

Corporate Health Physics
Corporate Occupational Medicine
Corporate Product Responsibility
Corporate Toxicology
3M Medical Department

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Determination of Serum Elimination Half-Lives of Ammonium Perfluorooctanoate, Perfluorooctane Sulfonic Acid, Perfluorohexane sulfonic acid and Total Organic Fluorine in Decatur Chemical Plant Retirees

The objective of this study is quantitate the human serum elimination half life of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), perfluorohexanesulfonate (PFOS) and total organic fluorine (TOF). Blood will be collected biannually from approximately 25 retired 3M Decatur employees for a period of 5 years. Upon collection and analysis of the third sample (baseline, 6 months and then 12 months) and thereafter, an interim draft half-life analysis report will be prepared.

PROTOCOL
Epidemiology,220-3W-05
Medical Department
3M Company
St. Paul, MN 55144

Date: October 14, 1998

Title: DETERMINATION OF SERUM ELIMINATION HALF-LIVES OF AMMONIUM
PERFLUOROOCTANOATE, PERFLUOROOCTANE SULFONIC ACID,
PERFLUOROHEXANE SULFONIC ACID AND TOTAL ORGANIC FLUORINE IN 3M
DECATUR CHEMICAL PLANT RETIREES.

Study	Protocol Number # 0007
Start Date:	IRB Approval # 98095
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Study Director::	Jeffrey Mandel, MD, MPH ¹
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Study Sponsor:	Medical Department 3M Company 220-3W-05 Saint Paul, MN 55144
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ABSTRACT

3M manufactures products which contain chemical compounds, either as intentional components or residual impurities, that have as a parent molecule, perfluorooctane sulfonyl fluoride (POSF). These compounds may be expected to transform metabolically, to an undetermined degree, to PFOS as an end-stage metabolite. Other compounds manufactured by 3M include ammonium perfluorooctanoate (PFOA) and perfluorohexane sulfonic acid (PFHS). These molecules enter a number of product applications (e.g., surfactants, food packaging additives, polymers).

The biological elimination rates of fluorochemicals have been studied in rats, pregnant rats, female rabbits and hamsters. Biological half-lives have been calculated from the studies in rats and dogs. In addition, the half-life of serum total organic fluorine in a fluorochemical worker was estimated using serial collections of serum total organic fluorine. These studies reveal that PFOS and PFOA may persist in the body for prolonged periods, and with continuing exposure, accumulate over time in biological systems. To date, the actual elimination half-life of perfluorooctane sulfonate (PFOS), ammonium perfluorooctanoate (PFOA), perfluorohexane sulfonic acid (PFHS), and total organic fluorine (TOF) in human serum is not completely understood. The objectives of this study are to quantitate the elimination half life in human serum of PFOA, PFOS, PFHS, and TOF. These half -lives will be calculated from declines in serum fluorochemical levels in 3M Decatur Chemical Plant Retirees.

INTRODUCTION

3M manufactures products which contain chemical compounds, either as intentional components or residual impurities, that have as a parent molecule, perfluorooctane sulfonyl fluoride (POSF). These chemicals include: perfluorooctane sulfonate (PFOS), N-ethyl perfluorooctanesulfonamide, N-ethyl perfluorooctanesulfonamido ethanol, N-methyl perfluorooctanesulfonamido ethanol and chemicals derived from it, and the mixture of mono-, di- and tri [N-ethyl perfluorooctane sulfonamidoethyl] phosphates. There may be other precursors in the workplace, including perfluorohexanesulfonyl fluoride (PHSF). These molecules enter a number of product applications (e.g., surfactants, food packaging additives, polymers). These compounds may be expected to transform metabolically, to an undetermined degree, to PFOS (and PFHS) as an end-stage metabolite. Potassium perfluorooctane sulfonate ($C_8F_{17}OSO_2K^+$) is itself, a surfactant used as a wetting and foaming agent in industrial and commercial processes. (Olsen, et al., 1998)

Subchronic studies in rats and primates suggest there may be a potential for cumulative toxicity with PFOS over time with the primary effect related to metabolic wasting. These molecules have long elimination half lives in biological systems. Although the mechanism of toxicity is not fully understood, toxicity may be due to an effect on peroxisome proliferation, fatty acid metabolism, membrane function, protein synthesis and/or mitochondrial bioenergetics. (Olsen, et al., 1998)

The biological elimination rates of fluorochemicals have been studied in rats, pregnant rats, female rabbits and hamsters. Biological half-lives have been calculated from the studies in rats and dogs. In addition, the half-life of serum total organic fluorine in a fluorochemical worker

was estimated using serial collections of serum total organic fluorine. These studies reveal that PFOS and PFOA may persist in the body for prolonged periods, and with continuing exposure, accumulate over time in biological systems. To date, the actual elimination half-life of perfluorooctane sulfonate (PFOS), ammonium perfluorooctanoate (PFOA), perfluorohexane sulfonic acid (PFHS), and total organic fluorine (TOF) in human serum is not completely understood.

The objectives of this study are to quantitate the elimination half life in human serum of PFOA, PFOS, PFHS, and TOF; these half lives will be calculated from declines in serum fluorochemical levels in 3M Decatur Chemical Plant Retirees. This information is necessary in order to: 1) plan the most appropriate periodicity of medical surveillance and 2) guide medical decision making of workers from fluorochemical production facilities when those workers' serum fluorochemical levels are determined to be unsafely elevated. A benefit of this research will be to understand the natural history of fluorochemical excretion.

LITERATURE REVIEW

AMMONIUM PERFLUOROOCTANOATE (PFOA or FC-143)

Excretion rates of PFOA have been observed in rats, and found to be different by gender and route of excretion. Following single i.v. doses of ^{14}C -FC-143 (carbonyl carbon labeled) in rats, Johnson et al, found that females excreted virtually all the administered ^{14}C within 1 day. Urinary excretion for males was about 50% of the dose by day 6 and 83% by day 36. Fecal ^{14}C excretion for females was 1.5% by 3 days and for males was 5.4% by 36 days. (Johnson, et al.,

1980) Rapid urinary excretion of ^{14}C following oral doses of ^{14}C -FC-143 was also shown to occur in pregnant rats. (Johnson, et al., 1983)

Excretion rates vary by species studied. Excretion of radiolabeled PFOA was studied in four species by Dupont in 1988. Excretion as a percentage of administered dose 120 hours after dosing was in the following order; female rat, male and female rabbit and male hamster (> 99%); female hamster (60%); male rat (39%); male and female mice (21%). (Dupont, 1988) Of note, the administered dose and routes of exposure and excretion were not specified in this study report.

Rats and dogs respond to pharmacologic interventions that change PFOA excretion rate. In male rats administered single i.v. doses of ^{14}C -FC-143 (carbonyl carbon labeled) cholestyramine (4% w/w in feed) increased cumulative 15 day fecal ^{14}C excretion 9.8-fold versus controls. Total ^{14}C excretion (feces plus urine) was also enhanced, although less dramatically (84.3% of dose vs. 71.8% for controls). (Johnson, et al., 1980a; Johnson JD, et al., 1984) There is no difference between the renal clearances of ^{14}C in male and female dogs either before or after probenecid. Probenecid significantly reduces PFOA clearance in dogs. Glomerular filtration rates of PFOA were similar in rats and dogs. (Hanhijarvi, et al., 1988)

Elimination half-life has been calculated under numerous conditions in rats and dogs. The results show the same differential elimination rate in rats by gender (i.e., female > male), as well as half-life differences by route of exposure and species. Following a single oral dose of ^{14}C -FC-143 (carbonyl carbon labeled) in male rats, the plasma half-life was 4.8 days. (Johnson, et al., 1979)

In female rats administered oral or i.v. doses of ^{14}C -FC-143 (carbonyl carbon labeled), over 90% of the administered dose was recovered in the urine within the first 12 hours. The whole body elimination half-life of PFOA in male and female rats was 15 days and less than one day, respectively, following a single 4-mg/kg intraperitoneal dose. (Vanden Heuvel, et al., 1991) (Johnson, et al., 1983; Johnson, et al., 1980) The half-life of PFOA in the liver was 60 hours for females and 210 hours for male rats. (Ylinen, et al., 1990) The decreased excretion rate (i.e., increased elimination half-life) in males is seen across at least two species: rats (as above) and dogs. The plasma half-life of PFOA was longer in male dogs (473 to 541 hours) than in females (202 to 305 hours). (Hanhijarvi, et al., 1988)

The elimination half-life appears to be similar in male rats exposed to either inhalational or dermal exposure. Following repeated inhalation exposures to FC-143 over a two-week period, blood organic fluoride levels in male rats showed a half-life of five to seven days. (Kennedy, et al., 1986) A blood half-life of five to seven days was seen following repeated dermal exposures in male rats. (Kennedy, 1985)

A calculation of the elimination half-life of an organic fluorine species has been reported in the human. The half-life of total serum organic fluorine in a fluorochemical worker who was removed from further exposure was greater than 18 months. This worker had a blood organic fluorine level of 40 parts per million over the period of one year. The worker's organic fluorine level rose to 70 parts per million for no apparent reason. The worker was then removed from the fluorochemical production area and his blood and urine samples were periodically checked for organic fluorine and PFOA over an 18-month period. A total of 18 months transpired before the

worker's blood organic fluorine level had returned to its base line of 40 parts per million. (Ubel, et al. 1980) Other estimates of serum fluorochemical half-lives have been calculated in the one thousand day range. ^ψ This anecdotal account does not actually track the serum PFOA level, but rather the serum total organic fluorine (TOF) level. It was found through empirical means that 90% of the blood organic fluorine existed as PFOA. (Ubel, et al. 1980)

PERFLUOROOCTANE SULFONIC ACID (PFOS or FC-95)

Perfluorooctane sulfonic acid appears to persist in biological systems, being excreted more slowly than PFOA. Single i.v. doses (mean 4.2 mg/kg) of ¹⁴C-FC-95 and 0.9% NaCl were administered to male rats. By 89 days after dosing, 30.2% of the administered ¹⁴C had been excreted in the urine and 12.6% had been excreted in the feces. (Johnson, et al., 1979)

Pharmacologic interventions do appear to increase the excretory rate of PFOS. Fecal and total excretion of ¹⁴C were markedly increased in male rats administered Cholestyramine (about 2.7 gm/kg per day) and their diet following single i.v. doses of ¹⁴C-FC-95. The results suggest that there was significant enterohepatic circulation of FC-95. (Johnson, et al., 1980a; Johnson, et al., 1984)

Much less is known concerning elimination half-life of PFOS than PFOA; only one calculation of the elimination half-life of PFOS has been attempted. The plasma elimination half-life of ¹⁴C following single oral administration of ¹⁴C-FC-95 (mean dose 4.2 mg/kg) to male rats was 7.5 days. (Johnson, et al. 1979a)

Ψ Mandel, J. Personal communication.

RESEARCH METHODS

The overall research design is prospective in nature, obtaining multiple serial blood samples from retirees throughout the course of a five-year period. We will track the decline in several serum fluorochemical levels in humans. The health outcomes that will be documented in this study are the half-life determinations of serum ammonium perfluorooctanoate (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorohexane sulfonic acid (PFHS), and serum total organic fluorine (TOF).

High-performance liquid chromatography mass spectrometry/mass spectrometry will be utilized to analyze all serum samples. The accuracy and reliability of this device will be the state of the art at the time of analysis. Periodically, split samples from one or two subjects will be utilized to assess reliability of the analysis.

Only individuals who have retired from 3M Decatur chemical plant from the 1st of January 1995 through the 1st of January 1998, will be included in the study. Using information received from the Human Resources Department of 3M Decatur, 34 individuals were identified to have retired during this time period. Of the 34 individuals identified, 7 have previously been enrolled in the fluorochemical medical surveillance program. Four of these individuals participated in the medical surveillance program in 1994 but not 1997; 3 individuals participated in 1997 only. We will attempt to enroll all 34 retirees into the study. Future retirees will not be enrolled.

Serum fluorochemical levels will be drawn every six months. All serum fluorochemical levels will be drawn within a one-month time frame (i.e., within one month of March and September).

