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EXPOSURE ASSESSMENT PROTOCOL

Work Request No.: 15076

Service Code No.: 1361

Performing Institution: Haskell Laboratory for Health and Environmental Sciences

Study Title: **Exposure Assessment of Measured Serum Perfluorinated Octanoic Acid Anion in the Manufacture of Ammonium Perfluorooctanoate (APFO) at Fayetteville, NC**

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BMP CRP 711-2216-C
[redacted]

Company Sanitized. Does not contain TSCA CBI

AUTHORIZATION FOR SERVICES

To:	From:
David M. Rurak SHE Manager Fluoroproducts	Joseph F. Viskocil, Jr. CIH Senior Industrial Hygienist Haskell Laboratory
Requested By:	**Date:** December 12, 2003

Title:
Exposure Assessment of Measured Serum Perfluorinated Octanoic Acid Anion in the Manufacture of Ammonium Perfluorooctanoate (APFO) at Fayetteville, NC

Authority is requested to undertake the study identified above and described as follows:
The primary purpose of this project is to measure worker exposure in the manufacture APFO in Fayetteville, NC. for assessment of the effectiveness of the industrial hygiene controls measures in place to minimize exposure to APFO.

The secondary purpose of this occupational exposure project is to characterize APFO exposure in workers engaged in the manufacture of APFO and to determine 1) whether the major route of exposure is via inhalation or dermal, 2) the length of time required for the compound to reach steady-state, and 3) if there is a sufficient population, determine the length of time for serum levels to return to baseline following cessation of exposure. This will be accomplished by longitudinal sampling and analysis of serum PFOA anion levels according to a repeated samples design, air monitoring, and estimating dermal and oral contributions to overall exposure. Correlations between external and internal measures of exposure will be calculated. The data will be examined for within- and between-subject variation associated with specific tasks on the process line.

AUTHORIZATION FOR SERVICES **Additional Support Required from the Plant:**

Air sampling equipment including pumps, calibrators, battery eliminators, ...
Personnel time – additional IH resources to conduct air sampling and investigate process upsets, data anomalies

INITIATION DATE: Immediately upon approval- Estimated approval date December 15, 2003	**ESTIMATED COMPLETION: December 31, 2009**
ESTIMATED COST: December 15, 2003 to December 31, 2004 cost [redacted]	**GENERAL LEDGER/SUBACCOUNT:** 9350-CVT91601002-89808800

Company Sanitized. Does not contain TSCA CBI

If this request meets with your approval, enter the general ledger and subaccount, sign below, and return.

AUTHORIZED APPROVAL: David B. Rush DATE: 12/15/03

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REVIEW AND APPROVAL

STUDY DIRECTOR AND COINVESTIGATORS

I attest that, to the best of my knowledge, this protocol is scientifically sound, describes the study and procedures to be used with appropriate regulatory requirements, Good Industrial Hygiene Practices, the research protocol and any amendments thereof.

Submitted:

Joseph F. Viskocil, Jr CIH
Study Director

Date: 12/15/03

Approved:

Janice L. Connell CIH
Operations Manager, Haskell Laboratory

Date: 12/15/03

INTRODUCTION

Abstract

This occupational exposure study will characterize APFO exposure by monitoring PFOA anion in workers engaged in the manufacture of APFO and to determine 1) whether the major route of exposure is via inhalation or dermal, 2) the length of time required for the compound to reach steady-state, and 3) if there is a sufficient population, determine the length of time for serum levels to return to baseline following cessation of exposure. This will be accomplished by sampling and analysis of serum PFOA anion levels according to a repeated samples design, air monitoring, and estimating dermal and oral contributions to overall exposure. Correlations between external and internal measures of exposure will be calculated. The data will be examined for within- and between-subject variation associated with specific tasks on the process line.

Definitions:

APFO – Ammonium Perfluorooctanoate
Biomonitoring –Industrial Hygiene serum samples analyzed for PFOA anion used as an indication of occupational exposure
LC – Liquid Chromatography
MS – Mass Spectrometry
PFOA – Perfluorooctanoic Acid
PFOA Anion –perfluorooctanoate anion, the analyte of both PFOA and APFO by LC/MS/MS

Objective:

The objective of this study is to monitor serum levels of PFOA anion levels in manufacturing and manufacturing support workers of APFO as a measure the effectiveness of industrial hygiene controls.

BACKGROUND

Despite the absence of any persuasive scientific evidence establishing a causal association between exposure to PFOA and specific health effects, there continues to be

questions raised on the health affects of PFOA. Epidemiological studies on the health effects of PFOA exposure have focused on occupational populations. A currently validated analytical method (with a limit of quantification as low as 0.5 ppb) allows increased understanding of background levels.

In Fayetteville APFO production began in the fall of 2003. Prior to start-up baseline blood serun samples were taken. With this background, we have a unique opportunity to monitor exposure as a function of process time.This PFOA anion in serum levels will be measured routinely as an indication of the effectiveness of industrial hygiene controls.

It is our goal to minimize employee exposure to APFO in the AFPO manufacturing process and to assure compliance with the DuPont Acceptable Exposure Limit (AEL) of 0.01 mg/m^3.

SURVEILLANCE METHODS

PARTICIPANT SELECTION

Biological monitoring evaluates the absorption of PFOA/APFO by measuring the chemical or its metabolites in body fluids. Serum sampling is the preferred method for biological monitoring because there is a validated analytical method for measuring PFOA anion, rather than a metabolite or the fluoride ion in this medium. Workers in the APFO manufacturing process are chosen because they are likely to have highest exposures at the Fayetteville Works, we were able to get a pre-start up baseline, and we can track serum levels with time from the process start-up. DuPont's goal is to minimize exposure.

Workers employed in the new APFO production unit at Fayetteville Works and for whom a baseline level of serum PFOA anion was determined before beginning work in the unit will be included in this surveillance study. Participants will be all APFO workers at the DuPont Fayetteville Works site who volunteer for participation in this study, and sign a consent form.

DATA COLLECTION

Every employee assigned to the APFO manufacturing area will asked to participate in the PFOA anion blood monitoring program. The employee will be asked to sign a consent form and a baseline blood sample will be taken before the employee begins work in the APFO manufacturing area. After the baseline sample is collected, the employee will participate in the normal 6-month sampling program. Once in the blood monitoring program, the employee is expected to have blood drawn and analyzed every 6-months for the duration of the study – whether or not they remain in the APFO manufacturing area.

For each participant, date of birth and date of hire will be collected. For each task with potential for exposure to APFO, job title, work area and begin and end dates will be recorded.

A total of 9 job titles and 5 work areas have been identified as having potential for exposure to APFO at the Fayetteville Plant. The number of unique task/work area combinations with potential for APFO exposure will be determined.

Blood Sampling

Standard Operating Procedure: Collecting and Processing Blood Samples – Appendix - 1

Standard Operating Procedure: Process for Analysis of Blood Biomonitoring Samples –Appendix –2

Standard Operating Procedure: Study-Specific Standard Operating Procedure: Documentation, Accountability, and Procedures for Consent Forms, and Collection & Processing of Serum Samples – Appendix – 3

Standard Operating Procedure: Documentation, Accountability, and Sample Chain of Custody Requirements for the Shipping of Serum Samples – Appendix – 4

Blood Collection Checklist – Appendix – 5

Air Sampling

Air Sampling Strategy - Appendix - 6

Standard Operating Procedure: Quality Assurance Procedures: Industrial Hygiene Laboratory and Field Work – Appendix - 7

Standard Operating Procedure: Sample Handling, Chain-of-Custody , Preservation and Shipping – Air Samples – Appendix – 8

Analytical Method for Air Samples – Appendix – 9

Air Monitoring Data Form – Appendix – 10

Wipe Test

Perfluorooctanoic Acid Wipe Test Analysis – Appendix - 11

DATA ANALYSIS

These exposure data will be analyzed as follows:

1. The general statistical procedures described below will be used in the analysis of the data. Analytical results of the regular samples will be analyzed using appropriate statistical methods.
2. Descriptive statistics will be generated on each exposure metric with all measurements pooled.
3. Where there is sufficient data, regression of serum PFOA anion for each individual will be done on gender, age, most frequent task ,
4. Analysis of variance for each time point measurement of serum PFOA anion will determine the between- and within-individual variation, and
5. Where there is sufficient data, estimate blood clearance rates of PFOA anion in workers by continued surveillance following cessation of exposure

EXPOSURE ASSESSMENT

Exposure will be measured by the following parameters:

1. Serum level of PFOA anion, prior to beginning work in the APFO Manufacturing Area and measured every six months (per the unit schedule)
2. Personal air monitoring of each participant, selected to cover all tasks and all shifts with potential for exposure to APFO
3. Area air monitoring for the laboratories, the manufacturing lines, and the packing and shipping of APFO
4. Estimate the dermal contribution to the biomonitoring result for each worker, and
5. If necessary estimate the contribution of other routes of exposure.

The investigators will compile a document for each area that will summarize data related to APFO exposure: (1) descriptions of processes where exposure to APFO may occur, including nature of and dates of engineering controls, changes in operating procedures and potential exposure points, (2) specific tasks performed in work areas where exposure to APFO may occur (3) work area names, job titles and time periods with potential for exposure, (4) documentation of personal protective equipment use, (5) air sampling data and analytical methods employed, (6) plant production records and (7) details on work conditions and practices gained from interviews and observations.

Because dermal contact may be a significant route of exposure, estimates of the amount and frequency of skin contact will be used to estimate a dermal dose using the surface area of the body covered, frequency of contact, length of contact and results of the surface wipe tests.

Personal air monitoring data will be used as a direct measure of inhalation exposure.

FACTORS THAT MAY INFLUENCE THE SURVEILLANCE DESIGN AND ANALYTIC METHODS

Analytic methods are new and recently validated. Only one laboratory is GLP certified to do the blood analysis and only one laboratory is currently AIHA accredited for the air sample analysis. Timing of results could become an issue.

The size of the surveillance population will not be large thus limiting the statistical power.

The wipe test analysis is complex. Sample turn around time will not allow wipe tests to be used as a quick check for contamination or the effectiveness of a decontamination procedure.

HUMAN SUBJECT PROTECTION

Haskell Quality Directive QD007-C-001 requires compliance with the U.S. Federal regulation known as the Health Insurance Probability and Accountability Act of 2003 (HIPAA) and is supported by standard operating procedures that specify privacy and confidentiality requirements.

PROTOCOL AMENDMENTS AND DEVIATIONS

Intended (planned) changes to the protocol will be made by protocol amendment. Unintended or unexpected divergence from this protocol will be documented in the field raw data as a deviation. The Study Director will be notified of all protocol amendments and deviations as soon as possible. All changes in or revisions of the approved protocol and the reasons for the changes will be formally prepared by the Study Director, signed and dated by the Study Director and Sponsor, and maintained with the protocol.

QUALITY CONTROL

Field staff will record observed exposure data on standardized forms and send the observed data to Haskell Laboratory within seven days of collection. Haskell staff will review forms and reconcile missing or discrepant information where appropriate. Sampled workers will be contacted if necessary to resolve discrepant, missing, or inconsistent data. Data will be transmitted electronically along with selected hardcopy data to the Study Director.

REPORTING OF RESULTS

Status reports will be reviewed by the Study Director following the receipt of the quarterly air monitoring results and upon receipt of blood results. A final summary report will be issued at the conclusion of the study. A communication of the final results will be to the site industrial hygienist who will communicate them to the study participants.

RESOURCES TO CONDUCT THE STUDY

Sampling Equipment – 10 Air Sampling Pumps, chargers, battery eliminator, calibrator, and maintenance log
Personal Assistance – An experienced industrial hygienist to conduct air sampling, provide field observations, ensure adherence to this protocol and investigate deviations from the protocol. See Air Sampling Strategy – Appendix 6

ARCHIVING OF STUDY DOCUMENTS

The protocol, amendments to the protocol, raw data, consent forms, analytical results, computer printouts, and final report will be archived through the standard procedures of the Haskell Laboratory Information Services Group.

BIBLIOGRAPHY

Reference Exygen Blood Method
Reference Clayton Air Method
Wipe Test Validation

Appendix-1

Standard Operating Procedure: Blood Collection

1. BLOOD COLLECTION SCHEDULE:

Personal communications will be maintained with each study participant to confirm the dates to begin sampling. Blood samples will be drawn every 6-months. To cover all shifts, blood samples will be collected within a 10-day period. Baseline samples will be collected prior to an employee working in the area. The employee will then join the normal 6-month rotation.

1.1. Field Laboratory for Blood Collection

Blood samples will be collected at Fayetteville Site Medical by the site nurse.

1.2. Aborted Blood Collection:

If an anticipated sampling is canceled, workers will be notified promptly and sampling will be re-scheduled as-soon as-possible after the originally scheduled day. If there are medical or physical reasons that contraindicate the drawing of a blood sample on a scheduled day, then that study subject's sampling will be rescheduled to occur on the next day that the same or similar tasks will be performed.

2. PROCESSING BLOOD SAMPLES:

At the field laboratory, blood samples will not sit ambient for more than 2 hours prior to serum separation.

3. TRAVEL BLANKS:

Travel Blanks will be an unused polypropylene tube supplied by Exygen. This blank will be shipped with collected blood samples to insure that the samples were not contaminated during shipping.

4. SAMPLE IDENTIFICATION:

The site nurse/industrial hygienist will create a sampling identification system that identifies the site and person sampled. The chain-of-custody form will record the shipping date.

6. PROCESSING SAMPLES FOR FROZEN STORAGE AND SHIPMENT:

6.1. SAMPLE STORAGE:

Appendix 3- Standard Operating Procedure: Documentation, Accountability, and Procedures for Consent Forms, and Collection & Processing of Serum Samples

6.2. SAMPLE SHIPMENT:

Appendix 4: Standard Operating Procedure: Documentation, Accountability, and Sample Chain of Custody Requirements for the Shipping of Serum Samples

7. SAMPLE ANALYSIS:

The detailed procedures for analysis of study samples for PFOA are presented in Attachment 3.

8. DATA ANALYSIS AND STATISTICAL METHODS:

Analytical results of the regular samples will be analyzed using appropriate statistical methods.

9. CONTROL OF BIAS:

Bias within the selection process for study participants and in the collection of blood samples should only arise from the voluntary nature of participation. Comparisons of the demographics, work histories, and usual tasks performed of the volunteers and the abstentions will be made in order to identify any systematic differences in the two groups.

10. REQUIRED FIELD DATA RECORDS:

All field notes and data sheets must be filled out promptly, accurately, and in indelible ink by study personnel. All pages must be signed. If more than one individual records data on a page, it must be clear which individual recorded specific data. All entries must be dated on the day of entry and signed or initialed by the person entering the data. The field staff will maintain original documents or legible verified copies of the following information:

1. A description of the tasks performed by the sampled participant, including personal protective equipment used.

2. A record of serum sample handling including time from collection to frozen storage.

3. A copy of the freezer temperature log for the period the serum samples were stored.

4. Personal work habits of each worker during his/her shift such as hand washing, sweating, accidental contamination, etc. Any observation that would provide an understanding of a potential route of exposure should be recorded.

11. PROTOCOL AMENDMENTS AND DEVIATIONS:

Intended (planned) changes to the protocol will be made by protocol amendment. Unintended or unexpected divergence from this protocol will be documented in the field raw data as a deviation. The Study Director will be notified of all protocol amendments and deviations as

soon as possible. All changes in or revisions of the approved protocol and the reasons for the changes will be formally prepared by the Study Director, signed and dated by the Study Director and maintained with the protocol.

12. REPORTING OF DATA:

Upon completion of the analytical and field portions of the study, analytical and field reports will be prepared by the Principal Investigators. Upon completion of these reports, a draft final report will be prepared for review and approval by the Study Director. Upon approval, a final report will be issued.

The report will contain narrative descriptions of the environmental conditions, work practices, worker background and experience, and other pertinent information collected during each replicate (campaign) of the study.

