38) Analytical method validation for determination of PFBS-K in freshwater, 454C-115

ANALYTICAL METHOD VALIDATION FOR THE DETERMINATION OF PERFLUOROBUTANE SULFONATE, POTASSIUM SALT (PFBS) IN FRESHWATER

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454C-115

3M Environmental Lab Project No. E00-1429

AUTHORS:

Raymond L. Van Hoven, Ph.D.
Willard B. Nixon, Ph.D.

STUDY INITIATION DATE: April 17, 2000

STUDY COMPLETION DATE: June 12, 2000

Submitted to:

3M Corporation
Environmental Laboratory
935 Bush Avenue
St. Paul, Minnesota 55106

Wildlife International, Ltd.
8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: 3M Corporation

TITLE: Analytical Method Validation for the Determination of Perfluorobutane Sulfonate, Potassium Salt (PFBS) in Freshwater

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454C-115

3M ENVIRONMENTAL LAB PROJECT NUMBER: E00-1429

STUDY COMPLETION: June 12, 2000

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Part 792, 17 August 1989 and OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17) with the following exceptions:

The test substance was not characterized in accordance with Good Laboratory Practice Standards.

The stability of the test substance under storage conditions at the test site was not determined in accordance with Good Laboratory Practice Standards.

STUDY DIRECTOR:

Raymond L. Van Hoven, Ph.D. — 6-12-00 DATE
Scientist
Wildlife International, Ltd.

SPONSOR APPROVAL:

Sponsor — 9/15/00 DATE

QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Part 792, 17 August 1989 and OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17). The dates of all inspections and audits and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

		DATE REPORTED TO:	
ACTIVITY:	DATE CONDUCTED:	STUDY DIRECTOR:	MANAGEMENT:
Matrix Fortification Preparation	April 18, 2000	April 18, 2000	April 20, 2000
Data and Draft Report	May 2 and 3, 200	May 3, 2000	May 4, 2000
Final Report	June 12, 2000	June 12, 2000	June 12, 2000

Susan L. Coleman

Susan L. Coleman, B.A.
Senior Quality Assurance Representative

6-12-00

DATE

REPORT APPROVAL

SPONSOR: 3M Corporation

TITLE: Analytical Method Validation for the Determination of Perfluorobutane Sulfonate, Potassium Salt (PFBS) in Freshwater

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454C-115

3M ENVIRONMENTAL LAB PROJECT NUMBER: E00-1429

STUDY DIRECTOR:

Raymond L. Van Hoven, Ph.D.
Scientist

6-12-00
DATE

MANAGEMENT:

Willard B. Nixon, Ph.D.
Manager, Analytical Chemistry

6/12/00
DATE

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SUMMARY

STUDY TITLE:	Analytical Method Validation for the Determination of Perfluorobutane Sulfonate, Potassium Salt (PFBS) in Freshwater
WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER:	454C-115
SPONSOR:	3M Corporation
TESTING FACILITY:	Wildlife International, Ltd. Easton, Maryland 21601
LOCATION OF STUDY, RAW DATA AND A COPY OF THE FINAL REPORT:	Wildlife International, Ltd. Easton, Maryland 21601

TEST SUBSTANCE:	PFBS
FORTIFIED TEST CONCENTRATIONS FOR METHOD VALIDATION:	0.500, 10.0, 100 and 1000 mg a.i./L
TEST DATES:	Experimental Start – April 18, 2000 Experimental Termination – April 19, 2000

TEST SYSTEM:	Freshwater

SUMMARY:	Freshwater samples were fortified with PFBS to reflect nominal concentrations of 0.500, 10.0, 100 and 1000 mg a.i./L. The samples were diluted into the instrumental calibration range with dilution solvent (50% methanol: 50% NANOpure® water) and analyzed by liquid chromatography mass spectrometry (LCMS). Recoveries of PFBS in freshwater yielded an overall mean percent recovery of 106% (SD = 5.72%; CV = 5.41%; N = 12). Matrix blank samples, consisting of unfortified freshwater, were devoid of interfering components when a base-acid wash on preparatory apparatus was incorporated into the methodology.

INTRODUCTION

This study was conducted by Wildlife International, Ltd. for 3M Corporation at the Wildlife International, Ltd. analytical chemistry facility in Easton, Maryland. Validation samples were fortified with Perfluorobutane Sulfonate, Potassium Salt (hereafter referred to as PFBS) and analyzed with liquid chromatography mass spectrometry (LCMS) to evaluate the performance of a method in freshwater. Validation samples were prepared and analyzed between April 18 and 19, 2000. Raw data generated by Wildlife International, Ltd. and a copy of the final report are filed under Project Number 454C-115 in archives located on the Wildlife International, Ltd. site.

PURPOSE

The purpose of this study was to verify the performance of a LCMS methodology for the analyses of PFBS in freshwater. The validated method will be used in support of aquatic toxicology and product chemistry tests to be conducted with the test substance.

EXPERIMENTAL DESIGN

Freshwater was fortified at four different concentrations in triplicate and analyzed based on LCMS methodology developed at Wildlife International, Ltd. The fortification levels bracketed the anticipated concentrations of samples from future aquatic toxicity tests. Matrix blank samples were analyzed concurrently to evaluate potential analytical method interferences. A calibration curve was generated from analyses of standard solutions of PFBS with each series of samples analyzed.

MATERIALS AND METHODS

This study was conducted according to the procedures outlined in the protocol, "Analytical Method Validation for the Determination of Perfluoro Butane Sulfonate, Potassium Salt (PFBS) in Freshwater" (Appendix I).

Test Substance

The test substance was received from 3M Environmental Technology and Safety Services on March 27, 2000, assigned Wildlife International, Ltd. Identification number 5216, and stored under ambient conditions. The test substance, a white powder, was identified as: Potassium Perfluorobutane Sulfonate (L-7038, Lot 2), expiration date: March 2010. The test substance was further identified with the 3M Environmental Laboratory test control and reference number TCR # 99131-39. After the protocol was issued, the test substance purity was re-determined and had a reported purity of 97.9% (Appendix II).

Reagents and Solvents

All solvents used in this study were HPLC grade or equivalent. All reagents were ACS reagent grade or equivalent. NANOpure® water (equivalent to ASTM Type II Designation D1193-91) was used (1).

Test System

The water used as matrix was freshwater obtained from a well approximately 40 meters deep located on the Wildlife International, Ltd. site. The water was passed through a sand filter and pumped into a 37,800-L storage tank where the water was aerated with spray nozzles. Prior to use, the water was filtered to 0.45 µm in order to remove fine particles. The freshwater is characterized as moderately-hard water. Specific conductance, hardness, alkalinity and pH of freshwater during the four-week period immediately preceding the test are presented in Appendix III. The results of analyses performed to measure the concentrations of selected contaminants in freshwater used by Wildlife International, Ltd. are presented in Appendix IV.

Analytical Validation Procedures

Wildlife International, Ltd. conducted a validation trial using procedures based on methodology developed at Wildlife International, Ltd. Validation samples were prepared in freshwater. Dilutions were performed with a solution of 50% methanol (HPLC grade, 99.9+%) and 50% NANOpure® water. Samples were then analyzed by direct injection. Concentrations of PFBS were determined by reverse-phase high performance liquid chromatography using a Hewlett-Packard Model 1100 High Performance Liquid Chromatograph (HPLC) with a Perkin-Elmer API 3000LC Mass Spectrometer equipped with a Perkin-Elmer TurboIonSpray ion source. Chromatographic separations were achieved using a Keystone Prism column (30 mm x 1.5 mm, 3-µm particle size). The instrument parameters are summarized in Table 1 and a method flow chart is provided in Figure 1.

