Study Title

Characterization Study of PFOS, Primary Standard - Test Control Reference #TCR-00017-046

PHASE: SOLUBILITY OF PFOS IN WATER

Data Requirement

40 CFR 160.105(b)

Author

Mark E. Ellefson

Phase Completion Date

Date of signing

Performing Laboratory

3M Environmental Laboratory

Building 2-3E-09

935 Bush Avenue

St. Paul, MN 55106

Project Identification

3M Environmental Laboratory Study # FACT-TCR002 (LIMS #E00-1716)

Centre Analytical Laboratories Study # 023-021

Total Number of Pages

67

GLP COMPLIANCE STATEMENT

Study Title: Characterization Study of PFOS, Primary Standard – Test Control Reference # TCR-00017-046, PHASE: Solubility of PFOS in Water.

Study Identification Number: FACT-TCR002, Centre Analytical Laboratories Study # 023-021

This phase of the study was conducted in compliance with Environmental Protection Agency (EPA) Good Laboratory Practice (GLP) Standards 40 CFR 160 with the exceptions in the bulleted list below.

Exceptions to GLP compliance:

- Some corrections were not made in the raw data per GLP requirements
- The electronic data systems in use have not been validated and there is not an electronic audit trail of corrections currently available (40 CFR 160.130 (e)). Authenticated hardcopies of chromatograms and associated documents will be considered as the original raw data.
- Not all data was dated on the day of entry and signed or initialed by the person entering the data.
- The SOPs (methods) used in the study were not approved by management prior to using them.

Mark E. Ellefson, Principal Investigator — 03/30/01 *Date*

William K. Reagen, Testing Facility Management — 03/30/01 *Date*

QUALITY ASSURANCE STATEMENT

Study Title: Characterization Study of PFOS, Primary Standard – Test Control Reference # TCR-00017-046, PHASE: Solubility of PFOS in Water.

Study Identification Number: FACT-TCR002, Centre Analytical Laboratories Study # 023-021

This phase of the study has been inspected by the 3M Environmental Laboratory Quality Assurance Unit (QAU) as indicated in the following table. The findings were reported to the study director and laboratory management.

Inspection Dates	Phase	Date Reported to Management	Study Director
11/28/00, 12/04,05/00	Data	12/11/00	12/11/00
01/23/01, 01/25/01, 01/26/01, 01/29/01-01/31/01	Draft Phase Report	02/01/01	02/01/01

Tanya Reid 3/30/01

QAU Representative *Date*

TABLE OF CONTENTS

LIST OF TABLES

STUDY INFORMATION

Study Director

John Flaherty
Centre Analytical Laboratory
3048 Research Drive
State College, PA 16801

Analytical Chemistry Laboratories

Solubility Testing

3M Environmental Technology
and Safety Services (ET&SS)
3M Environmental Laboratory
Bldg. 2-3E-09
935 Bush Avenue
St. Paul, MN 55106

Dr. William K. Reagen, *Laboratory Manager*
Mark E. Ellefson, *Principal Analytical Investigator*
Kristin L. Terrell, *Analytical Chemist*
Mark L. Anderson, *Analytical Chemist*
Cindy M. Carlson, *Analytical Chemist*

Certificate of Analysis

Centre Analytical Laboratory
3048 Research Drive
State College, PA 16801

John Flaherty, *Study Director*

Sponsor

Dr. William K. Reagen
3M Environmental Technology and Safety Services (ET&SS)
3M Environmental Laboratory
2-3E-09
935 Bush Avenue
St. Paul, MN 55106

Experimental Dates

Sample Analysis Initiation: 13 July 2000
Sample Analysis Completion: 29 August 2000

Archives

Reserve samples of the reference standards will be maintained by the 3M Environmental Laboratory. All study data for this phase and a copy of the phase report are archived by the 3M Environmental Laboratory.

SUMMARY

The approximate solubility of PFOS in water was determined by performing a solubility screen test, followed by a quantitative solubility determination via Shake Flask Method.

Results are presented in table 1:

Table 1. Summary Table of Solubility of PFOS TCR-00017-046 in Water.