In addition, the final report will include:

1. Name and address of the facilities performing the study and the data on which the study was initiated and completed
2. Objectives and procedures stated in the approved protocol, including any deviations and/or amendments in the original protocol
3. Identification and description of the test and reference substance(s)
4. A description of all methods used
5. A summary of the data generated while conducting the study and a description of all transformation, calculations, statistics, or other operations performed on the data
6. The name and dated signature of the Study Director
7. A description of all conclusions drawn from analysis of the samples
8. Complete description of the test system
9. Location of raw data, final report, and all specimens
10. A verified copy of the study protocol including protocol deviations and amendments
11. Location of the test site

Upon acceptance of the final report, all study specific original raw data will be submitted to the Sponsor along with exact copies of non-study specific support data. Original documents will be the property of the Sponsor. Subsequent support records shall be copied, certified as exact copies, and included with the original raw data and submitted to the Sponsor for archival.

13. QUALITY ASSURANCE:

The field phase of this protocol will be conducted and documented as completely as possible to ensure the quality and integrity of the data generated for this portion of the study.

The analytical portion of this study will be performed in compliance with the Good Laboratory Practice (GLP) as specified in 40 CFR §160. The analytical portion and the final report of this study will be inspected. The findings of these inspections will be made available to the Study Director and Test Facilities Management.

14. RECORDS RETENTION:

Reports, raw data, and records pertaining to this study will be archived by Haskell Information Sciences.

Appendix-2

Process for Analysis of Blood Biomonitoring Samples

Preparation Phase:

1. Plant site handle appropriate communications, scheduling of phlebotomy, and all other pre-sampling arrangements

2. Plant site requests supplies from Exygen at least 4 weeks in advance; estimated number of samples is needed at this time

3. Exygen arranges for supplies to be shipped and schedules samples for analysis

4. Supplies are shipped to plant site with instructions on processing and shipment

Sampling Phase:

5. Blood is sampled and processed into serum at the plant site

6. Serum samples are shipped directly from the plant site to Exygen laboratory with a sample listing and Chain-of-Custody records. The plant will cc S. Mark Kennedy on the sample listing.

Analysis and Analytical Reporting Phase:

7. Exygen analyzes the samples and prepares a draft report to review. The draft report and copies of the raw data are sent to S. Mark Kennedy for review

8. S. Mark Kennedy reviews the analytical data, makes any corrections/upgrades to the draft, and gives the contract lab permission to finalize the analytical report

9. The contract lab finalizes the analytical report and sends it to S. Mark Kennedy. S. Mark Kennedy sends a copy of the report to Study Director ,Joseph F. Viskocil who archives the original report at Haskell and informs the plant site industrial hygienist of the results

Completion Phase:

10. The site handles appropriate communications at the site

11. Upon receipt of the invoice from the contract lab, S. Mark Kennedy will provide the appropriate (plant-site-specific) charge code to have the bill paid against a standing purchase order

12. Samples will be retained for 12 months per the method validation. Samples will be discarded after 12 months upon the Study Director's approval.

Appendix-3

Specific Standard Operating Procedure:
Documentation, Accountability, and Procedures for Consent Forms, and Collection & Processing of Serum Samples

1. SCOPE AND APPLICABILITY

This SOP governs the documentation, accountability, and procedures for consent forms, and collection & processing of serum samples.

2. REPONSIBILITIES

2.1 The Study Director is responsible for conducting the necessary training covering proper documentation, accountability, and procedures for consent forms, and collection & processing of serum samples. .

2.2 The Phlebotomist who performs the blood collection is responsible for ensuring that the Consent Form is completely and correctly filled out by each participant prior to venipuncture.

2.3 The Phlebotomist & Site Industrial Hygienist are responsible for ensuring that forms and the samples are properly collected and labeled.

3. Names

Study Director: Joseph F. Viskocil, Jr CIH

Phlebotomist: Bonnie Wilson, RN- Site Nurse Fayetteville

Site Industrial Hygienist : Jennifer Locklear

4. MATERIALS AND EQUIPMENT

4.1. Consent Forms (duplicate or triplicate)

4.2. Serum Separator tubes (Red top) –(Supplied by Exygen)

4.3. Vacutainers, Needle holders and Needles–(Supplied by Exygen)

4.4. Centrifuges

4.5. Transfer Pipettes and Polypropylene Tubes–(Supplied by Exygen)

5. PROCEDURE

5.1. Have the participant complete the Consent form prior to initiation of venipuncture.

5.2. Ensure that the consent form completed by each participant receives the same code number as the blood sample from that participant.

5.3. Original consent for each participant will be sent to Study Director for storage.

5.4. Access to consent forms to be decided

5.5. Collect blood specimen using usual venipuncture technique. Fill tube completely.

5.6. Gently invert the serum separator tube 5 times to mix.

5.7. Allow blood to clot a minimum of 30 minutes but no longer than 1 hour.

5.8. Centrifuge for 15 minutes at 2500 to 3500 rpm

5.9. Remove from centrifuge

5.10. Serum portion of the collected sample in the SS tube is now ready to be pipetted into the specially provided polypropylene tubes.

5.11. Seal the polypropylene tubes

5.12. The polypropylene tubes (labeled with specimen ID number) are now ready for transport to the lab.

5.13. Please see Appendix 2 for details on how to complete the chain of custody form and prepare the sample for shipping.

5.14. Samples will need to be frozen immediately at –20°C and stored frozen.

5.15. Samples will also need to be shipped frozen. See Appenxix-4

6. REFERENCES – Appendix-4

7. FIGURES

Figure 1. Consent form

CONSENT TO PARTICIPATE IN PFOA EXPOSURE ASSESSMENT PROGRAM

Study title: **Exposure Assessment of Measured Serum Perfluorinated Octanoic Acid Anion in the Manufacture of Ammonium Perfluorooctanoate (APFO) at Fayetteville, NC**

Introduction: **You are being asked to participate in a research study. Your participation is voluntary.**

Your decision whether or not to participate will have no effect on your employment or the services you receive through DuPont's Occupational Health Program or Integrated Health Services, or Benefits Administration. Please ask questions of your site's OH Specialist or Nurse if there is anything you do not understand.

What is the purpose of this study?
The purpose of the program is to learn about the relationship between specific tasks and the exposure level of PFOA that may occur in workers at this plant site, and to identify ways to reduce this exposure to as low a level as possible.

Are there any benefits from participating in this study?
The benefit of participating in this study is that you may gain important knowledge about how exposures may vary depending on the ways certain tasks are carried out. This knowledge will help our OH Specialists, industrial hygienists, and engineers find ways to reduce these potential exposures.

What does this study involve?
Your participation in this study may last up to 5 years.

A blood sample will be drawn every 6-months and analyzed for the serum level of PFOA. No other blood levels will be determined.

Will the study be affected if you do not participate?
The more people that participate in this study, the more accurate and representative the results will be. In addition, we may be able to use these results to control exposures at other sites if we have enough participants to provide a good sample size.

What are the risks involved with being enrolled in this study?
The only risk of participating in this study would be the small risk of a hematoma (bruise) where the blood is drawn.

Other important items you should know:

- **Withdrawal from the study:** You may choose to stop your participation in

this study at any time. Your decision to stop your participation will have no effect on your employment or the services you receive through DuPont's Occupational Health Program or Integrated Health Services, or Benefits Administration.

- **New information:** To the best of our ability, any significant new findings during this research study will be made known to you.
- **Confidentiality:** Every effort will be taken to protect the names of the participants in this study. However, there is no guarantee that the information cannot be obtained by legal process or court order.
- **Funding:** Haskell Laboratory has been provided funding to conduct this study by the DuPont Fluoroproducts business.

Number of participants: We expect Approximately 30 participants.

Who should you call with questions about this study?
Questions about this study may be directed to the site OH Specialist, Jennifer Locklear; or the site Nurse, Bonnie Wilson, RN. You may also contact the Study Director, Joe Viskocil, at Haskell Laboratory: [redacted] during normal business hours.

Will you be paid to participate in this study?
No. Blood samples will be drawn during the work shift.

CONSENT

I have read the above information about (name of study) and have been given an opportunity to ask questions. I agree to participate in this study and I have been given a copy of this consent document for my own records.

Print name:_____Pass Number:_____

Your signature:_____ Date:_____

Witness: _____ Date: _____

Appendix-4

TITLE PAGE

Standard Operating Procedure:
Documentation, Accountability, and Sample Chain of Custody Requirements for the Shipping of Serum Samples

Analytical Facility:

Exygen Research
3058 Research Drive
State College, PA 16803
[redacted]

Analytical Facility Management:

John Flaherty, Vice President, Operations

Company Sanitized. Does not contain TSCA CBI

1. SCOPE AND APPLICABILITY

This SOP governs the documentation, accountability, and sample chain of custody requirements for the shipment of serum samples to the analytical facility.

2. REPONSIBILITIES

2.1 The phlebotomist who performs the blood collection is responsible for ensuring that the Chain of Custody/Analysis Request Form (COC) (Figure 1) is completely and correctly filled out and signed by staff members who package and ship the serum samples.

2.2 The Principal Investigator at the analytical facility is responsible for ensuring that the chain of custody remains intact after the samples are received by the analytical facility.

3. DEFINITIONS

Analytical Facility Management: John Flaherty, Vice President of Operations, Exygen Research 3058 Research Drive, State College, PA 16801 [redacted]

Principal Investigator: Emily Decker, Scientist
Exygen Research, 3058 Research Drive, State College, PA 16801 [redacted]

4. MATERIALS AND EQUIPMENT

4.1 Chain of Custody/Analysis Request Forms, triplicate design.

4.2. A readily available source of dry ice pellets. Also, necessary personal protective equipment appropriate for handling dry ice.

4.3 Coolers numbered and coded with a unique identification assigned for this project. They must be purchased from Uline Shipping Supplies Specialists. The Uline part number for the required coolers is S-7888. (This part number corresponds to a package which includes one 19" x 12" x 12.5" cooler and one shipping box).

4.4 Plastic bags for use as secondary sample packaging prior to shipping. These will also be obtained from Uline, part number S-1707 (3" x 5", 4 mil reclosable poly bags, 1000 per carton).

5. PROCEDURE

5.1.Before collecting any blood, the completed and signed consent form must be verified. When each sample is collected a unique alphanumeric identification code will be assigned to each sample. This number will be entered into the "Sample Identification" column of the COC sheet. Likewise, the date and time of each blood sample collection will be recorded, and the date and time that the serum sample preparation is completed will be entered into the appropriate column on the COC sheet.

5.2.Each sample cooler will be assigned a unique alphanumeric identification number, which will be written on the cooler.

5.3.Before packaging, the primary serum sample containers must be placed individually into small plastic bags as secondary packaging. The secondary packaging must have the unique sample code written on it in permanent black ink.

5.4.Before the samples are packed in dry ice, a person other than the packager will verify that the samples documented on the COC form match exactly with those being packed in the cooler. The verifier then initials and dates the "Cooler Contents Verified" prompt.

5.5.Samples are to be dispersed in a cooler full of dry ice pellets, arranged in such a way as to minimize the possibility of impact, and such that they are completely covered with the pellets. The cooler must be completely filled with dry ice pellets. No more than 20 samples are to be placed in one cooler. The cooler will be covered promptly after packing with dry ice pellets to minimize loss of dry ice to sublimation.

5.6. The time between the completion of serum preparation and packaging for shipment must be able to be accounted for on the COC page. **There must not be any unexplained gap in the chain of custody for the samples.**

5.7. After the serum samples are packaged, the Cooler ID# prompt will be completed. Section 2 ("Personnel Involved in Shipment of Samples") of the COC form also will be completed at this time, if it has not been previously. The printed name, signature, initials and date of signature need to be recorded for each person who entered information anywhere on the form. The page number prompts also need to be filled in.

5.8. The person who packaged the samples then completes the first line in the "Relinquished by" section, and verifies that any unused prompts are lined out.

5.9. The COC form is then faxed to Exygen Research Sample Receiving using the fax number at the top of the form. After it is faxed, the pink copy is removed and becomes part of the client's records. The white original and yellow copies are to be placed in an envelope, which is then placed into the shipping carton with the cooler that contains the samples to which the COC form corresponds.

5.10. The serum samples are required to be shipped to Exygen Research Sample Receiving at the address given at the top of the COC form by **FedEx overnight** delivery. This is the only carrier and method of shipment that is acceptable.

5.11. Upon receipt of the samples by Exygen Research, the chain of custody procedure will follow Exygen SOP V401, "Contract Research Sample Handling". After Exygen Sample Receiving completes the "Received by" section, a copy of the COC will be faxed back to the client.

5.12. Any problems with delivery or condition of samples upon arrival at Exygen will be documented. The Exygen Principal Investigator will notify the client of any problems immediately.

5.13. Correction and Clarification of Raw Data

5.13.1. As soon as any information has been entered on the COC form, it becomes raw data. At this point, this form is not to be discarded for any reason. If it becomes damaged, information may be transcribed onto another sheet, but the transcription process will be documented via footnote on the new page. The original damaged sheet must be attached to the transcribed page.

5.13.2. If errors are made in handwritten entries, or if clarifications are to be made, the errant information will be crossed out with a single line, so as not to obscure the original entry. Then, depending upon

the nature of the error, an appropriate error code is chosen (see Figure 2), and the error code is initialed and dated. If the error is more complex than what is covered by the error code list, a detailed explanation will be provided by footnote on the COC page.

6. REFERENCES

Exygen Research SOP V401, "Contract Research Sample Handling"

7. FIGURES

Figure 1. Chain of Custody/Analysis Request Form

Figure 2. List of Error Codes

8. SIGNATURE

John M Flaherty
John M. Flaherty.
Analytical Facility Management

12/3/03
Date

Figure 1. Chain of Custody/Analysis Request Form

Exygen RESEARCH

CHAIN OF CUSTODY

Exygen Research Sample Receiving • 3048 Research Drive • State College, PA 16801

COLLECTION SITE INFORMATION	STAFF INVOLVED IN SAMPLE SHIPMENT
Collection Site (name & address):	1. (Printed Name) Signature Initials/Date
Phone:	2. (Printed Name) Signature Initials/Date
Fax:	3. (Printed Name) Signature Initials/Date
Email:	

IMPORTANT: Specify AM or PM for time entries. Include month, day, and year for dates. Contents of package must be verified by someone other than the packager, and this verification must be attested to by initialing and dating the prompt.* Thoroughly document the reason for any time gap between the time the serum preparation is completed and the time packaged by adding a footnote to the bottom of the page. Each person who enters information on this form must add their name, signature, initials, and date above. Line out unused prompts.

Sample Identification	Blood Sample Collection Date & Time	Serum Sample Prep. Completed Date & Time

PLEASE FAX THE COMPLETED FORM to Exygen Sample Receiving prior to shipping samples.

KEEP THE PINK COPY and include the other two with sample shipment.

Cooler Contents Verified Initial & Date* →

Cooler ID #:

Relinquished by	Date	Time

Received by	Date	Time

Note: When samples arrive at Exygen, chain-of-custody procedures follow Exygen SOP V401 "Contract Research Sample Handling." Page ____ of ____

Company Sanitized. Does not contain TSCA CBI

Figure 2. List of Error Codes

EXPLANATION OF ERROR CODING

Correction of raw data is made by drawing a single line through the erroneous data and/or write-over, and filling in the correct data. Care must be taken not to obscure the original entry. The correction is initialed and dated by the individual making the correction, and an explanation is given for the correction.

The following error codes shall be used to explain corrections:

(wl)	Inadvertently recorded in Wrong Location
(cc)	Changed for greater Clarity
(wo)	Write Over (Technician inadvertently wrote over the error instead of drawing a line through the error)
(sp)	Spelling error
(ir)	Inadvertently not Recorded at time of initial observation
(ce)	Calculation Error
(re)	Recording Error
(ne)	Numerical transcription Error
(ee)	Entry not initialed and dated at time of entry
(te)	Transcription Error
(wd)	Wrong Date entry
(sm)	Stray Marks appearing on data with no other feasible explanation
(le)	Late Entry
(tl)	Technical error

Codes, other than those defined above may be used, if explained or defined by footnote on the COC form. Errors that cannot be explained using an error code must be fully detailed at the time of correction.

Error codes are to be circled and written above or close to the correction. If this is not feasible, the error should be footnoted with an asterisk or other symbol, and explained at the bottom of the page.