Primary Stock Solution, Calibration Standards and Curves

A primary stock solution of PFBS was prepared in methanol. Calibration standards were prepared in 50% methanol: 50% NANOpure® water by appropriate dilutions of this stock solution. Calibration standards of PFBS, ranging in concentration from 0.00100 to 0.0100 mg a.i./L, were analyzed with each sample set. Five calibration standards (different concentrations) were analyzed with the samples. The calibration standard series was injected at the beginning and end of each run, and one standard was injected, at a minimum, after every five samples. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. A typical calibration curve is presented in Figure 2. The concentration of PFBS in the samples was determined by substituting the peak area responses into the applicable linear regression equation. Representative ion chromatograms of low and high calibration standards are presented in Figures 3 and 4, respectively.

Fortification Stock Solutions

Freshwater was fortified with stock solutions of PFBS prepared in methanol. Each stock solution was assigned a unique identification code that was recorded on a stock preparation log sheet.

Limit of Quantitation

The method validation levels were well above the method capabilities for quantitation of the test substance. Therefore, the method limit of quantitation (LOQ) for the analyses was set at 0.100 mg a.i./L based upon the product of the lowest calibration standard (0.00100 mg a.i./L) and the dilution factor of the matrix blank samples (100).

Example Calculations

Sample number 454C-115-VMAS-2, nominal concentration of 0.500 mg a.i./L in freshwater.

Initial Volume: 0.100 mL

Final Volume: 10.0 mL

Dilution Factor: 100

PFBS Peak Area: 1166071

Calibration curve equation.

Slope: 214262096.0000

Intercept: 5379.9839

Curve is weighted (1/x).

$$\text{PFBS (mg a.i./L) in sample} = \frac{\text{peak area - (y-intercept)}}{\text{slope}} \times \text{dilution factor}$$

$$= \frac{1166071 - 5379.9839}{214262096.0000} \times 100$$

$$= 0.542$$

$$\text{Percent of Nominal Concentration} = \frac{\text{PFBS (mg a.i./L) in sample}}{\text{PFBS (mg a.i./L) nominal}} \times 100$$

$$= \frac{0.542}{0.500} \times 100 = 108\%$$

Instrument Software: MacQuan, version 1.6.

RESULTS

Matrix Blank Samples

Three matrix blank samples were analyzed to determine possible interferences. No interferences were observed at or above the LOQ (0.100 mg a.i./L) during the sample analyses (Table 2). However, a trace-level interfering component was evident in a preliminary trial that was eliminated through use of a base-acid wash procedure on preparatory apparatus. To prevent a potential source of bias in future definitive studies, the base-acid wash procedure was incorporated into the methodology. A representative ion chromatogram of a freshwater matrix blank sample is presented in Figure 5.

Method Validation Samples

Freshwater was fortified in triplicate at 0.500, 10.0, 100 and 1000 mg a.i./L resulting in mean recoveries of 112, 105, 103 and 103%, respectively (Table 3). Representative ion chromatograms of low and high-level freshwater matrix fortifications are presented in Figures 6 and 7, respectively.

CONCLUSIONS

Freshwater samples were fortified with PFBS to reflect nominal concentrations of 0.500, 10.0, 100 and 1000 mg a.i./L. The samples were diluted into the instrumental calibration range with dilution solvent (50% methanol/50% NANOpure® water) and analyzed by liquid chromatography mass spectrometry (LCMS). Recoveries of PFBS in freshwater yielded an overall mean percent recovery of 106% (SD = 5.72%; CV = 5.41%; N = 12). Matrix blank samples, consisting of unfortified freshwater, were devoid of interfering components when a base-acid wash on preparatory apparatus was incorporated into the methodology.

REFERENCES

1. American Society for Testing and Materials. 1991. Standard Specification for Reagent Water. D1193-91, ASTM Section II Water and Environmental Technology, Vol. 11.01:45-47.

Table 1

Typical LC/MS Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1100 High Performance Liquid Chromatograph with a Perkin-Elmer API 3000LC Mass Spectrometer equipped with a Perkin-Elmer TurboIonSpray ion source. Operated in selective ion monitoring mode (SIM).
ANALYTICAL COLUMN:	Keystone Prism Column (30 mm × 1.5 mm, 3-µm particle size)
OVEN TEMPERATURE:	40°C
STOP TIME:	3.00 minutes
FLOW RATE:	0.150 mL/minute
MOBILE PHASE:	75.0% Methanol : 25.0% NANOpure® Water containing 0.1% Ammonium Formate
INJECTION VOLUME:	2.5 µL
PFBS RETENTION TIME:	Approximately 1.5 minutes
PFBS MONITORED MASS:	299.0 amu

Table 2

Matrix Blanks Analyzed Concurrently During Sample Analysis

Sample		Measured PFBS Concentration[1] (mg a.i./L)
Number (454C-115-)	Type	
VMAB-4	Matrix Blank	< LOQ
VMAB-5	Matrix Blank	< LOQ
VMAB-6	Matrix Blank	< LOQ

[1] The limit of quantitation (LOQ) was 0.100 mg a.i./L based upon the product of the lowest calibration standard (0.00100 mg a.i./L) and the dilution factor of the matrix blank samples (100).

Table 3

Method Validation Recoveries for PFBS In Freshwater

Sample		PFBS Concentration (mg a.i./L)		Percent Recovery	Mean Measured (mg a.i./L)	Mean Percent Recovery
Number (454C-115-)	Type	Fortified	Measured			
VMAS-1	Matrix Fortification	0.500	0.596	119	0.558	112
VMAS-2	Matrix Fortification	0.500	0.542	108		
VMAS-3	Matrix Fortification	0.500	0.537	107		
VMAS-4	Matrix Fortification	10.0	9.89	98.9	10.5	105
VMAS-5	Matrix Fortification	10.0	10.5	105		
VMAS-6	Matrix Fortification	10.0	11.2	112		
VMAS-7	Matrix Fortification	100	106	106	103	103
VMAS-8	Matrix Fortification	100	102	102		
VMAS-9	Matrix Fortification	100	102	102		
VMAS-10	Matrix Fortification	1000	981	98.1	1030	103
VMAS-11	Matrix Fortification	1000	1039	104		
VMAS-12	Matrix Fortification	1000	1071	107		
			Overall Mean =	106		
			Standard Deviation =	5.72		
			CV =	5.41		
			N =	12		