Retirees will be recruited to the study through first, an introductory letter and second, a phone conversation from the principle investigator or his designee. Subsequent to the follow-up telephone conversation and if the retiree is willing to enter the study, a written informed consent form (Appendix A) and a medical questionnaire (Appendix B) will be sent to the retiree for his review. Once the retiree signs the consent form, completes the questionnaire, and returns the documents to the principle investigator, he will be entered into the study and scheduled for blood collection. Participation in the study is voluntary and retirees may drop out at any time.

Blood collection will be scheduled biannually, in April and October (to not conflict with Holiday and summer vacation travel). Letters announcing blood collection dates and times will be sent out to each study subject one month prior to each blood collection month. Retirees will update their medical questionnaire at each blood collection. All blood collections will be performed under the supervision of the 3M Decatur plant nurse, Cathy Simpson, RN. Two red-topped tubes will be obtained from each retiree; the 3M Occupational Medicine Service methodology for handling and shipping of fluorochemical blood samples will be applied to all blood samples. Samples will be analyzed by the 3M Environmental Laboratory or its designated contract laboratory. Retirees will receive written reports of their serum fluorochemical levels following each blood collection cycle.

Two methods of calculating the serum fluorochemical half life will be utilized. In the first method, a one compartment model will be assumed and the formula

$$q_t = q_0 (0.5)^{t/t_{1/2}}$$

will be utilized to estimate the half life of elimination for PFOS, PFOA, PFHS, and TOF. The terms q_t and q_0 refer to the serum concentrations at time t and time 0 , t is the elapsed time; $t_{1/2}$ is what we seek to solve.

The second method will be used to verify the first. It also assumes a one compartment model. Each retiree's fluorochemical levels will be graphed on a log-linear scale as serum fluorochemical vs. elapsed time from t_0 (in months) and entered into a database. Statistical analysis will be performed to determine the best-fit model that describes the slope of the log-linear line. (Appendix C)

The slope of the log-linear line is related to the elimination constant ($-k_{el}$) through the equation

$$Slope = -k_{el}(2.303)$$

Once the elimination constant is calculated, the half life can be calculated using the relationship

$$t_{1/2} = 0.693/k_{el}$$

Elimination half lives for TOF, PFOS, PFHS, and PFOA will be determined. (Benez, et al. 1985, Medinsky, et al. 1996.)

DISCUSSION

There are a number of limitations associated with the study, including:

1. The lack of pre-study serum fluorochemical levels in the majority of retirees, making it impossible to develop baseline levels for the majority of the study group. The t_0 values will therefore need to be their first fluorochemical level *post-retirement*.

2. Previous studies have performed serum total organic fluorine levels and not serum testing specific for serum PFOS or PFOA; comparability with previous work is thereby compromised.
3. Retirees on medications such as colestipol or cholestyramine, which can artificially increase the rate of elimination of fluorochemicals from the blood will artificially increase the elimination in unpredictable ways, thereby causing an underestimation of the elimination half life in the entire study population.
4. Retirees may unknowingly have medical conditions that will decrease their toxicant elimination rate unpredictably (e.g., renal failure, congestive heart failure, cholelithiasis, etc.), thereby causing an inaccurate estimation of the elimination half life in the entire study population.

For these reasons, the study will occur over the protracted period of five years instead of a shorter time period (i.e., three years). As the only human data plotting total organic fluorine suggest plasma elimination half-life of 18 months, a three year study would allow for verification of that estimate since three years would be equal to two half-lives. A five-year study, therefore, should include over three half-lives of elimination, and make it possible to use several years' data to calculate the elimination half life.

The overall results collected in this study may be used in publications or in public presentations. Retiree names or other individual data will not be revealed in any publication or other documentations intended for public examination. Individual results will be communicated only to the retiree. Individual results will be considered confidential information and will not be disclosed to anyone outside of the 3M Medical Department without the retiree's written consent.

Quality assurance will be performed per the guidelines set forth in the standard operating procedure for the creation, review, and approval of 3M epidemiology study protocols.

Archiving of study materials, upon completion of the study, will be performed per the standard operating procedure for the creation, auditing, review and approval of 3M epidemiology final reports.

The study will be communicated to the retirees, management, and the technical community.

Communications to the retirees will be in the form of a letter, which states the retiree's various blood fluorochemical levels. These letters will be sent out following each blood draw.

Communication to management will be in the form of a written document which will undergo the review process as set forth in the standard operating procedure for the creation, auditing, review and approval of 3M epidemiology final reports. The communication to the technical community will be in the form of a scientific paper, which will be submitted to a relevant peer reviewed scientific publication.

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Appendix A

CONSENT FORM FOR COLLECTION OF BLOOD FOR SERUM FLUORO-CHEMICAL LEVEL HALF-LIFE DETERMINATION

INTRODUCTION

You and your colleagues were involved in fluorochemical production at 3M Decatur prior to your retirement. You are invited to participate in a research study being conducted by 3M Occupational Medicine Services regarding fluorochemicals. Your participation would involve donating one red-topped test tube of blood approximately every six months for the next five years. Your blood will only be tested for the amount and kind of fluorochemicals it contains; it will not be used to test for any other substances or disease. Please review this consent form carefully and be sure your questions are answered before you make a decision to participate. The plant nurse will be available to provide you with additional information at the time of your medical surveillance blood donation.

PURPOSE OF STUDY

The purpose of the study is to determine how much and what types of fluorochemicals are eliminated from the human body over time.

STUDY PROCEDURES

Your blood will be drawn with one needle stick and require two test tubes. This will occur about every six months for the next five years. A letter will be sent to you in advance informing you of the proper date, time, and place to report to for blood collection.

POTENTIAL RISKS/BENEFITS

The only discomfort you may feel is from the needle stick. You may also have some temporary redness/bruising/swelling in this area after blood collection.

BENEFITS

There will be no direct benefit from your participation in this study. However, the information gained from this study will further help us understand human exposures to fluorochemicals. Your individual results as well as the overall results will be communicated to you only. Your individual results will be considered confidential information and will not be disclosed to anyone outside the 3M Medical Department without your written consent.

COMPENSATION

If you suffer injury or a medical condition that appears to be the result of participating in this study, you will be referred to another health care professional at no cost to you. In the event of a research related injury, compensation will be determined on a case by case basis by 3M.

CONFIDENTIALITY

The overall results collected in this study may be used in publications or public presentation. Your name will not be revealed in any publication or other documents intended for publication examination. Your individual results will be communicated to you only. Your individual results will be considered confidential information and will not be disclosed to anyone outside the 3M Medical Department without your written consent.

SUBJECT RIGHTS/AVAILABILITY OF INFORMATION

If you have any questions about the study now, or later, or in the event of a research related injury or emergency, contact Dr. Jeffrey Mandel (651-733-8670) or Jean Burris, R.N. (651-737-7867). For answers to questions about your rights in regard to this research, you may contact Dr. Larry Zobel, Chair, 3M Institutional Review Board at 651-733-5181.

VOLUNTARY PARTICIPATION AND WITHDRAWAL

Participation in this study is voluntary. Refusal to participate will involve no penalty of loss of benefits to which you are otherwise entitled. You are free to withdraw from the study at any time for any reason. The investigator may stop your participation in this study should it be determined that continued participation may be detrimental to your health. Following each semi-annual blood collection, you will receive a check for \$50.00 as compensation for your time and effort.

SUBJECT CONSENT

By signing the consent form, I certify that I am at least 18 years old. I confirm that I have read this consent form, and that I have been given adequate opportunity to ask any questions I may have about this consent form or the study. I also confirm that I understand the scope of my participation in this study, and that all of my questions have been answered to my satisfaction. I am signing this consent form voluntarily, and I desire to participate in the study. I understand that I will receive a copy of this signed consent form. I understand that I will receive a check for \$50.00 immediately following each semi-annual collection of my blood.

Signature

Date

Printed Name

Witness

Appendix B

MEDICAL HISTORY QUESTIONNAIRE

Please list the names of the **ALL** the medications you take at least once a day.

Please circle the conditions you now suffer from or have suffered from in the last three years.

Kidney Failure

Gall Bladder Disease

Hepatitis/Jaundice

Congestive Heart Failure

Liver Disease

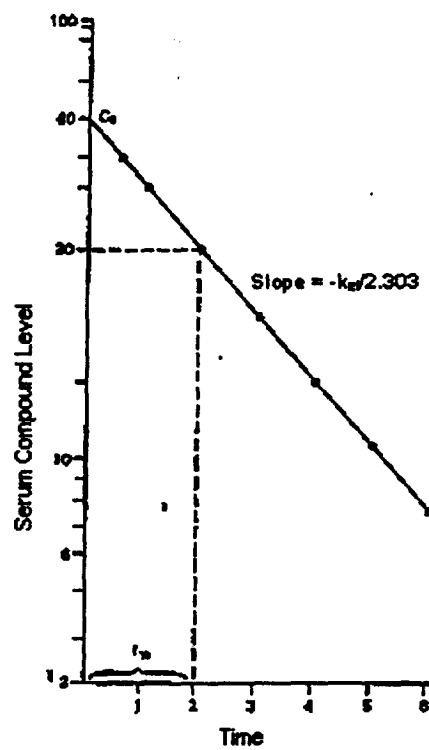
Inflammatory Bowel Disease

Crohn's Disease

Pernicious Anemia

Appendix C

Example of a log-linear plot that will be used to estimate the serum fluorochemical elimination half life in Decatur retirees.



3M ENVIRONMENTAL LABORATORY

METHOD

EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUORO-CHEMICAL COMPOUNDS FROM SERUM FOR ANALYSIS USING HPLC- ELECTROSPRAY/MASS SPECTROMETRY

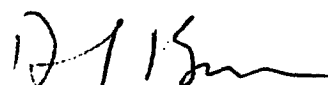
Method Number: ETS-8-4.1

Adoption Date: 03/01/99

Revision Date: 4/27/99

Author: Lisa Clemen, Glenn Langenburg

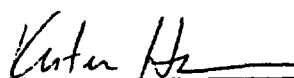
Approved By:



Laboratory Manager

4/27/99

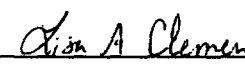
Date



Group Leader

4/26/99

Date



Technical Reviewer

04/26/99

Date

1.0 SCOPE AND APPLICATION

- 1.1 **Scope:** This method is for the extraction of potassium perfluorooctanesulfonate (PFOS) or other fluorochemical compounds from serum.
- 1.2 **Applicable compounds:** Fluorochemical surfactants or other fluorinated compounds.
- 1.3 **Matrices:** Rabbit, rat, bovine, monkey, and human serum or other fluids as designated in the validation report.

2.0 SUMMARY OF METHOD

- 2.1 This method describes the procedure for extracting potassium perfluorooctanesulfonate (PFOS) or other fluorochemical surfactants from serum, or other fluids, using an ion pairing reagent and methyl-*tert*-butyl ether (MtBE). In this method, seven fluorochemicals were extracted: PFOS, PFOSA, PFOSAA, EtFOSE-OH, PFOSEA, M556, and surrogate standard (see 3.0 *Definitions*). An ion pairing reagent is added to the sample and the analyte ion pair is partitioned into MtBE. The MtBE extract is removed and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 1.0 mL of methanol, then filtered through a 3 cc plastic syringe attached to a 0.2 μ m nylon filter into glass autovials.
- 2.2 These sample extracts are analyzed following method ETS-8-5.1 or other appropriate methods.

3.0 DEFINITIONS

- 3.1 PFOS: perfluorooctanesulfonate (anion of potassium salt) $C_8F_{17}SO_3^-$
- 3.2 PFOSA: perfluorooctane sulfonylamide $C_8F_{17}SO_2NH_2$
- 3.3 PFOSAA: perfluorooctane sulfonylamido (ethyl)acetate $C_8F_{17}SO_2N(CH_2CH_3)CH_2CO_2^-$
- 3.4 EtFOSE-OH: 2(N-ethylperfluorooctane sulfonamido)-ethyl alcohol
 $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$
- 3.5 PFOSEA: perfluorooctane sulfonyl ethylamide $C_8F_{17}SO_2N(CH_2CH_3)H$
- 3.6 M556: $C_8F_{17}SO_2N(H)(CH_2COOH)$
- 3.7 Surrogate standard: 1H-1H-2H-2H perfluorooctane sulfonic acid

4.0 WARNINGS AND CAUTIONS

4.1 Health and safety warnings

- 4.1.1 Use universal precautions, especially laboratory coats, goggles, and gloves when handling animal tissue, which may contain pathogens.