SAMPLING EVENT	PFOS SOLUBILITY IN WATER (CORRECTED FOR PURITY)	Standard Deviation	% CV
Day 1	621 µg/mL	23	4%
Day 2	717 µg/mL	91	13%
Day 3	702 µg/mL	2	0.3%
Average Value	**680 µg/mL**	43	6%

The solubility of PFOS TCR-00017-046 in water was determined to be 680 µg/mL at 24-25 °C.

PURPOSE

The purpose of this phase of the study was to determine the solubility of PFOS in water as part of the characterization of this test, control, and reference substance.

TEST SUBSTANCE

PFOS, Test Control Reference #TCR-00017-046

Table 2. Characterization of the Test Substance and Analytical Reference Substances

TEST SUBSTANCE	PFOS	THPFOS
Source	Re-crystallized by George Moore, 3M Specialty Materials, Bldg. 236-1B-10	SynQuest Labs
Expiration Date	01-01-2010	Not Available
Storage Conditions	Frozen	Frozen
3M Laboratory Identification Number	TCR-00017-046	TCR-00017-047 Lot #Q75-30
Physical Description	White Powder	White Powder
Purity	97.9%	*

**The purity of THPFOS (currently being determined by Centre Analytical Laboratory) will not affect any analytical findings. The same lot/source of THPFOS was used throughout the entire study.*

TEST SYSTEM

Table 3. Description of test systems used in this study

	WATER
Source	Millipore System
Expiration Date	NA
Storage Conditions	Ambient
3M Laboratory Identification Number	NA
Purity/Grade	ASTM Type I

METHOD SUMMARIES

The solubility determination of PFOS TCR-00017-046 in water was performed according to United States Environmental Protection Agency OPPTS 830.7840, "Water Solubility: Column Elution Method; Shake Flask Method" and OECD 105, "Water Solubility" Guidelines, using the 3M Environmental Laboratory methods ETS-8-170.1 "Solubility Screen Test: Approximate Solubility Determination of a Test Substance in Various Solvents;" and ETS-8-172.1 "Shake Flask Method; Solubility Determination of a Test Substance in Various Solvents". All analyses were performed according to the 3M Environmental Laboratory method ETS-8-155.0 "Analysis of Perfluorooctanesulfonate or Other Fluorochemicals in Waste Stream or Water Extracts Using HPLC-Electrospray/Mass Spectrometry".

The Solubility Screen Test Method (ETS-8-170.1) was used to determine the solubility range of PFOS TCR-00017-046 in water. Incremental amounts of water were added to PFOS neat material and a qualitative determination of the solubility point was made. The screen test indicated a solubility of PFOS in water of greater than 5,000 µg/mL. The Shake Flask Method was used for the quantitative determination of PFOS solubility. Detailed descriptions of the Solubility Screen Test Method and Shake Flask methods used in this study are located in Attachment A.

PREPARATORY METHODS

- **ETS-8-170.1 "Solubility Screen Test: Approximate Solubility Determination of a Test Substance in Various Solvents."** This method is a pre-requisite to the shake flask and column elution methods and gives an estimate of the solubility point of the test substance in the solvent of choice. Approximately ten milligrams of PFOS was weighed into a glass vial, and varying amounts of water were added in a step-wise fashion. After each water addition, the vial was shaken, sonicated, and observed for particulates. The screen test estimated the solubility of PFOS in water to be greater than 5,000 µg/mL. This concentration estimation was used as a starting point for the shake flask method, and as a guide to determine the amount of dilution necessary to bring the final sample concentration into the analytical range of method ETS-8-155.0 (2.5 – 1002 ng PFOS/mL).