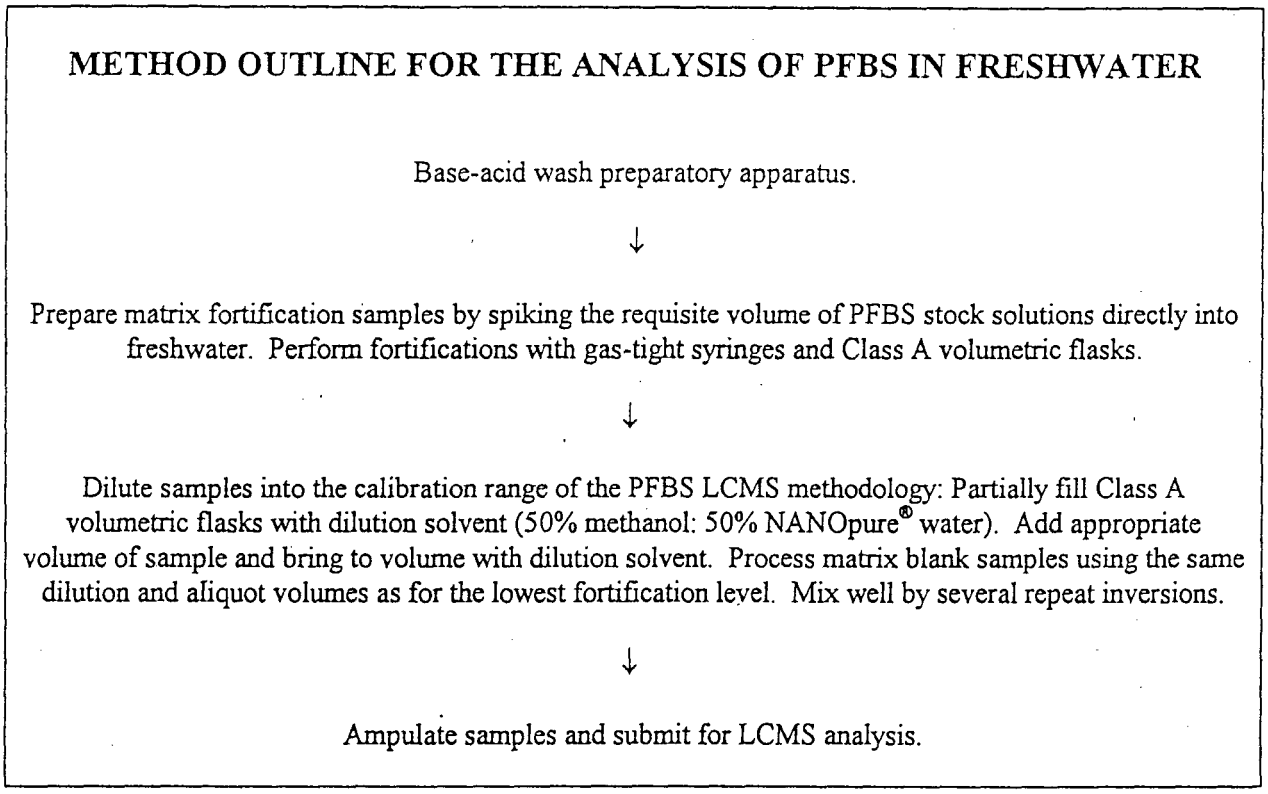

Figure 1. Analytical method flow chart for the analysis of PFBS in freshwater.

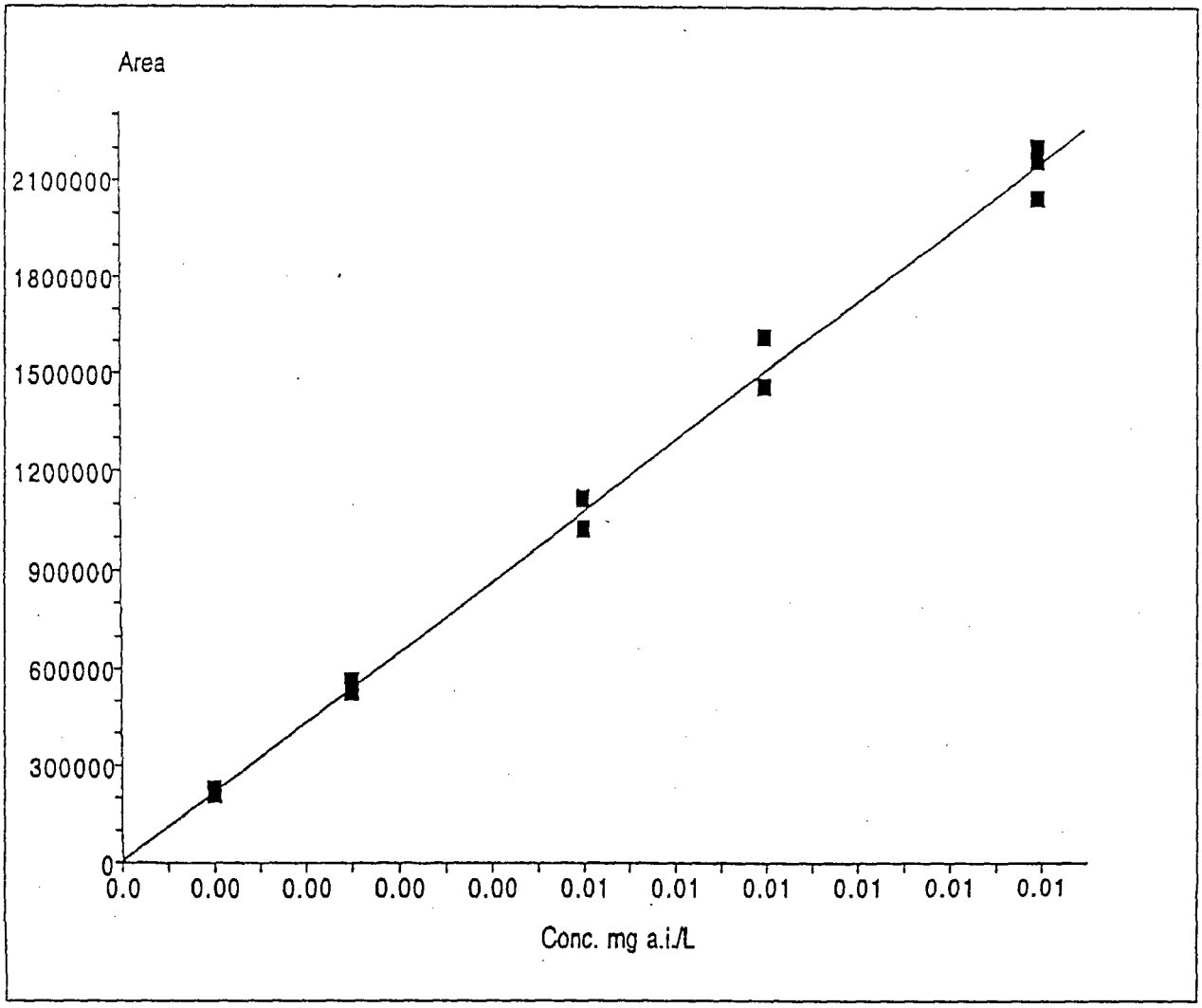

Figure 2. A typical calibration curve for PFBS. Slope = 214262096.0000; Intercept = 5379.9839; r = 0.9985.

