3.5-4.0

days. In monkeys, urinary excretion was a major route of elimination of unchanged perfluorobutanesulfonate.

6.0 Record Archives

All raw data pertaining to the conduct of this study, and all samples/specimens collected in this study, will be stored in the Archives at Southern Research Institute for up to 1 year after acceptance of the final report by the Sponsor. After 1 year and with the permission of the Sponsor's Monitor, the data and any samples/specimens will be shipped to the Sponsor or to the Sponsor's designated archival facility. If materials are to be retained in the archives beyond this date, such continued storage will be for a specific fee determined with the Sponsor. A copy of the final report will be retained in the central archives at Southern Research.

7.0 References

1. Institute of Animal Laboratory Resources, Commission on Life Sciences, National Research Council; National Academy Press; Washington D.C.; 1996.

Table 1

A Pharmacokinetic Study of Perfluorobutanesulfonate in the Cynomolgus Monkey

Body Weights

Bodyweights (kg)

Day numbers relative to Start Date

Group Sex	Animal Number	0	4	7	14
1M	2052	5.8	5.9	5.9	6.0
	2054	5.6	5.7	5.7	5.8
	2211	4.4	4.4	4.4	4.5
1F	2058	3.3	3.4	3.4	3.4
	2059	3.4	3.4	3.3	3.4
	2061	3.9	3.9	3.9	4.0

Nominal Dose: Group 1 - 10 mg/kg

Table 2

A Pharmacokinetic Study of Perfluorobutanesulfonate in the Cynomolgus Monkey

Serum Concentrations of Perfluorobutanesulfonate

	Serum Concentration (ng/mL)					
	Males			Females		
Timepoint	2052	2054	2211	2058	2059	2061
0	1.6	1.1	BQL[a]	BQL[a]	BQL[a]	BQL[a]
2 hrs	19,628	39,400	37,560	61,420	61,740	41,920
4 hrs	29,225	39,540	29,060	40,580	30,160	22,990
8 hrs	20,695	4,311	9,952	29,420	17,350	12,530
24 hrs	10,410	2,406	4,224	19,278	5,930	3,460
48 hrs	6,506	463	1,293	8,172	2,278	948
Day 4	1,999	15.3	52.4	2,683	326	58.7
Day 7	71.7	4.1	11.6	881.6	13.9	10.2
Day 11	6.8	2.7	2.5	43.8	1.9	BQL[a]
Day 14	5.1	1.6	BQL[a]	7	1.7	1.1
Day 31[b]	BQL[b]	BQL[b]	BQL[b]	BQL[b]	BQL[b]	BQL[b]

[a] Below the quantitation limit (<1 ng/mL)

[b] Below the quantitation limit (<0.5 ng/mL); Day 31 samples were analyzed during a separate analytical run.

Table 3

A Pharmacokinetic Study of Perfluorobutanesulfonate in the Cynomolgus Monkey

Pharmacokinetic Parameters Calculated from Serum Concentrations of Perfluorobutanesulfonate

	Male					Female				
Parameters	2052	2054	2211	Mean	SD	2058	2059	2061	Mean	SD
$t_{1/2\alpha}$ (day)[a]	0.01	0.04	0.05	0.04	0.02	0.04	0.06	0.06	0.06	0.01
$t_{1/2\beta}$ (day)[b]	0.79	0.42	0.44	0.55	0.21	0.30	0.66	0.45	0.47	0.18
$t_{1/2\gamma}$ (day)[c]	3.8	6.0	2.1	4.0	1.9	1.2	6.9	2.3	3.5	3.1
AUC $_{0\text{-inf}}$ (ng•day/mL)[d]	41352	13862	17561	24258	14918	61338	27548	17318	35401	23037
Cl (mL/day/kg)[e]	242	721	569	511	245	163	363	577	368	207
Vd_{ss} (mL/kg)[f]	245	228	289	254	31.5	234	243	289	255	29.5

[a]Half-life of the α-phase
[b]Half-life of the β-phase
[c]Half-life of the γ-phase
[d]Area under the serum concentration versus time curve calculated from 0 to infinity
[e]Total body clearance
[f]Volume of distribution at steady state

Table 4

A Pharmacokinetic Study of Perfluorobutanesulfonate in the Cynomolgus Monkey

Urinary Excretion of Perfluorobutanesulfonate

Animal ID	Sex	Urine Concentration (ng/mL)	Urine Volume (mL)	Total (μg)	Dose (μg)	Percent of Dose in Urine (%)
Day 0						
2052	M	2	290	0.58	58,000	--
2054	M	BQL	770	--	56,000	--
2211	M	BQL	255	--	44,000	--
2058	F	BQL	340	--	33,000	--
2059	F	BQL[a]	875	--	34,000	--
2061	F	[b]	300	--	39,000	--
Day 1						
2052	M	125000[c]	242	30,250.0	58,000	52.2
2054	M	51,425	660	33,940.5	56,000	60.6
2211	M	125000[c]	119	14,875.0	44,000	33.8
2058	F	32,950	461	15,190.0	33,000	46.0
2059	F	38,325	770	29,510.3	34,000	86.8
2061	F	1,958	108	211.5	39,000	0.542
Day 7						
2052	M	219	725	158.8	58,000	0.274
2054	M	123	508	62.5	56,000	0.112
2211	M	120	191	22.9	44,000	0.052
2058	F	414[a]	328	136.0	33,000	0.412
2059	F	142	190	27.0	34,000	0.079
2061	F	89.8	168	15.1	39,000	0.039
Day 14						
2052	M	8.5[a]	879	7.5	58,000	0.013
2054	M	5.6	572	3.2	56,000	0.006
2211	M	3.1	400	1.2	44,000	0.003
2058	F	10.2	215	2.2	33,000	0.007
2059	F	2.3	210	0.5	34,000	0.001
2061	F	46.9	335	15.7	39,000	0.040

BQL = Below the quantitation limit (<1 ng/mL)

[a] Sample vial was received broken but frozen.

[b] Sample was contaminated.

[c] Diluted sample concentration was out of calibration range. Estimated concentration was based on the highest standard in the calibration curve × the dilution factor of the sample. The actual concentration was most likely greater than this amount.