Example COC Sheet and Use of Error Codes

Exygen® RESEARCH CHAIN OF CUSTODY

Exygen Research Sample Receiving • 3048 Research Drive • State College, PA 16801

CLINIC INFORMATION	STAFF INVOLVED IN SAMPLE SHIPMENT
Clinic (name & address): Name of Clinic Address (Street, P.O. Box) City, State Zip code **Clinic Identification Code:** A **Phone:** (area code) 7-digit # **Fax:** (area code) 7-digit # **Email:** email@clinic.com	1. B.C. Nurse (Printed Name) — B.C. Nurse (Signature) — BCN 07/18/03 (Initials/Date) 2. John Doc Staff (Printed Name) — John Doc Staff (Signature) — JDS 07/18/03 (Initials/Date) 3. J. Smith (Printed Name) — J. Smith (Signature) — JS 07-18-03 (Initials/Date)

IMPORTANT: Specify AM or PM for time entries. Include month, day, and year for dates. Contents of package must be verified by someone other than the packager, and this verification must be attested to by initialing and dating the prompt.* Thoroughly document the reason for any time gap between the time the serum preparation is completed and the time packaged by adding a footnote to the bottom of the page. Each person who enters information on this form must add their name, signature, initials, and date above. Line out unused prompts.

Clinic Sample Identification	Blood Sample Collection Date & Time	Serum Sample Prep. Completed Date & Time
A001	07/18/03 11:30 AM	07/18/03 2:00 PM
A002	07/18/03 11:57 AM	07/18/03 2:00 PM
A003	07/18/03 12:25 PM	07/18/03 2:00 PM
~~A003~~ A004 (fe) BCN 07/18/03	07/18/03 12:50 PM	07/18/03 2:00 PM
	①	

PLEASE FAX THE COMPLETED FORM to Exygen Sample Receiving prior to shipping samples.

Cooler Contents Verified Initial & Date* → JS 07-18-03

KEEP THE PINK COPY and include the other two with sample shipment. Cooler ID #: 1A

① BCN 07/18/03

Relinquished by	Date	Time
John Doc Staff	07/18/03	2:30 pm

Received by	Date	Time
(Completed at Exygen)		

Note: When samples arrive at Exygen, chain-of-custody procedures follow Exygen SOP V401, "Contract Research Sample Handling."

REV 1 7-03

Company Sanitized. Does not contain TSCA CBI

DRAFT

Appendix-5

BLOOD COLLECTING CHECKLIST

Employees Name _____

Employees Job Title_____

Date and Time Blood Sample taken _____

Collecting Blood Sample

Red Capped Collection Tube Completely Filled	Yes	No
Filled Tube inverted 5 times	Yes	No

Centrifuge for 15 minutes @ 2500-3500 rpm

Start_____ Stop_____ (military time) *

Pipet Serum into Polypropylene tube with specimen ID

Specimen ID Number _____

Complete Chain-of-Custody (COC) Form Yes ____ No____

There must not be any unexplained gap in the COC for the samples.

Serum Placed in –20°C Freezer at _____ (military time) ±5 YES ___ NO ___

Serum Samples must be shipped frozen - Sample is frozen YES NO

Sample Shipped to:

Exygen Research
3058 Research Drive
State College, PA 16801
[redacted]

Shipped **FedEx overnight ONLY.** (per Standard Operating Procedure: Documentation, Accountability, and Sample Chain of Custody Requirements for the Shipping of Serum Samples – Appendix – 4)

Phlebotomist (Site Nurse) Name)Print)_____

Signature _____

Date_____

Please Send the Original Blood Collecting Checklist to

Joseph F. Viskocil, Jr. CIH
[redacted]
Stine-Haskell Research Center
PO Box 50, 1090 Elkton Road
Newark, DE 19714-0050

Company Sanitized. Does not contain TSCA CBI

Appendix – 6

AIR SAMPLING STRATEGY

The goal of exposure assessment is to recognize all exposures and to minimize all exposures. Workers may be exposed while performing tasks, by incidental contact from background contamination, or because of nearby tasks performed by others. Although the highest exposures usually result from a worker's own task, exposures significant can occur from the other two sources.

Serum levels of PFOA anion are an indication of overall exposure.

Air sampling results are an indication of airborne exposure.

The significance of exposure by incidental contact cannot be assessed until the airborne exposure is controlled. Since the number of job assignments at Fayetteville is small, we will sample each job assignment quarterly. Where job assignments extend into evenings and weekends, accommodations will be made to monitor workers at these times.

Two APFO Field/CCR Technicians are on duty for each 12-hour shift. One technician monitors the Control Room and the other technician does the fieldwork. Since the fieldwork technician is more likely to have an exposure, the majority of APFO/CCR technician samples in the field.

Proposed Air Sampling Plan for the first 12-Months

Collect six (6) personal samples Time-Weighted-Average (TWA) quarterly for each 12-hour shift schedule (APFO Field/CCR technician)	24 samples
Collect six (6) personal samples Time-Weighted-Average (TWA) quarterly for 8-hour shift schedule (APFO Lab and Mechanical Technician)	12 samples
Collect three (3) personal samples Time-Weighted-Average (TWA) quarterly for 8-hour shift schedule for APFO Supervisors	3 samples
Collect three (3) personal samples Time-Weighted-Average (TWA) quarterly for 8-hour shift schedule for APFO FLS	3 samples
Collect six (6) personal samples (duration of task) quarterly for technical/professional personnel (chemist and engineers)	6 samples

Total 48 regularly scheduled personal samples per quarter
Sampling Strategy to be re-assessed after 2 -quarters

Sampling will be scheduled to ensure that off-shifts and weekends will be proportionally represented

For an 8-hour shift; a minimum of 6- hours sampling is required to qualify as a 8-hour TWA

For a 12-hour shift; a minimum of 10- hours sampling is required to qualify as a 12-hour TWA

APPENDIX 7

QUALITY ASSURANCE PROCEDURES

INDUSTRIAL HYGIENE LABORATORY AND FIELD WORK

This procedure specifies quality assurance procedures for industrial hygiene field work.

Laboratory Standard Operating Procedures

To minimize the possibility of contamination, external surfaces of adapters, tubing and the air sampling pumps should be wiped down with a 50/50 solution of isopropyl alcohol. The wipe should be assumed to be contaminated and disposed of properly. Minimum personal protective equipment should include safety glasses with side shields and nitrile gloves.

This procedure must be followed to assure equipment calibration and maintenance is done properly.

Relevant quality control procedures are incorporated into this procedure.

Sample Numbering System

Each field sample must be assigned a unique number. The industrial hygienist will assign a number for each survey. The hygienist will add a unique sequential number for each sample taken on a survey.

Example: 82-24-01

82 Year of Survey
24 Survey number
01 Sequential sample number

Field Sampling

Seal the OVS cassettes or other similar sampling devices in plastic bags for transport to and from use site. Calibrate sample pumps before and after each sample. Calibrate sample pumps with counters, or rotometers at least before and after each survey. Before and after flow rates should be within 10%.

Instruct test subjects not to tamper with personal samplers and observe for compliance. Void any samples which are suspect.

Place sampler inlet in downward orientation to prevent contamination.

Immediately after a sample is taken, place caps into filter cassettes.

Pressure and temperature corrections to 25°C and 760 mmHg must be made to actual air sample volumes to calculate ppb concentrations of gases and vapors, for comparison with exposure limits.

Pressure and temperature corrections are not made, but actual air sample volumes are used to calculate mg/m3 of particulates for comparison with exposure limits.

Control, Known and Duplicate Samples

Control, known and duplicate samples must be submitted to the analytical laboratory, along with air samples.

Control samples are collection devices which have been opened at the site, immediately closed and sealed, and assigned a sequential number. They provide a check on the analytical zero.

Known samples are collection devices, which have been spiked with a known quantity of a chemical at Haskell Laboratory (or at the site under the hygienist's direction), carried on the survey, and assigned a sequential number. They provide a check on analytical accuracy. In some cases, it may not be feasible to prepare reliable known samples and they may be omitted upon concurrence of the Study Director.

Duplicate samples are area air samples taken from six to twelve inches apart and assigned sequential numbers. They provide a check on sampling and analytical precision.

Results of control, known and duplicate samples must be evaluated with the chemist in view of the specific analytical method used to determine adequacy. Generally, known sample results should be within 25% of the spiked amount and duplicate sample values should be 25% or less apart. Reasons for deviations should be determined and corrective actions taken.

Control results should be less than 5% of the exposure limit dose on the collection device at the average survey sample volume or non-detectable (whichever is less stringent). Sample results should be corrected for control sample results. Reasons for deviations should be determined and corrective actions taken.

Minimum required numbers of control, known and duplicate samples which must be submitted, are given in the following table.

Number of Samples

Field	<10	11-20	21-40	>41
Control	1	3	3	4
Known	2*	2	4**	4****
Duplicate Pairs	0	1	2***	2****

Two at one level **
Two at each of two levels ***
One pair at each of two levels ****
Additional pairs may be needed if a wide range of concentrations is expected.

INDUSTRIAL HYGIENE SURVEY RECORDS -

Quantitative Survey Data

Air Sampling Data - All air sampling data must be entered on Air Monitoring Data Form which must be sent to the Study Director for permanent retention.

Other Quantitative Survey Data - Other data, such as ventilation, room and equipment dimensions, or other data which will be used in a quantitative manner for calculations, must be entered on the Air Monitoring Data Form.

Survey Field Notes (other than quantitative data)

These notes are taken only as an informal key to memory. Significant information will be summarized in the survey report. These field notes may be discarded when no longer needed. However, if in the industrial hygienist's judgment, original non-quantitative notes need to be retained, the information should be attached to Air Monitoring Data Form for permanent retention.

Analysis and Analytical Reporting Phase:

Clayton analyzes the samples and prepares a draft report to review. The draft report and copies of the raw data are sent to Mary A. Kaiser, Ph.D. for review.

1. Mary A. Kaiser, PhD reviews the analytical data, makes any corrections/upgrades to the draft, and gives the contract lab permission to finalize the analytical report

2. The contract lab finalizes the analytical report and sends it to Mary A. Kaiser, PhD

3. Mary A. Kaiser, PhD sends a copy of the report to Study Director, Joseph F. Viskocil who archives the original report at Haskell and informs the plant industrial hygienist of the results

Appendix – 8

SAMPLE HANDLING, CHAIN-OF-CUSTODY, PRESERVATION and SHIPPING – AIR SAMPLES

The person collecting the sample should wear nitrile disposable lab or exam gloves and should limit his/her contact with the samples.

Sample tubes (OVS) will be provided by the contracted laboratory and should be stored in a clean, dry location. Sample tubes and collected samples are not required to be chilled, however sample tubes and collected samples should not be subjected to temperature extremes.

In order to minimize the possibility of introducing APFO contamination into samples, the following protocol should be followed:

- ❑ Avoid polytetrafluoroethylene (PTFE).
- ❑ Avoid aluminum foil.
- ❑ Do not use self-stick memo notes.
- ❑ Avoid blue ice (the OVS tubes are shipped at ambient temperature).
- ❑ Avoid pre-wrapped foods or snacks.
- ❑ Wear clothing that has been washed at least six times.
- ❑ Use only containers supplied by contract laboratory.

Following sample collection, the OVS tubes are capped and placed in a zip-loc bag. Seal the zip-loc bag.

Complete the sample label and chain-of custody (COC) form, including the volume of air sampled through the tube. The completed sample label is attached to the outside of the zip-loc bag.

Pack the samples in a small box or cooler with bubble wrap. Include the completed COC form.

Complete a mailing label and airbill and ship by overnight carrier to:

Clayton Group Services

22345 Roethel Drive

Novi, MI 46375

Contact at the laboratory is Karen Coonan [redacted]

[redacted]

Samples should be stored and shipped refrigerated to the laboratory.

Appendix – 9

December 4, 2003

Mary A. Kaiser, Ph.D.
Research Fellow
Corporate Center for Analytical Services
Dupont Central Research & Development
DUPONT
Experimental Station
P.O. Box 80302
Wilmington, DE 19880-0302

Clayton Work Order No. 03010080
Clayton Proposal No. 03DETLAB015.REV1

Dear Dr. Kaiser:

We are pleased to provide the following **Draft** report on the sampling and analytical method conditions followed for the method validation for [redacted]. This draft report is based on our proposal dated July 18, 2003.

The sampling parameters may change as we progress with the method validation. Not all of the parameters outlined in this report have been fully validated.

If you have any questions, please call me at (248) 344-1770.

Sincerely,

Kem Charron
Program Manager-Special Projects
Laboratory Services

KC
Enclosure

22345 Roethel Drive
Novi, MI 48375
248.344.1770
Fax 248.344.2654

Clayton GROUP SERVICES

Clayton Group Services, Inc.

Perfluorooctanoic Acid (PFOA)

Empirical Formula: $CF_3(CF_2)_6CO_2H$
Molecular Weight: 414.06
CAS Number: 335-67-1

Clayton Work Order No.: 03070834
Draft Report Date: February 9, 2004

OSHA: None
NIOSH: None
ACGIH: None
AEL: 0.56 ppb (9.48 µg/m³)

Properties
Appearance: White Powder
Melting Point: 55-56 °C

Synonyms: Pentadecafluorooctanoic acid

Sampling and Storage

Sampler: OSHA Versatile Sampler, SKC Cat. No. 226-30-16

Flow Rate: Area or personal monitoring: 1.0 L/min for up to 480 minutes for a sample volume of 480 L.
Fence line monitoring: 15 L/min for up to 480 minutes for a sample volume of 7200 L.

Storage: Refrigerated or ambient temperatures

Shipping: Samples can be shipped at ambient temperature

Sample Stability: 60 days at refrigerated or ambient temperatures

Equipment

1. SKC OSHA Versatile Sampler (OVS) Cat. # 226-30-16
2. HPLC System: LC pump with automated gradient controller, autosampler, optional tunable UV absorbance detector
3. Mass Spectrometer: Triple quadrupole mass spectrometer 2 – 1000 amu mass range minimum, API interface with Electrospray probe, negative and positive ionization mode capability, and data acquisition/reduction software
4. Column: Agilent Hypersil ODS, 2.1 × 100 mm, 3 µm particle size, Cat. # 79916OD-352, or equivalent
5. 16 mL amber glass vial with screw cap and polytetrafluorethylene/rubber liner. The polytetrafluorethylene side of the liner is positioned in the cap towards the sample
6. 4 mL amber glass vial with screw cap and polytetrafluorethylene/rubber liner. The polytetrafluorethylene side of the liner is positioned in the cap towards the sample
7. Waters total recovery glass autosampler vials with polytetrafluorethylene/rubber liner. The polytetrafluorethylene side of the liner is positioned in the cap towards the sample
8. .Syringe: 10, 25, 50, & 100 µL
9. Disposable glass test tube, 16 × 125 mm with caps

Reagents and Standards

1. Perfluorooctanoic Acid (PFOA), Aldrich 171468-5G, Lot # 04702MA
2. 1,2-di-^{13}C-Perfluorooctanoic Acid (^{13}C-PFOA), Internal Standard, Dupont, date of synthesis January 2, 2003
3. Water (H_2O), Direct-Q
4. Methanol (MeOH), HPLC grade, B&J Cat. # 230-4
5. Ammonium Acetate, Reagent grade, J.T. Baker Cat. # 0599-08
6. 2 mM Ammonium Acetate: dissolve 0.1388 g Ammonium Acetate in 900 mL H_2O
7. Extraction Solvent: 100% MeOH
8. Stock Analytical Standard Solution (SAS):
   Dissolve 56.9 mg ± 0.1 mg of PFOA (corrected for material purity) in 10 mL of MeOH
   Concentration: ~ 5690 µg/mL
9. Calibration Standards:
   Dilutions of SAS in MeOH resulting in PFOA concentrations of 11.4, 28.4, 45.5, 56.9, 114, and 227 ng/mL
10. Stock Spike Standard Solution (SSS):
    Dissolve 56.8 mg ± 0.1 mg of PFOA (corrected for material purity) in 10 mL of MeOH
    Concentration: ~ 5680 µg/mL
11. Spiking Standard Solutions:
    Dilutions of SSS in MeOH resulting in PFOA concentrations of 568 and 56.8 µg/mL
12. Stock Internal Standard Solution:
    Dissolve 7.2 mg ± 0.1 mg 1,2-di-^{13}C-Perfluorooctanoic Acid (corrected for material purity) in 10 mL of MeOH
    Concentration: ~ 720 µg/mL
13. Internal Standard Solution: Added to calibration standards and samples prior to analysis.
    Dilution of the Stock Internal Standard Solution in MeOH resulting in a ^{13}C-PFOA concentration of 1.4 µg/mL