5.0 INTERFERENCES

- 5.1 There are no interferences known at this time.

6.0 EQUIPMENT

- 6.1 The following equipment is used while performing this method. Equivalent equipment is acceptable.
- 6.1.1 Vortex mixer, VWR, Vortex Genie 2
- 6.1.2 Centrifuge, Mistral 1000 or IEC
- 6.1.3 Shaker, Eberbach or VWR

6.1.4 Nitrogen evaporator, Organomation

6.1.5 Balance (± 0.100 g)

7.0 SUPPLIES AND MATERIALS

- 7.1 Gloves
- 7.2 Eppendorf or disposable pipettes
- 7.3 Nalgene bottles, capable of holding 250 mL and 1 L
- 7.4 Volumetric flasks, glass, type A
- 7.5 I-CHEM vials, glass, 40 mL glass
- 7.6 Centrifuge tubes, polypropylene, 15 mL
- 7.7 Labels
- 7.8 Oxford Dispenser – 3.0 to 10.0 mL
- 7.9 Syringes, capable of measuring 5 μ L to 50 μ L
- 7.10 Graduated pipettes
- 7.11 Syringes, disposable plastic, 3 cc
- 7.12 Syringe filters, nylon, 0.2 μ m, 25 mm
- 7.13 Timer
- 7.14 Crimp cap autovials and caps
- 7.15 Crimpers

Note: Prior to using glassware and bottles, rinse 3 times with methanol and 3 times with Milli-Q™ water. Rinse syringes a minimum of 9 times with methanol, 3 rinses from 3 separate vials.

8.0 REAGENTS AND STANDARDS

- 8.1 Type I reagent grade water, Milli-Q™ or equivalent; all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus™ system
- 8.2 Sodium hydroxide (NaOH), J.T Baker or equivalent
- 8.3 Tetrabutylammonium hydrogen sulfate(TBA), Kodak or equivalent
- 8.4 Sodium carbonate (Na₂CO₃), J.T. Baker or equivalent
- 8.5 Sodium bicarbonate (NaHCO₃), J.T. Baker or equivalent
- 8.6 Methyl-T-Butyl Ether, Omnisolv, glass distilled or HPLC grade
- 8.7 Methanol, Omnisolv, glass distilled or HPLC grade
- 8.8 Serum or blood, frozen from supplier
- 8.9 **Fluorochemical standards**
 - 8.9.1 PFOS (3M Specialty Chemical Division), molecular weight = 538
 - 8.9.2 PFOSA (3M Specialty Chemical Division), molecular weight = 499

- 8.9.3 PFOSAA (3M Specialty Chemical Division), molecular weight = 585
- 8.9.4 EtFOSE-OH (3M Specialty Chemical Division), molecular weight = 570
- 8.9.5 PFOSEA (3M Specialty Chemical Division), molecular weight = 527
- 8.9.6 M556 (3M Specialty Chemical Division), molecular weight = 557
- 8.9.7 Surrogate standard: 4-H, perfluorooctane sulfonic acid (1-H, 1-H, 2-H, 2-H $C_8F_{13}SO_3H$) molecular weight = 428
- 8.9.8 Other fluorochemicals, as appropriate

8.10 Reagent preparation

NOTE: When preparing larger volumes than listed in reagent, standard, or surrogate preparation, adjust accordingly.

- 8.10.1 10 N sodium hydroxide (NaOH): Weigh approximately 200 g NaOH. Pour into a 1000 mL beaker containing 500 mL Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.
- 8.10.2 1 N sodium hydroxide (NaOH): Dilute 10 N NaOH 1:10. Measure 10 mL of 10 N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 mL Nalgene bottle.
- 8.10.3 0.5 M tetrabutylammonium hydrogen sulfate (TBA): Weigh approximately 169 g of TBA into a 1 L volumetric containing 500 mL Milli-Q™ water. Adjust to pH 10 using approximately 44 to 54 mL of 10 N NaOH (While adding the last mL of NaOH, add slowly because the pH changes abruptly). Dilute to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.
 - 8.10.3.1 TBA requires a check prior to each use to ensure pH = 10. Adjust as needed using 1 N NaOH solution.
- 8.10.4 0.25 M sodium carbonate/sodium bicarbonate buffer ($Na_2CO_3/NaHCO_3$): Weigh approximately 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

8.11 Standards preparation

- 8.11.1 Prepare PFOS standards for the standard curve.
- 8.11.2 Prepare other fluorochemical standards, as appropriate. Multicomponent fluorochemical standards are acceptable (for example, one working standard solution containing 1.00 ppm PFOS, 1.02 ppm PFOSA, 0.987 ppm PFOSAA, and 1.10 ppm EtFOSE-OH.)
- 8.11.3 Weigh approximately 100 mg of PFOS into a 100 mL volumetric flask and record the actual weight.
- 8.11.4 Bring to volume with methanol for a stock standard of approximately 1000 ppm ($\mu g/mL$).
- 8.11.5 Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm.
- 8.11.6 Dilute working standard 1 with methanol for a working standard 2 solution of approx. 5.0 ppm.

8.11.7 Dilute working standard 1 with methanol for a working standard 3 solution of approx. 0.50 ppm.

8.12 Surrogate stock standard preparation

8.12.1 Weigh approximately 50-60 mg of surrogate standard 1-H,1-H, 2-H, 2-H, $C_8F_{13}SO_3H$ into a 50 mL volumetric flask and record the actual weight.

8.12.2 Bring to volume with methanol for a surrogate stock of approximately 1000-1200 ppm.

8.12.3 Prepare a surrogate working standard. Transfer approximately 1 mL of surrogate stock to a 10 mL volumetric flask and bring to volume with methanol for a working standard of 100 ppm. Record the actual volume transferred.

9.0 SAMPLE HANDLING

9.1 All samples are received frozen and must be kept frozen until the extraction is performed.

9.2 Allow samples to thaw to room temperature prior to extraction.

10.0 QUALITY CONTROL

10.1 Solvent Blanks, Method blanks and matrix blanks

10.1.1 An aliquot of 1.0 mL methanol is used as a solvent blank.

10.1.2 Extract two 1.0 mL aliquots of Milli-Q™ water following this procedure and use as method blanks.

10.1.3 Extract two 1.0 mL aliquots of the serum following this procedure and use as matrix blanks. See 11.1.4.

10.2 Matrix spikes

10.2.1 Prepare and analyze matrix spike and matrix spike duplicate samples to determine the accuracy of the extraction.

10.2.2 Prepare each spike using a sample chosen by the analyst, usually the control matrix received with each sample set.

10.2.3 Expected concentrations will fall in the mid-range of the initial calibration curve. Additional spikes may be included and may fall in the low-range of the initial calibration curve.

10.2.4 Prepare one matrix spike and matrix spike duplicate per 40 samples, with a minimum of 2 matrix spikes per batch.

10.3 Continuing calibration checks

10.3.1 Prepare continuing calibration check samples to ensure the accuracy of the initial calibration curve.

10.3.2 Prepare, at a minimum, one continuing check per group of 10 samples. For example, if a sample set = 34, four checks are prepared and extracted.

10.3.3 Prepare each continuing calibration check from the same matrix used to prepare the initial curve.

- 10.3.4 The expected concentrations will fall within the mid-range of the initial calibration curve. Additional spikes may be included that fall in the low-range of the initial calibration curve. This is necessary if the analyst must quantitate using only the low end of the calibration curve (for example, 5 ppb – 100 ppb, rather than 5 ppb – 1000 ppb).

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare matrix calibration standards

- 11.1.1 Transfer 1 mL of serum to a 15 mL centrifuge tube.
- 11.1.2 If most sample volumes are less than 1.0 mL, extract standards with matrix volumes equal to the sample volumes. Do not extract less than 0.50 mL of matrix. Record each sample volume on the extraction sheet.
- 11.1.3 While preparing a total of twenty aliquots in 15 mL centrifuge tubes, mix or shake between aliquots.
- 11.1.4 Two 1 mL aliquots, or other appropriate volume, serve as matrix blanks. Typically use the standard concentrations and spiking amounts listed in Table 1, at the end of this section, to spike, in duplicate, two standard curves, for a total of eighteen standards, two matrix blanks, and two method blanks.
- 11.1.5 Refer to validation report ETS-8-4.0 & ETS-8-5.0-V-1, which lists the working ranges and the Linear Calibration Range (LCR) for calibration curves.
- 11.1.6 Use Attachment D as an aid in calculating the concentrations of the working standards. See Section 13.0 to calculate actual concentrations of PFOS in calibration standards.
- 11.2 To each standard, blank, or continuing check, add appropriate amount of surrogate working standard for the concentration to fall within the calibration curve range 5 ppb - 1000 ppb.
- 11.3 Extract spiked matrix standards following 12.6-12.16 of this method. Use these standards to establish each initial curve on the mass spectrometer.

Table 1 Approximate spiking amounts for standards and spikes Using 1.0 mL of matrix		
Working standard (approx. conc.)	μL	Approx. final conc. of analyte in matrix
-	-	Blank
0.500 ppm	10	0.005 ppm
0.500 ppm	20	0.010 ppm
5.00 ppm	5	0.025 ppm
5.00 ppm	10	0.050 ppm
5.00 ppm	20	0.100 ppm
50.0 ppm	5	0.250 ppm
50.0 ppm	10	0.500 ppm
50.0 ppm	15	0.750 ppm
50.0 ppm	20	1.00 ppm

12.0 PROCEDURE

- 12.1 Obtain frozen samples and allow to thaw at room temperature or in a lukewarm waterbath.
- 12.2 Vortex mix for 15 seconds, then transfer 1.0 mL or other appropriate volume to a 15 mL polypropylene centrifuge tube.
- 12.3 Return unused samples to freezer after extraction amounts have been removed.
- 12.4 Record the initial volume on the extraction worksheet.
- 12.5 Label the tube with the study number, sample ID, date and analyst initials. See attached worksheet for documenting the remaining steps.
- 12.6 Spike all samples, including blanks and standards, ready for extraction with surrogate standard as described in 11.2.
- 12.7 Spike each matrix with the appropriate amount of standard as described in 11.1, or Table 1 in that section, for the calibration curve standards. Also prepare matrix spikes and continuing calibration standards.
- 12.8 Vortex mix the standard curve samples, matrix spike samples, and continuing calibration samples for 15 seconds.
- 12.9 Check to ensure the 0.5 M TBA reagent is at pH 10. If not, adjust accordingly.
- 12.10 To each sample, add 1 mL 0.5 M TBA and 2 mL of 0.25M sodium carbonate/sodium bicarbonate buffer.
- 12.11 Using an Oxford Dispenser, add 5 mL methyl-*tert*-butyl ether.
- 12.12 Cap each sample and put on the shaker at a setting of 300 rpm, for 20 minutes.
- 12.13 Centrifuge for 20 to 25 minutes at a setting of 3500 rpm, or until layers are well separated.

- 12.14 Label a fresh 15 mL centrifuge tube with the same information as in 12.5.
- 12.15 Remove 4.0 mL of the organic layer to this clean 15 mL centrifuge tube.
- 12.16 Put each sample on the analytical nitrogen evaporator until dry, approximately 1 to 2 hours.
- 12.17 Add 1.0 mL of methanol to each centrifuge tube using a graduated pipette.
- 12.18 Vortex mix for 30 seconds.
- 12.19 Attach a 0.2 µm nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 mL glass autovial or low-volume autovial when necessary.
- 12.20 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) performing the extraction.
- 12.21 Cap and store extracts at room temperature or at approximately 4 °C until analysis.
- 12.22 Complete the extraction worksheet, attached to this document, and tape in the study notebook or include in study binder, as appropriate.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations

- 13.1.1 Calculate actual concentrations of PFOS, or other applicable fluorochemical, in calibration standards using the following equation:

$$\frac{\text{mL of standard} \times \text{concentration of standard } (\mu\text{g/mL})}{\text{mL of standard} + \text{mL of surrogate standard} + \text{initial matrix volume (mL)}} =$$

Final Concentration (µg/mL) of PFOS in matrix

14.0 METHOD PERFORMANCE

- 14.1 The method detection limit (MDL) is analyte and matrix specific. Refer to MDL report for specific MDL and limit of quantitation (LOQ) values (see **Attachments B and C**).
- 14.2 The following quality control samples are extracted with each batch of samples to evaluate the quality of the extraction and analysis.
 - 14.2.1 Method blanks and matrix blanks.
 - 14.2.2 Matrix spike and matrix spike duplicate samples to determine accuracy and precision of the extraction.
 - 14.2.3 Continuing calibration check samples to determine the continued accuracy of the initial calibration curve.
- 14.3 Refer to section 14 of ETS-8-5.1 for method performance criteria.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

- 15.1 Sample waste is disposed in biohazard containers, flammable solvent waste is disposed in high BTU containers, and used glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 RECORDS

- 16.1 Complete the extraction worksheet attached to this method, and tape in the study notebook or include in the 3-ring study binder, as appropriate.