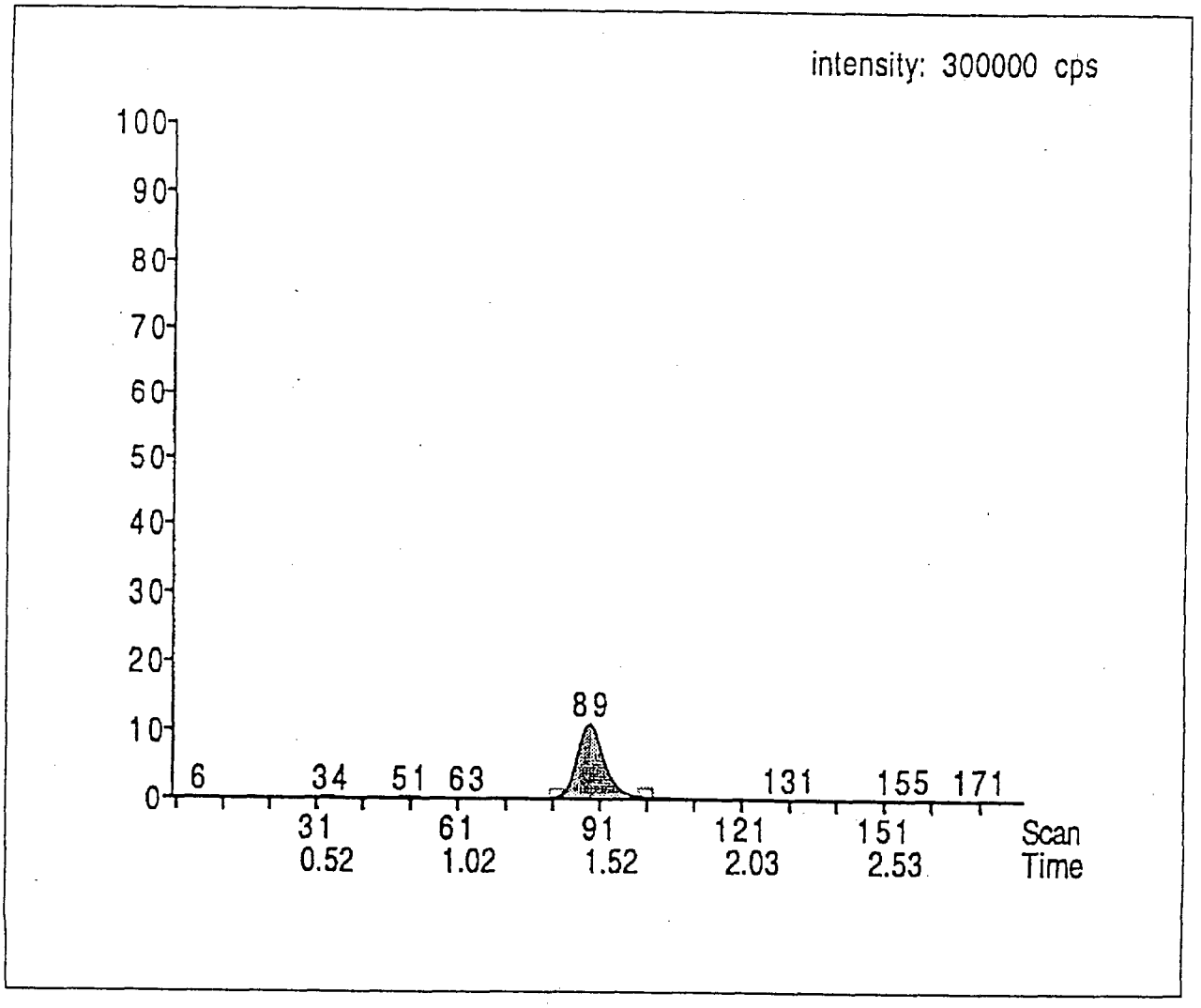

Figure 3. A representative ion chromatogram of a low-level (0.00100 mg a.i./L) standard.

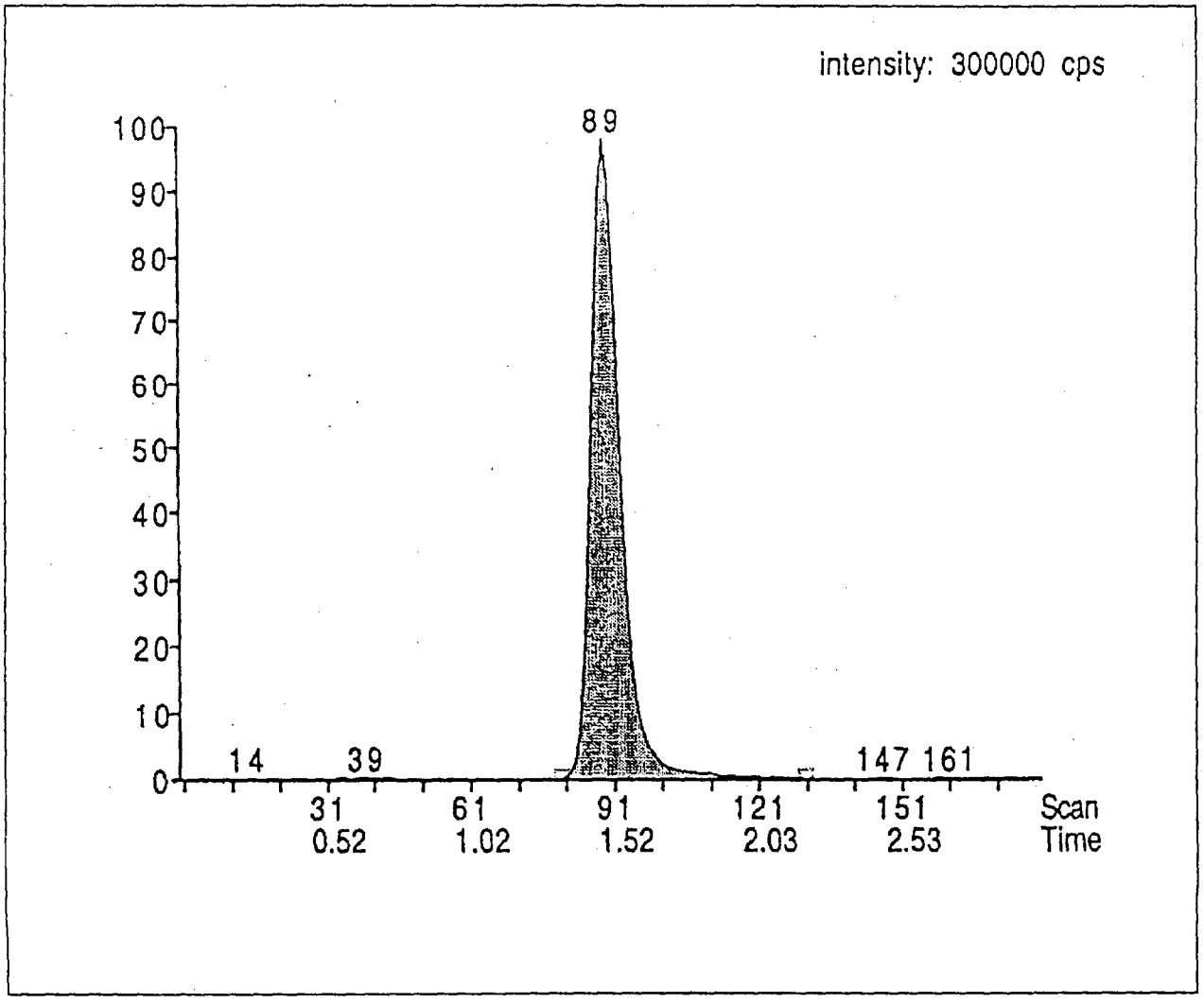

Figure 4. A representative ion chromatogram of a high-level (0.0100 mg a.i./L) standard.

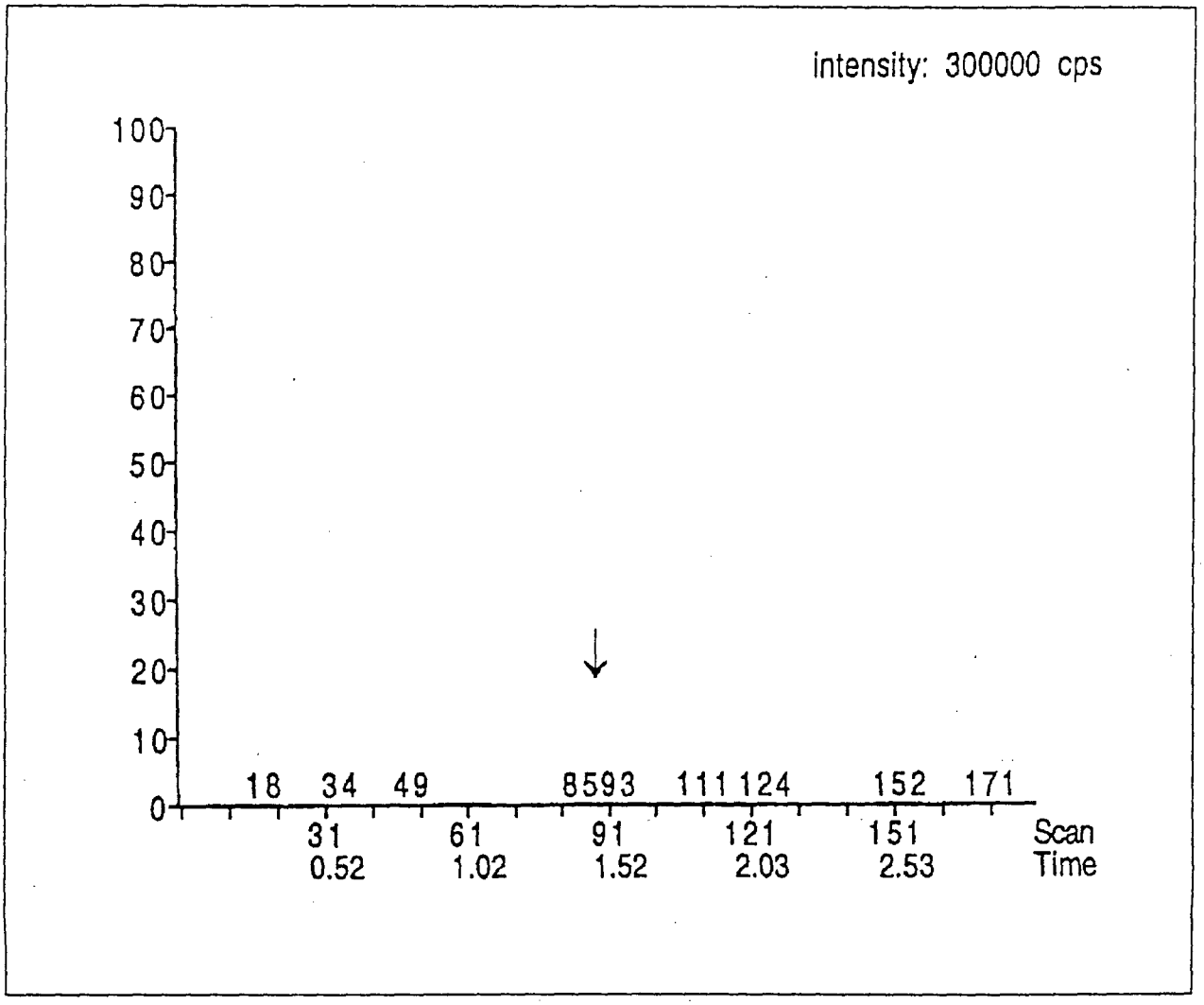

Figure 5. A representative ion chromatogram of a freshwater matrix blank sample (454C-115-VMAB-4). The arrow indicates the retention time of PFBS.