Measurement

Technique: High Performance Liquid Chromatography (HPLC) coupled with Tandem Mass Spectrometry

Detectors: Triple Quadrupole Mass Spectrometer (Micromass Quattro LC, or equivalent)

Extraction: 4 mL of extraction solvent with shaking for 60 minutes on a flatbed mechanical shaker

Instrument Conditions

HPLC Conditions

Column: Agilent Hypersil ODS, 2.1 × 100 mm, 3 µm particle size

Column Temp:	30 °C
Flow Rate:	0.3 mL/minute
Injection Volume:	5 µL
Run Time:	14 minutes
Retention Time:	6.4 minutes

Mobile Phase: Gradient

Time (min)	%A	%B	Curve
0	90	10	-
5	10	90	-
9	10	90	-
10	90	10	-
14	90	10	-

A = 2 mM Ammonium Acetate
B = Methanol

Wavelength (UV): Not Applicable

Mass Spectrometer Conditions

Ionization Mode: Electrospray Negative

Nebulizer Gas (L/hr):	96
Desolvation Gas (L/hr):	690
Source Block Temperature (°C):	120
Desolvation Temperature (°C):	350
Collision Gas Pressure (mBar):	3.1e-3
Multiplier:	650

	ES -Ion
Source Settings	
Capillary Voltage (kV):	1.5
Extractor (V):	1
RF Lens (V):	0.5
Analyzer Settings	
MS1 LM&HM Resolution:	14
MS1 Ion Energy (V):	0.5
MS2 LM&HM Resolution:	15
MS2 Ion Energy (V):	1.5
Entrance (V):	0
Exit (V):	0

Mass Spectrometer Method

MS Function: Multiple Reaction Monitoring (MRM) ESP-
Inter Channel Delay for MRM (s): 0.1
Span (Daltons): 0.5
Start time: 0 min End time: 14 min

Analyte Specific Settings:	Transition (amu)	Dwell (s)	Cone Voltage (V)	Collision Energy (eV)
PFOA	412.9 – 368.9 (Q)	0.5	10	10
Internal Standard (^{13}C-PFOA)	414.9 – 369.9 (Q)	0.5	10	10

(Q) = Transition used for quantitation

Sampling

1. Samplers (SKC 226-30-16) are received from the manufacturer (SKC).
2. Calibrate each sampling pump with a representative collection device in line.
3. Depending on the application, there are two flow rate options:
   (1) For area or personal monitoring, sample at a flow rate of 1.0 L/min for up to 480 minutes to obtain a sample volume of 480 L.
   (2) For fence line monitoring, sample at a flow rate of 15 L/min for up to 480 minutes to obtain a sample volume of 7200 L.
4. Label the sample with appropriate sample identification.
5. At the completion of sampling, re-calibrate each pump with a representative collection device in line.
6. Cap the ends of each sampler.
7. Samples are stored at ambient temperature.
8. A chain of custody form is filled out and sent with the samplers under ambient conditions to the Laboratory for analysis.

Sample Preparation

Note: Samples are extracted just prior to analysis.

1. Extract each OVS sampling tube in three separate sections. Place each section in a separate disposable glass test tube.
   Section 1: GFF Filter
   Section 2: XAD-2 Front portion of media plus foam plug
   Section 3: XAD-2 Back portion of media
2. Add 4 mL of Methanol to each test tube and shake for 60 minutes on a mechanical flatbed shaker set at ~2 cycles per second.
3. Transfer approximately 3.5 mL of each extract to a 4 mL amber glass vial by filtering through a Gelman 13-mm GHP Acrodisc. Extracts should be stored at refrigerated temperatures (2 to 6 °C).
4. For LC/MS/MS analysis, transfer 200 µL each sample extract to a glass autosampler vial. Add 10 µL of a 1.4 µg/mL internal standard solution (1,2-di-^{13}C-Perfluorooctanoic Acid).

Calibration and Quality Control

1. Calibrate daily with six working standards over the range of 11.4 to 227 ng/mL for PFOA.

   a) Calibration standards are prepared by transferring 200 µL each PFOA standard solution to a glass autosampler vial. Add 10 µL of a 1.4 µg/mL internal standard solution (1,2-di-^{13}C-Perfluorooctanoic Acid).

   b) Analyze standards together with samples and blanks. A calibration curve is analyzed at the beginning and end of each run, and Continuing Calibration Verification (CCV) standards are analyzed a minimum of every ten samples. Acceptance criteria for a CCV is ± 20%.

   c) Generate an internal standard, linear calibration curve from the observed peak area response factors and standard concentrations, applying 1/X weighting. The correlation coefficient (R) for the calibration curve must be ≥ 0.992 ($R^2 \geq 0.985$).

2. Determine the Extraction Efficiency (EE) at least once with each lot of samplers. Prepare at least one level in duplicate within the expected sampling range. Include one media blank per lot.

   a) Inject µL amounts of PFOA spiking standard solution onto the GFF portion of an OVS tube.

   b) Allow the tube to air-dry at ambient temperature.

   c) Cap the tube and store at **refrigerated** temperatures (2 to 6 °C) until extraction.

   d) Extract the PFOA-spiked and blank tubes as described in Sample Preparation. Analyze an aliquot from each sample along with standards. Transfer 200 µL each extract solution to a glass autosampler vial. Add 10 µL of a 1.4 µg/mL Internal Standard solution (1,2-di-^{13}C-Perfluorooctanoic Acid).

   e) Calculate the ratio for the ng of PFOA found on the media spike to the theoretical amount spiked. The concentrations found in each of the three sections must be summed to obtain the total amount of PFOA found.

   The ratio reflects the recovery of PFOA from the media. The mean recovery of the replicates equals the EE for PFOA.

Measurement

1. Set the liquid chromatograph and mass spectrometer conditions in accordance with those specified on page 3, or with suitable adjustments to optimize chromatography and sensitivity.

2. Inject the specified aliquot of each standard and sample.

3. Record the peak area response factor for PFOA and Internal Standard peaks. The observed retention time is 6.4 minutes for both PFOA and ^{13}C-PFOA.

Calculations

1. Calculate the response factor (RF) for PFOA.

   RF = (PFOA Area / ^{13}C-PFOA Area)

2. Determine the mass (ng) of PFOA collected using a first order linear regression curve plotting RF versus PFOA concentration.

   a) Analyte found: ng = (ng/mL × FV)

   Where: ng/mL = obtained by interpolation of the PFOA peak RF on the calibration curve

   FV = the Extraction Final volume (mL)

   b) Concentration (mg/m^3) = (μg_{Sample} - μg_{Blank}) /V

   Where: μg_{Sample} = µg amount found in the sample (ng found / 1000 ng/µg)

   μg_{Blank} = µg amount found in the blank sample (ng found / 1000 ng/µg)

   V = air sample volume (L)

This report prepared by:

Kristine Kurtz, Ph.D.
Supervisor, Method Validations
Laboratory Services

This report reviewed by:

Robert Lieckfield, Jr., CIH
Senior Vice President
Director, Laboratory Services

February 9, 2004

Clayton Group Services, Inc.

[redacted]

Empirical Formula: [redacted]
Molecular Weight: [redacted]
CAS Number: [redacted]

Clayton Work Order No.: 03070834
Draft Report Date: December 4, 2003

OSHA: None
NIOSH: None
ACGIH: None
AEL: 0.56 ppb

Properties
Appearance: White Powder
Odor:
Melting Point: [redacted]

Synonyms: Pentadecafluorooctanoic acid

Sampling and Storage

Sampler: OSHA Versatile Sampler SKC Cat. No. 226-30-16
Storage: To be determined
Shipping: Samples should be shipped at **refrigerated** temperatures (2-6 °C)
Sample Stability: To be determined

Equipment

1. SKC OSHA Versatile Sampler (OVS) Cat. No. 226-30-16
2. HPLC System: LC pump with automated gradient controller, autosampler, optional tunable UV absorbance detector
6. Mass Spectrometer: Triple quadrupole mass spectrometer 2 – 1000 amu mass range minimum, API interface with Electrospray probe, negative and positive ionization mode capability, and data acquisition/reduction software
7. Column: Agilent Hypersil ODS, 2.1 × 100 mm, 3 µm particle size, Cat. # 79916OD-352 or equivalent
8. 16 mL amber glass vial with screw cap and polytetrafluorethylene/rubber liner. The polytetrafluorethylene side of the liner is positioned in the cap towards the sample
9. 4 mL amber glass vial with screw cap and polytetrafluorethylene/rubber liner. The polytetrafluorethylene side of the liner is positioned in the cap towards the sample
10. Waters total recovery glass autosampler vials with polytetrafluorethylene/rubber liner. The polytetrafluorethylene side of the liner is positioned in the cap towards the sample
12. Syringe: 10, 25, 50, & 100 µL
13. Disposable glass test tube, 16 x 125 mm with caps

Measurement

Technique: High Performance Liquid Chromatography (HPLC) coupled with Tandem Mass Spectrometry

Detectors: Triple Quadrupole Mass Spectrometer (Micromass Quattro LC, or equivalent)

Extraction: 4 mL of extraction solvent with shaking for 60 minutes on a flatbed mechanical shaker

Instrument Conditions:

HPLC Conditions

Column: Agilent Hypersil ODS, 2.1 × 100 mm, 3 µm particle size

Column Temp: 30 °C
Flow Rate: 0.3 mL/minute
Injection Volume: 5 µL
Run Time: 14 minutes
Retention Time: 6.4 minutes

Mobile Phase: Gradient

Time (min)	%A	%B	Curve
0	90	10	-
5	10	90	-
9	10	90	-
10	90	10	-
14	90	10	-

A = 2 mM Ammonium Acetate
B = Methanol

Wavelength (UV): Not Applicable

Mass Spectrometer Conditions

Ionization Mode: Electrospray Negative

Nebulizer Gas (L/hr): 96
Desolvation Gas (L/hr): 690
Source Block Temperature (°C):120
Desolvation Temperature (°C):350
Collision Gas Pressure (mBar):3.1e-3
Multiplier: 650

	ES -Ion
Source Settings	
Capillary Voltage (kV):	1.5
Extractor (V):	1
RF Lens (V):	0.5
Analyzer Settings	
MS1 LM&HM Resolution:	14
MS1 Ion Energy (V):	0.5
MS2 LM&HM Resolution:	15
MS2 Ion Energy (V):	1.5
Entrance (V):	0
Exit (V):	0

Mass Spectrometer Method

MS Function: Multiple Reaction Monitoring (MRM) ESP-
Inter Channel Delay for MRM (s): 0.1
Span (Daltons): 0.5
Start time: 0 min End time: 14 min

Analyte Specific Settings:	Transition (amu)	Dwell (s)	Cone Voltage (V)	Collision Energy (eV)
[redacted]	[redacted] (Q)	0.5	10	10
Internal Standard [redacted]	[redacted] (Q)	0.5	10	10

(Q) = Transition used for quantitation

Sampling

1. Samplers (SKC 226-30-16) are received from the manufacturer (SKC).
2. Calibrate each sampling pump with a representative collection device in line.
3. Depending on the application, there are two flow rate options:
   (1) For area or personal monitoring, sample at a flow rate of 1.0 L/min for up to 480 minutes to obtain a sample volume of 960 L.
   (2) For fence line monitoring, sample at a flow rate of 15 L/min for up to 1440 minutes to obtain a sample volume of 21600 L.
4. Label the sample with appropriate sample identification.
5. At the completion of sampling, re-calibrate each pump with a representative collection device in line.
6. Cap the ends of each sampler.
7. Samples are stored at refrigerated temperatures (2 to 6 °C).
8. A chain of custody form is filled out and sent with the samplers under refrigerated conditions to the Laboratory for analysis

Sample Preparation

Note: Samples are extracted just prior to analysis.

1. Extract each OVS sampling tube in three separate sections. Place each section in a separate disposable glass test tube.
   Section 1: GFF Filter
   Section 2: XAD-2 Front portion of media plus foam plug
   Section 3: XAD-2 Back portion of media
2. Add 4 mL of Methanol to each test tube and shake for 60 minutes on a mechanical flatbed shaker set at ~2 cycles per second.
3. Transfer approximately 3.5 mL of each extract to a 4 mL amber glass vial by filtering through a Gelman 13-mm GHP Acrodisc. Extracts should be stored refrigerated temperatures (2 to 6 °C).
4. For LC/MS/MS analysis, transfer 200 µL each sample extract to a glass autosampler vial. Add 10 µL of a 1.4 µg/mL Internal Standard solution ([redacted]).

Calibration and Quality Control

1. Calibrate daily with six working standards over the range of 11.4 to 227 ng/mL for [redacted].
   a) Calibration standards are prepared by transferring 200 µL each [redacted] standard solution to a glass autosampler vial. Add 10 µL of a 1.4 µg/mL Internal Standard solution ([redacted]).

b) Analyze standards together with samples and blanks. A calibration curve is analyzed at the beginning and end of each run, and Continuing Calibration Verification (CCV) standards are analyzed a minimum of every ten samples. Acceptance criteria for a CCV is ± 20%.

c) Generate an internal standard, linear calibration curve from the observed peak area response factors and standard concentrations.

2. Determine the Extraction Efficiency (EE) at least once with each lot of samplers. Prepare at least one level in duplicate within the expected sampling range. Include one media blank per lot.

a) Inject µL amounts of [redacted] spiking standard solution onto the GFF portion of an OVS tube.

b) Allow the tube to air-dry at ambient temperature.

c) Cap the tube and store at **refrigerated** temperatures (2 to 6 °C) until extraction.

d) Extract the PFOA-spiked and blank tubes as described in Sample Preparation. Analyze an aliquot from each sample along with standards. Transfer 200 µL each extract solution to a glass autosampler vial. Add 10 µL of a 1.4 µg/mL Internal Standard solution ([redacted]).

e) Calculate the ratio for the ng of [redacted] found on the media spike to the theoretical amount spiked. The concentrations found in each of the three sections must be summed to obtain the total amount of [redacted] found.

The ratio reflects the recovery of [redacted] from the media. The mean recovery of the replicates equals the EE for [redacted].

Measurement

1. Set the liquid chromatograph and mass spectrometer conditions in accordance with those specified on page 2, or with suitable adjustments to optimize chromatography and sensitivity.

2. Inject the specified aliquot of each standard and sample.

3. Record the peak area response factor for [redacted] and Internal Standard peaks. The observed retention time is 6.4 minutes for both [redacted] and Internal Standard.

DUPONT

Appendix-10 Air Monitoring Data Form

Sample Number	**Date**	**Monitoring Type** ___ Personal ___ Area
Chemical Agent(s)		
Sample Type ___ Personal ___ Source ___ Bulk		
Sample Duration ___ TWA ___ STEL ___ Ceiling		
PPE* – Respirator Air Supplied ,Full Face Air Purifying w/ov/acid gas/high Efficiency Particulate Filter, none - Full Acid Suit : nitrile or laytex gloves ; other		
Type of Control ___ Closed System ___ Local Exhaust ___Mechanical Exhaust ___ Natural Ventilation		
Employee Information		
Name		**SSN/Employee Number**
Unit	**Area**	**Job Name**
Shift	**Supervisor**	
Sample Information		
Building	**Floor**	**Area**
Description of Location		
Tasks- See Personal Monitoring Log		
Sample Collection Information		
Pump Manufacturer	**Pump Model**	**Pump Number**
Start Time	**Stop Time**	**Total Time (min)**

Initial Flow Rate	**Final Flow Rate**	**Average Flow Rate**	**Sample Volume**
Sample Media		**Lot Number**	
Environmental Data			
Temperature (F)	**Humidity (%)**	**Wind Direction**	**Wind Speed (mph)**
Results			
Agent	**Analytical Result**		**Final Result**
Method		**Limit of Detection**	
Sample Analyzed By		**Sample Collected By**	

* Standard PPE includes Safety Glasses, Hard Hat, Steel Toe Acid Suit Boots

FAYETTEVILLE WORKS PERSONNEL AIR MONITORING ACTIVITY INFORMATION WORKSHEET

Date:

Employee Name:

Work Area:

Sample Number:

Pump Number:

Time	Comments

Appendix – 11 Wipe Test Procedure

Materials:

- WYPALL L40 disposable wipers; Kimberly-Clark #05701, VWR #21908-000
- Cheesecloth wipes; VWR PK-200 4X4 #21910-107
- Cotton Pads; VWR PK-100 4X4 #21902-985
- Templates for Wipe Sampling 10 X 10cm paper; SKC Inc. #225-2415
- Isopropyl alcohol Omnisolv 4L 99.9%; VWR #EM-PX1834-1
- Water Omnisolv 4L; VWR #EM-WX0004-1
- Scintillation vials, 20 mL, disposable, glass w/polyethylene cap VWR #66020-326

Safety:

- The minimum PPE for this procedure is safety eyewear with sideshields and nitrile gloves. All generated waste is to be disposed in an appropriate manner.