17.0 ATTACHMENTS

- 17.1 Attachment A, Extraction worksheet
17.2 Attachment B, MDL/LOQ values and summary
17.3 Attachment C, Calibration standard concentration worksheet

18.0 REFERENCES

- 18.1 The validation report associated with this method is ETS-8-4.0 & 5.0-V-1.
18.2 FACT-M-3.1, "Analysis of Serum or Other Fluid Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

19.0 AFFECTED DOCUMENTS

- 19.1 ETS-8-5.1, "Analysis of Serum or Other Fluid Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

20.0 REVISIONS

<u>Revision Number</u>	<u>Reason For Revision</u>	<u>Revision Date</u>
1	Section 12.21 Changed to include sample storage at room temperature. Section 12.13 Added the shaker speed. Section 12.17 Final volume is 1.0 mL; not adjusted for initial volumes less than 1.0 mL.	04/02/99

Extraction Worksheet ETS-8-4.1

[illegible]

MDL/LOQ values for rabbit serum

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR)
			Approximate concentrations to be used for preparing the Standard Calibration Curve
PFOS	1.74	5.55	5 ppb - 1000 ppb
PFOSA	1.51	4.79	5 ppb - 1000 ppb
PFOSAA	3.46	20.5	5 ppb - 1000 ppb
EtFOSE-OH	11.4	36.2	5 ppb - 1000 ppb
M556	6.03	19.2	5 ppb - 1000 ppb
PFOSEA	5.71	18.2	5 ppb - 1000 ppb

MDL/LOQ values in rat, bovine, monkey, and human serum, and monkey plasma were not statistically determined. Two curves in each of these matrices were extracted and analyzed with the rabbit serum curves to determine equivalence. Responses in the rat, bovine, monkey, and human were equivalent to the rabbit responses, therefore, their MDL and LOQ will be the same values as determined in rabbit serum.

Please see LOQ Summary and MDL study in ETS-8-4.0 & 5.0-V-1 for further information.

Compound: PFOS

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.995 - 978	24.8 - 978	83-108	4.67-11.0
Low Curve	4.94 - 248	4.94 - 248	85-104	5.34-12.0
High curve	97.8 - 978	97.8 - 978	85-106	4.84-9.80
1/X	0.995 - 978	4.94 - 978	94-111	4.60-10.5

Compound: PFOSA

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.993 - 976	4.93 - 976	88-103	5.10-14.7
Low Curve	4.93 - 97.6	4.93 - 97.6	87-105	9.85-14.7
High curve	24.8 - 976	24.8 - 978	93-102	5.08-13.9
1/X	0.993 - 976	4.93 - 976	94-103	5.10-14.5

Compound: PFOSAA

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.991 - 974	24.7 - 974	81-111	4.18-10.6
Low Curve	4.92 - 247	9.74 - 247	97-107	6.38-21.8
High curve	49.2 - 974	97.4 - 974	85-108	4.33-12.5
1/X	0.991 - 974	9.74 - 974	95-115	4.11-23.2

Compound: EtFOSE-OH

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.993 - 976	49.3 - 976	77-110	11.2-25.5
Low Curve	4.93 - 97.6	9.76 - 97.6	97-107	14.1-21.3
High curve	49.3 - 976	97.6 - 976	90-109	11.5-19.6
1/X	0.993 - 493	9.76 - 976	86-111	11.1-21.2

Compound: PFOSEA

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.993 - 976	24.8 - 976	96-106	10.1-16.2
Low Curve	4.93 - 248	9.76 - 248	91-110	11.8-19.5
High curve	49.3 - 976	49.3 - 976	86-106	10.2-18.2
1/X	0.993 - 976	9.76 - 976	95-117	10.1-19.1

Compound: M556

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.993 - 976	24.8 - 976	88-106	4.82-17.9
Low Curve	4.93 - 97.6	9.76 - 97.6	100-105	5.95-18.2
High curve	97.6 - 976	97.6 - 976	81-111	5.11-9.74
1/X	0.993 - 976	9.76 - 976	97-110	4.77-19.5

Ion Pair Standard Curves – Fluids

Prep date(s):

Standard number:

Analyte(s):

Equipment number:

Sample matrix:

Final solvent and TN:

Blank fluid/identifier:

Method/revision:

Target analyte(s):

FC mix std approx. 0.500 ppm:

FC mix std approx. 5.00 ppm:

FC mix std approx. 50.0 ppm:

Surrogate std approx. 100 ppm:

Actual concentrations of standards in the FC mix

PFOS Std conc ug/mL	PFOSA Std conc ug/mL	PFOSAA Std conc ug/mL	EtFOSE Std conc ug/mL	PFOSEA Std conc ug/mL	M556 Std conc ug/mL	All Am't spiked mL	All Final vol mL
0.500	0.507	0.532	0.501	0.521	0.501	0.010	1.015
0.500	0.507	0.532	0.501	0.521	0.501	0.020	1.025
5.00	5.07	5.32	5.01	5.21	5.01	0.005	1.010
5.00	5.07	5.32	5.01	5.21	5.01	0.010	1.015
5.00	5.07	5.32	5.01	5.21	5.01	0.020	1.025
50.0	50.1	53.2	50.1	52.1	50.1	0.005	1.010
50.0	50.1	53.2	50.1	52.1	50.1	0.010	1.015
50.0	50.1	53.2	50.1	52.1	50.1	0.015	1.020
50.0	50.1	53.2	50.1	52.1	50.1	0.020	1.025

Calculated concentrations of standards in the sample matrix

PFOS Final conc ng/mL	PFOSA Final conc ng/mL	PFOSAA Final conc ng/mL	EtFOSE Final conc ng/mL	PFOSEA Final conc ng/mL	M556 Final conc ng/mL	Surrogate Std conc ng/mL	All Am't spiked mL
4.93	5.00	5.24	4.94	5.01	5.13	100	0.005
9.76	9.89	10.4	9.78	9.93	10.2	Surrogate Final conc ng/mL 500	
24.8	25.1	26.3	24.8	25.2	25.8		
49.3	50.0	52.4	49.4	50.1	51.3		
97.6	98.9	104	97.8	99.3	102		
248	251	263	248	252	258		
493	500	524	494	501	513		
735	746	782	737	749	766		
976	989	1038	978	993	1017		

Validated ranges – approximate concentrations

Serum	PFOS	PFOSA	PFOSAA	EtFOSE-OH	PFOSEA	M556
Rabbit	5.00-1000	5.00-1000	5.00-1000	5.00-1000	5.00-1000	5.00-1000
Bovine	Estimates only. Use values for rabbit.					
Rat	Estimates only. Use values for rabbit.					
Monkey & Plasma	Estimates only. Use values for rabbit.					
Human	Estimates only. Use values for rabbit.					

3M ENVIRONMENTAL LABORATORY

METHOD

ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROchemicals IN SERUM EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

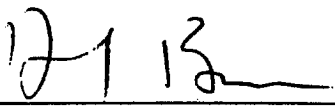
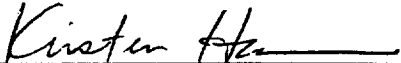
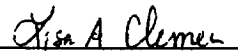
Method Number: ETS-8-5.1

Adoption Date: 03/01/99

Revision Date: 4/26/99

Author: Lisa Clemen, Robert Wynne

Approved By:

	4/26/99
Laboratory Manager	Date
	4/26/99
Group Leader	Date
	04/26/99
Technical Reviewer	Date

1.0 SCOPE AND APPLICATION

- 1.1 Scope:** This method describes the analysis of serum extracts for fluorochemical surfactants using HPLC-electrospray/mass spectrometry.
- 1.2 Applicable Compounds:** Fluorochemical surfactants or other fluorinated compounds, or other ionizable compounds.
- 1.3 Matrices:** Rabbit, rat, bovine, monkey, and human serum, or other fluids as designated in the validation report.

2.0 SUMMARY OF METHOD

- 2.1** This method describes the analysis of fluorochemical surfactants extracted from serum or other fluids, using HPLC-electrospray/mass spectrometry, or similar system as appropriate. The analysis is performed by monitoring a single ion characteristic of a particular fluorochemical, such as the perfluorooctanesulfonate (PFOS) anion, $m/z = 499$. Additionally, samples may be analyzed using a tandem mass spectrometer to further verify the identity of a compound by detecting daughter ions of the parent ion.

3.0 DEFINITIONS

- 3.1 Atmospheric Pressure Ionization (API):** The Micromass Quattro II triple quadrupole systems allow for various methods of ionization by utilizing various sources, probes, and interfaces. These include but are not limited to: Electrospray Ionization (ESI), Atmospheric Pressure chemical Ionization (APCI), Thermospray, etc. The ionization process in these techniques occurs at atmospheric pressure (i.e., not under a vacuum).
- 3.2 Electrospray Ionization (ES, ESI):** a method of ionization performed at atmospheric pressure, whereby ions in solution are transferred to the gas phase via tiny charged droplets. These charged droplets are produced by the application of a strong electrical field.
- 3.3 Mass Spectrometry, Mass Spectrometer (MS), Tandem Mass Spectrometer (MS/MS):** The API Quattro II triple quadrupole systems are equipped with quadrupole mass selective detectors. Ions are selectively discriminated by mass to charge ratio (m/z) and subsequently detected. A single MS may be employed for ion detection or a series (MS/MS) for more specific fragmentation information.
- 3.4 Conventional vs. Z-spray probe interface:** The latest models of Micromass Quattro II triple quadrupole systems (post 1998) utilize a "Z-spray" conformation. The spray emitted from a probe is orthogonal to the cone aperture. In the conventional conformation it is aimed directly at the cone aperture, after passing through a tortuous pathway in the counter electrode. Though the configuration is different, the methods of operation, cleaning, and maintenance are the same. However, Z-spray components and conventional components are not compatible with one another, but only with similar systems (i.e., Z-spray components are compatible with some other Z-spray systems, etc.)
- 3.5 Mass Lynx Software:** System software designed for the specific operation of these Quattro II triple quadrupole systems. Currently MassLynx has Windows 95 and WindowsNT 4.0 versions. All versions are similar. For more details see the manual specific to the instrument (Micromass Quattro II triple quadrupole MassLynx or MassLynx NT User's Guide).

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

- 4.1.1** Use caution with the voltage cables for the probe. When engaged, the probe employs a voltage of approximately 5000 Volts.
- 4.1.2** When handling samples or solvents wear appropriate protective gloves, eyewear, and clothing.

4.2 Cautions:

- 4.2.1 Do not operate solvent pumps above capacity of 400 bar (5800 psi) back pressure. If the back pressure exceeds 400 bar, the HP1100 will initiate automatic shutdown.
- 4.2.2 Do not run solvent pumps to dryness.

5.0 INTERFERENCES

- 5.1 To minimize interferences when analyzing samples, teflon should not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

6.0 EQUIPMENT

- 6.1 Equipment listed below may be modified in order to optimize the system. Document any modifications in the raw data as method deviations.
 - 6.1.1 Micromass Quattro II triple quadrupole Mass Spectrometer equipped with an electrospray ionization source
 - 6.1.2 HP1100 low pulse solvent pumping system, solvent degasser, column compartment, and autosampler

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

- 7.1.1 High purity grade nitrogen gas regulated to approximately 100 psi (House air system)
- 7.1.2 HPLC analytical column, specifics to be determined by the analyst and documented in the raw data.
- 7.1.3 Capped autovials or capped 15 mL centrifuge tubes

8.0 REAGENTS AND STANDARDS

8.1 Reagents

- 8.1.1 Methanol, HPLC grade or equivalent
- 8.1.2 Milli-Q™ water, all water used in this method should be Milli-Q™ water or equivalent, and may be provided by a Milli-Q TOC Plus system or other vendor
- 8.1.3 Ammonium acetate, reagent grade or equivalent

8.2 Standards

- 8.2.1 Typically two method blanks, two matrix blanks, and eighteen matrix standards are prepared during the extraction procedure. See ETS-8-4.1.