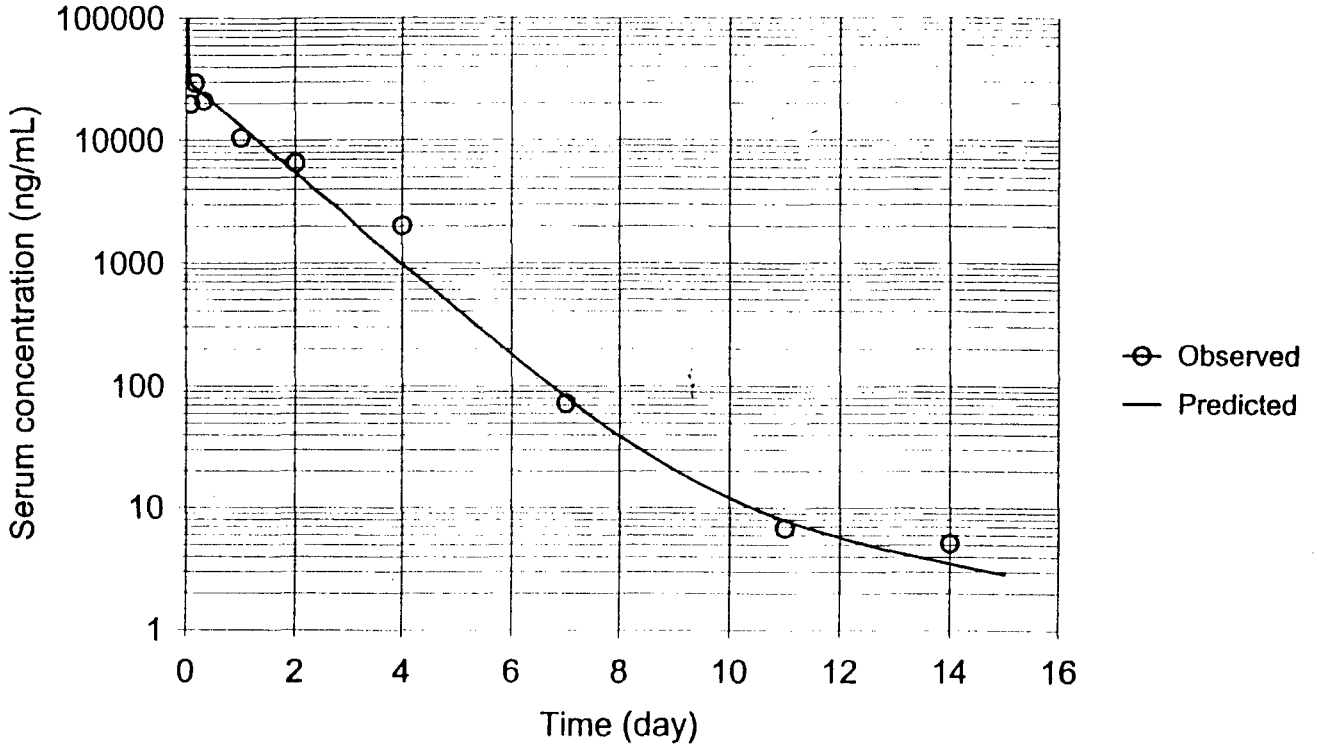


Figure 1

A Pharmacokinetic Study of Perfluorobutanesulfonate in the Cynomolgus Monkey

Serum Concentration Profile of Perfluorobutanesulfonate in 1M2052

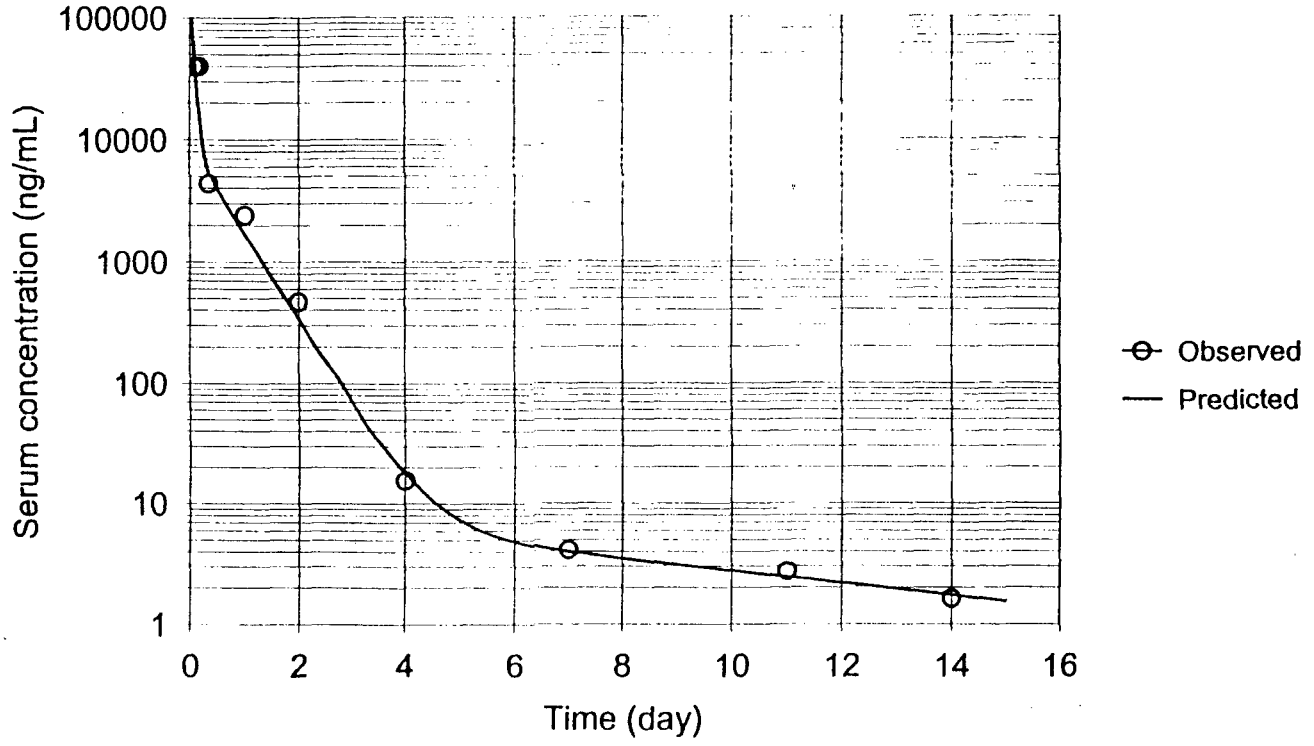


Figure 2

A Pharmacokinetic Study of Perfluorobutanesulfonate in the Cynomolgus Monkey

Serum Concentration Profile of Perfluorobutanesulfonate in 1M2054

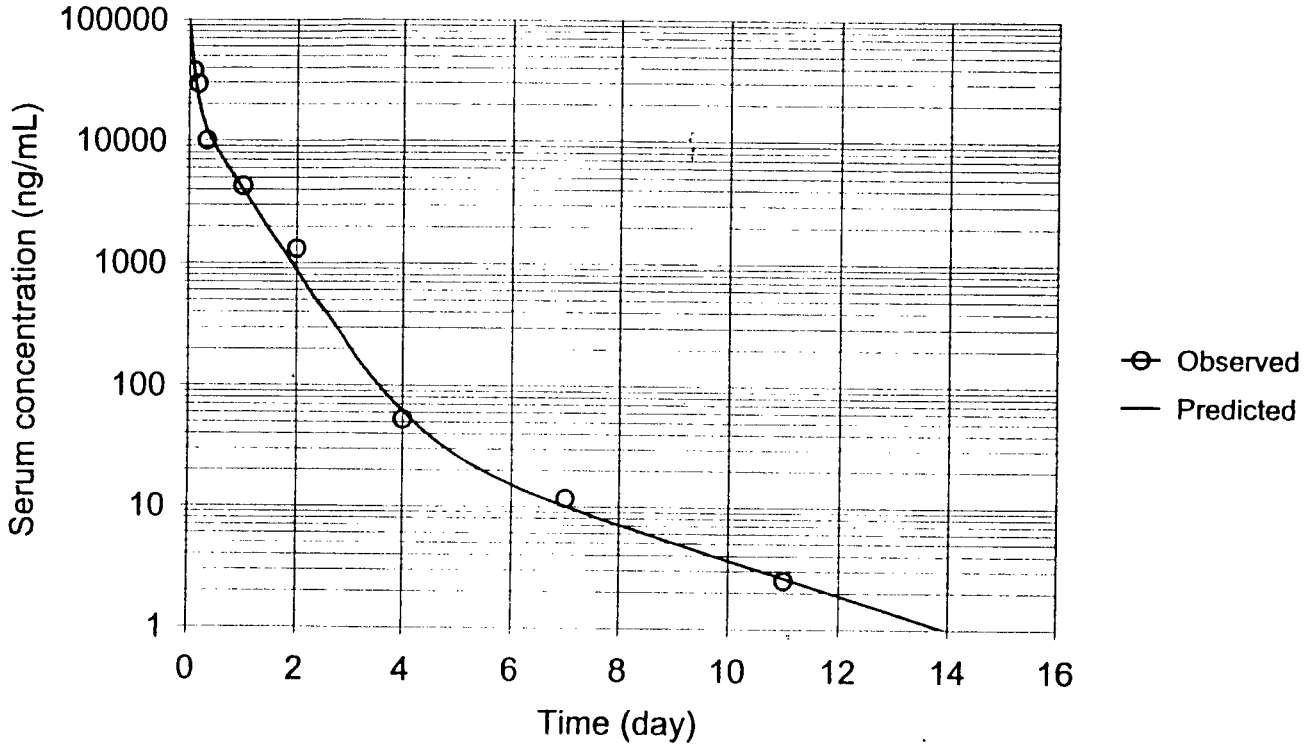


Figure 3

A Pharmacokinetic Study of Perfluorobutanesulfonate in the Cynomolgus Monkey

Serum Concentration Profile of Perfluorobutanesulfonate in 1M2211

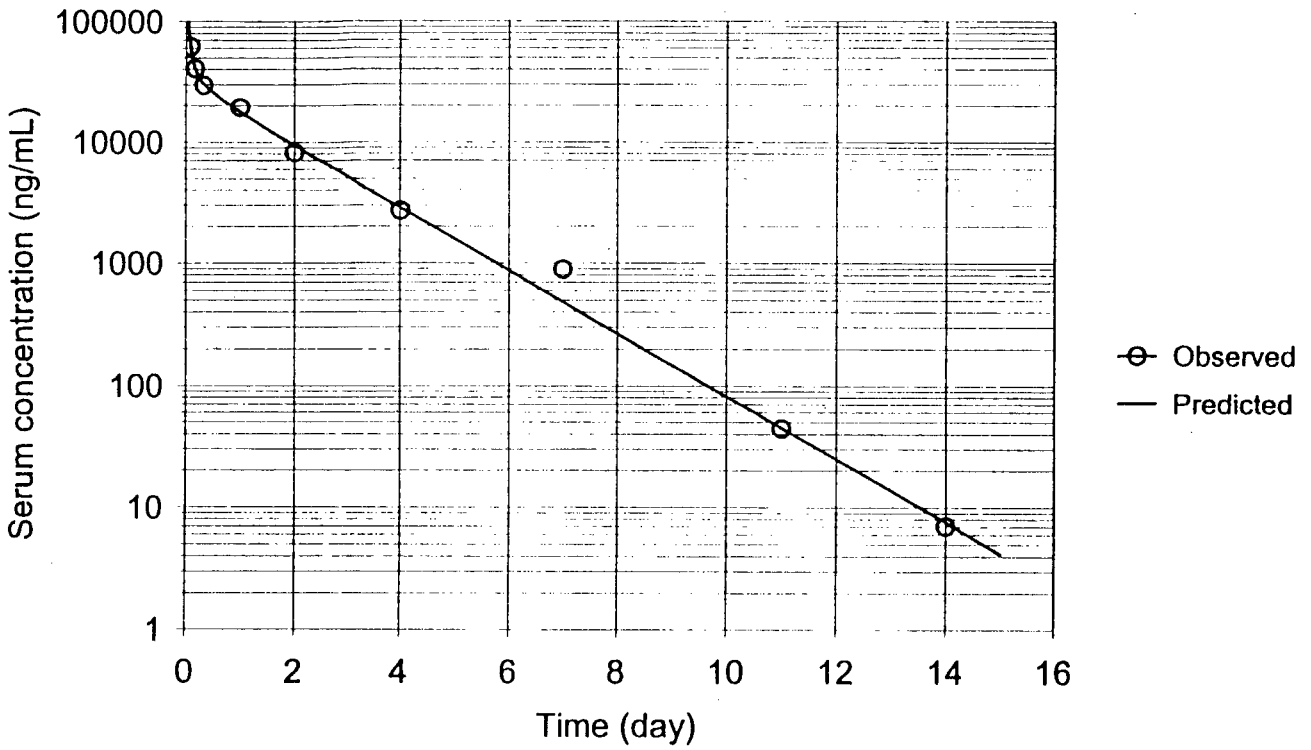


Figure 4

A Pharmacokinetic Study of Perfluorobutanesulfonate in the Cynomolgus Monkey

Serum Concentration Profile of Perfluorobutanesulfonate in 1F2058

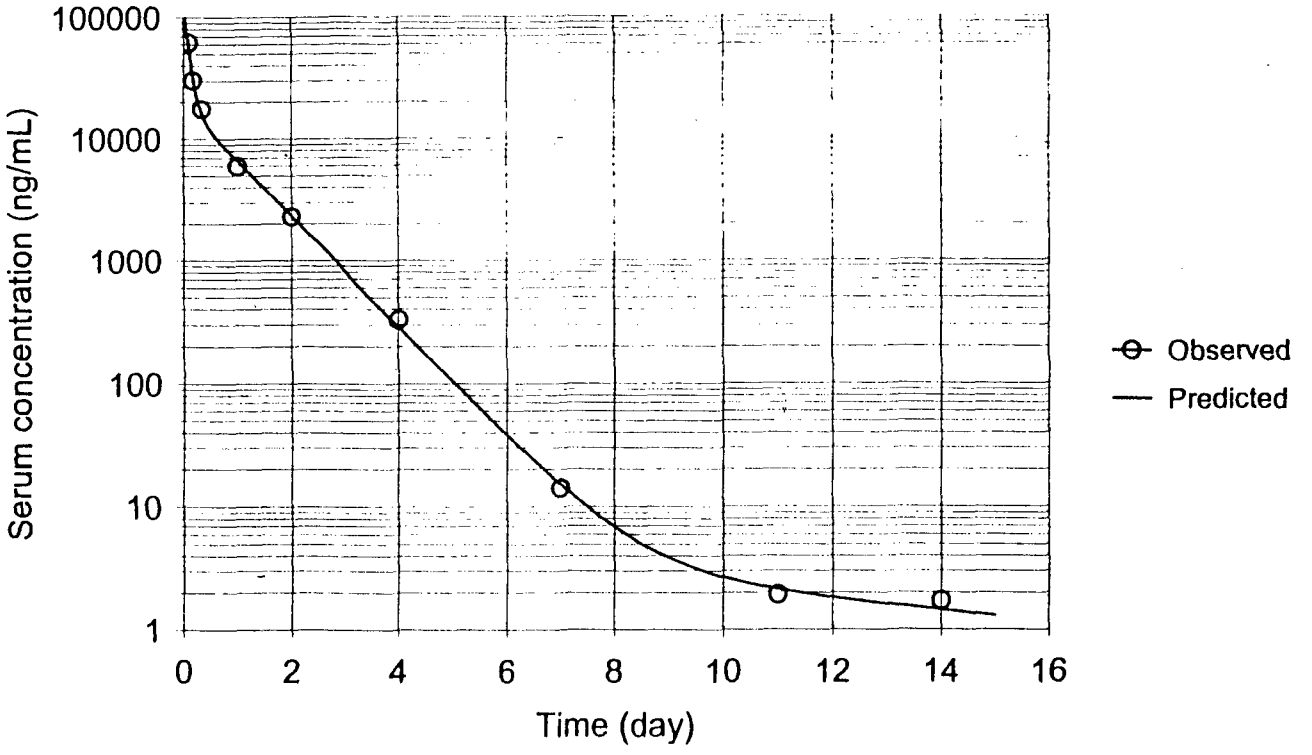


Figure 5

A Pharmacokinetic Study of Perfluorobutanesulfonate in the Cynomolgus Monkey

Serum Concentration Profile of Perfluorobutanesulfonate in 1F2059

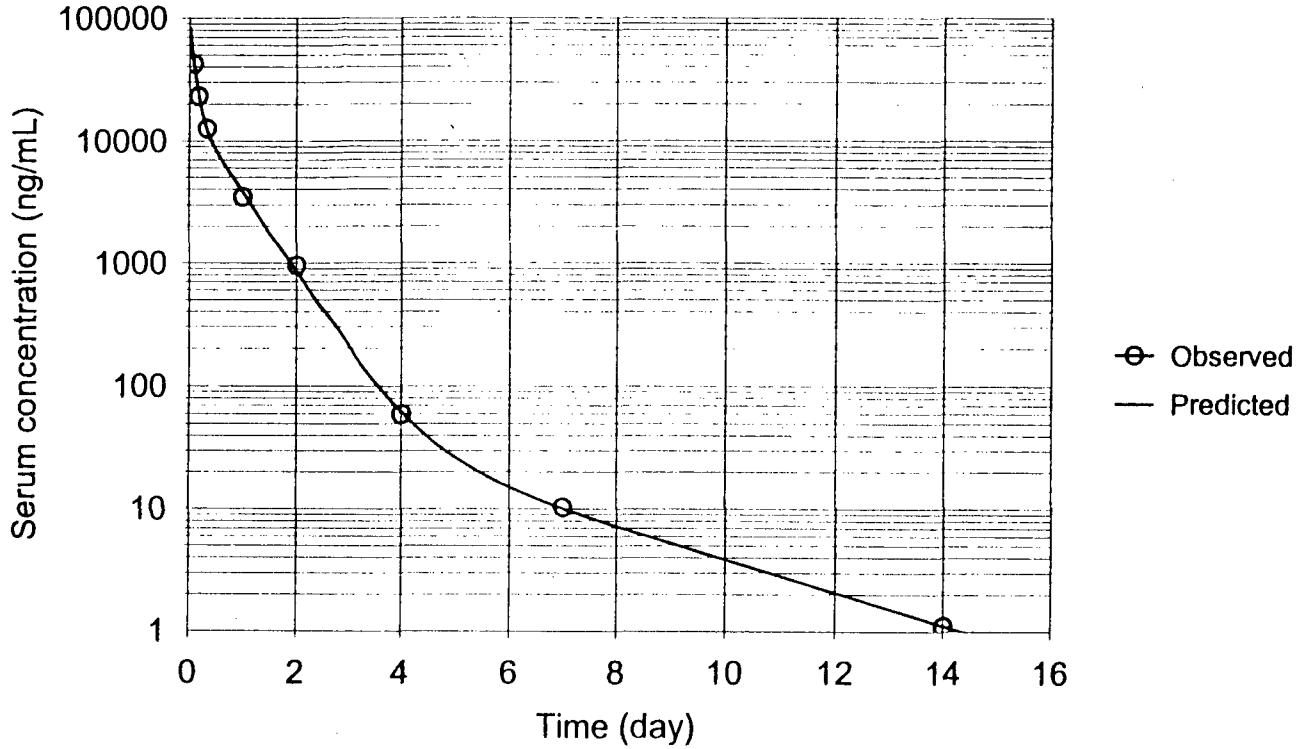


Figure 6

A Pharmacokinetic Study of Perfluorobutanesulfonate in the Cynomolgus Monkey

Serum Concentration Profile of Perfluorobutanesulfonate in 1F2061

Appendix A

Study Protocol and Amendments

Study Protocol:

A Pharmacokinetic Study of Perfluorobutanesulfonate in the Cynomolgus Monkey

Serquest Study ID: 9921.1

March 17, 2000

serquest

a division of Southern Research Institute

1.0 SPONSOR REPRESENTATIVE AND CONTACTS:

Sponsor:

3M Corporation
P.O. Box 33327 • 55133-3327
3M Center, 224-IN-04
St. Paul, Minnesota 55144-1000

Sponsor's Representative & Study Monitor:

Paul H. Lieder, Ph.D., D.A.B.T.
P.O. Box 33327 • 55133-3327
St. Paul, Minnesota 55144-1000
(651) 737-2678; FAX: (651) 733-1773

Protocol Approval:
(Initial last page also)