Procedure:

1. Make 1 L of mopping solvent (MS) by mixing 500 mL of isopropyl alcohol (IPA) with 500 mL of water (MILLI-Q or other water purification system water may be substituted for EM Omnisolv water). MS is stored in a 1 L Nalgene® volumetric flask.
2. Identify the area to be wipe tested, Using the 10 X 10 cm Template for Wipe Sampling to define the 100 square centimeter area to be tested.
3. Fill a transfer pipette with MS from the 1 min. scintillation vial. Evenly drip half of the contents (~0.75 mL) on a folded cheesecloth wipe and return the balance of the MS to the vial. Remove the paper template and mop the test area with the wipe. Insert wipe into the vial, gently push the wipe to the bottom with the transfer pipette and close the cap tightly. Dispose of the pipette in the waste jug.
4. In order to minimize the possibility of introducing APFO contamination into samples, the following protocol should be followed:
   Avoid polytetrafluoroethylene (PTFE).
   Avoid aluminum foil.
   Do not use self-stick memo notes.
   Avoid blue ice (the samples are shipped at ambient temperature).
   Avoid pre-wrapped foods or snacks.
   Wear clothing that has been washed at least six times.
5. Following sample collection, the vials are capped and placed in a zip-loc bag. Seal the zip-loc bag.
6. Complete the sample label . The completed sample label is attached to the outside of the zip-loc bag.
7. Pack the samples in a small box or cooler with bubble wrap.

TITLE

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Serum and Plasma by LC/MS/MS

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1. SUMMARY

This report details a method of analysis for perfluorooctanoic acid (PFOA) in serum and plasma.

PFOA is extracted from serum and plasma by protein precipitation in acetonitrile by means of a robotic liquid sample handler. Quantification of PFOA is accomplished by liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis using selected reaction monitoring (SRM). The chemical formula of PFOA is given in section 2 of this method.

The lower limit of quantitation (LLOQ) for this method is 0.5 ppb for PFOA in serum and plasma.

Quantification is performed using extracted calibration standards containing an internal standard.

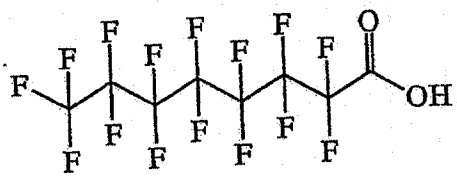
This method was developed using rabbit serum and rabbit plasma. The overall percent recovery ± standard deviation for PFOA in rabbit serum at 0.5, 3, and 40 ppb was 95% ± 8.5% (**Table I**). The overall percent recovery ± standard deviation for PFOA in rabbit plasma at 0.5, 3, and 40 ppb was 99% ± 6.3% (**Table II**).

A representative calibration curve for PFOA in rabbit serum is shown in **Figure 1.** Representative chromatograms for PFOA in rabbit serum are shown in **Figures 2 to 4.** A representative calibration curve for PFOA in rat plasma is shown in **Figure 5.** Representative chromatograms for PFOA in rat plasma are shown in **Figures 6 to 8.**

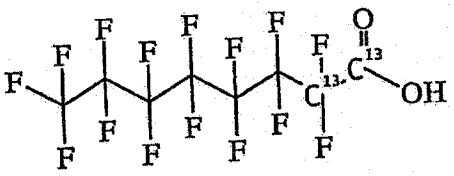
2. EXPERIMENTAL COMPOUNDS

The structure of perfluorooctanoic acid (PFOA) and the internal standard are given below.

PFOA
Chemical Name = perfluorooctanoic acid
Molecular weight = 414



^{13}C – PFOA
Chemical Name = perfluorooctanoic acid, [1,2-di-^{13}C]
Molecular weight = 416



3. CHEMICALS AND SUPPLIES

3.1. CHEMICALS

Chemical	Grade	Source	Catalog No.
Methanol (MeOH)	HPLC	EM Science	JT9093-2
Acetonitrile (ACN)	HPLC	EM Science	AX0145-1
Ammonium Acetate	Reagent	Sigma-Aldrich	A-7330
OmniSolv Water	HPLC	EM Science	WX0004-1

3.2. STANDARDS

Standard	Lot Number	Purity (%)	Source
perfluorooctanoic acid (PFOA)	21002	97	Oakwood Products
perfluorooctanoic acid, [1,2-di-^{13}C]	NA	96.35	DuPont

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3.3. EQUIPMENT AND SUPPLIES

Equipment	Supplier
Balance, analytical (display at least 0.0001 g)	Mettler
Disposable micropipets (50-100uL, 100-200uL)	Drummond (VWR)
Class A pipets and volumetric flasks	various suppliers
Hypercarb drop-in guard column (4 mm) (part # 844017-400)	Keystone
Stand-alone drop-in guard cartridge holder	Keystone
125-mL LDPE narrow-mouth bottles	Nalgene
2 mL clear HPLC vial kit	VWR
Standard lab equipment (graduated cylinders, disposable tubes etc.)	various suppliers
Mulitprobe II Ex, Robotic liquid handling system	Packard
MBP Disposable Conductive Tips (1 mL) - (part #6000655)	Packard
Array protein precipitation columns, 2 mL (part #120-2020-T)	Argonaut
Isolute array collection plate, 1 mL (part #121-5202)	Argonaut
12x75 mm disposable culture tubes	VWR
LC/MS/MS and HPLC systems	As described in section 4.5.

Note: Equivalent materials may be substituted for those specified in this method if they can be shown to produce satisfactory results.

Notes:

1. In order to avoid contamination, the use of disposable labware is highly recommended (tubes, pipets, etc.).
2. PTFE or PTFE lined containers or equipment, including PTFE-lined HPLC vials for the HPLC autosampler must not be used.
3. It is necessary to check the solvents (methanol) for the presence of contaminants by LC/MS/MS before use. Certain lot numbers have been found to be unsuitable for use.
4. Use disposable micropipets or pipets to aliquot standard solutions to make calibration standards and sample fortifications.
5. The hypercarb cartridges should be changed when the system contaminants (elevated baseline) move to within 1 minute from the elution of PFOA.

3.4. SOLUTIONS

(1) 2 mM ammonium acetate solution is prepared by weighing 0.15 g of ammonium acetate and dissolving in 1 L of water.

Note: The aforementioned example is provided for guidance, alternative volumes may be prepared as long as the ratios of the solvent to solute are maintained.

3.5. PREPARATION OF STANDARDS AND FORTIFICATION SOLUTIONS

Analytical standards are used for three purposes:

1. Calibration Standards – These standards are prepared in control rabbit serum or rabbit plasma and are used to calibrate the response of the detector used in the analysis.
2. Laboratory Control Spikes – These fortifications are usually prepared at concentrations corresponding to the LLOQ and 10x LLOQ and are used to determine analytical recovery. Laboratory control spikes are prepared in control rabbit serum or rabbit plasma.
3. Matrix Spikes – These fortifications are prepared by spiking into the field samples at known concentrations. Matrix spikes are used to evaluate the effect of the sample matrix on analytical recovery.

The absolute volumes of the standards may be varied by the analyst as long as the correct proportions of solute to solvent are maintained.

3.5.1. Stock solution

Prepare individual stock solutions of 100 µg/mL of PFOA and ^{13}C-PFOA by weighing out 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 100-mL volumetric flask. Each stock solution (in 125-mL LDPE bottles) is to be stored in a refrigerator at 2°C to 6°C and is stable for a maximum period of 1 year from the date of preparation.

3.5.2. Fortification Solutions

a. Prepare a fortification standard of 1.0 µg/mL (1000 ng/mL) for PFOA by diluting 1.0 mL of the fortification solution described in 3.5.1 to 100 mL with acetonitrile in a volumetric flask.
b. Prepare a fortification standard of 1.0 µg/mL for ^{13}C-PFOA by diluting 1.0 mL of the ^{13}C-PFOA solution described in 3.5.1 to 100 mL with acetonitrile in a volumetric flask.
c. Prepare a fortification standard of 0.1 µg/mL (100 ng/mL) for PFOA by diluting 10.0 mL of the fortification solution described in 3.5.2.a to 100 mL with acetonitrile in a volumetric flask.
d. Prepare a fortification standard of 0.005 µg/mL for PFOA containing 0.001 µg/mL of ^{13}C-PFOA by diluting 5.0 mL of the PFOA solution

described in 3.5.2.c and 0.1 mL of the ^{13}C-PFOA solution described in 3.5.2.b to 100 mL with acetonitrile in a volumetric flask.

e. Prepare a fortification standard of 0.002 µg/mL for PFOA containing 0.001 µg/mL of ^{13}C-PFOA by diluting 2.0 mL of the PFOA solution described in 3.5.2.c and 0.1 mL of the ^{13}C-PFOA solution described in 3.5.2.b to 100 mL with acetonitrile in a volumetric flask.

f. Prepare a fortification standard of 0.001 µg/mL for PFOA containing 0.001 µg/mL of ^{13}C-PFOA by diluting 1.0 mL of the PFOA solution described in 3.5.2.c and 0.1 mL of the ^{13}C-PFOA solution described in 3.5.2.b to 100 mL with acetonitrile in a volumetric flask.

g. Prepare a fortification standard of 0.0005 µg/mL for PFOA containing 0.001 µg/mL of ^{13}C-PFOA by diluting 0.5 mL of the PFOA solution described in 3.5.2.c and 0.1 mL of the ^{13}C-PFOA solution described in 3.5.2.b to 100 mL with acetonitrile in a volumetric flask.

h. Prepare a fortification standard of 0.0001 µg/mL for PFOA containing 0.001 µg/mL of ^{13}C-PFOA by diluting 0.1 mL of the PFOA solution described in 3.5.2.c and 0.1 mL of the ^{13}C-PFOA solution described in 3.5.2.b to 100 mL with acetonitrile in a volumetric flask.

i. Prepare a fortification standard of 0.00005 µg/mL for PFOA containing 0.001 µg/mL of ^{13}C-PFOA by diluting 0.05 mL of the PFOA solution described in 3.5.2.c and 0.1 mL of the ^{13}C-PFOA solution described in 3.5.2.b to 100 mL with acetonitrile in a volumetric flask.

j. Prepare a fortification standard of 0.001 µg/mL of ^{13}C-PFOA by diluting 0.1 mL of the ^{13}C-PFOA solution described in 3.5.2.b to 100 mL with acetonitrile in a volumetric flask.

Store all fortification standard solutions in a refrigerator (in 125-mL LDPE bottles) at 2°C to 6°C for a maximum period of 1 year from the date of preparation, after which time it is necessary to make new standards using the stock solution.

3.5.3. Calibration Standards

The calibration standards are processed through the extraction procedure, identical to the samples. The fortification of the standards before extraction is done according to the following table:

Conc. Of Mixed Fortification Solution (ng/mL)	Fortification Volume (µL)	Volume of Control Sample (mL)	Conc. of Extracted Calibration Standard (ppb)
0.05	500	0.05	0.5
0.1	500	0.05	1
0.5	500	0.05	5
1.0	500	0.05	10
2.0	500	0.05	20
5.0	500	0.05	50

4. METHOD

4.1. FLOW DIAGRAM

The flow diagram of the method is given below, followed by a detailed description of each step.

Method Flow Diagram

Measure 0.05 mL of sample (using disposable micropipette) into protein precipitation column

↓

↓

Protein precipitation in acetonitrile using Multiprobe

↓

↓

LC/MS/MS analysis

4.2. SAMPLE PROCESSING

No sample processing is needed for serum or plasma samples. However, frozen samples must be allowed to completely thaw, un-aided, at room temperature. Samples stored refrigerated should also be allowed to equilibrate to room temperature. All samples must be thoroughly mixed before being sampled for extraction.

4.3. Sample Preparation

a. Each batch of samples analyzed (typically 35 or less) must include at least one reagent control (acetonitrile blank), one matrix control (method blank), two matrix controls fortified at known concentrations to verify procedural recovery for the batch.
b. At least one sample per batch should be extracted in duplicate
c. At least one sample extracted should be separately fortified at a known concentration and carried through the procedure to verify recovery. Additional matrix spikes may be performed at the sponsor's request.

4.4. Extraction

a. Configure the Multiprobe deck with the following labware:
    1. 1000 µL disposable tips
    2. 12 x 75 mm test tube rack
    3. SPE manifold with protein precipitation columns and 1 mL collection tray (plug any unused holes in the SPE manifold)
    4. Tip chute with disposal bag.
b. Load 0.05 mL of sample into the appropriate protein precipitation column.
c. Place at least 1 mL of Acetonitrile containing the appropriate concentration of analyte and internal standard into a disposable 12 x 75 mm test tube.
d. Using the Multiprobe's control software, Winprep, create a program that performs the following steps in sequence:
    1. Get clean pipette tip(s).
    2. Go to the appropriate 12 x 75 mm test tube(s) and aspirate an air plug followed by 500 µL of ACN solution followed by a transport air plug.
    3. Go to the appropriate protein precipitation column(s) on the SPE manifold and dispense the ACN solution.
    4. Go to the tipchute and drop the tip(s).
    5. Repeat Multiprobe procedure steps 1-4 until ACN solutions have been added to all required protein precipitation columns.
    6. Activate the SPE manifold vacuum system in 10 to 20 second cycles until all ACN solution has been drawn through the protein precipitation column (usually 10 to 15 cycles). *Note: Additional vacuum cycles can be initiated by opening the vacuum node of the Winprep method and selecting the vacuum step "Preview."*
e. Transfer ~ 400µL to an autosampler vial with a pipette for analysis.

4.5. Quantitation

4.5.1. LC/MS/MS System and Operating Conditions

Instrument:	PE SCIEX API 4000 Biomolecular Mass Analyzer SCIEX Turbo Ion Spray Liquid Introduction Interface
Computer:	Dell OptiPlex GX 110

Software: PE Sciex Analyst 1.2

HPLC Equipment: Hewlett Packard (HP) Series 1100
Quatpump G1311A
Vacuum Degasser G1322A
Autoinjector G1313A
Column Compartment G1316A

Note: Two 4 × 10 mm hypercarb drop-in guard cartridge (Keystone, part # 844017-400) are attached in-line after the purge valve and before the sample injector port to trap any residue contaminants that may be in the mobile phase and/or HPLC system.

HPLC Column: Genesis C_8 (JonesChromatography), 2.1 mm x 50 mm, 4µ
Column Temperature: 30° C
Injection Volume: 5 µL
Mobile Phase (A): 2 mM Ammonium Acetate in Water
Mobile Phase (B): Methanol

Time	% A	% B	Flow (mL/min)
0.0	40	60	0.3
3	40	60	0.3
3.5	0	100	0.3
3.7	0	100	0.5
7	0	100	0.5
7.5	40	60	0.5
9	40	60	0.5
9.5	40	60	0.3
12	40	60	0.3

It may be necessary to adjust the HPLC gradient in order to optimize instrument performance.

Ions monitored:

Analyte	Mode	Transition Monitored	Approximate Retention Time
PFOA	Negative	413 → 369	~2.8 min.
^{13}C-PFOA	Negative	415 → 370	~2.8 min.

The retention times may vary, on a day-to-day basis, depending on the column, batch of mobile phase, etc. Drift in retention times is acceptable within an analytical run, as long as the drift continues through the entire analysis and the standards are interspersed throughout the analytical run.

Note: An alternative LC/MS/MS system may be used once demonstrated to be equivalent.