9.0 SAMPLE HANDLING

- 9.1 Fresh matrix standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials or capped 15 mL centrifuge tubes until analysis.

- 9.2 If analysis will be delayed, extracted standards and samples can be refrigerated at approximately 4° C, or at room temperature, until analysis can be performed.

10.0 QUALITY CONTROL

10.1 Solvent Blanks, Method Blanks and Matrix Blanks

10.1.1 Solvent blanks, method blanks and matrix blanks are prepared and analyzed with each batch to determine contamination or carryover.

10.1.2 Analyze a method blank and a matrix blank prior to each calibration curve.

10.2 Matrix Spikes

10.2.1 Matrix spikes are prepared and analyzed to determine the matrix effect on the recovery efficiency.

10.2.2 Matrix spike duplicates are prepared and analyzed to measure the precision and the recovery for each analyte.

10.2.3 Analyze a matrix spike and matrix spike duplicate per forty samples, with a minimum of 2 spikes per batch.

10.2.4 Matrix spike and matrix spike duplicate concentrations will fall in the mid-range of the initial calibration curve. Additional spike concentrations may fall in the low-range of the initial calibration curve.

10.3 Continuing Calibration Verifications

10.3.1 Continuing calibration verifications are analyzed to verify the continued accuracy of the calibration curve.

10.3.2 Analyze a mid-range calibration standard after every tenth sample, with a minimum of one per batch.

11.0 CALIBRATION AND STANDARDIZATION

11.1 Analyze the extracted matrix standards prior to and following each set of extracts. The average of two standard curves will be plotted by linear regression ($y = my + b$), weighted $1/x$, not forced through zero, using MassLynx or other suitable software.

11.2 If the curve does not meet requirements, perform routine maintenance or reextract the standard curve (if necessary) and reanalyze.

11.3 For purposes of accuracy when quantitating low levels of analyte, it may be necessary to use the low end of the calibration curve rather than the full range of the standard curve. Example: when attempting to quantitate approximately 10 ppb of analyte, generate a calibration curve consisting of the standards from 5 ppb to 100 ppb rather than the full range of the curve (5 ppb to 1000 ppb). This will reduce inaccuracy attributed to linear regression weighting of high concentration standards.

12.0 PROCEDURES

12.1 Acquisition Set up

- 12.1.1 Click on start button in the Acquisition Control Panel. Set up a sample list. Assign a filename using MO-DAY-last digit of year-sample number, assign a method (MS) for acquiring, and type in sample descriptions.
- 12.1.2 To create a method click on scan button in the Acquisition control panel and select SIR (Single Ion Recording) or MRM. Set Ionization Mode as appropriate and mass to 499 or other appropriate masses. A full scan is usually collected along with the SIRs. Save acquisition method. If MS/MS instruments are employed, additional product ion fragmentation information may be collected. See Micromass MassLynx GUIDE TO DATA ACQUISITION for additional information and MRM (Multiple Reaction Monitoring).
- 12.1.3 Typically the analytical batch run sequence begins with a set of extracted matrix standards and ends with a set of extracted matrix standards.
- 12.1.4 Samples are analyzed with a continuing calibration check injected after every tenth sample. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered samples but may be included as such.

12.2 Using the Autosampler

- 12.2.1 Set up sample tray according to the sample list prepared in Section 12.1.1.
- 12.2.2 Set-up the HP1100/autosampler at the following conditions or at conditions the analyst considers appropriate for optimal response. Record actual conditions in the instrument logbook:
 - 12.2.2.1 Sample size = 10 μ L injection
 - 12.2.2.2 Inject/sample = 1
 - 12.2.2.3 Cycle time = 13.5 minutes
 - 12.2.2.4 Solvent ramp =

Time	MeOH	2.0 mM Ammonium acetate
0.00 min.	40%	60%
8.50 min.	90%	10%
11.0 min.	90%	10%
12.0 min.	40%	60%

- 12.2.2.5 Press the "Start" button.

12.3 Instrument Set-up

- 12.3.1 Refer to ETS-9-24.0 for more details.
- 12.3.2 Check the solvent level in reservoirs and refill if necessary.

- 12.3.3 Check the stainless steel capillary at the end of the probe. Use an eyepiece to check the tip. The tip should be flat with no jagged edges. If the tip is found to be unsatisfactory, disassemble the probe and replace the stainless steel capillary.
- 12.3.4 Set HPLC pump to "On". Set the flow to 10 - 500 uL/min or as appropriate. Observe droplets coming out of the tip of the probe. Allow to equilibrate for approximately 10 minutes.
- 12.3.5 Turn on the nitrogen. A fine mist should be expelled with no nitrogen leaking around the tip of the probe. Readjust the tip of the probe if no mist is observed.
- 12.3.6 The instrument uses these parameters at the following settings. These settings may change in order to optimize the response:
 - 12.3.6.1 Drying gas 250-400 liters/hour
 - 12.3.6.2 ESI nebulizing gas 10-15 liters/hour
 - 12.3.6.3 HPLC constant flow mode, flow rate 10 – 500 µL/min
 - 12.3.6.4 Pressure <400 bar (This parameter is not set, it is a guide to ensure the HPLC is operating correctly.)
- 12.3.7 Carefully guide the probe into the opening. Insert probe until it will not go any further. Connect the voltage cables to the probe.
- 12.3.8 Print the tune page, with its parameters, and store it in the study binder with a copy taped into the instrument log.
- 12.3.9 Using the cross-flow counter electrode in the ES/MS source is recommended for the analysis of biological matrices.
- 12.3.10 Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, see appropriate MassLynx USER'S GUIDE). Press the start button. Ensure start and end sample number includes all samples to be analyzed.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

- 13.1.4 Calculate matrix spike percent recoveries using the following equation:

$$\% \text{ Recovery} = \frac{\text{Observed Result} - \text{Background Result}}{\text{Expected Result}} \times 100$$

- 13.1.5 Calculate percent difference using the following equation:

$$\% \text{ Difference} = \frac{\text{Expected Conc.} - \text{Calculated Conc.}}{\text{Expected Conc.}} \times 100$$

- 13.1.6 Calculate actual concentration of PFOS, or other fluorochemical, in matrix (µg/mL):

$$\frac{(\text{ng of PFOS calc. from std. Curve} \times \text{Dilution Factor})}{(\text{Initial Volume of matrix (mL)} + \text{mL of Surrogate Standard})} \times \frac{1 \mu\text{g}}{1000 \text{ ng}} \times \text{Final Volume (mL)}$$

14.0 METHOD PERFORMANCE

- 14.1** Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Please see ETS-8-4.1, Attachment B, for a listing of current validated MDL and LOQ values.
- 14.2 Solvent Blanks, Method Blanks, and Matrix Blanks**
- 14.2.1 Solvent blanks, method blanks, and matrix blanks values are must be below the lowest standard in the calibration curve
- 14.3 Calibration Curves**
- 14.3.1 The r^2 value for the calibration curve must be 0.980 or better.
- 14.4 Matrix Spikes**
- 14.4.1 Matrix spike percent recoveries are must be within $\pm 30\%$ of the spiked concentration.
- 14.5 Continuing Calibration Verifications**
- 14.5.1 Continuing calibration verification percent recoveries must be $\pm 30\%$ of the spiked concentration.
- 14.6** If criteria listed in this method performance section isn't met, maintenance may be performed on the system and samples reanalyzed or other actions as determined by the analyst. Document all actions in the appropriate logbook.
- 14.7** If data are to be reported when performance criteria have not been met, the data must be footnoted on tables and discussed in the text of the report.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

- 15.1** Sample extract waste and flammable solvent is disposed in high BTU containers, and glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 RECORDS

- 16.1** Each page generated for a study must have the following information included either in the header or hand written on the page: study or project number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst.
- 16.2** Print the tune page, sample list, and acquisition method from MassLynx to include in the appropriate study folder. Copy these pages and tape into the instrument runlog.
- 16.3** Plot the calibration curve by linear regression, weighted $1/x$, then print these graphs and store in the study folder.
- 16.4** Print data integration summary, integration method, and chromatograms, from MassLynx, and store in the study folder.

- 16.5 Summarize data using suitable software (Excel 5.0) and store in the study folder, see **Attachment A** for an example of a summary spreadsheet.
- 16.6 Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

- 17.1 Attachment A: ETS-8-5.1 Data summary spreadsheet.

18.0 REFERENCES

- 18.1 FACT-M-4.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"
- 18.2 ETS-9-24.0, "Operation and Maintenance of the Micromass Atmospheric Pressure Ionization/Mass Spectrometer Quattro II triple quadrupole Systems"
- 18.3 The validation report associated with this method is **ETS-8-4.0 & 5.0-V-1**.

19.0 AFFECTED DOCUMENTS

- 19.1 ETS-8-4.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"

20.0 REVISIONS

<u>Revision Number.</u>	<u>Reason For Revision</u>	<u>Revision Date</u>
1	Section 6.1.2 Clarification of HP1100 system components. Section 11.1 Average of two curves, not standard values, are used for plotting linear regression and added the 1/x weighting of the curve. Section 12.2.2.4 Clarification of solvent ramp. Section 17.1 Changed from attachment B to A.	04/02/99

Laboratory Study

Study:
 Test Material:
 Matrix/Final Solvent:
 Method/Revision:
 Analytical Equipment System Number:
 Instrument Software/Version:
 Filename:
 R-Squared Value:
 Slope:
 Y Intercept:
 Date of Extraction/Analyst:
 Date of Analysis/Analyst:

Group Dose	Sample#	Concentration ug/mL	Initial Vol. mL	Dilution Factor	Final Conc. ug/mL

Slope: Taken from linear regression equation.

Group/Dose: Taken from the study folder.

Sample#: Taken from the study folder.

Concentration (ug/mL): Taken from the MassLynx integration summary.

Initial Volume (mL): Taken from the study folder.

Dilution Factor: Taken from the study folder.

Final Conc. (ug/mL): Calculated by dividing the initial volume from the concentration

3M ENVIRONMENTAL LABORATORY

METHOD

EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUORO-CHEMICAL COMPOUNDS FROM LIVER FOR ANALYSIS USING HPLC- ELECTROSPRAY/MASS SPECTROMETRY

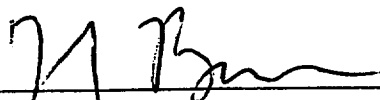
Method Number: ETS-8-6.0

Adoption Date: 07/22/99

Revision Date: NA

Author: Lisa Clemen, Robert Wynne

Approved By:



Laboratory Manager

7/22/99

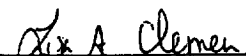
Date



Group Leader

7/14/99

Date



Technical Reviewer

07/14/99

Date

1.0 SCOPE AND APPLICATION

1.1 Scope: This method is for the extraction of potassium perfluorooctanesulfonate (PFOS) or other fluorochemical compounds from liver.

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds.

1.3 Matrices: Rabbit, rat, bovine, and monkey livers or other tissues as designated in the validation report.

2.0 SUMMARY OF METHOD

- 2.1 This method describes the procedure for extracting potassium perfluorooctanesulfonate (PFOS) or other fluorochemical surfactants from liver, or other tissues, using an ion pairing reagent and methyl-*tert*-butyl ether (MtBE). In this method, seven fluorochemicals can be extracted: PFOS, PFOSA, PFOSAA, EtFOSE-OH, PFOSEA, M556, and surrogate standard. An ion pairing reagent is added to the sample and the analyte ion pair is partitioned into MtBE. The MtBE extract is transferred to a centrifuge tube and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 1.0 mL methanol then filtered through a 3 cc plastic syringe attached to a 0.2 μ m nylon filter into glass autovials.
- 2.2 These sample extracts are analyzed following method ETS-8-7.0 or other appropriate methods.