Paul H. Lieder Date

Test Article:

Perfluorobutanesulfonate, potassium salt

Ship Unused Test Article to:

D. Hakes
Building B236
3M Corporation
P.O. Box 33327 • 55133-3327
3M Center, 224-IN-04
St. Paul, Minnesota 55144-1000

2.0 TITLE:

A Pharmacokinetic Study of Perfluorobutanesulfonate in the Cynomolgus Monkey

3.0 OBJECTIVE:

The objectives of this study are to determine the concentration of perfluorobutanesulfonate in serum and to estimate urinary clearance at various times following administration of a single intravenous dose to monkeys.

4.0 TESTING LABORATORY:

Serquest
(a division of Southern Research Institute)
2000 Ninth Avenue South • 35205
P.O. Box 55537
Birmingham, AL 35255-5537
(205) 581-2335; FAX: (205) 581-2044

5.0 KEY STUDY DATES:

Event	Sequence (Day)	Date(s) – Year 2000
Day of Treatment	0	**TBD**
Urine and Feces Collections	-1 0 – 0-24 hours 7 14	TBD
Serum Drug Levels	Baseline 0 – 0, 2, 4, 8 hours postdose 1 – 24 hours postdose 2 – 48 hours postdose 4 7 11 14	TBD
Draft Report Due	60 Calendar days after completion of the in-life phase	**TBD**
Final Report Due	15 days after final Sponsor comments received	TBD

6.0 STUDY PERSONNEL:

The following are the primary contributors and supervisory personnel participating in this study.

Study Director:	**Patricia E. Noker**	**Ph.D., D.A.B.T.**
Clinical Pathologist & Manager, Clinical Pathology:	Ronna Fulton	D.V.M., Ph.D., D.A.C.V.P.
Director, Safety Assessment:	Ward R. Richter	D.V.M., M.S., D.A.C.V.P.
Associate Director:	Norman D. Jefferson	B.A.
Manager, Bioanalytical Chemistry	James D. Johnson	M.S., M.B.A.
Supervisor, In-Life Laboratories	Deborah S. Bailey	B.S.
Supervisor, Study Coordination	Donna B. Hogan	B.S.
Veterinarian:	Darrell E. Hoskins	D.V.M., A.C.L.A.M. (Dipl.)