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4.5.2. Tune File Parameters

The mass spectrometer is tuned for each analyte by infusing a ~ 1 µg/mL standard solution of PFOA (at 10 µL/min, using an infusion pump) via a "T" into a stream of mobile phase containing 60% methanol and 40% 2mM ammonium acetate in water at 0.3 mL/min flow rate. The analyte is initially tuned for the parent ion and then tuned for the product ion. Once the instrument is tuned, the optimized parameters are saved as a tune file. This tune file is then used during routine analysis.

4.5.3. Calibration Procedures

a. Inject the same aliquot (5 µL) of each calibration standard into the LC/MS/MS.

b. Use linear standard curves for quantitation. Linear standard curves are generated for each analyte by linear regression using 1/x weighting of the ratio analyte peak area/internal standard peak area versus the ratio of the concentration of analyte/concentration of internal standard using Analyst 1.2 (or equivalent) software system. Any calibration standard found to be a statistical outlier by using the appropriate outlier test (e.g. Huge Outlier Test), may be excluded from the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected and at least one calibration standard at the LLOQ must be retained.

c. The correlation coefficient (R) for calibration curves generated must be ≥ 0.9925 ($R^2 \geq 0.985$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

4.5.4. Sample Analysis

a. Inject the same aliquot (5 µL) of each standard, sample, recovery, control, etc. into the LC/MS/MS system.

b. Standards corresponding to at least five or more concentration levels must be included in an analytical set.

c. An entire set of calibration standards should be injected at the beginning of a set followed by calibration standards interspersed every 5-10 samples (to account for a second set of standards). As an alternative, an entire set of calibration standards may be included at the beginning and at the end of a sample set. In either case, calibration standards must be the first and last injection in a sample set.

d. The concentration of each sample/fortification/control is determined from the standard curve, based on the peak area of each analyte. The standard responses should bracket responses of the residue found in each sample set.

If necessary, dilute the samples in 50:50 methanol: water containing 1 ng/mL of ^{13}C-PFOA to give a response within the standard curve range

e. Fortification recoveries falling within 85 to 115% (80 to 120% for levels at the LLOQ) are considered acceptable.

f. Extracted samples must be stored refrigerated between 2°C to 6°C until analysis.

g. Samples in which no peaks are detected (i.e. signal : noise ratio < 3:1) at the corresponding analyte retention times will be reported as ND (not detected). Samples in which peaks are detected at the corresponding analyte retention times but are less than the lowest concentration of the calibration standards (0.5 ppb where LLOQ = 0.5 ppb) will be reported as < 0.5 ppb.

4.6. Acceptance Criteria

The following criteria must be met to ensure the presence of PFOA and ^{13}C-PFOA:

1. The chromatograms must show the following peaks:
   PFOA: a daughter ion at 369 amu from a parent of 413 amu
   ^{13}C-PFOA: a daughter ion at 370 amu from a parent of 415 amu

2. Any analyte present in method blanks must be at least 3-fold lower than the LLOQ. Any analyte present in the reagent blank must be at least 5-fold lower than the LLOQ.

3. Recoveries of lab control spikes and matrix spikes must be between 85-115% (80-120% for levels at the LLOQ) of their known values. Any method fortification (lab control spike) falling outside the acceptable limits warrants re-extraction of the entire analytical set. Any matrix spike outside the acceptable range should be evaluated by the analyst to determine if re-extraction is warranted.

4. Any calibration standard found to be a statistical outlier by using an appropriate outlier test, may be excluded from the curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected and at least one calibration standard at the LLOQ must be retained.

5. The correlation coefficient (R) for calibration curves generated must be ≥0.9925 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

4.7. PERFORMANCE CRITERIA

The following two criteria must be performed as a system suitability test, before the commencement of analysis, when using an instrumentation set-up that has not been used for this method.

First Criterion:
Run a standard solution on LC/MS/MS corresponding to the estimated LLOQ and obtain a signal-to-noise ratio of at least 9:1, compared to a reagent blank. If this criterion cannot be met, optimize and change instrument operating parameters.

Second Criterion:
Run a set of standards of five or more concentration levels, spanning a range starting at or below the LLOQ up to the highest concentration level to be included in the analysis. Generate a calibration curve for the analyte and obtain a linear regression with a coefficient of determination (R^2) of at least 0.985. Once this criterion is met, samples may be analyzed with standards interspersed.

4.8. TIME REQUIRED FOR ANALYSIS

A set of 35 samples (1 reagent control, 1 matrix control, 1 laboratory spike, 2 laboratory control spikes, and 30 samples) can be taken through the extraction procedure in approximately 2 hours by one person. The LC/MS/MS analysis (standards and 35 samples) will take approximately 9 hours.

5. CALCULATIONS

a. Use Equation 1 to calculate the amount of analyte found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program.

Equation 1:

$$\text{Analyte found (ppb)} = \frac{(\text{Analyte Peak area/IS peak area}) - \text{intercept})) \times \text{IS conc. (ppb)}}{\text{slope}}$$

b. For samples fortified with known amounts of analyte prior to extraction, use Equation 2 to calculate the percent recovery.

Equation 2:

$$\text{Recovery (\%)} = \frac{\text{Analyte found (ppb)} - \text{avg. Analyte found in the corresponding sample (ppb)}}{\text{Amount Analyte added (ppb)}} \times 100\%$$

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6. SAFETY

The analyst should read the material safety data sheets for all standards and reagents before performing this method. Use universal precautions when handling standards and reagents, including working in fume hoods and wearing laboratory coats, safety glasses, and gloves. Use blood-borne pathogen handling precautions when handing serum and plasma.

Table I. Recovery of PFOA from Fortifications In Rabbit Serum

Summary of PFOA Recoveries for 0.5 ppb Fortification in Rabbit Serum

Exygen ID	Sponsor ID	Extraction Date	Analysis Date	Fort. Level (ppb)	% Recovery
0300808 Fort. 1	Lot 99H8400	05/22/03	05/23/03	0.5	95
0300808 Fort. 2	Lot 99H8400	05/22/03	05/23/03	0.5	85
0300808 Fort. 3	Lot 99H8400	05/22/03	05/23/03	0.5	88
				AVERAGE:	**89**
				STANDARD DEVIATION:	**5.1**
				RELATIVE STANDARD DEVIATION:	**5.7**

Summary of PFOA Recoveries for 3 ppb Fortification in Rabbit Serum

Exygen ID	Sponsor ID	Extraction Date	Analysis Date	Fort. Level (ppb)	% Recovery
0300808 Fort. 4	Lot 99H8400	05/22/03	05/23/03	3	108
0300808 Fort. 5	Lot 99H8400	05/22/03	05/23/03	3	102
0300808 Fort. 6	Lot 99H8400	05/22/03	05/23/03	3	107
				AVERAGE:	**106**
				STANDARD DEVIATION:	**3.2**
				RELATIVE STANDARD DEVIATION:	**3.0**

Summary of PFOA Recoveries for 40 ppb Fortification in Rabbit Serum

Exygen ID	Sponsor ID	Extraction Date	Analysis Date	Fort. Level (ppb)	% Recovery
0300808 Fort. 7	Lot 99H8400	05/22/03	05/23/03	40	90
0300808 Fort. 8	Lot 99H8400	05/22/03	05/23/03	40	91
0300808 Fort. 9	Lot 99H8400	05/22/03	05/23/03	40	90
				AVERAGE:	**90**
				STANDARD DEVIATION:	**0.6**
				RELATIVE STANDARD DEVIATION:	**0.6**
				OVERALL AVERAGE:	**95**
				OVERALL STANDARD DEVIATION:	**8.5**
				OVERALL RELATIVE STANDARD DEVIATION:	**8.9**

Table II. Recovery of PFOA from Fortifications In Rabbit Plasma

Summary of PFOA Recoveries for 0.5 ppb Fortification in Rabbit Plasma

Exygen ID	Sponsor ID	Extraction Date	Analysis Date	Fort. Level (ppb)	% Recovery
0300922 Fort. 1	Lot 40K7400	05/22/03	05/23/03	0.5	99
0300922 Fort. 2	Lot 40K7400	05/22/03	05/23/03	0.5	100
0300922 Fort. 3	Lot 40K7400	05/22/03	05/23/03	0.5	100
				AVERAGE:	**100**
				STANDARD DEVIATION:	**0.6**
				RELATIVE STANDARD DEVIATION:	**0.6**

Summary of PFOA Recoveries for 3 ppb Fortification in Rabbit Plasma

Exygen ID	Sponsor ID	Extraction Date	Analysis Date	Fort. Level (ppb)	% Recovery
0300922 Fort. 4	Lot 40K7400	05/22/03	05/23/03	3	107
0300922 Fort. 5	Lot 40K7400	05/22/03	05/23/03	3	106
0300922 Fort. 6	Lot 40K7400	05/22/03	05/23/03	3	105
				AVERAGE:	**106**
				STANDARD DEVIATION:	**1.0**
				RELATIVE STANDARD DEVIATION:	**0.9**

Summary of PFOA Recoveries for 40 ppb Fortification in Rabbit Plasma

Exygen ID	Sponsor ID	Extraction Date	Analysis Date	Fort. Level (ppb)	% Recovery
0300922 Fort. 7	Lot 40K7400	05/22/03	05/23/03	40	91
0300922 Fort. 8	Lot 40K7400	05/22/03	05/23/03	40	92
0300922 Fort. 9	Lot 40K7400	05/22/03	05/23/03	40	92
				AVERAGE:	**92**
				STANDARD DEVIATION:	**0.6**
				RELATIVE STANDARD DEVIATION:	**0.6**
				OVERALL AVERAGE:	**99**
				OVERALL STANDARD DEVIATION:	**6.3**
				OVERALL RELATIVE STANDARD DEVIATION:	**6.3**

Figure 1. Calibration curve for PFOA in Control Rabbit Serum

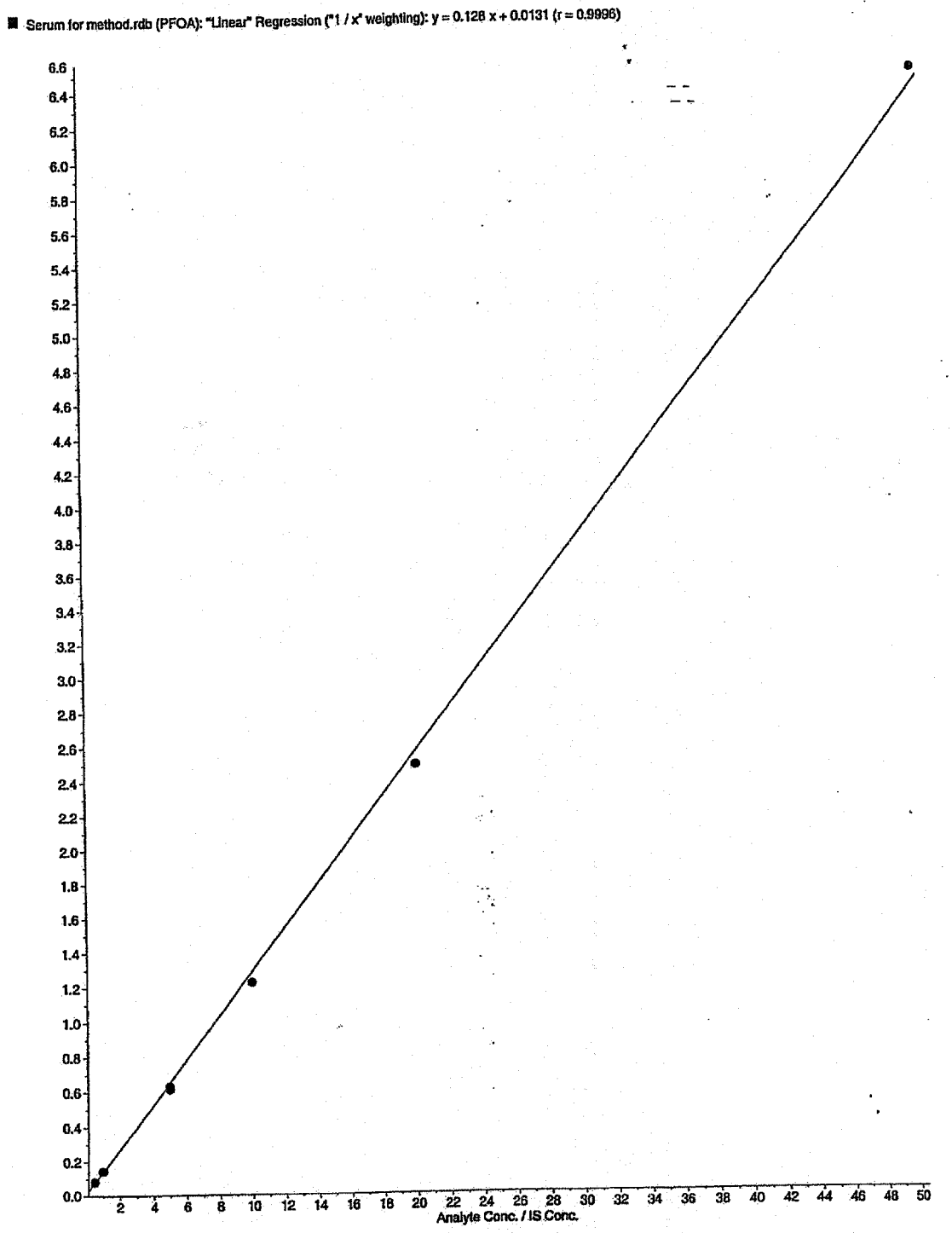


Figure 2. Representative Chromatogram of a Control Rabbit Serum Sample for PFOA with ~ 1 ng/mL of ^{13}C-PFOA

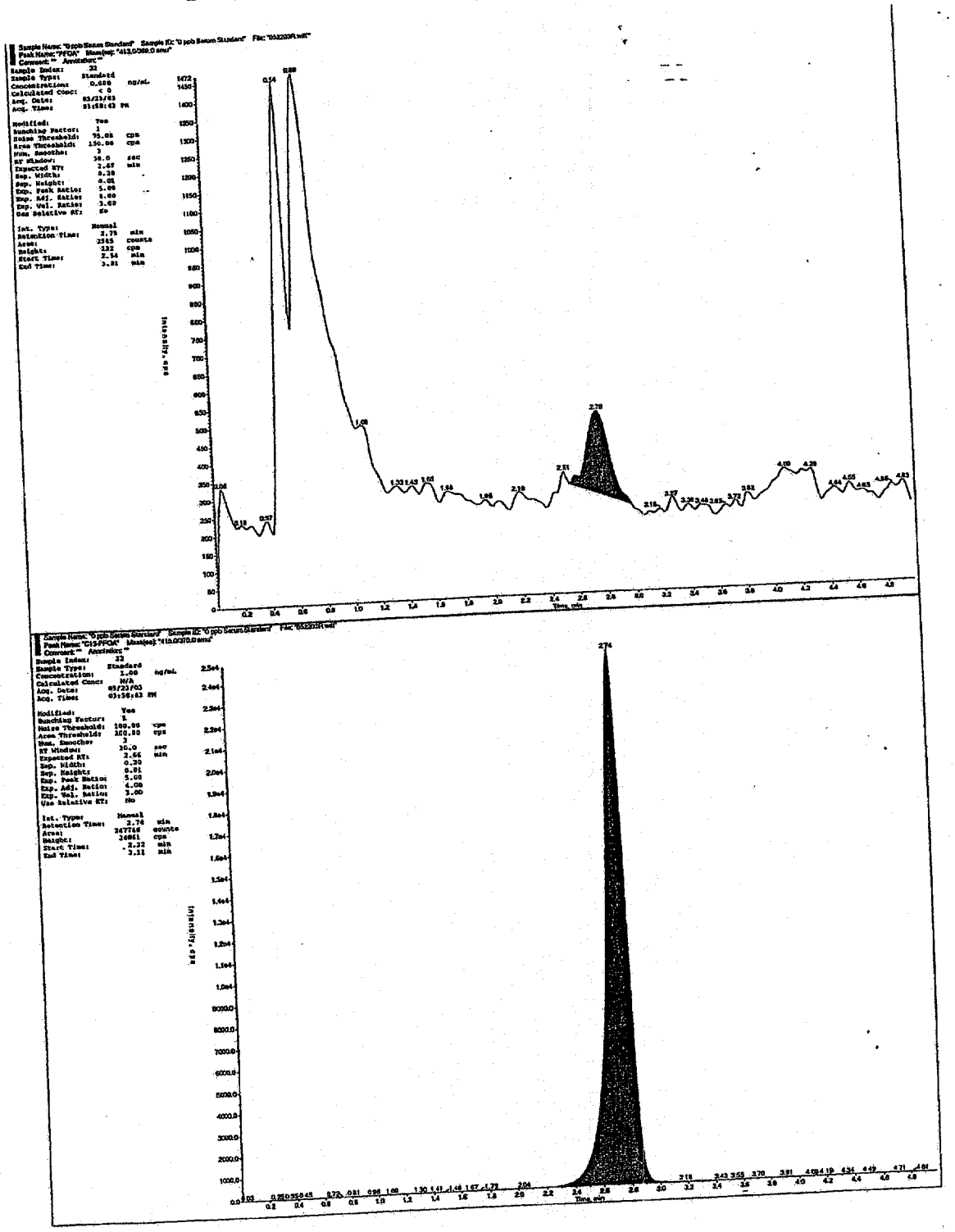


Figure 3. Representative Chromatogram of a 0.5 ppb Standard for PFOA in Control Rabbit Serum with ~ 1 ng/mL of ^{13}C-PFOA