3.0 DEFINITIONS

- 3.1 PFOS: perfluorooctanesulfonate (anion of potassium salt) $C_8F_{17}SO_3$
- 3.2 PFOSA: perfluorooctane sulfonylamide $C_8F_{17}SO_2NH_2$
- 3.3 PFOSAA: perfluorooctane sulfonylamido (ethyl)acetate $C_8F_{17}SO_2N(CH_2CH_3)CH_2CO_2$
- 3.4 EtFOSE-OH: 2(N-ethylperfluorooctane sulfonamido)-ethyl alcohol $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$
- 3.5 PFOSEA: perfluorooctane sulfonyl ethylamide $C_8F_{17}SO_2N(CH_2CH_3)H$
- 3.6 M556: $C_8F_{17}SO_2N(H)(CH_2COOH)$
- 3.7 Surrogate standard: 1H-1H-2H-2H perfluorooctane sulfonic acid

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

- 4.1.1 Use universal precautions, especially laboratory coats, goggles, and gloves when handling animal tissue, which may contain pathogens.

5.0 INTERFERENCES

- 5.1 There are no interferences known at this time.

6.0 EQUIPMENT

- 6.1 The following equipment is used while performing this method. Equivalent equipment is acceptable.
- 6.1.1 Ultra-Turrax T25 Grinder for grinding liver samples
- 6.1.2 Vortex mixer, VWR, Vortex Genie 2
- 6.1.3 Centrifuge, Mistral 1000 or IEC
- 6.1.4 Shaker, Eberbach or VWR

6.1.5 Nitrogen Evaporator, Organomation

6.1.6 Balance (sensitivity to 0.100 g)

7.0 SUPPLIES AND MATERIALS

- 7.1 Gloves
- 7.2 Dissecting scalpels
- 7.3 Eppendorf or disposable pipettes
- 7.4 Nalgene bottles, capable of holding 250 mL and 1 L
- 7.5 Volumetric flasks, glass, type A
- 7.6 I-CHEM vials, 40 mL glass
- 7.7 Plastic sample vials, Wheaton, 6 mL (or appropriate size)
- 7.8 Centrifuge tubes, polypropylene, 15 mL
- 7.9 Labels
- 7.10 Oxford Dispensor – 3.0 to 10.0 ml
- 7.11 Syringes, capable of measuring 5 μ L to 50 μ L
- 7.12 Graduated pipettes
- 7.13 Syringes, disposable plastic, 3 cc
- 7.14 Syringe filters, nylon, 0.2 μ m, 25 mm
- 7.15 Timer
- 7.16 Crimp cap autovials and caps
- 7.17 Crimpers

Note: Prior to using glassware and bottles, rinse 3 times with methanol and 3 times with Milli-Q™ water. Rinse syringes a minimum of 9 times with methanol, 3 rinses from 3 separate vials.

8.0 REAGENTS AND STANDARDS

- 8.1 Type I reagent grade water, Milli-Q™ or equivalent; all water used in this method should be Milli-Q™ water and be provided by a Milli-Q TOC Plus™ system
- 8.2 Sodium hydroxide (NaOH), J.T Baker or equivalent
- 8.3 Tetrabutylammonium hydrogen sulfate(TBA), Kodak or equivalent
- 8.4 Sodium carbonate (Na_2CO_3), J.T. Baker or equivalent
- 8.5 Sodium bicarbonate (NaHCO_3), J.T. Baker or equivalent
- 8.6 Methyl-*tert*-butyl ether, Omnisolv, glass distilled or HPLC grade
- 8.7 Methanol, Omnisolv, glass distilled or HPLC grade
- 8.8 Liver, frozen from supplier
- 8.9 Dry ice from supplier
- 8.10 Fluorochemical standards
 - 8.10.1 PFOS (3M Specialty Chemical Division), molecular weight = 538

- 8.10.2 PFOSA (3M Specialty Chemical Division), molecular weight = 499
- 8.10.3 PFOSAA (3M Specialty Chemical Division), molecular weight = 585
- 8.10.4 EtFOSE-OH (3M Specialty Chemical Division), molecular weight = 570
- 8.10.5 PFOSEA (3M Specialty Chemical Division), molecular weight = 527
- 8.10.6 M556 (3M Specialty Chemical Division), molecular weight = 557
- 8.10.7 Surrogate standard: 4-H, perfluorooctane sulfonic acid (1-H, 1-H, 2-H, 2-H $C_8F_{13}SO_3H$) molecular weight = 428
- 8.10.8 Other fluorochemicals, as appropriate

8.11 Reagent preparation

NOTE: When preparing larger volumes than listed in reagent, standard, or surrogate preparation, adjust accordingly.

- 8.11.1 10 N sodium hydroxide (NaOH): Weigh approximately 200 g NaOH. Pour into a 1000 mL beaker containing 500 mL Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.
- 8.11.2 1 N sodium hydroxide (NaOH): Dilute 10 N NaOH 1:10. Measure 10 mL of 10 N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 mL Nalgene bottle.
- 8.11.3 0.5 M tetrabutylammonium hydrogen sulfate (TBA): Weigh approximately 169 g of TBA into a 1 L volumetric containing 500 mL Milli-Q™ water. Adjust to pH 10 using approximately 44 to 54 mL of 10 N NaOH (While adding the last mL of NaOH, add slowly because the pH changes abruptly). Dilute to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.
 - 8.11.3.1 TBA requires a check prior to each use to ensure pH = 10. Adjust as needed using 1 N NaOH solution.
- 8.11.4 0.25 M sodium carbonate/sodium bicarbonate buffer ($Na_2CO_3/NaHCO_3$): Weigh approximately 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

8.12 Standards preparation

- 8.12.1 Prepare PFOS standards for the standard curve.
- 8.12.2 Prepare other fluorochemical standards, as appropriate. Multicomponent fluorochemical standards are acceptable (for example, one working standard solution containing 1.00 ppm PFOS, 1.02 ppm PFOSA, 0.987 ppm PFOSAA, and 1.10 ppm EtFOSE-OH.)
- 8.12.3 Weigh approximately 100 mg of PFOS into a 100 mL volumetric flask and record the actual weight.
- 8.12.4 Bring to volume with methanol for a stock standard of approximately 1000 ppm ($\mu g/mL$).
- 8.12.5 Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm.

8.12.6 Dilute the stock solution with methanol for a working standard 2 solution of approx. 5.0 ppm.

8.12.7 Dilute the stock solution with methanol for a working standard 3 solution of approx. 0.50 ppm.

8.13 Surrogate stock standard preparation

8.13.1 Weigh approximately 50-60 mg of surrogate standard 1-H,1-H, 2-H, 2-H, $C_8F_{13}SO_3H$ into a 50 ml volumetric flask and record the actual weight.

8.13.2 Bring to volume with methanol for a surrogate stock of approximately 1000-1200 ppm.

8.13.3 Prepare a surrogate working standard. Transfer approximately 1.0 ml of surrogate stock to a 10 ml volumetric flask and bring to volume with methanol for a working standard of 10-20 ppm. Record the actual volume transferred.

9.0 SAMPLE HANDLING

9.1 All samples are received frozen and must be kept frozen until the extraction is performed.

10.0 QUALITY CONTROL

10.1 Matrix blanks and method blanks

10.1.1 An aliquot of 1.0 mL methanol is used as a solvent blank.

10.1.2 Extract two 1.0 mL aliquots of Milli-Q™ water following this procedure and use as method blanks.

10.1.3 Extract two 1.0 mL aliquots of liver homogenate following this procedure and use as matrix blanks. Refer to 11.1.6.

10.2 Matrix spikes

10.2.1 Prepare and analyze matrix spike and matrix spike duplicate samples to determine the accuracy of the extraction.

10.2.2 Prepare each spike using a sample chosen by the analyst, usually a control liver received with each sample set.

10.2.3 Expected concentrations will fall in the mid-range of the initial calibration curve. Additional spikes may be included and may fall in the low-range of the initial calibration curve.

10.2.4 Prepare one matrix spike and matrix spike duplicate per 40 samples, with a minimum of 2 matrix spikes per batch.

10.3 Continuing calibration verifications

10.3.1 Prepare continuing calibration verification samples to ensure the accuracy of the initial calibration curve.

10.3.2 Prepare, at a minimum, one continuing calibration verification sample per group of 10 samples. For example, if a sample set = 34, four verifications are prepared and extracted.

- 10.3.3 Prepare each continuing calibration verification from the same matrix used to prepare the initial curve.
- 10.3.4 The expected concentrations will fall within the mid-range of the initial calibration curve. Additional spikes may be included that fall in the low-range of the initial calibration curve. This is necessary if the analyst must quantitate using only the low end of the calibration curve (for example, 5 ppb – 100 ppb, rather than 5 ppb – 1000 ppb).

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare matrix calibration standards

- 11.1.1 Weigh approximately 40 g of liver into a 250 mL Nalgene bottle containing 200 mLs Milli-Q™ water. Grind to a homogeneous solution.
 - 11.1.2 If 40 g is not available, use appropriate amounts of liver and water to ensure a 1:5 ratio.
 - 11.1.3 Refer to 13.0 to calculate the actual density of liver homogenate and the concentration of solid liver tissue dispersed in 1.0 mL of homogenate solution.
 - 11.1.5 Add 1 mL of homogenate to a 15 mL centrifuge tube. Re-suspend solution by shaking between aliquots while preparing a total of eighteen 1 mL aliquots of homogeneous solution in 15 mL centrifuge tubes.
 - 11.1.6 Two 1 mL aliquots, or other appropriate volume, serve as matrix blanks.
 - 11.1.7 Typically use the standard concentrations and spiking amounts listed in Table 1, at the end of this section, to spike, in duplicate, two standard curves, for a total of eighteen samples, two matrix blanks, and two method blanks.
 - 11.1.8 Refer to validation reports ETS-8-6.0 and ETS-8-7.0-V-1 or Attachment B, which lists the working ranges and the Linear Calibration Range (LCR) for calibration curves.
 - 11.1.9 Use Attachment C as an aid in calculating the concentrations of the working standards. Refer to 13.0 to calculate actual concentrations of PFOS in calibration standards.
- 11.2 To each working standard, blank, or continuing verification, add appropriate amount of surrogate working standard for the concentration to fall within the calibration curve range 5 ppb – 1000ppb.

- 11.3 Extract spiked liver homogenates following 12.14-12.25 of this method. Use these standards to establish each initial curve on the mass spectrometer.

Table 1 Approximate Spiking Amounts for Calibration Standards		
Working Standard (Approx. Conc.)	μl	Approx. final conc. of PFOS in liver
-	-	Blank
0.50 ppm	2	0.005 ppm
0.50 ppm	4	0.010 ppm
0.50 ppm	10	0.025 ppm
0.50 ppm	20	0.050 ppm
0.50 ppm	40	0.100 ppm
5.0 ppm	10	0.250 ppm
5.0 ppm	20	0.500 ppm
5.0 ppm	30	0.750 ppm
50 ppm	4	1.00 ppm

12.0 PROCEDURE

- 12.1 Obtain frozen liver samples.
- 12.2 Cut approximately 1 g of liver using a dissecting scalpel. This part of the procedure is best performed quickly, not allowing the liver to thaw.
- 12.3 Weigh the sample directly into a tared plastic sample vial.
- 12.4 Record the liver weight in the study notebook.
- 12.5 Return unused liver portions to freezer.
- 12.6 Add 2.5 mLs of water to sample vial.
- 12.7 Grind the sample. Put the grinder probe in the sample and grind for about 2 minutes, or until the sample is homogeneous.
- 12.8 Rinse the probe into the sample with 2.5 mLs water using a pipette.
- 12.9 Take the grinder apart and clean it with methanol after each sample. Refer to AMDT-EP-22.
- 12.10 Cap the sample and vortex for 15 seconds. Label the sample vial with the study number, weight, liver ID, date and analyst initials.