7.0 TEST & CONTROL ARTICLES:

The test article will be supplied by the Sponsor, who will be responsible for documentation of stability, as well as methods of synthesis, fabrication, or derivation. Stability of the test article formulated in the vehicle will be assessed prior to treatment initiation. Upon completion of the study, residual bulk test article will be returned to the Sponsor.

7.1 IDENTITY OF THE TEST ARTICLE:

Name:	**Perfluorobutanesulfonate, potassium salt**
Identification:	CAS No. 29420-49-3
Supplier:	3M Corporation
Lot Number(s):	To be documented in the study data; the minimum number of lots necessary to complete the study will be used
Special Handling:	None
Characterization:	Documentation of characterization, including identity, purity, strength, and composition, as well as methods of synthesis, fabrication, or derivation, is the responsibility of the Sponsor. [Copies of these data will be provided to the testing laboratory.]
Stability & Storage:	The bulk test article will be stored at room temperature. Stability of the bulk test article is the responsibility of the Sponsor. Copies of these data have been provided to the testing facility.

7.2 IDENTITY OF THE VEHICLE:

Name:	**Sterile saline**
Supplier:	Commercial supplier
Lot Number(s):	To be documented in the study data; the minimum number of lots necessary will be used on the study.
Special Handling:	None

Characterization: Documentation of characterization of the vehicle may be attained by recording all pertinent information from the container labels, or by retaining the container labels, or copies thereof, in the study data. The vehicle is a commercially available product.

Stability & Storage: Sterile saline is considered stable through the date(s) of expiration provided by the manufacturer when stored appropriately. The bulk vehicle will be stored in accordance with the manufacturer's instructions.

7.3 FORMULATIONS:

Preparation: The test article will be formulated in sterile saline at a concentration of 5 mg/mL for intravenous administration; briefly, the required amount of test article will be mixed with the required amount of sterile saline, and the mixture will be stirred until the test article is visibly in solution. Formulations will be stored refrigerated until used for dosing; formulations of the test article in sterile saline are expected to be stable for weeks when so stored.

Dose Formulation Concentration and Homogeneity Analyses: No analysis of dose formulation concentration and homogeneity will be conducted.

8.0 TEST SYSTEM:

Species & Strain:	**Cynomolgus monkeys** *(Macaca fascicularis)*
Supplier:	Charles River BRF, Inc. (Houston, TX)
Age on Day 0:	2.5-3 years of age (estimated)
Weight at randomization:	3-7 kg
Number on Study:	Males -3 Females -3

8.1 JUSTIFICATION:

Primates are commonly used in preclinical pharmacological and toxicological evaluations of compounds used or intended for use in humans, or to which humans might be exposed.

8.2 HOUSING:

During quarantine/acclimation and study, animals will be individually housed in stainless steel, slat-bottom cages. All animals will be housed in a room that provides a minimum of 10 air exchanges per hour. Controls will be set to maintain the animal room at a temperature of 64-84 °F and a relative humidity of 30-70%. A 12-hour light/12-hour dark cycle will be routinely maintained. Animals will be acclimated in the same room used for study.

8.3 BEDDING:

None required for caging equipped with flushable pans. For cages equipped with excrement absorption pans, commercial heat-treated hardwood chip bedding will be used for excrement absorption. Analyses of the bedding, supplied by the vendor, will be reviewed by the Department of Veterinary Medicine and Bioresources (DVMB) of Serquest to assure that no known contaminants are present that could interfere with or affect the outcome of the study.

8.4 DIET:

Diet will be commercial Certified Primate Chow #5048 (PMI Feeds, Inc.; St. Louis, MO). The primates will be offered feed twice daily, with approximately the recommended daily ration available at each feeding interval. In addition, the diet will be supplemented with fresh fruit offered daily and treats offered several times each week. The quantity of the daily ration will be sufficient to meet nutritional requirements. Analyses of the feed, supplied by the vendor, will be reviewed by the DVMB of Serquest to assure that no known contaminants are present that could interfere with and affect outcome of the study.

8.5 WATER:

Water (Birmingham public water supply) will be supplied *ad libitum* during the quarantine and study periods via an automatic watering system. Samples of water from the animal facility will be periodically analyzed, and the analyses will be reviewed by the DVMB of Serquest to assure that no known contaminants are present that could interfere with or affect the outcome of the study.

8.6 QUARANTINE:

All primates will be selected from stock animals that will have been quarantined for a minimum of 35 days prior to study start. No prophylactic or therapeutic treatments will be administered during the quarantine period. Standard procedures to be conducted during the quarantine period are as follows:

a) A complete physical examination including a fecal examination for internal parasites, complete blood count (CBC), body weight, and body (rectal) temperature will be performed; b) three tuberculin tests at 2-week intervals (administered intrapalpebrally, using alternate eyelids for each test) will be performed on each primate (primates must test negative to all three tests prior to release from quarantine; primates that respond positively to a tuberculin test will be euthanized immediately, while non-responding primates housed in the same room will be held in quarantine for an additional 90 days); c) the blood sample drawn for CBC will also be used for measuring of B virus titer (primates found to have a positive B virus titer will be euthanized immediately); d) a fecal sample for culture (screening for *Salmonella* and *Shigella*) will be obtained and submitted to an independent laboratory for analysis (if any primates are found to be positive for *Salmonella, Shigella*, or parasites, the Study Director, in conjunction with the staff veterinarian, will determine the course of action); and e) all primates will be examined at least once weekly by a veterinarian and observed (cage-side observations) twice daily for abnormal clinical observations and mortality/moribundity.

b) A baseline serum sample will be collected from each monkey and stored at or below –20 °C.

8.7 PSYCHOLOGICAL WELL-BEING AND SOCIALIZATION:

Nonhuman primates will be provided a psychological well-being program for social enrichment as directed by a veterinarian and approved by the IACUC and in accordance with the appropriate SOP. Nonhuman primates will be provided cage and feeding regimen modifications daily for their psychological well-being. The modifications include, but are not limited to: swings, perches, Kong toys, clean 2-liter soft drink bottles, puzzle feeders, nutritionally sound primate treats, unshelled peanuts, and raw fruit. Where possible, primates will be housed proximate to one another for visual and vocal contact.

8.8 ANIMAL IDENTIFICATION:

During quarantine, the primates will be individually identified by chest tattoo number or letter combination. Positive identification will be required after every cage change and prior to blood sampling, dose administration, and observation.

9.0 EXPERIMENTAL DESIGN:

As only one treatment group will be used in this study, no formal randomization will be required.

Doses will be administered by intravenous injection to determine the pharmacokinetics of the test article. Each primate (3 males, 3 females) will receive a single dose of perfluorobutanesulfonate by injection into a superficial arm or leg vein.

Blood samples for serum drug level determinations will be collected from each primate at seven time points during the study.

A synopsis of the study design is presented in the following table.

	Day of Study															
Study Procedures	-1[a]	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Health Check	x															
Dose Preparation		x														
Dosing		x														
Clinical Observations		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Body Weights	x					x			x							x
Urine & Feces	x	x[b]	x						x							x
Serum Drug Levels		x[c]	x	x		x			x				x			x

[a] Denotes Week

[b] 0-24 hours

[c] 0 minutes (predose); 2, 4, and 8 hours.

9.1 RANDOMIZATION & GROUP ASSIGNMENT:

As only one treatment group will be used in this study, no formal randomization will be required.

9.2 DOSE PROCEDURE:

On Day 0, each primate (3 males, 3 females) will receive a single intravenous (IV) dose of perfluorobutanesulfonate at 10 mg/kg by injection into a superficial arm or

leg vein. Doses will be based upon the most recent individual body weights. Doses will be administered at a volume of 2 mL/kg.

9.3 CLINICAL OBSERVATIONS:

Daily Observations: All monkeys will be observed once daily during quarantine and twice daily, morning and afternoon, at least 4 hours apart, during the study for signs of mortality/moribundity. Animals found *in extremis* will be humanely sacrificed by an overdose of barbiturate followed by exsanguination with appropriate approval.

Detailed Observations: Each primate will be examined shortly after dose administration for clinical signs of toxicity. All findings will be recorded. Additional clinical observations (cage-side) will be performed daily; any adverse findings will be recorded.

9.4 BODY WEIGHTS:

Each primate will be weighed on Days -1, 4, 7, and 14.

9.5 URINE AND FECES COLLECTIONS:

Urine and feces will be collected prior to dose administration (baseline), on Day 0 (0-24 hours postdose), and on Days 7 and 14. The volume of each urine sample will be measured upon collection. Samples will be stored frozen prior to analysis. Fecal samples will be collected and stored frozen, but samples will not be analyzed unless specifically requested by and at additional cost to the Sponsor.

9.6 SERUM DRUG LEVELS:

Blood samples (approximately 2 mL) will be collected from each primate at approximately 0 (predose) minutes; 2, 4, 8, 24, and 48 hours; and on Days 4, 7, 11, and 14 postdose. Samples will be collected into tubes without anticoagulant and will be allowed to clot at room temperature. The blood samples will then be centrifuged, and the serum will be separated and stored frozen until analyzed.