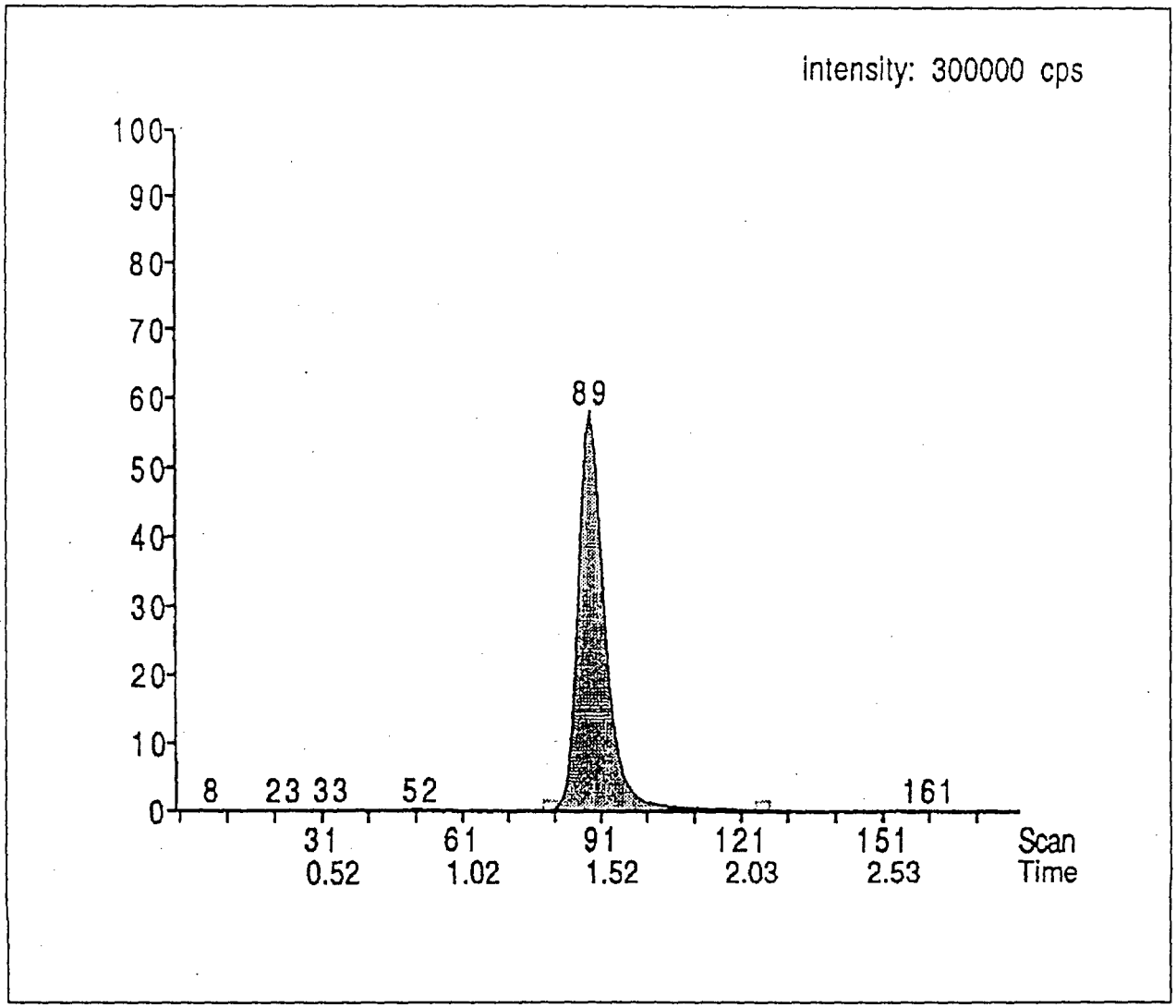

Figure 6. A representative ion chromatogram of a low-level freshwater matrix fortification sample (454C-115-VMAS-2, nominal concentration of 0.500 mg a.i./L).

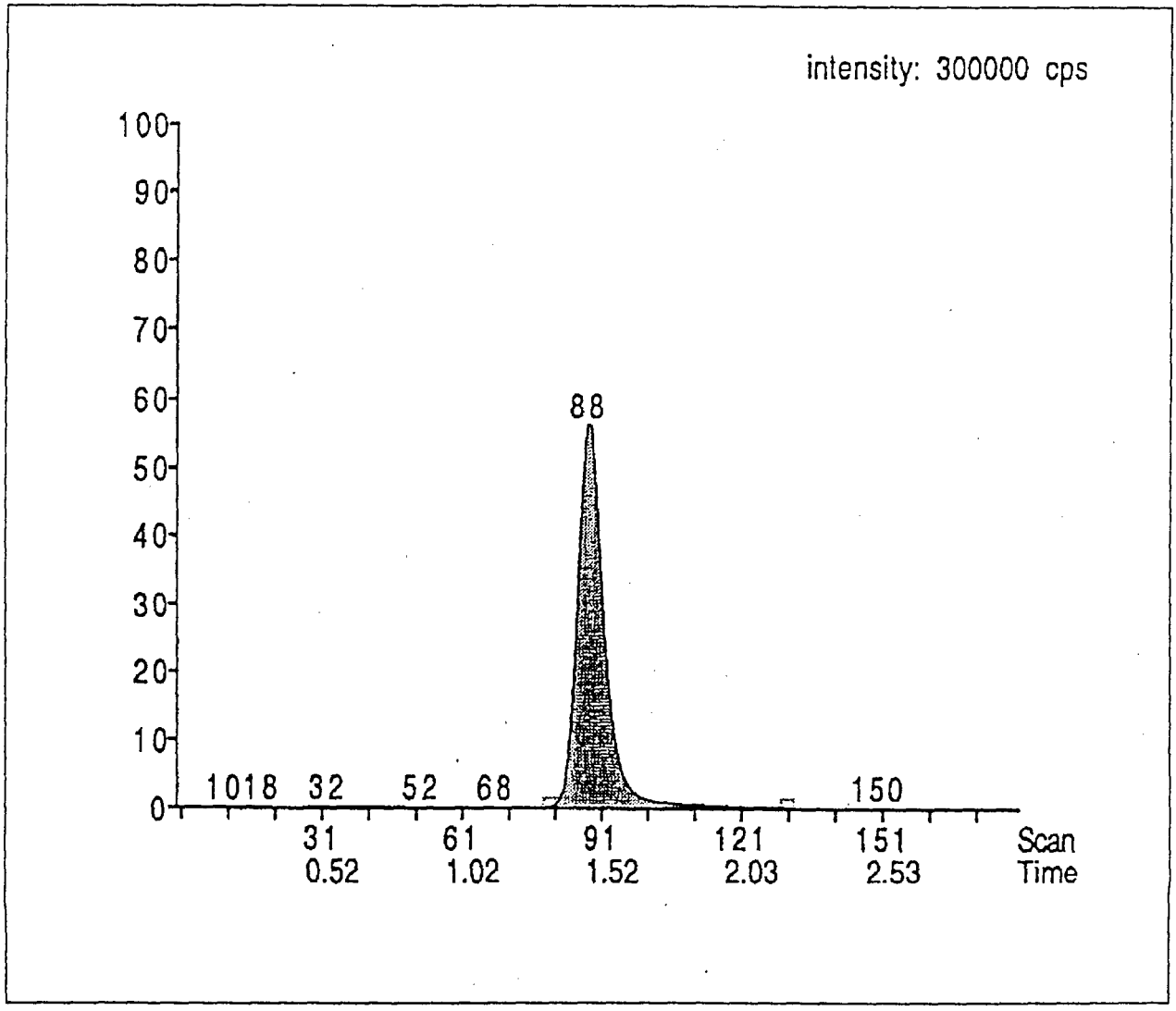

Figure 7. A representative ion chromatogram of a high-level freshwater matrix fortification sample (454C-115-VMAS-12, nominal concentration of 1000 mg a.i./L).