- 12.11 Pipette 1.0 mL, or other appropriate volume, of homogenate into a 15 mL polypropylene centrifuge tube. Label the centrifuge tube with the identical information as the sample vial. Refer to attached worksheet for documenting the remaining steps.
- 12.12 Pipette two 1 mL aliquots of Milli-Q™ water to centrifuge tubes. These will serve as method blanks.
- 12.13 Spike all samples, including blanks and standards ready for extraction with surrogate standard as described in section 11.2.
- 12.14 Spike each matrix with the appropriate amount of standard as described in 11.1, or Table 1 of that section, for the calibration curve standards. Also prepare matrix spikes and continuing calibration standards.
- 12.15 Vortex mix the standard curve samples, matrix spike samples, and continuing calibration samples for 15 seconds.
- 12.16 Check to ensure 0.5 M TBA reagent is at pH 10. If not, adjust accordingly.
- 12.17 To each sample, add 1 mL 0.5 M TBA and 2 mL of the 0.25 M sodium carbonate/sodium bicarbonate buffer.
- 12.18 Using an Oxford Dispenser, add 5 mL methyl-*tert*-butyl ether.
- 12.19 Cap each sample and put on the shaker at a setting of 300 rpm, for 20 minutes.
- 12.20 Centrifuge for 20 to 25 minutes at a setting of 3500 rpm, or until layers are well separated.
- 12.21 Label a fresh 15 mL centrifuge tube with the same information as in 12.10.
- 12.22 Remove 4.0 mL of the organic layer to the fresh 15 mL centrifuge tube.
- 12.23 Put each sample on the analytical nitrogen evaporator until dry, approximately 1 to 2 hours.
- 12.24 Add 1.0 mL to each centrifuge tube using a graduated pipette.
- 12.25 Vortex mix for 30 seconds.
- 12.26 Attach a 0.2 µm nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 mL glass autovial or low-volume autovial when necessary.
- 12.27 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) performing the extraction.
- 12.28 Cap and store extracts at room temperature or at approximately 4 °C until analysis.
- 12.29 Complete the extraction worksheet, attached to this document, and tape in study notebook or include in study binder, as appropriate.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

- 13.1.1 Calculate the average density of the liver homogenate by recording each mass of ten separate 1.0 mL aliquots of homogenate.

$$\text{Average density (mg/mL)} = \frac{\text{Average mass (mg) of the aliquots}}{1.0 \text{ mL aliquot}}$$

- 13.1.2 Calculate the amount of liver (mg) per 1.0 mL homogenate (or concentration of dispersed solid tissue per mL of homogenate suspension) using the following equation:

$$\frac{\text{g of Liver} \times \text{Average density* of homogenate (mg/mL)}}{(\text{g of Liver} + \text{g of Water})}$$

* refer to 13.1.1 for details.

- 13.1.3 Calculate actual concentrations of PFOS and other fluorochemicals in calibration standards using the following equation:

$$\frac{\mu\text{L of Standard} \times \text{Concentration } (\mu\text{g /mL})}{\text{mg Liver / 1 mL homogenate*}} = \text{Final Concentration } (\mu\text{g/g or mg/kg}) \text{ of PFOS in Liver}$$

*refer to 13.1.2 for details.

14.0 METHOD PERFORMANCE

- 14.1 The method detection limit (MDL) is analyte and matrix specific. Refer to MDL report for specific MDL and limit of quantitation (LOQ) values (refer to **Attachments B and C**).
- 14.2 The following quality control samples are extracted with each batch of samples to evaluate the quality of the extraction and analysis.
- 14.2.1 Method blanks and matrix blanks.
 - 14.2.2 Matrix spike and matrix spike duplicate samples to determine accuracy and precision of the extraction.
 - 14.2.3 Continuing calibration verification samples to determine the continued accuracy of the initial calibration curve.
- 14.3 Refer to section 14 of ETS-8-7.0 for method performance criteria.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

- 15.1 Sample waste is disposed in biohazard containers, flammable solvent waste is disposed in high BTU containers, and used glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 RECORDS

- 16.1 Complete the extraction worksheet attached to this method, and tape in the study notebook or include in the 3-ring study binder, as appropriate.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

- 17.1 Attachment A, Extraction worksheet
- 17.2 Attachment B, MDL/LOQ values and summary
- 17.3 Attachment C, Calibration standard calculation and concentration worksheet

18.0 REFERENCES

- 18.1 The validation report associated with this method is ETS-8-6.0 & 7.0-V-1.
- 18.2 AMDT-EP-22, "Routine Maintenance of Ultra-Turrax T-25"
- 18.3 FACT-M-1.1, "Extraction of PFOS or Other Anionic Fluorochemical Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

19.0 AFFECTED DOCUMENTS

- 19.1 ETS-8-7.0, "Analysis of Liver Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

20.0 REVISIONS

<u>Revision Number.</u>	<u>Reason For Revision</u>	<u>Revision Date</u>
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[illegible]

MDL/LOQ values for rabbit liver

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate concentrations to be used for preparing the Standard Calibration Curve
PFOS	8.45	26.9	30 ppb – 1200 ppb
PFOSA	3.50	11.1	12 ppb – 1200 ppb
PFOSAA	24.6	78.3	30 ppb – 1200 ppb
EtFOSE-OH	108	345	60 ppb – 900 ppb*
M556	82.3	262	60 ppb – 1200 ppb
PFOSEA	33.9	108	30 ppb- 1200 ppb

MDL/LOQ values in rat, bovine, and monkey liver were not statistically determined. Two curves in each of these matrices were extracted and analyzed with the rabbit liver curves to determine equivalence. Responses in the rat, bovine, and monkey liver curves were equivalent to the rabbit responses, therefore, their MDL and LOQ will be assumed to be equivalent to those values as determined for the rabbit liver.

Refer to LOQ Summary and MDL study in ETS-8-6.0 & 7.0-V-1 for further information

* EtFOSE-OH estimates only for MDL and LOQ. Did not meet criteria for validation.

Compound: PFOS

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.19 - 1237	12 - 1200	12 - 1200	6 - 300	12 - 300	60 - 1200	60 - 1200

Compound: PFOSA

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.19 - 1237	12 - 1200	12 - 1200	12 - 300	12 - 300	60 - 1200	60 - 1200

Compound: PFOSAA

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.16 - 1232	12 - 1200	30 - 1200	30 - 900	60 - 900	N/A	N/A

Compound: EtFOSE-OH

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.17 - 1235	31 - 900	31 - 900	N/A	N/A	N/A	N/A

Compound: PFOSEA

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.17 - 1235	31 - 1200	31 - 1200	N/A	N/A	N/A	N/A

Compound: M556

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.17 - 1235	31 - 1200	60 - 1200	N/A	N/A	N/A	N/A

Ion Pair Standard Curves – Tissue

Prep date(s):

Analyte(s):

Sample matrix:

Standard number:

Equipment number:

Final solvent and TN:

Blank liver/identifier:

Method/revision:

Target analyte(s):

FC mix std approx. 0.500 ppm:

FC mix std approx. 5.00 ppm:

FC mix std approx. 50.0 ppm:

Surrogate std approx. 100 ppm:

Actual concentrations of standards in the FC mix

PFOS Std conc ug/mL	PFOSA Std conc ug/mL	PFOSAA Std conc ug/mL	EtFOSE Std conc ug/mL	PFOSEA Std conc ug/mL	M556 Std conc ug/mL	Std conc ug/mL	All Am't spiked mL	All Density g
0.500	0.500	0.500	0.500	0.500	0.500		0.002	0.167
0.500	0.500	0.500	0.500	0.500	0.500		0.004	0.167
0.500	0.500	0.500	0.500	0.500	0.500		0.010	0.167
0.500	0.500	0.500	0.500	0.500	0.500		0.020	0.167
0.500	0.500	0.500	0.500	0.500	0.500		0.040	0.167
5.00	5.00	5.00	5.00	5.00	5.00		0.010	0.167
5.00	5.00	5.00	5.00	5.00	5.00		0.020	0.167
5.00	5.00	5.00	5.00	5.00	5.00		0.030	0.167
50.0	50.0	50.0	50.0	50.0	50.0		0.004	0.167

Calculated concentrations of standards in the sample matrix

PFOS Final conc ng/g	PFOSA Final conc ng/g	PFOSAA Final conc ng/g	EtFOSE Final conc ng/g	PFOSEA Final conc ng/g	M556 Final conc ng/g	Std conc ng/g	Surrogate Std conc ng/mL	All Am't spiked mL
5.99	5.99	5.99	5.99	5.99	5.99		100	0.005
12.0	12.0	12.0	12.0	12.0	12.0		Surrogate Final conc ng/mL	
29.9	29.9	29.9	29.9	29.9	29.9			
59.9	59.9	59.9	59.9	59.9	59.9			
120	120	120	120	120	120			
299	299	299	299	299	299			
599	599	599	599	599	599		0.500	
898	898	898	898	898	898			
1198	1198	1198	1198	1198	1198			

Validated ranges – approximate concentrations

Liver	PFOS	PFOSA	PFOSAA	EtFOSE-OH	POAA	PFOSEA
Rabbit	5-1000 ppb	5-1000 ppb	5-1000 ppb	5-1000 ppb	5-1000 ppb	5-1000 ppb
Bovine	Estimates only, use rabbit values.					
Rat	Estimates only, use rabbit values.					
Monkey	Estimates only, use rabbit values.					

3M ENVIRONMENTAL LABORATORY

METHOD

ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROchemicals IN LIVER EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

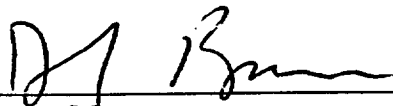
Method Number: ETS-8-7.0

Adoption Date: 07/22/99

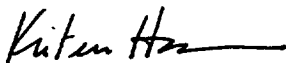
Revision Date: NA

Author: Lisa Clemen, Glenn Langenburg

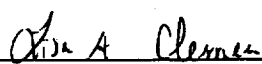
Approved By:


Laboratory Manager

7/22/99
Date


Group Leader

7/14/99
Date


Technical Reviewer

07/14/99
Date

1.0 SCOPE AND APPLICATION

1.1 Scope: This method is for the analysis of liver extracts for fluorochemical surfactants using HPLC-electrospray/mass spectrometry.

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds, or other ionizable compounds.

1.3 Matrices: Rabbit, rat, bovine, monkey liver, or other tissues as designated in the validation report.

2.0 SUMMARY OF METHOD

- 2.1** This method describes the analysis of fluorochemical surfactants extracted from liver using HPLC-electrospray/mass spectrometry, or similar system as appropriate. The analysis is performed by monitoring a single ion characteristic of a particular fluorochemical, such as the perfluorooctanesulfonate (PFOS) anion, $m/z = 499$. Additionally, samples may be analyzed using a tandem mass spectrometer to further verify the identity of a compound by detecting daughter ions of the selected parent ion.

3.0 DEFINITIONS

- 3.1 Atmospheric Pressure Ionization (API):** The Micromass Quattro II triple quadrupole systems allow for various methods of ionization by utilizing various sources, probes, and interfaces. These include but are not limited to: Electrospray Ionization (ESI), Atmospheric Pressure chemical Ionization (APCI), Thermospray, etc. The ionization process in these techniques occurs at atmospheric pressure (i.e. not under a vacuum).
- 3.2 Electrospray Ionization (ES, ESI):** a method of ionization performed at atmospheric pressure, whereby ions in solution are transferred to the gas phase via tiny charged droplets. These charged droplets are produced by the application of a strong electrical field.
- 3.3 Mass Spectrometry, Mass Spectrometer (MS), Tandem Mass Spectrometer (MS/MS):** The API Quattro II triple quadrupole mass spectrometer is equipped with two quadrupole mass selective detectors and a collision cell. Ions are selectively discriminated by mass to charge ratio (m/z) and subsequently detected. A single MS may be employed for ion detection or an ion may be selected in the first quadrupole, fragmented in the collision cell, and these fragments may be analyzed in the second quadrupole.
- 3.4 Conventional vs. Z-spray probe interface:** The latest models of Micromass Quattro II triple quadrupole (post 1998) utilize a "Z-spray" conformation. The spray emitted from a probe is orthogonal to the cone aperture. In the conventional conformation it is aimed directly at the cone aperture, after passing through a tortuous pathway in the counter electrode. Though the configuration is different, the methods of operation, cleaning, and maintenance are the same. However, Z-spray components and conventional components are not compatible with one another, but only with similar systems (i.e. Z-spray components are compatible with other Z-spray systems, etc.)
- 3.5 Mass Lynx Software:** System software designed for the specific operation of these Quattro II triple quadrupole systems. Currently MassLynx has Windows 95 and WindowsNT 4.0 versions. All versions are similar. For more details refer to the manual specific to the instrument (Micromass Quattro II triple quadrupole MassLynx or MassLynx NT User's Guide).