9.7 BIO-ANALYTICAL METHOD DEVELOPMENT:

Bio-analytical method(s) are to be developed for the analysis of the compound and its likely metabolites in serum and urine matrices. The methods should be validated for accuracy and be sensitive to the 1 ppb or less level. The Sponsor will provide initial input on analytical methodology used for compound identification. If a

method for feces analysis needs to be developed, such a decision would result in additional cost.

9.8 BIO-ANALYTICAL SAMPLES ANALYSIS:

The serum and urine samples from all monkeys will be analyzed for the concentrations of perfluorobutanesulfonate using the previously validated method. Feces will be analyzed only if requested by the Sponsor.

9.9 ANIMAL DISPOSITION:

At the end of the study, monkeys will be maintained in the stock colony.

10.0 DATA ANALYSIS:

Pharmacokinetic parameters (e.g., AUC, half-life, clearance) will be estimated from serum concentrations of unchanged perfluorobutanesulfonate and metabolites, as appropriate and feasible, using a standard pharmacokinetic program.

The urinary clearance of perfluorobutanesulfonate (and metabolites) at each collection interval will be calculated.

Mean values and standard deviations will be calculated for each time point and sample type, as appropriate. No other statistical analyses of the data will be performed.

11.0 RECORDS:

All raw data pertaining to the conduct of this study, and all samples/specimens collected in this study, will be stored in the Archives at Southern Research Institute for up to 1 year after acceptance of the final report by the Sponsor. After 1 year and with the permission of the Sponsor's Monitor, the data and any samples/specimens will be shipped to the Sponsor or to the Sponsor's designated archival facility. If materials are to be retained in the archives beyond this date, such continued storage will be for a specific fee determined with the Sponsor. A copy of the final report will be retained in the central archives at Southern Research.

12.0 FINAL REPORT:

A draft final report will be issued within 60 calendar days after completion of the in-life aspects of the study. The final report (electronic and hard copies) will be issued within 15 working days after receipt of the Sponsor's final review comments on the draft report. The final report for the present study will include, but not necessarily be limited to the following:

Dose formulation preparation
Clinical observations
Body weight data
Serum drug level data
Pharmacokinetic parameters
Urine excretion data

13.0 REGULATORY REFERENCES:

This study will be conducted in accordance with the protocol and the Standard Operating Procedures of Southern Research Institute, and in accordance with the applicable regulatory requirements, as addressed below.

13.1 PROTOCOL AMENDMENTS AND DEVIATIONS:

Amendments: All changes in or revisions of the approved protocol and the reasons thereof will be documented, signed, and dated by the Study Director, and the Sponsor's Monitor. Amendments will be maintained with the protocol. Written approval (a fax signature or electronic communication, such as email) for changes in the protocol may be granted by the Sponsor's Monitor, but a written amendment will follow.

Deviations: All operations pertaining to this study, unless specifically defined in this protocol, will be performed according to the Standard Operating Procedures (SOPs) of Southern Research and/or the protocol, and any deviations from protocol or SOP will be documented.

13.2 REGULATORY COMPLIANCE:

Good Laboratory Practices: This nonclinical laboratory study will be conducted in the spirit of, but will not require strict compliance with, the U.S. Food and Drug Administration's (FDA) Good Laboratory Practice (GLP) regulations (21 CFR Part 58). Data from this study may be submitted to the FDA in support of an IND/NDA application.

Quality Assurance Review: As this study will not be conducted in strict compliance with FDA's GLP regulations, neither the in-life activities nor the final report will be audited by the Quality Assurance Unit at Southern Research Institute.

13.3 FACILITIES MANAGEMENT AND ANIMAL HUSBANDRY:

Animal care will be in compliance with the Standard Operating Procedures (SOPs) of Southern Research Institute, the *Guidelines for the Care and Use of Laboratory*

Animals, 7th Edition (Institute of Animal Resources, Commission on Life Sciences, National Research Council; National Academy Press; Washington, DC; 1996), and the U.S. Department of Agriculture through the Animal Welfare Act (Public Law 99-198). Southern Research Institute is fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

13.4 ANIMAL WELFARE ACT COMPLIANCE:

By signing this protocol, the Sponsor signifies that there are no generally accepted alternatives to the use of animals, and that the study described by this protocol does not unnecessarily duplicate previously conducted or reported experiments.

Procedures used in this protocol are designed to conform to accepted practices and to minimize or avoid causing pain, distress, or discomfort in the animals. In those circumstances in which required study procedures are likely to cause more than momentary or slight pain or distress, the animals will receive appropriate analgesics or anesthetics unless the withholding of these agents has been justified in writing by the Study Director and/or Sponsor and approved by the IACUC.

The number of animals selected for use in this study is considered to be the minimum number necessary to meet scientific and regulatory guidelines for this type of study.

This study design was reviewed by the IACUC at Southern Research Institute and was approved on xx/xx/xxx; it was assigned IACUC tracking number **xx-xx-xxx**.

14.0 PROTOCOL APPROVALS:

This protocol has been reviewed and approved.

Study Director: Patricia E. Noker 3/17/00

Patricia E. Noker, Ph.D., D.A.B.T. — Date
Study Director

Sponsor's Monitor: Paul W. Luder, DVM 11/13/00

INITIALS ONLY (See page 2) — Date

Management Approval: Ward R Richter 3/17/00

Ward R. Richter, M.S., D.V.M., D.A.C.V.P. — Date
Director, Safety Assessment, Serquest

PROTOCOL AMENDMENT: 9921.1A$_1$

April 10, 2000

Protocol 9921.1 is amended as follows:

1. Page 3 — Section 5.0 (Key Study Dates): Key study dates were unavailable at the time of protocol finalization. This table has been amended as follows:

Event	Sequence (Day)	Date(s) – Year 2000
Day of Treatment	0	**4/10**
Urine and Feces Collections	-1	4/9
	0 – 0-24 hours	4/10 – 4/11
	7	4/17
	14	4/24
Serum Drug Levels	Baseline	4/10
	0 – 0, 2, 4, 8 hours postdose	4/10
	1 – 24 hours postdose	4/11
	2 – 48 hours postdose	4/12
	4	4/14
	7	4/17
	11	4/21
	14	4/24
Draft Report Due	60 calendar days after completion of the in-life phase	**6/23**
Final Report Due	15 days after final Sponsor comments received	TBD

2. Page 9 — Section 9.5 (Urine and Feces Collection): During protocol development, details on the freezing conditions for the urine and fecal samples collected were inadvertently omitted from the protocol. All samples collected will be stored frozen at -20 °C or below prior to analysis (urine) or until further notice by the Sponsor (fecal).

3. Page 9 — Section 9.6 (Serum Drug Levels): During protocol development, details on the freezing conditions for the serum samples collected were inadvertently omitted from the protocol. All serum samples collected will be stored frozen at -20 °C or below prior to analysis.

4. Page 12 — Section 13.4 (Animal Welfare Act Compliance): IACUC approval had not been received at the time of protocol finalization. Approval was received on March 24, 2000 (IACUC Tracking No. 00-03-019), and the last sentence of this section has been amended accordingly.

Effective Date: April 7, 2000

PROTOCOL AMENDMENT: 9921.1A$_1$

Approval Signatures

Study Director: Patricia E. Noker 4/10/00

Patricia E. Noker, Ph.D., D.A.B.T. — Date
Study Director

Sponsor's Monitor: Paul H. Lieder 11/13/00

Paul H. Lieder, Ph.D., D.A.B.T. — Date
Sponsor's Monitor

Management Approval: Ward R Richter 4/12/00

Ward R. Richter, M.S., D.V.M., D.A.C.V.P. — Date
Director, Safety Assessment, Serquest

Appendix B

Method Validation Report: Validation of Analytical Methods for Determination of Perfluorobutanesulfonate in Monkey Serum Using HPLC/MS/MS

METHOD VALIDATION REPORT

VALIDATION OF ANALYTICAL METHODS FOR DETERMINATION OF PERFLUOROBUTANESULFONATE IN MONKEY SERUM USING HPLC/MS/MS

STUDY ID: 9921.1

Serquest
2000 Ninth Avenue South
P.O. Box 55537
Birmingham, AL 35255-5537

SUMMARY

Serquest has successfully validated for 3M an analytical method (BACG 3523) entitled "Determination of Perfluorobutanesulfonate in Monkey Serum: Sample Preparation and Analysis by HPLC Mass Spectrometry/Mass Spectrometry (HPLC/MS/MS)". Calibration standards were prepared by spiking samples of blank monkey serum with known amounts of test article, perfluorobutanesulfonate (PFBS) and internal standard, perfluoropentanoic acid (PFPA). The concentration of test article covered a range of 0.5 to 500 ng/mL. Quantitation limits were set at 1 ng/mL. Standard curves generated using internal standard quantitation produced correlation coefficients of 0.9919 or greater. A total of 39 standards in two composite calibration curves prepared on different days over a concentration range of 0.5 to 100 ng/mL produced standard curves with a correlation coefficient of 0.9962 and 0.9979. A third composite curve containing a total of 33 standards and encompassing a range of 0.5 to 500 ng/mL produced a correlation coefficient of 0.9919 thereby extending the validated calibration range.

KEY PERSONNEL

James D. Johnson, M.S.
Manager
Bioanalytical Chemistry Group

Gregory S. Gorman, Ph.D.
Research Chemist III
Bioanalytical Chemistry Group

Lester Williams, B.S.
Associate Chemist II
Bioanalytical Chemistry Group

1. OBJECTIVE

The objective of this study was to provide a validated analytical method for the determination of perfluorobutanesulfonate in monkey serum.

2. SAFETY

All necessary procedures to ensure safety of the analysts were based on information contained in the Material Safety and Data Sheets (MSDS), provided by 3M for the test article used in this study.