APPENDIX I

Protocol, Amendment and Deviation

PROTOCOL

ANALYTICAL METHOD VALIDATION FOR THE DETERMINATION OF PERFLUORO BUTANE SULFONATE, POTASSIUM SALT (PFBS) IN FRESHWATER, SALTWATER AND ALGAL MEDIA

3M Environmental Lab Project No. E00-1429

Submitted to

3M Corporation
Environmental Laboratory
935 Bush Avenue
St. Paul, Minnesota 55106

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

March 29, 2000

Wildlife International, Ltd.

ANALYTICAL METHOD VALIDATION FOR THE DETERMINATION OF
PERFLUORO BUTANE SULFONATE, POTASSIUM SALT (PFBS)
IN FRESHWATER, SALTWATER AND ALGAL MEDIA

SPONSOR: 3M Corporation
Environmental Laboratory
935 Bush Avenue
St. Paul, Minnesota 55106

SPONSOR'S REPRESENTATIVE:

TESTING FACILITY: Wildlife International, Ltd.
8598 Commerce Drive
Easton, Maryland 21601

STUDY DIRECTOR: Raymond L. VanHoven, Ph.D.
Scientist

LABORATORY MANAGEMENT: Willard B. Nixon, Ph.D.
Manager of Analytical Chemistry

FOR LABORATORY USE ONLY

Proposed Dates:

Experimental Start Date: 04-18-00 Experimental Termination Date: 05-18-00

Project No.: 454C-115 Test Concentrations: Negative Control, 0.500, 10.0, 100, and 1000 mg a.i./L

Test Substance No.: 5916 Reference Substance No.: NA

PROTOCOL APPROVAL

STUDY DIRECTOR — DATE: 4-17-00

LABORATORY MANAGEMENT — DATE: 4/19/00

SPONSOR'S REPRESENTATIVE — DATE: 3/30/00

Wildlife International, Ltd.

INTRODUCTION

Wildlife International, Ltd. will conduct analytical trials to validate the performance of a liquid chromatography mass spectrometry (LCMS) method for the determination of Perfluoro Butane Sulfonate, Potassium Salt (PFBS) in freshwater, filtered saltwater, and algal media. The study will be performed at the Wildlife International, Ltd. analytical chemistry facility in Easton, Maryland. The method will be verified by fortifying each water matrix with the test substance and determining recoveries of PFBS. Raw data for all work performed at Wildlife International, Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International, Ltd. site, or at an alternative location to be specified in the final report.

OBJECTIVE

The objective of this study is to verify the performance of LCMS methodology for analyses of PFBS in freshwater, filtered salt water, and algal media. The method to be validated will be used in support of aquatic toxicology and product chemistry tests to be conducted with the test substance.

EXPERIMENTAL DESIGN

Wildlife International, Ltd. freshwater, filtered saltwater, and algal media will each be fortified at four different concentrations and analyzed using LCMS methodology based upon that developed at Wildlife International, Ltd. Modifications to the methodology will be made as necessary to achieve quantitation of PFBS in each of the test matrices. Matrix and/or reagent blanks will be analyzed concurrently to evaluate potential analytical interferences. The levels of fortification will bracket the anticipated concentrations of samples from aquatic toxicity tests. One or more calibration curves will be generated from analyses of standard solutions of PFBS to be analyzed with each series of matrix fortification samples.

MATERIALS AND METHODS

Test Substance

Information on the characterization of test, control and reference substances is required by Good Laboratory Practice Standards (GLP), 40 CFR Parts 160.31 and 792. The Sponsor is responsible for providing Wildlife International, Ltd. written validation that the test substance has been characterized according to GLPs prior to its use in the study. If written validation of GLP

test substance characterization is not provided to Wildlife International, Ltd., it will be noted in the compliance statement of the final report. The attached form IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR (Appendix I) is to be used to provide information necessary for GLP compliance.

The Sponsor is responsible for all information related to test and reference substances and agrees to accept any unused test or reference substance and/or test or reference substance containers remaining at the end of the study.

Reagents and Solvents

All solvents used in the method or procedure will be HPLC grade or equivalent. All reagents will be ACS reagent grade or higher quality. Nanopure water (equivalent to ASTM Type II Designation D1193-91) will be used (1). The solvents and reagents are not expected to contain contaminants capable of interfering with the purpose of this study.

Algal Culture Medium

Culture medium to be used for the method validation will be prepared according to Wildlife International, Ltd. Standard Operating Procedures. The concentrations of the components in the medium are presented in Table 1 (2). Stock nutrient solutions will be prepared by adding reagent-grade or better chemicals to Wildlife International, Ltd. well water purified by reverse-osmosis. Appropriate volumes of the stock nutrient solutions will then be diluted with purified well water to prepare the medium. The medium will be filter sterilized (0.2 µm) or autoclaved prior to use. The pH of the medium should be 7.5 ± 0.1.

Freshwater

Freshwater to be used for the method validation will be obtained from a well approximately 40-meters deep located on the Wildlife International, Ltd. site. The water will be passed through a sand filter and pumped into a 37,800-L storage tank where the water will be aerated with spray nozzles. Prior to use, the water will be filtered to 0.45 µm in order to remove fine particles. The resulting water is characterized as moderately hard. Approximate values for hardness, alkalinity, pH and specific conductance are:

Hardness, mg/L as $CaCO_3$	145
Alkalinity, mg/L as $CaCO_3$	190
pH	8.1
Specific Conductance, µmhos/cm	330

Hardness, alkalinity, pH and specific conductance will be measured weekly to monitor the consistency of the well water. Means and ranges of the measured parameters for the four-week period preceding the test will be provided in the final report.

Saltwater

Natural seawater collected at Indian River Inlet, Delaware will be filtered through a sand filter prior to delivery to a 37,800-L holding tank. The salinity of the seawater will be diluted to approximately 20 ‰ (parts per thousand) with Wildlife International, Ltd. well water in the holding tank to produce the saltwater matrix. The 20 ‰ saltwater will be aerated using spray nozzles and filtered (0.45 µm) prior to its use in the method validation.

Salinity and pH will be measured weekly to monitor the consistency of the saltwater. Means and ranges of the measured parameters for the four-week period preceding the test will be provided in the final report.

Analyses will be performed at least once annually to determine the concentrations of selected organic and inorganic constituents of the well water and seawater. Results of the most recent GLP-compliant analyses will be summarized in the final report. Specifications for acceptable levels of contaminants in culture medium, well water, and seawater have not been established. However, there are no known levels of contaminants reasonably expected to be present in these well matrices that are considered to interfere with the purpose or conduct of the test.

Analytical Validation Procedures

Wildlife International, Ltd. will conduct validation trials using procedures based on LC/MS methodology developed at Wildlife International, Ltd. The method may be modified to yield a method capable of quantitating PFBS at the desired concentrations in freshwater, filtered salt water, and algal media. The method used, including any modifications, will be documented in the raw data and summarized in the final report.