Table 4. Solubility Screen Test Sample Preparation

STEP	CENTRIFUGE TUBES PREPARATION
1	10 mg test substance weighed into tared 4.0 mL glass screw-capped vial
2	0.1 mL of water added to glass screw-capped vial
3	Following the addition of water, vial was capped, shaken vigorously and vortexed, and sonicated. Mixing and sonication times recorded on the worksheet.
4	Sample checked visually for undissolved particles of the test substance. (If all of the test substance is dissolved, the solubility is determined to be greater than 10% (mass/volume) and no additional solubility testing is required. If a portion of the test substance remains undissolved, additional dilutions are performed using the appropriate solvent.) Observation made: undissolved particles present… continue to step 5.
5	Step 2 repeated with total volume of water of 0.5, 1, and 2 mL, respectively. Following water addition, vial was shaken, sonicated, and observed for particulate.
6	Following the final water addition to bring the volume of water to 2 mL, it appeared as though the PFOS had dissolved. The concentration was approximately 5,000 μg PFOS/mL and used as a starting point for the shake flask method.

- **ETS-8-172.1 "Shake Flask Method: Solubility Determination of a Test Substance in Various Solvents."** Greater than 0.045 g PFOS was weighed into a tared 15 mL plastic centrifuge tube. Water was added gravimetrically to approximately 10 g. Nine centrifuge tubes were prepared, along with three method blanks (tubes with 10 g ± 0.5 g water only). The centrifuge tubes were placed in a temperature-controlled orbital shaker set at approximately 30°C. See the following table documenting the shake flask method preparation:

Table 5. Shake Flask Method Preparation for Study (FACT-TCR002)

STEP	CENTRIFUGE TUBE PREPARATION	REPLICATES	DATE(S) PERFORMED
1	>0.045 g PFOS weighed into tared 15 mL centrifuge tube	9	07-31-00
2	Water added to centrifuge tube such that total weight of PFOS and water is > 9.9-10.4 g.	9	07-31-00
3	Method Blank Preparation- Approximately 10 mL water added to a tared centrifuge tube gravimetrically.	3	07-31-00
4	Samples sealed with tape, vortex-mixed, and placed in a temperature-controlled orbital shaker set at approximately 30.0°C, rotation speed set at approximately 225 rpm.	12	07-31-00

SAMPLE COLLECTION AND ANALYSIS

On each of Days 1, 2, and 3 (approximately 24, 48 and 72 hours after initial sample preparation) three centrifuge tubes (containing PFOS) and a method blank were removed for analysis. The centrifuge tubes with PFOS and method blanks were allowed to equilibrate at 24-25 °C for 24 hours, centrifuged, and aliquots of supernatant were collected. The aliquots were diluted serially using two 100X dilutions (990 μL methanol:10 μL sample) to a final dilution factor of 10,000X. The final diluted samples were spiked with internal standard (11μL of 22.2 μg

THPFOS/mL) and analyzed via HPLC/ES/MS according to analytical method ETS-8-155.0. Four injections of each final diluted sample were performed.

The following table describes the sample collection and preparation regimen:

Table 6. Sample Collection and Preparation for Study FACT-TCR002*

STEP	PROCEDURE	REPLICATES		DATE(S) PERFORMED
1	At 24, 48, and 72 hours, 3 PFOS+ water, and 1 water (method blank) centrifuge tubes are removed from the incubator and equilibrated at 4-25 °C for approximately 24 hours.	4 centri-fuge tubes per day	Day1	8-1-00
			Day2	8-2-00
			Day3	8-3-00
2	Aliquots of the supernatant (water containing dissolved PFOS or water only from the method blanks) were collected and dispensed into autovials.	4 per centrifuge tube	Day1	8-2-00
			Day2	8-3-00
			Day3	8-4-00
3	The aliquots were diluted 100X with methanol (using a dual syringe diluter). A second 100X dilution in methanol was performed to produce a final dilution factor of 10,000X.	Day 1		8-27-00
		Day 2		8-27-00
		Day 3		8-4-00
4	Internal Standard was added to the 10,000X sample dilutions (11 µL THPFOS solution at 22.2 µg /mL in methanol).	Day 1		8-27-00
		Day 2		8-27-00 (method blks only), and 8-29-00 (samples only)
		Day 3		8-7-00
5	Samples were analyzed via HPLC/ES/MS on HP 1100 LC/MSD	Day 1		8-27-00
		Day 2		8-27-00, and 8-29-00
		Day 3		8-25-00

*Information pertains to sample preparation and dates of final reported data only.