CAUTION: Since primates may carry a number of zoonoses, all unpreserved tissues, including blood, plasma and serum, are to be considered as biohazards and handled with **universal precautions.** Refer to SOP number SRI 2-5-5 for a description of safety procedures to be used when handling unpreserved primate tissue.

3. EXPERIMENTAL

3.1 Analytical Procedures

The sample preparation and analysis procedures as described in the analytical method entitled "Determination of Perfluorobutanesulfonate in Monkey Serum: Sample Preparation and Analysis by HPLC Mass Spectrometry/Mass Spectrometry (HPLC/MS/MS)" were employed for all analyses. For the preparation of the calibration standards, a known volume of a blank serum sample (e.g., 500 µL) was spiked with a known amount of test article (PFBS) and internal standard (PFPA). To the standard was added 500 µL of TBA ion-pairing solution, 1 mL of carbonate/bicarbonate buffer, and 1 mL of deionized water. This mixture was vortexed for approximately 5 seconds. Then 2.5 mL of ethyl acetate was added and the mixture was placed on a horizontal shake at a low speed for 1 hour. The mixture was then centrifuged at approximately 2500 rpm for 5 minutes. The ethyl acetate layer was transferred to a second tube and evaporated to dryness at approximately 55°C under a stream of dry nitrogen. The residue was reconstituted in mobile phase (70 % 5mM ammonium acetate in deionized water : 30% methanol) and filtered through a 0.2 µm syringe filter.

3.2 Method Validation

Validation for BACG 3523 "Determination of Perfluorobutanesulfonate in Monkey Serum: Sample Preparation and Analysis by HPLC Mass Spectrometry/Mass Spectrometry (HPLC/MS/MS)" consisted of analyzing standard curves prepared in monkey serum over two different concentration ranges: 0.5 – 100 ng/mL and 0.5 – 500 ng/mL.

0.5 – 100 ng/mL

Two composite calibration curves comprising a total of 5 analytical runs in the 0.5 – 100 ng/mL concentration range were generated from a total of 39 standards prepared on different days. The correlation coefficient for these composite curves are 0.9962 and 0.9979 respectively. One data point was dropped (0.5 ng/mL) from the first curve due to interference from matrix. An example total and extracted ion chromatogram for each component is shown in Figure 1. A statistical summary for each composite curve is shown below:

STANDARDS COMPOSITE CURVE 1 (ng/mL)

Concen. (ng/mL)	0.5	1	2	5	10	20	50	100
# of Standards	2	3	3	3	3	3	3	3
Mean % Accur.	93.6	99.8	92.2	113.1	108.6	88.1	102.6	99.7
Std. Deviation	0.014	0.085	0.192	0.523	0.717	0.474	3.44	6.28

STANDARDS COMPOSITE CURVE 2 (ng/mL)

Concen. (ng/mL)	0.5	1	2	5	10	20	50	100
# of Standards	2	2	2	2	2	2	2	2
Mean % Accur.	81.8	101.2	113.1	112.4	94.6	95.3	102.1	99.7
Std. Deviation	0.131	0.146	0.182	0.308	0.211	1.01	3.14	3.91

0.5 – 500 ng/mL

A single composite curve consisting of 37 standards taken from an analytical run encompassing a concentration range of 0.5 – 500 ng/mL produced a correlation coefficient of 0.9919 (Figure 2). Of these data three points were dropped, 2 at the 0.5 ng/mL level and one at the 500 ng/mL level. This was due to matrix interference for one standard (0.5 ng/mL) and insufficient vial volume for the remaining two (0.5, 500 ng/mL). A statistical summary for the composite curve is given below:

STANDARDS COMPOSITE CURVE (ng/mL)

Conc. (ng/mL)	0.5	1	2	5	10	20	50	100	200	500
# of Stds	2	4	4	4	4	4	4	4	4	3
Mean % Accur.	97.7	96.5	108	108	102	98.2	95.3	88.7	100	109
Std. Deviation	0.01	0.14	0.16	0.16	0.72	1.70	4.71	12.4	23.5	26.0

These results demonstrates the reproducibility and reliability of the sample preparation and analysis methodologies used to analyze serum samples.

3.3 Calculations

Calculations were performed using TurboQuan (Version 1.0). The amount of analyte in the serum extracts (ng/mL) was back calculated using a calibration curve generated from a set of calibration standards. The calibration curve was generated by a regression analysis to determine the best fit curve (e.g., linear, quadratic etc.) and amount of weighting. A quadratic fit with 1/X weighting was determined to be the best fit :

$$y = ax^2 + bx + c$$

where:

y = Peak height response of PFBS divided by peak height response of the IS (PFPA) in standards.

x = Concentration of the PFBS in standards.

a, b, c = Constants derived from the regression analysis.

3.4 Compound Stability

Blank serum samples spiked with PFBS at the 5 ng/mL level were subjected to two complete freeze thaw cycle then extracted and analyzed as described in BACG 3523. The results of the analysis are listed in the table below:

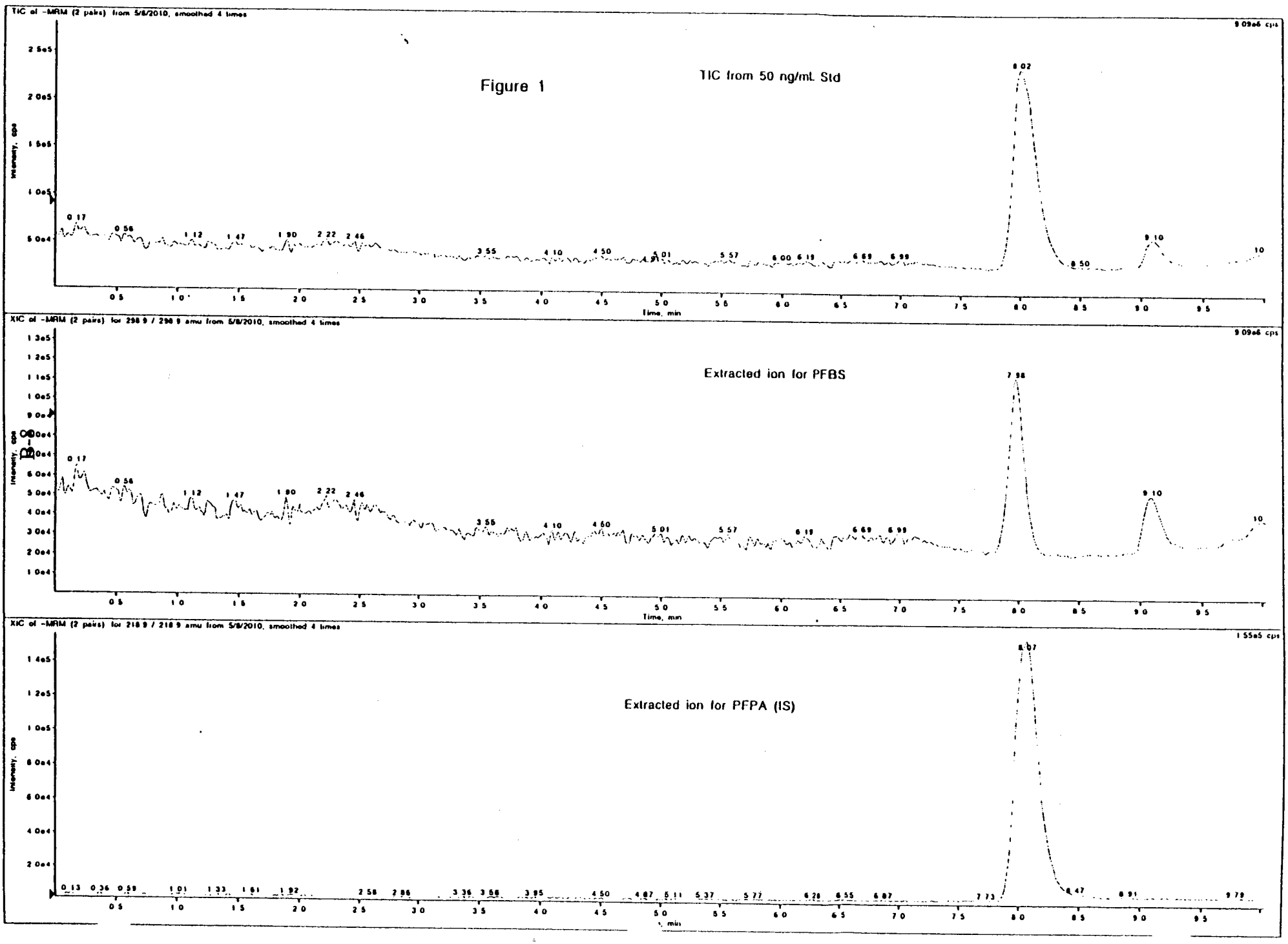
Spike Concentration (ng/mL)	Found Amount (ng/mL)	% Recovery
5	5.3	106
5	5.3	106
5	4.1	82
Average	4.9	98