Reference Stock Solution(s), Calibration Standards and Curves

A primary stock solution(s) will be prepared from PFBS reference substance, if available, or directly from the test substance in an appropriate solvent. Calibration standards will be prepared by appropriate dilution of this primary stock solution(s). A minimum of five concentrations of calibration standards will be prepared and analyzed along with each analysis set of validation samples. The calibration standard series will be injected at the beginning and end of the analytical run with, in addition, a minimum of one mid-level standard injected following every five samples. One or more calibration curves will be derived from regression analysis of the instrumental responses of the standards.

Fortification Stock Solution(s)

Freshwater, saltwater, and algal media will each be fortified with a stock solution(s) of PFBS. Each stock solution will be assigned a unique identification code which will be recorded on a stock preparation log sheet.

Evaluation of Interferences

Matrix and reagent blanks, if applicable, will be analyzed to assess the presence of potential interferences. Matrix blanks will consist of freshwater, saltwater, and algal media without addition of test substance. Reagent blanks, if applicable, will consist of reagents processed through the method.

Validation Analyses - Method Performance

Matrix fortifications (fortified freshwater, saltwater, and algal media) prepared at known concentrations of PFBS, will be analyzed to determine recoveries and to evaluate method performance. The anticipated validation series will consist of the following analyses:

SUMMARY OF PROPOSED VALIDATION ANALYSIS SCHEME

Analysis Type	Concentration	Number of Samples		
		Algal Medium	Freshwater	Saltwater
Reagent Blank (if applicable)	0 (Control)	1	1	1
Matrix Blank	0 (Control)	3	3	3
Matrix Fortifications	Level 1 - Low[1]	3	3	3
	Level 2	3	3	3
	Level 3	3	3	3
	Level 4 - High	3	3	3
Total Number of Analyses		48		

[1] Matrix fortifications will be prepared at four concentrations, the lowest and highest of which will bracket the anticipated treatment range for environmental effects studies.

Matrix fortifications will span the range of concentrations that are anticipated to be used in subsequent environmental effects tests. Individual fortification samples (identified by project number and a unique sample identification number) will be prepared and analyzed.

If difficulties arise in the validation process (e.g., low recoveries or interferences), the Sponsor will be notified and the need for additional validation and/or method development will be determined through discussions with the Sponsor. Upon completion of the method validation, the Sponsor will review the results and authorize the use of the methodology for analysis of samples, or will authorize further method development trials. Recovery values in the range of 80 to 120% will be used as criteria for method acceptability.

Data Analysis

One or more calibration curves for each analytical run will be derived from the instrumental responses of PFBS standards of known concentration using regression analysis. The concentrations of PFBS in fortified samples will be determined by substituting the respective instrumental response into the regression equation

If the fortification range and calibration curve standard concentrations are significantly higher than method capabilities for quantitation, the limit of quantitation (LOQ) will be defined

as the concentration equivalent to the lowest standard accounting for dilutions and other manipulations in the method for matrix blanks. If the fortification range is near the method capability for accurate quantitation of the analyte, the LOQ will be determined from the responses of replicate control samples. The mean (Sb) and standard deviation (σ) of responses will be determined for the replicate control sample and converted to ppm equivalents using a standard curve and method dilution factors. The LOQ will be expressed as the concentration equivalent of Sb + 10σ. The limit of detection (LOD), defined as the lowest concentration of analyte which can be injected and produce a measurable response above background, will be expressed as the amount equivalent to Sb +3σ.

RECORDS TO BE MAINTAINED

Records, to be maintained for data generated by Wildlife International, Ltd., will include:

1. A copy of the signed protocol.
2. Identification and characterization of test and reference substances, if provided by the Sponsor.
3. Dates of initiation and termination of the test.
4. Storage conditions for test and reference substances, and samples.
5. Test and reference substance use logs.
6. Concentration calculations and records of solution preparation.
7. Instrument operating conditions and chromatograms.
8. Statistical calculations.
9. A copy of the final report.

FINAL REPORT

The report will summarize the findings of the validation, the fortification recoveries obtained, and method(s) and instrumentation employed. Upon receipt of these findings, the Sponsor will review the methods and results, and evaluate the results for acceptability.

The final report will include, but not be limited to, the following:

1. Name and address of the facility performing the study.
2. Dates on which the study was initiated and completed.

3. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
4. Objectives and procedures stated in the approved protocol, including any changes in the original protocol or deviations from the protocol.
5. Identification of test and reference substances, including name, chemical abstract number or code number, strength, purity, composition, date of receipt, lot number, storage conditions, physical characteristics, stability and method of preparation of test concentrations, if provided by the Sponsor.
6. A brief summary of the analytical methodology to include, where applicable, a description of the experimental measurements, example calculations, sample preparation (sample weights and/or dilutions), instrumentation employed, reagents used, purity of reagents and solvents, and any major modifications to the method.
7. A description of any circumstances that may have affected the quality or integrity of the data.
8. The name of the Study Director, the names of other scientists or professionals, and the names of all supervisory personnel involved in the study.
9. A description of the transformations, calculations, or operations performed on the data.
10. The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.
11. The location where raw data and final report are to be stored.
12. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and the dates of any findings reported to the Study Director and Management.

CHANGING OF PROTOCOL

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor's Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. Any other changes will be in the form of written deviations signed by the Study Director and filed with the raw data. All changes to the protocol will be indicated in the final report.

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GOOD LABORATORY PRACTICES

This study will be conducted in accordance with Good Laboratory Practice Standards for EPA (40 CFR Part 160 and/or 792) and OECD Principles of Good Laboratory Practices (ENV/MC/CHEM (98) 17). Each study conducted by Wildlife International, Ltd. is routinely examined by the Wildlife International, Ltd. Quality Assurance Unit for compliance with Good Laboratory Practices, Standard Operating Procedures and the specified protocol. A statement of compliance with Good Laboratory Practices will be prepared for all portions of the study conducted by Wildlife International, Ltd. The Sponsor will be responsible for compliance with Good Laboratory Practices for procedures performed by other laboratories.

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REFERENCES

1 American Society for Testing and Materials. 1991. Standard Specification for Reagent Water. D1193-91, ASTM Section II Water and Environmental Technology, Vol. 11.01

2 American Society for Testing and Materials. 1990. Standard Guide for Conducting Static 96-Hour Toxicity Tests with Microalgae. E 1218-90. ASTM Section II Water and Environmental Technology, Vol. 11.05.

3 American Society for Testing and Materials. 1991. Standard Guide for Conducting Static Toxicity Tests with *Lemna gibba* G-3. E 1415-91. ASTM Section II Water and Environmental Technology, Vol. 11.05.

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TABLE 1

FRESHWATER ALGAL MEDIUM CONSTITUENTS

Component	Nominal Concentration
$MgCl_2 \cdot 6H_2O$	12.16 mg/L
$CaCl_2 \cdot 2H_2O$	4.40 mg/L
H_3BO_3	0.1856 mg/L
$MnCl_2 \cdot 4H_2O$	0.416 mg/L
$ZnCl_2$	3.28 µg/L
$FeCl_3 \cdot 6H_2O$	0.1598 mg/L
$CoCl_2 \cdot 6H_2O$	1.428 µg/L
$Na_2MoO_4 \cdot 2H_2O$	7.26 µg/L
$CuCl_2 \cdot 2H_2O$	0.012 µg/L
$Na_2EDTA \cdot 2H_2O$	0.300 mg/L
$NaNO_3$	25.50 mg/L
$MgSO_4 \cdot 7H_2O$	14.70 mg/L
K_2HPO_4	1.044 mg/L
$NaHCO_3$	15.00 mg/L

The pH of the medium will be adjusted, as necessary, to 7.5 ± 0.1 using 0.1 N NaOH or 10% HCl.

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APPENDIX I

IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR

To be Completed by Sponsor

I. Test Substance Identity (name to be used in the report) Perfluoro Butane Sulfonate, Potassium Salt (PFBS)

Test Substance Sample Code or Batch Number: _____

Test Substance Purity (% Active Ingredient): 99.82 Expiration Date: March, 2010

II. Test Substance Characterization

Have the identity, strength, purity and composition or other characteristics which appropriately define the test substance been determined prior to its use in this study in accordance with GLP Standards? Yes _____ No X

III. Test Substance Storage Conditions

Please indicate the recommended storage conditions at Wildlife International, Ltd.