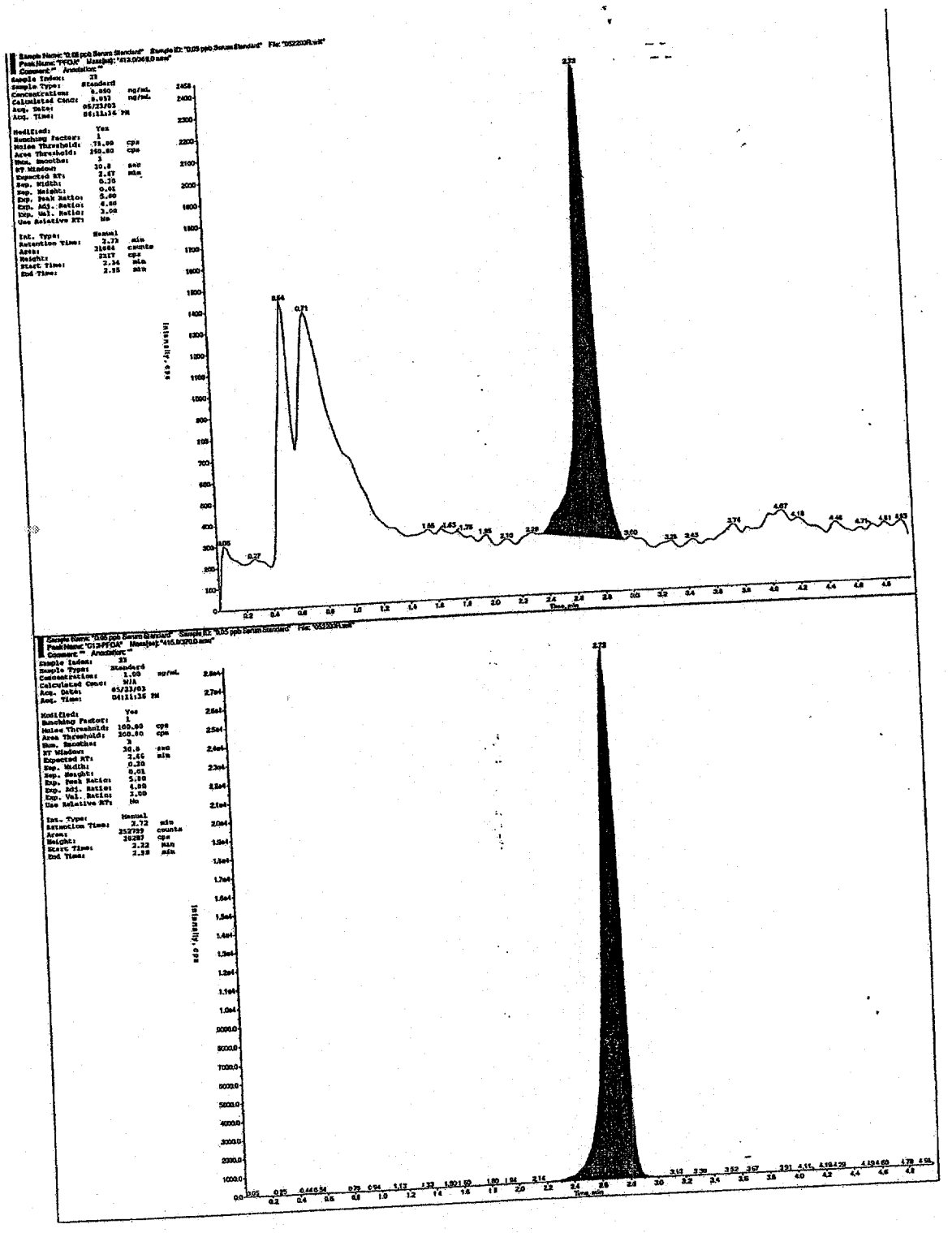


Figure 4. Representative Chromatogram of Control Rabbit Serum Fortified at 0.5 ppb with PFOA and with ~ 1 ng/mL of ^{13}C-PFOA

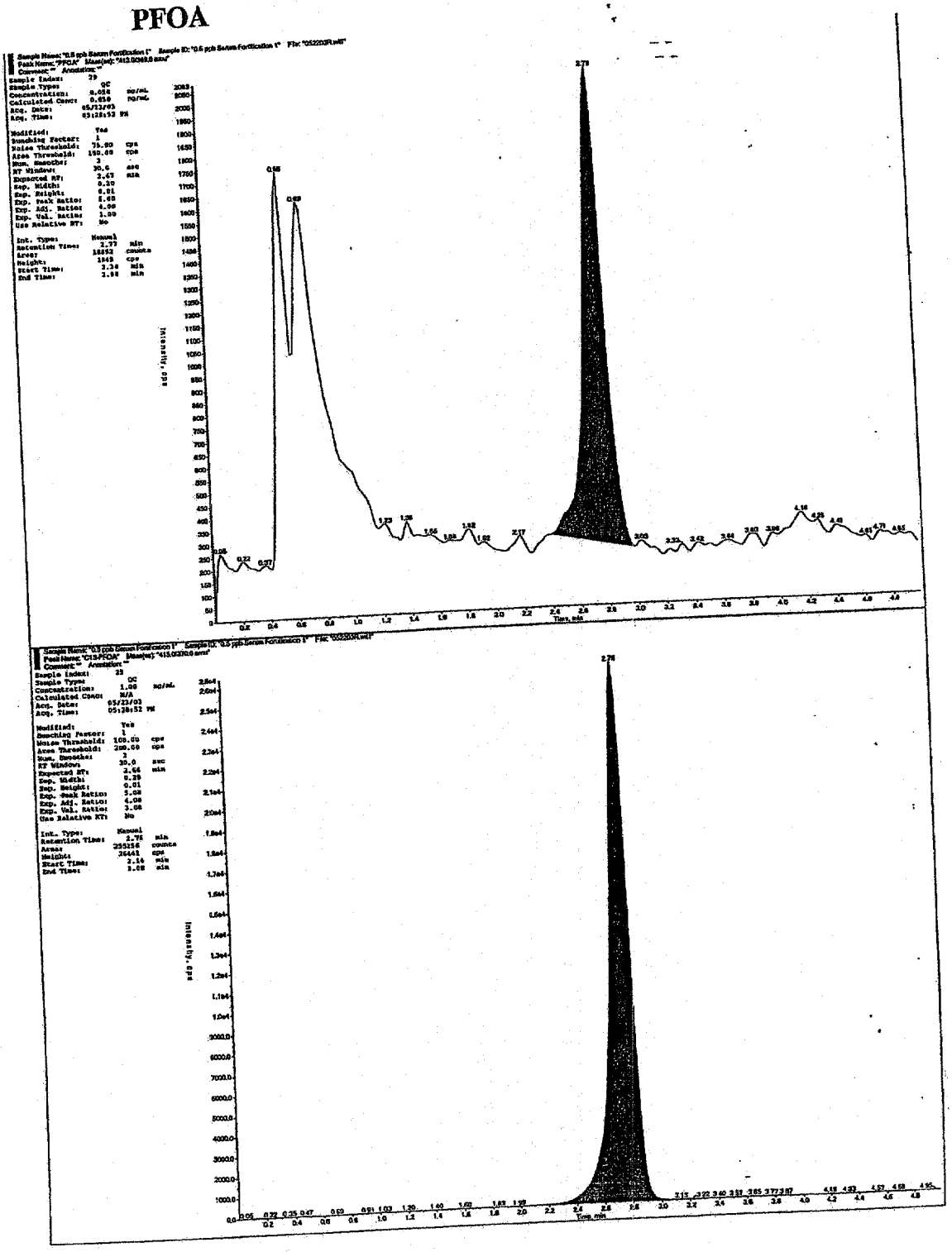


Figure 5. Calibration curve for PFOA in Control Rabbit Plasma

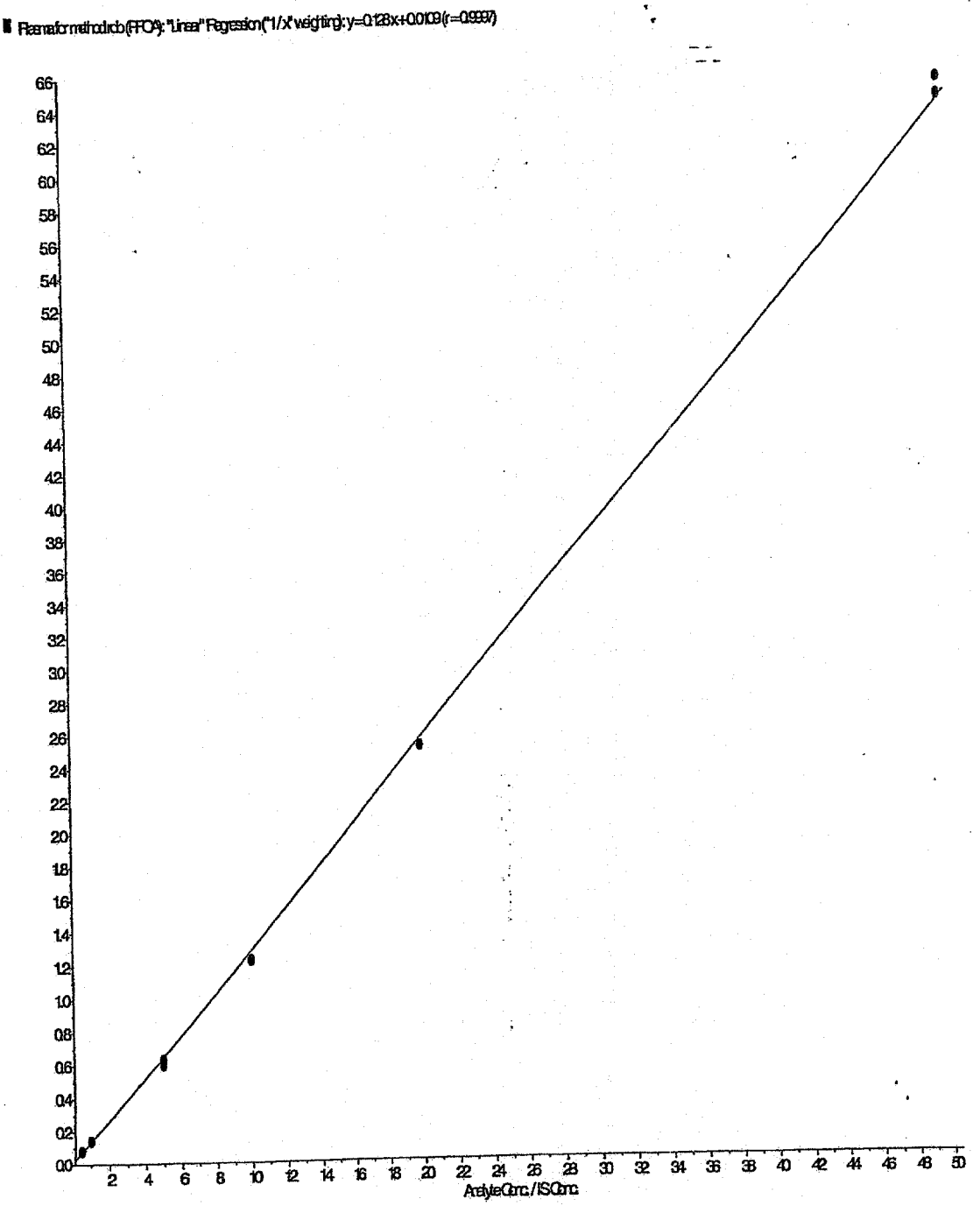


Figure 6. Representative Chromatogram of a Control Rabbit Plasma Sample for PFOA with 1 ng/mL of ^{13}C-PFOA

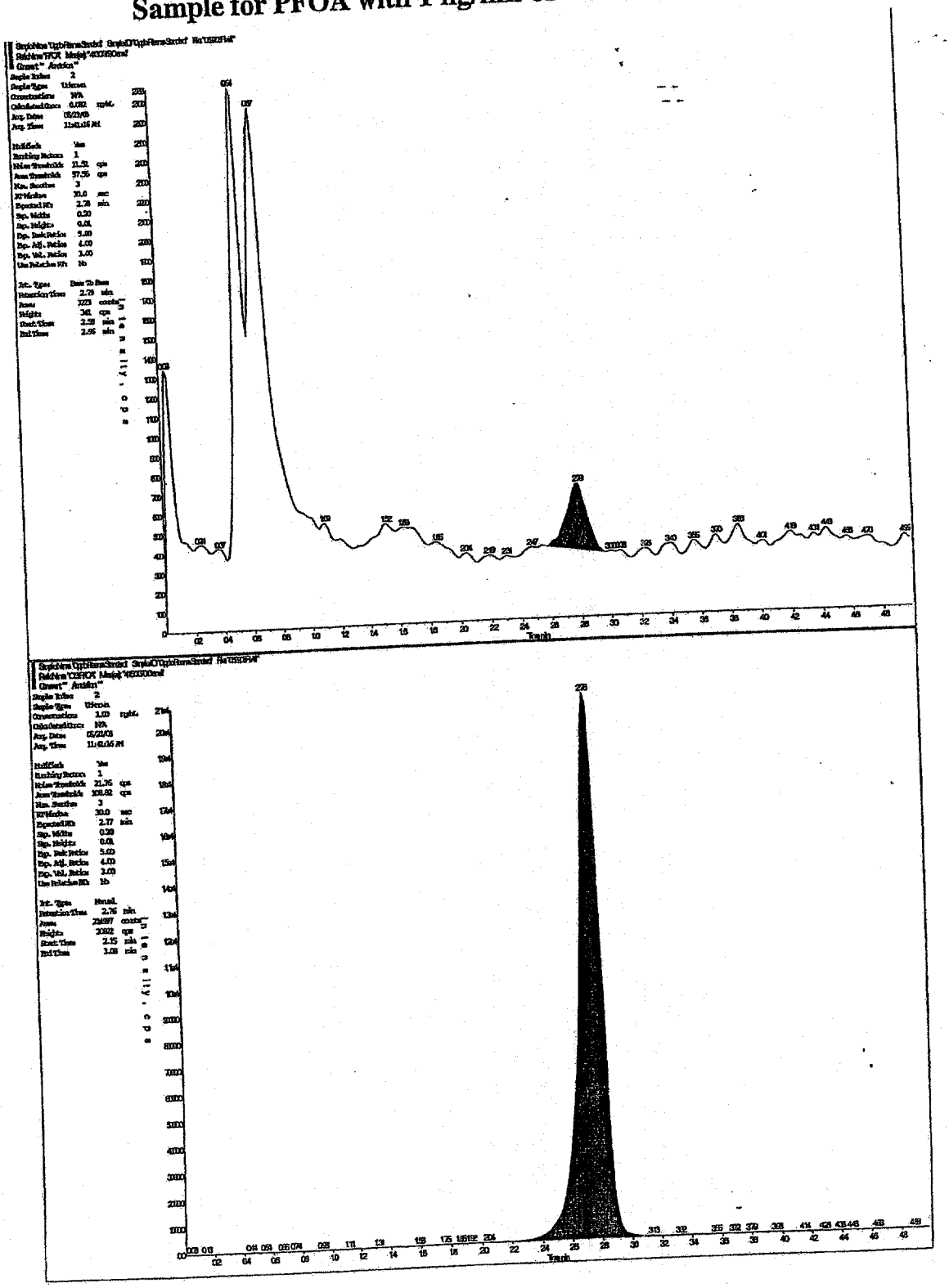


Figure 7. Representative Chromatogram of a 0.5 ppb Standard for PFOA in Control Rabbit Plasma with ~ 1 ng/mL of ^{13}C-PFOA