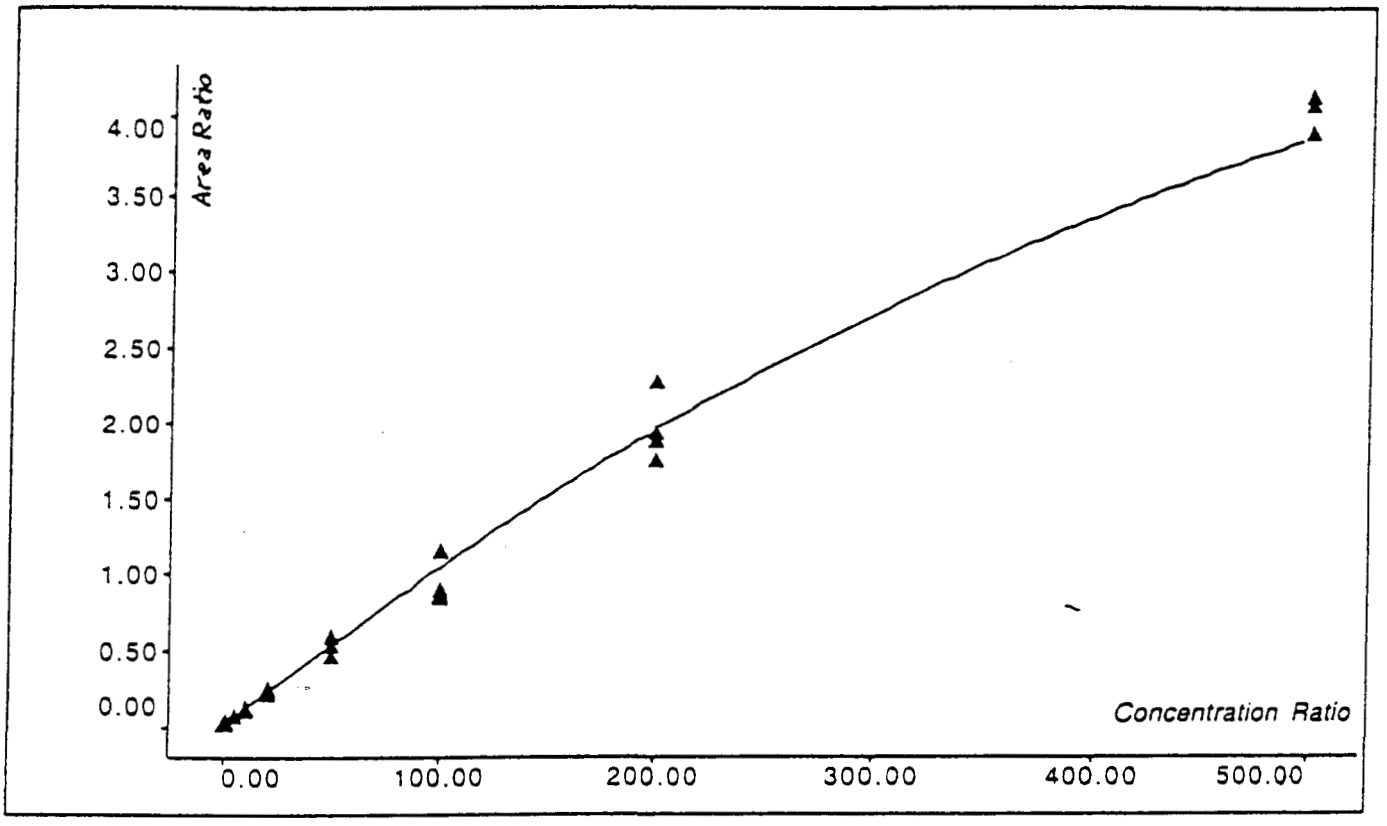
4.0 Conclusion

A quantitative method (BAGC 3523) has been developed and validated for the determination of perfluorobutanesulfonate in monkey serum utilizing LC/MS/MS. Quantitation for this method is based on an internal standard (PFPA) which produces standard curves with a correlation coefficients of 0.9919 or greater over a concentration range of 0.5 to 500 ng/mL. Analysis of

spiked monkey serum after two complete freeze thaw cycles produced an average quantitative recovery of 98 %

Figure 1


TIC of -MRM (2 pairs) from 5/8/2010, smoothed 4 times
TIC from 50 ng/mL Std
Intensity, cps
Time, min
XIC of -MRM (2 pairs) for 298.9 / 298.9 amu from 5/8/2010, smoothed 4 times
Extracted ion for PFBS
XIC of -MRM (2 pairs) for 218.9 / 218.9 amu from 5/8/2010, smoothed 4 times
Extracted ion for PFPA (IS)



Compound:	PFBS
Internal Standard:	IS
Fit:	Quadratic
Fit Parameter:	Area Ratio
Weighting:	1÷X*X
Equation:	y = - 6.7074e-6 * x * x +0.01105 * x + 0.00789
Correlation Coefficient (r):	0.991897
Chi Square:	0.00005

Appendix C

Analytical Method for the Analysis of Perfluorobutanesulfonate in Serum

ANALYTICAL METHOD

Method No.: BACG-3523

Title: Determination of Perfluorobutanesulfonate in Monkey Serum: Sample Preparation and Analysis by HPLC Mass Spectrometry/Mass Spectrometry (HPLC/MS/MS)

1.0 PRINCIPLE

Serum samples are obtained from cynomolgus monkeys treated with perfluorobutanesulfonate (PFBS). Serum (e.g., 0.5 mL) containing (PFBS) is fortified with an internal standard (IS), perfluoropentanoic acid (PFPA). The serum samples are then mixed with an ion-pairing reagent and buffer, followed by extraction with ethyl acetate. The ethyl acetate layer is removed, evaporated to dryness, reconstituted in mobile phase, filtered, and transferred to autosampler vials, and analyzed by HPLC Mass Spectrometry/Mass Spectrometry (HPLC/MS/MS). The range of reliable results extends from about 0.5 to 500 ng/mL of PFBS in serum. Serum containing PFBS at concentrations greater than 500 ng/mL may be diluted with control blank serum so that the concentration of PFBS will be within the range of reliable results prior to analysis.

The mass spectrometry of PFBS and PFPA is accomplished in the negative ion mode. The ion spray source voltage is set at - 2000 volts which is low enough to greatly reduce the formation of other potentially interfering ions extracted from the serum. In addition, the collision energy is set rather high to reduce potential interferences from other matrix compounds. In order to maximize sensitivity, this method is based on mixed reaction monitoring of the negative parent ion (M-1) for both PFBS and PFPA.

CAUTION: Since primates may carry a number of zoonoses, all unpreserved tissues, including blood, plasma and serum, are to be considered as biohazards and handled with **universal precautions.** Refer to SOP number SRI 2-5-5 for a description of safety procedures to be used when handling unpreserved primate tissue.

2.0 REAGENTS AND SOLUTIONS

The listed reagents or their equivalents may be used.

2.1 Neat Reagents

2.1.1 Water, deionized and organic free (from in-house purification system; e.g., Ingalls 210N)

ANALYTICAL METHOD

Method No.: BACG-3523

Title: Determination of Perfluorobutanesulfonate in Monkey Serum: Sample Preparation and Analysis by HPLC Mass Spectrometry/Mass Spectrometry (HPLC/MS/MS)

2.1.2 Methanol, HPLC grade

2.1.3 Perfluorobutanesulfonate (analyte), as provided by the client

2.1.4 Perfluoropentanoic acid (internal standard), 97%

2.1.5 Ammonium acetate, HPLC grade

2.1.6 Blank control monkey serum

2.1.7 Sodium Carbonate, Certified ACS Grade or equivalent

2.1.8 Sodium Bicarbonate, Certified ACS Grade or equivalent

2.1.9 Ethyl Acetate, HPLC grade

2.1.10 Tetrabutylammonium Hydrogen Sulfate

2.1.11 Sodium Hydroxide 50% solution, Certified grade or equivalent

2.2 **Prepared Solutions**

Appropriate changes in the solutions may be made at the discretion of the analyst

2.2.1 5 mM Ammonium acetate in organic free water

2.2.1.1 For example, to prepare 4 liters, measure out ammonium acetate (e.g., 1.542 g) and add in organic-free water (e.g., 4 L). Mix well and filter through HPLC mobile phase filtration apparatus.

2.2.2 TBA Ion-Pairing Solution (0.5 M tetrabutylammonium hydroxide)

2.2.2.1 For example, to prepare 25 mL, dissolve 4.24 g of tetrabutylammonium hydrogen sulfate in deionized water and adjust the pH to 10 with 50% NaOH solution.

ANALYTICAL METHOD

Method No.: BACG-3523

Title: Determination of Perfluorobutanesulfonate in Monkey Serum: Sample Preparation and Analysis by HPLC Mass Spectrometry/Mass Spectrometry (HPLC/MS/MS)

2.2.3 Carbonate/Bicarbonate Buffer Solution

2.2.3.1 For example, to prepare 100 mL, dissolve approximately 2.65 g of sodium carbonate and approximately 2.10 g of sodium bicarbonate in 100 mL of deionized water. Mix well to ensure complete dissolution.

3.0 INSTRUMENTS, MATERIALS, AND APPARATUS

The following or their equivalents may be used.

3.1 HPLC pump(s), autosampler, and triple quadrupole mass spectrometer

3.2 Autosampler vials with inserts

3.3 Vortex mixers (e.g., touch mixer and IKA-Vibrax ® platform mixer)

3.4 Solvent-concentration apparatus (e.g., Zymark Turbo-Vap ® with source of nitrogen)

3.5 HPLC mobile phase filtration apparatus

3.6 Filters for HPLC mobile phase filtration apparatus (e.g., Nylon-66, 0.20 μm)

3.7 Analytical balance

3.8 Volumetric flasks (e.g., 10 and 25 mL)

3.9 Disposable Pasteur pipets

3.10 Micropipettor(s) with tips

3.11 Culture tubes with teflon-lined caps

3.12 Centrifuge

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3.13 Assorted glassware and syringes

3.14 Culture tubes (vials) for use with solvent-concentration apparatus

3.15 0.2 μm PVDF syringe filters

3.16 Variable speed horizontal platform shaker

4.0 PREPARATION OF STOCKS AND WORKING STOCKS

Appropriate changes in the concentrations of the solutions may be made at the discretion of the analyst. Actual dilutions will be documented on the preparation sheets.