Room temp.

Has the stability of the test substance under these storage conditions been determined in accordance with GLP Standards? Yes _____ No X

Other pertinent stability information: _____

IV. Toxicity Information:

Mammalian: _____

Aquatic: None available

Other Toxicity Information (including findings of any chronic and subchronic tests): _____

V. Chemical Classification: Surfactant

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: Analytical Method Validation for the Determination of Perfluoro Butane Sulfonate, Potassium Salt (PFBS) in Freshwater, Saltwater and Algal Media

PROTOCOL NO: 454/032900/MVAL/SUB454 AMENDMENT NO.: 1

SPONSOR: 3M Corporation PROJECT NO.: 454C-115

EFFECTIVE DATE: April 17, 2000 3M LAB REQUEST NO.: E00-1429

AMENDMENT: Page 1, Study Title

Change: Analytical Method Validation for the Determination of Perfluoro Butane Sulfonate, Potassium Salt (PFBS) in Freshwater, Saltwater and Algal Media

To: Analytical Method Validation for the Determination of Perfluoro Butane Sulfonate, Potassium Salt (PFBS) in Freshwater

REASON: At the Sponsor's request, the protocol was changed to encompass validation in freshwater only.

AMENDMENT: Pages 1-13

Delete: All references to Saltwater and Algal Media.

REASON: At the Sponsor's request, the protocol was changed to encompass validation in freshwater only.

AMENDMENT: Page 13, Appendix I

Change: Test Substance Purity (% Active Ingredient): 99.82

To: Test Substance Purity (% Active Ingredient): 97.90

REASON: The Certificate of Analysis for the Test Substance was revised

STUDY DIRECTOR — DATE 4-17-00

LABORATORY MANAGEMENT — DATE 4/17/00

SPONSOR'S REPRESENTATIVE — DATE 5/30/00

Reviewed by QA SLC 4-17

WILDLIFE INTERNATIONAL, LTD

WLI Project No.: 454C-115
Page 1 of 1

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: ANALYTICAL METHOD VALIDATION FOR THE DETERMINATION OF PERFLUORO BUTANE SULFONATE, POTASSIUM SALT (PFBS) IN FRESHWATER

PROTOCOL NO.: 454/032900/MVAL/SUB454 DEVIATION NO.: 1

SPONSOR: 3M Corporation PROJECT NO.: 454C-115

DATE OF DEFACTO DEVIATION: April 18, 2000

DEVIATION: The protocol states that the calibration standard series will be injected at the beginning and end of the analytical run with, in addition, a minimum of one mid-level standard injected following every five samples. After the first five samples, a low-level standard was injected. After the next five samples a high-level standard was injected.

REASON: Typically the standard level injected after five samples depends on the total number of samples required in between samples. For example, if only one standard is required, the mid-level standard is injected; if two standards are required, the low and high-level standards are injected; if three standards are required, the low, mid and high-level standards are injected, etc.

IMPACT: This deviation had no impact on the study since all sample results were bracketed by time and concentration. The standards injected in between samples serve the purpose of demonstrating instrument stability in the presence of the matrix.

STUDY DIRECTOR
Raymond L. Van Hoven, Ph.D.

5-01-00
DATE

LABORATORY MANAGEMENT
Willard B. Nixon, Ph.D.

5/1/00
DATE

APPENDIX II

Certificate of Analysis

$C_4F_9SO_3^-K^+$; PFBS)
L-7038, Lot 2 (1999)

April 17, 2000
(Supercedes Certificate of Analysis dated April 11, 2000)

This sample was analyzed using LC/MS, ^{1}H-NMR, ^{19}F-NMR, and elemental analyses techniques. The results of these tests show the sample to contain the following weight percent composition:

	0.13 %
$C_4F_9SO_3^-K^+$	97.90%
	1.96 %

Additionally, the isomer distribution of the sample was determined using ^{19}F-NMR techniques and found to contain the following weight percent composition:

$CF_3(CF_2)_3$-$SO_3^-K^+$	97.86 %
	0.04 %

Analytical Study Number: 60488

APPENDIX III

Specific Conductance, Hardness, Alkalinity and pH of Freshwater Measured During the 4-Week Period Immediately Preceding the Test

	Mean	Range
Specific Conductance (μmhos/cm)	315 (N = 4)	310 – 320
Hardness (mg/L as $CaC0_3$)	131 (N = 4)	128 – 132
Alkalinity (mg/L as $CaC0_3$)	172 (N = 4)	170 – 174
pH	8.2 (N = 4)	8.1 – 8.3

APPENDIX IV
Analyses of Pesticides, Organics, Metals and Other Inorganics
in Wildlife International, Ltd. Freshwater[1]

ANALYSIS	MEASURED CONCENTRATION		
Miscellaneous Measurements			
Total Dissolved Solids		286	mg/L
Ammonia Nitrogen	<	0.050	mg/L
Total Organic Carbon[2]	<	1.0	mg/L
Total Cyanide	<	10.0	µg/L
Organochlorines and PCBs			
Aldrin	<	0.005	µg/L
Alpha BHC	<	0.005	µg/L
Beta BHC	<	0.005	µg/L
Delta BHC	<	0.005	µg/L
Gamma BHC (Lindane)	<	0.006	µg/L
Chlordane	<	0.025	µg/L
DDD, pp'	<	0.006	µg/L
DDE, pp'	<	0.005	µg/L
DDT, pp'	<	0.008	µg/L
Dieldrin	<	0.005	µg/L
Endosulfan, A	<	0.005	µg/L
Endosulfan, B	<	0.005	µg/L
Endosulfan Sulfate	<	0.018	µg/L
Endrin	<	0.010	µg/L
Endrin Aldehyde	<	0.005	µg/L
Heptachlor	<	0.005	µg/L
Methoxychlor	<	0.007	µg/L
Heptachlor Epoxide	<	0.005	µg/L
Toxaphene	<	0.500	µg/L
PCB-1016	<	0.260	µg/L
PCB-1221	<	0.260	µg/L
PCB-1232	<	0.260	µg/L
PCB-1242	<	0.720	µg/L
PCB-1248	<	0.720	µg/L
PCB-1254	<	0.720	µg/L
PCB-1260	<	0.720	µg/L
Metals and Other Inorganics			
Aluminum[3]	<	100	µg/L
Arsenic[3]	<	25.0	µg/L
Beryllium[3]	<	0.50	µg/L
Cadmium[3]	<	1.0	µg/L
Calcium[3]		35.0	mg/L
Chromium[3]	<	2.0	µg/L
Cobalt[3]	<	1.0	µg/L
Copper[3]	<	20.0	µg/L
Iron[3]	<	100	µg/L
Lead[3]	<	10.0	µg/L
Magnesium[3]		13.5	mg/L
Manganese[3]	<	1.0	µg/L
Mercury	<	0.20	µg/L
Molybdenum[3]	<	2.0	µg/L
Nickel[3]	<	2.0	µg/L
Potassium[3]		6.62	mg/L
Selenium[3]	<	25.0	µg/L
Silver[3]	<	1.0	µg/L
Sodium[3]		21.3	mg/L
Zinc[3]	<	20.0	µg/L

[1] Analyses performed by QST Environmental, Gainesville, Florida for samples collected on November 3 through November 7, 1997.
[2] Analyses performed by Wildlife International, Ltd. for the sample collected on November 5, 1997.
[3] Analyses performed by Wildlife International, Ltd. for samples collected on November 5 through 7, 1997.

APPENDIX V

Personnel Involved in the Study

The following key Wildlife International, Ltd. personnel were involved in the conduct or management of this study:

1. Willard B. Nixon, Ph.D., Manager, Analytical Chemistry
2. Raymond Van Hoven, Ph.D., Scientist
3. Jon MacGregor, B.S., Scientist
4. Keith Keller, B.S., Chemist