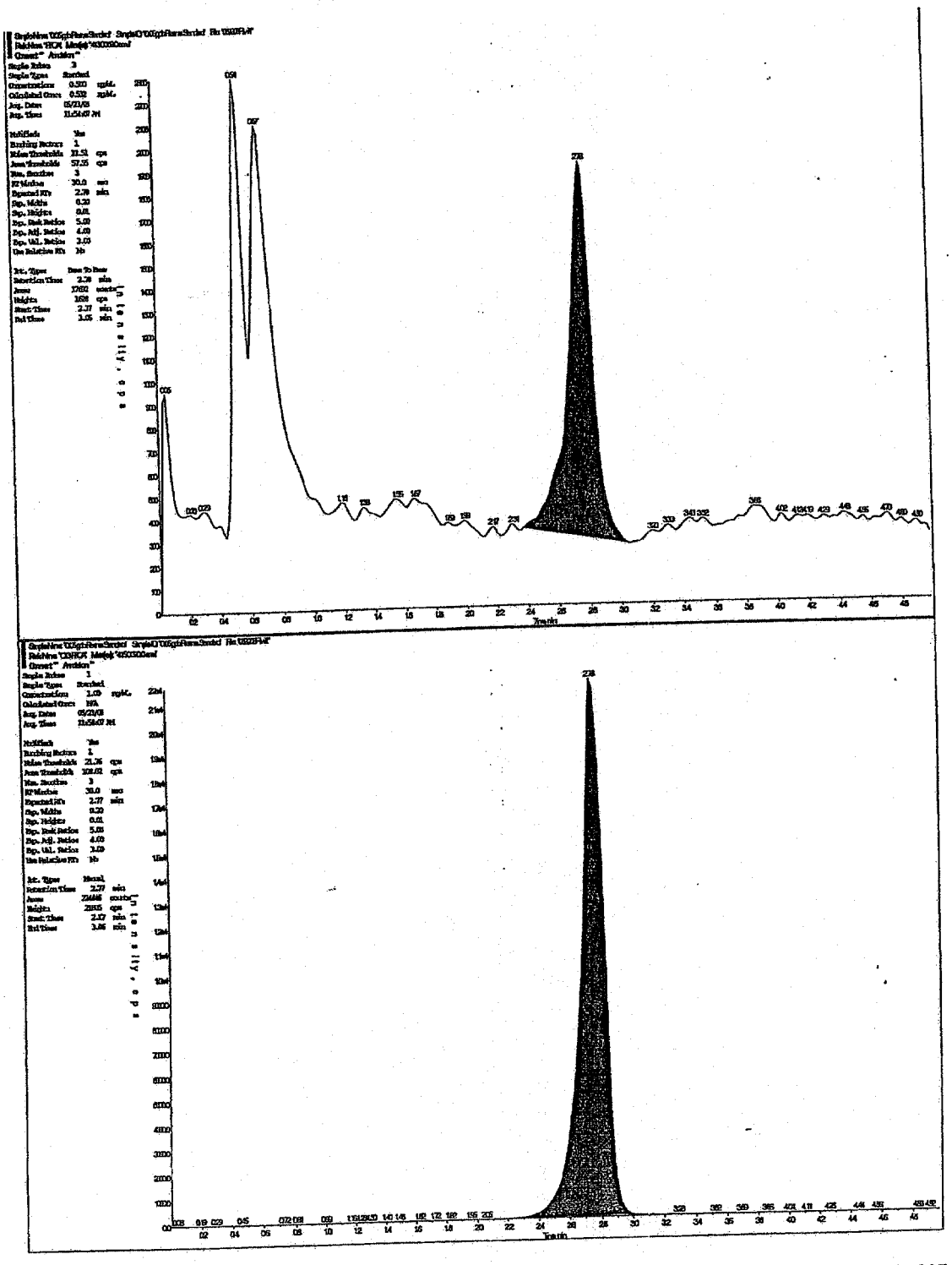
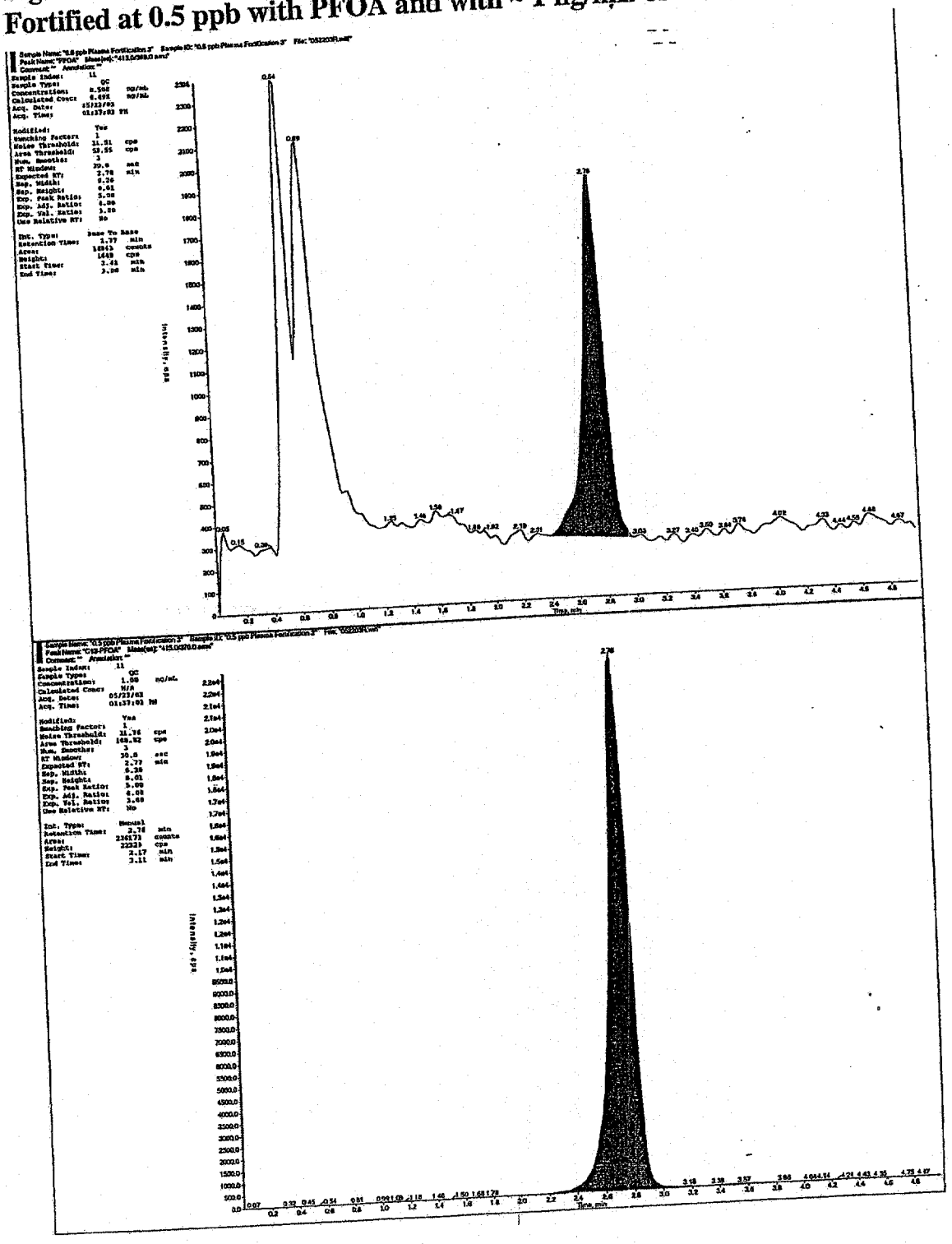


Figure 8. Representative Chromatogram of Control Rabbit Plasma Fortified at 0.5 ppb with PFOA and with ~ 1 ng/mL of ^{13}C-PFOA



December 4, 2003

Mary A. Kaiser, Ph.D.
Research Fellow
Corporate Center for Analytical Services
Dupont Central Research & Development
DUPONT
Experimental Station
P.O. Box 80302
Wilmington, DE 19880-0302

Clayton Work Order No. 03010080
Clayton Proposal No. 03DETLAB015.REV1

Dear Dr. Kaiser:

We are pleased to provide the following **Draft** report on the sampling and analytical method conditions followed for the method validation for [redacted]. This draft report is based on our proposal dated July 18, 2003.

The sampling parameters may change as we progress with the method validation. Not all of the parameters outlined in this report have been fully validated.

If you have any questions, please call me at (248) 344-1770.

Sincerely,

Kem Charron
Program Manager-Special Projects
Laboratory Services

KC
Enclosure

Clayton Group Services, Inc.

[redacted]

Empirical Formula: [redacted]
Molecular Weight: [redacted]
CAS Number: [redacted]

Clayton Work Order No.: 03070834
Draft Report Date: December 4, 2003

OSHA: None
NIOSH: None
ACGIH: None
AEL: 0.56 ppb

Properties
Appearance: White Powder
Odor:
Melting Point: [redacted]

Synonyms: Pentadecafluorooctanoic acid

Sampling and Storage

Sampler: Osha Versatile Sampler SKC Cat. No. 226-30-16

Storage: To be determined

Shipping: Samples should be shipped at **refrigerated** temperatures

Sample Stability: To be determined

Equipment

1. SKC OSHA Versatile Sampler (OVS) Cat. No. 226-30-16
2. HPLC System: LC pump with automated gradient controller, autosampler, optional tunable UV absorbance detector
6. Mass Spectrometer: Triple quadrupole mass spectrometer 2 – 1000 amu mass range minimum, API interface with Electrospray probe, negative and positive ionization mode capability, and data acquisition/reduction software
7. Column: Agilent Hypersil ODS, 2.1 × 100 mm, 3 µm particle size, Cat. # 79916OD-352 or equivalent
8. 16 mL amber glass vial with screw cap and Teflon/rubber liner
9. 4 mL amber glass vial with screw cap and Teflon/rubber liner
10. Waters total recovery glass autosampler vials with Teflon/rubber liner
12. Syringe: 10, 25, 50, & 100 µL
13. Disposable glass test tube, 16 x 125 mm with caps

Measurement

Technique: High Performance Liquid Chromatography (HPLC) coupled with Tandem Mass Spectrometry

Detectors: Triple Quadrupole Mass Spectrometer (Micromass Quattro LC, or equivalent)

Extraction: 4 mL of extraction solvent for 60 minutes

Instrument Conditions:

HPLC Conditions

Column: Agilent Hypersil ODS, 2.1 × 100 mm, 3 µm particle size

Column Temp: 30 °C
Flow Rate: 0.3 mL/minute
Injection Volume: 5 µL
Run Time: 14 minutes
Retention Time: 6.4 minutes

Mobile Phase: Gradient

Time (min)	%A	%B	Curve
0	90	10	-
5	10	90	-
9	10	90	-
10	90	10	-
14	90	10	-

A = 2 mM Ammonium Acetate
B = Methanol

Wavelength (UV): Not Applicable

Mass Spectrometer Conditions

Ionization Mode: Electrospray Negative

Nebulizer Gas (L/hr): 96
Desolvation Gas (L/hr): 690
Source Block Temperature (°C):120
Desolvation Temperature (°C):350
Collision Gas Pressure (mBar):3.1e-3
Multiplier: 650

	ES -Ion
Source Settings	
Capillary Voltage (kV):	1.5
Extractor (V):	1
RF Lens (V):	0.5
Analyzer Settings	
MS1 LM&HM Resolution:	14
MS1 Ion Energy (V):	0.5
MS2 LM&HM Resolution:	15
MS2 Ion Energy (V):	1.5
Entrance (V):	0
Exit (V):	0

Mass Spectrometer Method

MS Function: Multiple Reaction Monitoring (MRM) ESP-
Inter Channel Delay for MRM (s): 0.1
Span (Daltons): 0.5
Start time: 0 min End time: 14 min

Analyte Specific Settings:	Transition (amu)	Dwell (s)	Cone Voltage (V)	Collision Energy (eV)
[redacted]	412.9 – 368.9 (Q)	0.5	10	10
Internal Standard [redacted]	414.9 – 369.9 (Q)	0.5	10	10

(Q) = Transition used for quantitation

Sampling

1. Samplers (SKC 226-30-16) are received from the manufacturer (SKC).
2. Calibrate each sampling pump with a representative collection device in line.
3. Depending on the application, there are two flow rate options:
   (1) For area or personal monitoring, sample at a flow rate of 1.0 L/min for up to 480 minutes to obtain a sample volume of 960 L.
   (2) For fence line monitoring, sample at a flow rate of 15 L/min for up to 1440 minutes to obtain a sample volume of 21600 L.
4. Label the sample with appropriate sample identification.
5. At the completion of sampling, re-calibrate each pump with a representative collection device in line.
6. Cap the ends of each sampler.
7. Samples are stored at refrigerated temperatures.
8. A chain of custody form is filled out and sent with the samplers under refrigerated conditions to the Laboratory for analysis

Sample Preparation

Note: Samples are extracted just prior to analysis. Samples are stable for X days at X temperatures.

1. Extract each OVS sampling tube in three separate sections. Place each section in a separate disposable glass test tube.
   Section 1: GFF Filter
   Section 2: XAD-2 Front portion of media plus foam plug
   Section 3: XAD-2 Back portion of media
2. Add 4 mL of Methanol to each test tube and shake for 60 minutes on a mechanical shaker.
3. Transfer approximately 3.5 mL of each extract to a 4 mL amber glass vial by filtering through a Gelman 13-mm GHP Acrodisc. Extracts should be stored refrigerated.
4. For LC/MS/MS analysis, transfer 200 µL each sample extract to a glass autosampler vial. Add 10 µL of a 1.4 µg/mL Internal Standard solution ([redacted]).

Calibration and Quality Control

1. Calibrate daily with six working standards over the range of 11.4 to 227 ng/mL for [redacted].
   a) Calibration standards are prepared by transferring 200 µL each [redacted] standard solution to a glass autosampler vial. Add 10 µL of a 1.4 µg/mL Internal Standard solution ([redacted]).

b) Analyze standards together with samples and blanks. A calibration curve is analyzed at the beginning and end of each run, and Continuing Calibration Verification (CCV) standards are analyzed a minimum of every ten samples. Acceptance criteria for a CCV is ± 20%.

c) Generate an internal standard, linear calibration curve from the observed peak area response factors and standard concentrations.

2. Determine the Extraction Efficiency (EE) at least once with each lot of samplers. Prepare at least one level in duplicate within the expected sampling range. Include one media blank per lot.

a) Inject µL amounts of [redacted] spiking standard solution onto the GFF portion of an OVS tube.

b) Allow the tube to air-dry at ambient temperature.

c) Cap the tube and store at **refrigerated** temperatures until extraction.

d) Extract the [redacted]-spiked and blank tubes as described in Sample Preparation. Analyze an aliquot from each sample along with standards. Transfer 200 µL each extract solution to a glass autosampler vial. Add 10 µL of a 1.4 µg/mL Internal Standard solution [redacted].

e) Calculate the ratio for the ng of [redacted] found on the media spike to the theoretical amount spiked. The concentrations found in each of the three sections must be summed to obtain the total amount of [redacted] found.

The ratio reflects the recovery of [redacted] from the media. The mean recovery of the replicates equals the EE for [redacted].

Measurement

1. Set the liquid chromatograph and mass spectrometer conditions in accordance with those specified on page 2, or with suitable adjustments to optimize chromatography and sensitivity.

2. Inject the specified aliquot of each standard and sample.

3. Record the peak area response factor for [redacted] and Internal Standard peaks. The observed retention time is 6.4 minutes for both [redacted] and Internal Standard.