4.1 Main Stock Solution of PFBS ~1000 μg/mL

4.1.1 Prepare an ~1000 μg/mL solution of PFBS in deionized organic-free water (e.g., accurately weigh about 10 mg PFBS into a 10-mL volumetric flask). Add deionized organic-free water to dissolve. Dilute to the mark. Alternatively, weigh the compound into an appropriate vessel (e.g., culture tube) and add 10 mL of deionized organic-free water. Mix well. Transfer the solution to a clean vessel if desired.

4.2 Stock Solution of Internal Standard (PFPA), ~200 μg/mL

4.2.1 Prepare an ~200 μg/mL solution of PFPA in deionized organic-free water (e.g., accurately weigh about 10 mg into a 50-mL volumetric flask). Add deionized organic-free water to dissolve and dilute to the mark with deionized organic-free water. Alternatively, weigh the compound into a an appropriate vessel (e.g., culture tube) and add 50 mL of deionized organic-free water. Mix well. Transfer the solution to a clean vessel if desired.

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4.3 **Working Stock Solutions of PFBS**

4.3.1 To prepare working stock solutions, make the proper dilutions as shown in the following table. Prepare in 10-mL volumetric flasks or other appropriate glassware. If desired a modified dilution scheme can be used and documented in the study records.

Working Stock Level (WSL) Approximate Concentration (ng/mL)	Volume of PFBS solution	Final Volume in deionized organic free water (mL)
5000	50 µL of 1000 µg/mL stock	10
2500	5 mL of 5000 ng/mL stock	10
1000	4 mL of 2500 ng/mL stock	10
500	5 mL of 1000 ng/mL stock	10
250	5 mL of 500 ng/mL stock	10
100	4 mL of 250 ng/mL stock	10
50	5 mL of 100 ng/mL stock	10
25	5 mL of 50 ng/mL stock	10
12.5	5 mL of 25 ng/mL stock	10
0	0 mL of 6.25 stock	10

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4.3.2 Summary of concentrations of serum standards:

Standard Level	Approximate Concentration of PFBS in serum (ng/mL)
A2	500
A1	200
A	100
B	50
C	20
D	10
E	5
F	2
G	1
H	0.5
I	0.25

5.0 PREPARATION OF SPIKED STANDARDS AND BLANKS

Appropriate changes in the concentrations of the solutions may be made at the discretion of the analyst.

5.1 Multiple (e.g., about three) sets of serum standards and a serum blank (blank + IS) are analyzed with each set of unknown samples. A serum double blank (blank-IS) may also be analyzed if desired.

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5.2 Into individual ~20-mL culture tubes, pipet blank serum (e.g., 0.5 mL). Pipet in 50, 20, and 10 µL of the 5000 ng/mL working stock for the 500, 200, and 100 ng/mL standard respectively. For the remaining standards pipet 10 µL of each subsequent working stock standard solution into separate tubes that contain the blank serum. For the blanks, pipet 10 µL of organic-free water instead of the working stock solution. Add the 5 µL of internal standard stock (~200 µg/mL) to each tube except the blank-IS (pipet 10 µL of organic-free water instead) and vortex for ~5 seconds.

5.3 To each tube add 500 µL of the TBA ion-pairing solution, 1 mL of carbonate/bicarbonate buffer, and 1 mL of deionized organic free water. Vortex each tube for about 5 seconds.

5.4 Add 2.5 mL of ethyl acetate and extract on horizontal mixer for 1 hour at a low speed setting.

5.5 Remove the tube from the shaker and place in a centrifuge (e.g., 2500 rpm) for about 5 minutes.

5.6 Take off the upper ethyl acetate layer and put it into a clean tube and evaporate to dryness (e.g., ~ 50 minutes in the Turbo-Vap ®) with a gentle stream of nitrogen and moderate heat (e.g., 50 °C).

5.7 Reconstitute the residue in 500 µl of mobile phase(70% 5 mM ammonium acetate: 30% methanol) and vortex briefly to mix: Filter the samples through 0.2 µm PVDF syringe filters into autosampler vials.

6.0 PREPARATION OF SAMPLES

6.1 Allow each serum sample to thaw to room temperature. Vortex each sample briefly, but vigorously. Pipet an aliquot of each sample (e.g., 0.5 mL) into individual ~20-mL culture tubes. If necessary, dilute an aliquot of the serum sample with blank serum so that the expected concentration of the test article being analyzed will fall within the concentration range of the standard curve. Add 5 µL of internal standard stock (~ 200 µg/mL) to each tube and vortex for a couple of seconds.

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6.2 To each tube add 500 µL of the TBA ion-pairing solution, 1 mL of carbonate/bicarbonate buffer, and 1 mL of deionized organic free water. Vortex each tube for about 5 seconds.

6.3 Add 2.5 mL of ethyl acetate and extract on horizontal mixer for 1 hour at a low speed setting.

6.4 Remove the tube from the shaker and place in a centrifuge (e.g., 2500 rpm) for about 5 minutes.

6.5 Take off the upper ethyl acetate layer and put it into a clean tube and evaporate to dryness (e.g., ~ 50 minutes in the Turbo-Vap ®) with a gentle stream of nitrogen and moderate heat (e.g., 50 °C).

6.6 Reconstitute the residue in 500 µL of mobile phase (70% 5 mM ammonium acetate: 30% methanol) and vortex briefly to mix. Filter the samples through 0.2 µm PVDF syringe filters into autosampler vials. Cap vials for analysis.

7.0 ANALYSIS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY MASS SPECTROMETRY/MASS SPECTROMETRY (HPLC/MS/MS)

7.1 Conditions are to be optimized if necessary.

7.1.1 HPLC Conditions

Analytical Column:	Keystone Scientific Fluofix 120E, 5 micron, 150 mm x 2 mm ID, or equivalent
Guard Column:	Fluofix 120E or Fluophase RP 10 mm x 2 mm
Elution Flow rate:	200 µL/min.
Injection volume:	50 µL
Mobile phase:	A: 5mM ammonium acetate buffer B: methanol

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Gradient Profile:	0 - 2 min. 70% A : 30% B 2 - 6 min. 30% A : 70% B linear gradient 6-11 min. 30%A : 70 % B 11-15 min. 70%A : 30% B step gradient
Temperature:	Ambient

7.1.2 PE Sciex API 3000 Triple Quadrupole Mass Spectrometer Conditions
Software: PE Sciex TurboQuan

Turboion Spray Source
Note: Values listed under "MS/MS Acquisition Conditions" override parameters in this table.

Auxiliary Gas: Air (e.g., Grade 0.1) at 85 pounds per square inch

Parameter	Value
IS	-2000
NC	0
TEM	450
OR	-20
RNG	-120
Q0	18
IQ1	19
ST	23
RO1	19
IQ2	20
RO2	65
ST3	75

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Parameter	Value
RO3	65
DF	250
CEM	2600
NEB	15
CUR	6
CAD	5
QPE	0
POL	0
VCM	0
IPE	0

MS/MS Acquisition Conditions

Scan type: MRM
Polarity: Positive
Acquisition mode: Profile
Pause time: 5 milliseconds

Masses requested:

PFBS:

Q1 Mass (amu)	Q3 Mass (amu)	Dwell Time (ms)
298.9	298.9	200
Parameter	Start Value	Stop Value
RO2	65	65
RO3	75	75
ST3	65	65

PFPA (IS)

Q1 Mass (amu)	Q3 Mass (amu)	Dwell Time (ms)
218.9	218.9	200
Parameter	Start Value	Stop Value
RO2	45	45
RO3	55	55
ST3	45	45

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8.0 CALCULATIONS

8.1 At the end of the analytical run, review each chromatogram to ensure the retention time, peak shape, and peak height and peak area determination of the test article and the IS are acceptable. The data may be smoothed as appropriate. For quantitation, use the ion profiles at the following mass-to-charge ratios:

Analyte	Ion Profile
PFBS	298.9 to 298.9
PFPA	218.9 to 218.9

8.2 Plot the peak area response of PFBS divided by the peak area response of the IS (PFPA) from all standards versus the concentration of the test article in the standards. Alternatively, the peak heights may be used instead of peak areas. Obtain the best curve fit of the data (e.g., quadratic fit weighted with 1/concentration of the test article or a quadratic fit). Note: The best curve fit may be dependent on the range of the standard curve and it may be necessary to have more than one standard curve for various concentration ranges using the following:

$$y = ax^2 + bx + c$$

where y = Peak height response of PFBS divided by peak height response of the IS (PFPA) in standards.
x = Concentration of the PFBS in standards.
a, b, c = Constants derived from the regression analysis.

8.3 Using the standard curve, calculate the level of PFBS in each unknown sample. Correct the results of samples for any dilutions.

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NOTE: Due to unresolvable interferences with the test article and/or internal standard from the matrix, external standard quantitation may be used at the discretion of the supervising mass spectrometrist.

9.0 ACCEPTANCE AND REJECTION CRITERIA

9.1 Refer to SOP SRI 91-3 for acceptance/rejection criteria except acceptable accuracy for standards is 80-120% of theoretical.

10.0 REPORTING

10.1 Results of all analyses are tabulated, and the raw data, original chromatograms, and reports are to be filed in the appropriate study file.

Authors: Gregory S. Gorman 5-11-2000

Gregory S. Gorman, Ph.D., Research Chemist III Date

Approved by: James D. Johnson 5/11/2000

James D. Johnson MS, MBA, Manager Date
Bioanalytical Chemistry Group