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

- 4.1.1** Use caution with the voltage cables for the probe. When engaged, the probe employs a voltage of approximately 5000 Volts.

- 4.1.2 When handling samples or solvents wear appropriate protective gloves, eyewear, and clothing.

4.2 Cautions:

- 4.2.1 Operate the solvent pumps below a back pressure of 400 bar (5800 psi). If the back pressure exceeds 400 bar, the HP1100 will initiate automatic shutdown.
- 4.2.2 Do not run solvent pumps to dryness.

5.0 INTERFERENCES

- 5.1 To minimize interferences when analyzing samples, Teflon shall not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

6.0 EQUIPMENT

- 6.1 Equipment listed below may be modified in order to optimize the system. Document any modifications in the raw data as method deviations.
 - 6.1.1 Micromass Quattro II triple quadrupole Mass Spectrometer equipped with an electrospray ionization source.
 - 6.1.2 HP1100 low pulse solvent pumping system, solvent degasser, column compartment, and autosampler

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

- 7.1.1 High purity grade air regulated to approximately 100 psi (house air system)
- 7.1.2 HPLC analytical column, specifics to be determined by the analyst and documented in the raw data
- 7.1.3 Capped autovials or capped 15 ml centrifuge tubes

8.0 REAGENTS AND STANDARDS

8.1 Reagents

- 8.1.1 Methanol, HPLC grade or equivalent
- 8.1.2 Milli-Q™ water (ASTM type I), all water used in this method should be ATSM type I, or equivalent, and be provided by a Milli-Q TOC Plus system or other vendor
- 8.1.3 Ammonium acetate, reagent grade or equivalent
 - 8.1.3.1 When preparing different amounts than those listed, adjust accordingly.
 - 8.1.3.2 2.0 mM ammonium acetate solution: Weigh approximately 0.300 g ammonium acetate. Pour into a 2000 mL volumetric container containing 2000 mL Milli-Q™ water, mix until all solids are dissolved. Store at room temperature.

8.2 Standards

- 8.2.1** Typically two method blanks, two matrix blanks, and eighteen matrix standards are prepared during the extraction procedure. Refer to ETS-8-6.0.

9.0 SAMPLE HANDLING

- 9.1** Fresh matrix standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials or capped 15 ml centrifuge tubes until analysis.
- 9.2** If analysis will be delayed, extracted standards and samples may be stored at room temperature, or refrigerated at approximately 4° C, until analysis can be performed.

10.0 QUALITY CONTROL

10.1 Method Blanks and Matrix Blanks

- 10.1.1** Solvent blanks, method blanks, and matrix blanks are prepared and analyzed with each batch to determine contamination or carryover.
- 10.1.2** Analyze a method blank and a matrix blank prior to each calibration curve.

10.2 Matrix Spikes

- 10.2.1** Matrix spikes are prepared and analyzed to determine the matrix effect on the recovery efficiency.
- 10.2.2** Matrix spike duplicates are prepared and analyzed to measure the precision and the recovery for each analyte.
- 10.2.3** Analyze a matrix spike and matrix spike duplicate per forty samples. With a minimum of 2 spikes per batch.
- 10.2.4** Matrix spike and matrix spike duplicate concentrations will fall in the mid-range of the initial calibration curve. Additional spike concentrations may fall in the low-range of the initial calibration curve.

10.3 Continuing Calibration Checks

- 10.3.1** Continuing calibration verifications are analyzed to verify the continued accuracy of the calibration curve.
- 10.3.2** Analyze a mid-range calibration standard every tenth sample, with a minimum of one per batch.

11.0 CALIBRATION AND STANDARDIZATION

- 11.1** Analyze the extracted matrix standards prior to and following each set of sample extracts. The average of two standard curves will be plotted by linear regression ($y = mx + b$), weighted $1/x$, not forced through the origin, using MassLynx or other suitable software.
- 11.2** If the curve does not meet requirements perform routine maintenance or reextract the standard curve (if necessary) and reanalyze.

- 11.3 For purposes of accuracy when quantitating low levels of analyte, it may be necessary to use the low end of the calibration curve rather than the full range of the standard curve. Example: when attempting to quantitate approximately 10 ppb of analyte, generate a calibration curve consisting of the standards from 5 ppb to 100 ppb rather than the full range of the curve (5 ppb to 1000 ppb). This will reduce inaccuracy attributed to linear regression weighting of high concentration standards.

12.0 PROCEDURES

12.1 Acquisition Set up

12.1.1 Set up the sample list.

12.1.1.1 Assign a sample list filename using MO-DAY-last digit of year-increasing letter of the alphabet starting with a

12.1.1.2 Assign a method (MS file) for acquiring

12.1.1.3 Assign an HPLC program (Inlet file)

12.1.1.4 Type in sample descriptions and vial position numbers

12.1.2 To create a method click on method in the Acquisition control panel then mass spectrometer headings and select SIR (Single Ion Recording) or MRM (Multiple Reaction Monitoring). Set Ionization Mode as appropriate and mass to 499 or other appropriate masses. A full scan is usually collected along with the SIRs. Save acquisition method. If MS/MS instruments are employed, additional product ion fragmentation information may be collected. Refer to Micromass MassLynx GUIDE TO DATA ACQUISITION for additional information and MRM.

12.1.3 Typically the analytical batch run sequence begins and ends with a set of extracted matrix standards.

12.1.4 Samples are analyzed with a continuing calibration verification injected standard after every tenth sample. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered samples but may be included as such.

12.2 Using the Autosampler

12.2.1 Set up sample tray according to the sample list prepared in Section 12.1.1.

12.2.2 Set-up the HP1100/autosampler at the following conditions or at conditions the analyst considers appropriate for optimal response. Record actual conditions in the instrument logbook:

12.2.2.1 Sample size = 10 μ L injection

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 9 minutes

12.2.2.4 Solvent ramp conditions

Time	MeOH	2.0 mM Ammonium acetate
0.00 min.	40%	60%
1.0 min.	40%	60%
4.5 min.	95%	5%
6.5 min.	95%	5%
7.0 min.	40%	60%
9.0 min.	40%	60%

12.2.2.5 Press the "Start" button.

12.3 Instrument Set-up

12.3.1 Refer to ETS-9-24.0, "Operation and Maintenance of the Micromass Quattro II Triple Quadrupole Mass Spectrometer Fitted with an Atmospheric Pressure Ionization Source," for more details.

12.3.2 Check the solvent level in reservoirs and refill if necessary.

12.3.3 Check the stainless steel capillary at the end of the probe. Use an eyepiece to check the tip. The tip should be flat with no jagged edges. If the tip is found to be unsatisfactory, disassemble the probe and replace the stainless steel capillary.

12.3.4 Turn on the nitrogen.

12.3.5 Open the tune page. Clicks on operate to initiate source block and desolvation heaters.

12.3.6 Open the Inlet Editor.

12.3.6.1 Set HPLC pump to "On"

12.3.6.2 Set the flow to 10 - 500 μ L/min or as appropriate

12.3.6.3 Observe droplets coming out of the tip of the probe. A fine mist should be expelled with no nitrogen leaking around the tip of the probe. Readjust the tip of the probe if no mist is observed

12.3.6.4 Allow to equilibrate for approximately 10 minutes.

12.3.7 The instrument uses these parameters at the following settings. These settings may change in order to optimize the response:

12.3.7.1 Drying gas 250-400 liters/hour

12.3.7.2 ESI nebulizing gas 10-15 liters/hour

12.3.7.3 HPLC constant flow mode flow rate 10 - 500 μ L/min

12.3.7.4 Pressure <400 bar (This parameter is not set, it is a guide to ensure the HPLC is operating correctly.)

12.3.7.5 Source block temperature 150°

12.3.7.6 Desolvation temperature 250°

- 12.3.8 Print the tune page, with its parameters, and store it in the study binder with a copy taped into the instrument log.
- 12.3.9 Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, refer to appropriate MassLynx User's Guide). Ensure start and end sample number includes all samples to be analyzed.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

- 13.1.4 Calculate matrix spike percent recoveries using the following equation:

$$\% \text{ Recovery} = \frac{\text{Observed Result} - \text{Background Result}}{\text{Expected Result}} \times 100$$

- 13.1.5 Calculate percent difference using the following equation:

$$\% \text{ Difference} = \frac{\text{Expected Conc.} - \text{Calculated Conc.}}{\text{Expected Conc.}} \times 100$$

- 13.1.6 Calculate actual concentrations in matrix (µg/g):

$$\frac{(\text{ng of PFOS calc. from std. Curve} \times \text{Dilution Factor})}{\frac{(\text{Initial Weight of Liver (g)})}{\text{Final Volume (mL)}}} \times \frac{1 \mu\text{g}}{1000 \text{ ng}}$$

14.0 METHOD PERFORMANCE

- 14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Refer to ETS-8-6.0, Attachment B for a listing of current validated MDL and LOQ values.
- 14.2 Solvent Blanks, Method Blanks and Matrix Blanks
- 14.2.1 Solvent blanks, method blanks, and matrix blanks must be below the lowest standard in the calibration curve.
- 14.3 Calibration Curves
- 14.3.1 The r^2 value for the calibration must be 0.980 or better.
- 14.4 Matrix Spikes
- 14.4.1 Matrix spike percent recoveries must be within $\pm 30\%$ of the spiked concentration.
- 14.5 Continuing Calibration Verification
- 14.5.1 Continuing calibration verification percent recoveries must be within $\pm 30\%$ of the spiked concentration.
- 14.6 If criteria listed in the method performance section are not met, maintenance may be performed on the system and samples reanalyzed or other actions as determined by the analyst. Document all actions in the appropriate logbook.

- 14.7 If data are to be reported when performance criteria have not been met, the data must be footnoted on tables and discussed in the text of the report.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

- 15.1 Sample extract waste and flammable solvent is disposed in high BTU containers, and glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 RECORDS

- 16.1 Each page generated for a study must have the following information included either in the header or hand written on the page: study or project number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst.
- 16.2 Print the tune page, sample list, and acquisition method from MassLynx to include in the appropriate study folder. Copy these pages and tape into the instrument runlog.
- 16.3 Plot the calibration curve by linear regression, weighted 1/x, then print these graphs and store in the study folder.
- 16.4 Print data integration summary, integration method, and chromatograms from MassLynx and store in the study folder.
- 16.5 Summarize data using suitable software (Excel 5.0+) and store in the study folder, refer to **Attachment A** for an example of a summary spreadsheet.
- 16.6 Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

- 17.1 Attachment A: ETS-8-7.0 Data summary spreadsheet

18.0 REFERENCES

- 18.1 FACT-M-2.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"
- 18.2 ETS-9-24.0, "Operation and Maintenance of the Micromass Atmospheric Pressure Ionization/Mass Spectrometer Quattro II triple quadrupole Systems"
- 18.3 The validation report associated with this method is ETS-8-6.0 & 7.0-V-1

19.0 AFFECTED DOCUMENTS

- 19.1 ETS-8-6.0, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver or Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry"

20.0 REVISIONS

Revision
Number

Reason For Revision

Revision
Date

Laboratory Study

Study:
 Test Material:
 Matrix/Final Solvent:
 Method/Revision:
 Analytical Equipment System Number:
 Instrument Software/Version:
 Filename:
 R-Squared Value:
 Slope:
 Y Intercept:
 Date of Extraction/Analyst:
 Date of Analysis/Analyst:

Group Dose	Sample#	Concentration ng/g	Initial Wt. g	Dilution Factor	Final Conc. ug/g

Slope: Taken from linear regression equation.

Group/Dose: Taken from the study folder.

Sample#: Taken from the study folder.

Concentration (ng/g): Taken from the MassLynx integration summary.

Initial Wt. (g): Taken from the study folder.

Dilution Factor: Taken from the study folder.

Final Conc. (ug/g): Calculated by dividing the initial volume from the concentration