ANALYTICAL METHOD

- ETS-8-155.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Waste Stream or Water Extracts Using HPLC-Electrospray/Mass Spectrometry". Diluted samples (i.e. diluted supernatants) were analyzed using HPLC/ES/MS in the negative ion mode. PFOS levels were evaluated versus calibration standards ranging in concentration from 2.5-1002 ng PFOS/mL. Internal Standard quantification was used to normalize the data. Target ions were 499 m/z (PFOS anion), and 427 m/z (deprotonated THPFOS).

Analytical Equipment

Liquid Chromatograph: Hewlett-Packard® Series 1100 Liquid Chromatograph system
Analytical column: Keystone® Betasil™ C_{18} 2x50mm, 5µm particle size
Column temperature: Ambient
Cycle Time: 10.5 minutes
Flow rate: 300µL/min
Injection volume: 5µL
Mobile phase components:
Solvent A: 2.0 mM ammonium acetate in water

Solvent B: Methanol

Solvent Gradient:

Time	% B
0.00	40 %
1.00	40 %
4.50	95 %
7.50	95 %
8.00	40 %
10.50	Stop

Mass Spectrometer: Hewlett-Packard® Series 1100 API/Mass Spectrometer Detector
Software: HP ChemStation™ 6.0
Fragmentor Voltage: m/z 427=100 V; m/z 499= 140 V
Capillary Voltage: 3500 V
Gain = 2.0 EMV
Mode: Electrospray Negative
Gas Temperature: 350 °C
Drying Gas: 10.0 L /min.
Nebulizer Pressure: 25 psig
Analysis Type: Single Ion Monitoring (SIM)

DATA SUMMARY, ANALYSES, AND RESULTS

ANALYTICAL RESULTS

Data quality objectives outlined in the 3M Environmental Laboratory method were met (see Appendix A).

- **Regressions.** Quadratic curve fit was applied to calibration standards and sample data to improve quantitation over the concentration range appropriate to the data. All calibration curves had least-square fits of 0.990 or greater (R^2 *values ranged from 0.9960 - 0.9998*).
- **Calibration Standards.** Eleven standards ranging in concentration from approximately 2.5 to 1000 ng PFOS/mL methanol were used for the calibration curves. Calibration curves were run before and after every analytical sequence.
- **Sample Replicates.** Samples 073100-wPFOS-1.1.4, 073100-wPFOS-2.2.4, and 073100-wPFOS-2.3.4 were excluded from the reported data using Dixon's Q-Test. Day 2, sample 1 was barely above the warning limit (RSD of 15.9%).
- **Continuing Calibration Verification.** For quantitative determinations, a mid-level matrix calibration check was analyzed every five samples to monitor instrumental drift, with a limit of ±15% deviation of the target concentrations.
- **Limit of Quantitation (LOQ).** The LOQ is equal to the lowest standard in the calibration curve (either 2.5 or 5.0 ng/mL).

Table 7. Calibration Standard Reprocessing Summary

DATA SUB BATCH, ANALYSIS DATE	CALIBRATION STANDARDS	STANDARDS NOT USED, REASON FOR EXCLUSION
Day 3 data: 08-25-00	5 – 500 ng/mL	Std 1 (2.5 ng/mL), and Std 11 (1002 ng/mL) were excluded to better fit the data at the low end of the curve.
Day 1 data: 08-27-00	2.5 – 500 ng/mL	Std 11 (1002 ng/mL) was excluded to better fit the data at the low end of the curve.
Day 2 data: 08-29-00	5 – 500 ng/mL	Std 1 (2.5 ng/mL), and Std 11 (1002 ng/mL) were excluded to better fit the data at the low end of the curve.

- **Blanks.**
  - Method blanks (Milli-Q water taken through the entire sample preparation, dilution, and analysis process) provided a measure of laboratory contamination. Acceptable values for the blanks were less than 50% of the limit of quantitation (LOQ).
  - Solvent blanks (methanol injections) provided a measure of instrument contamination. Acceptable solvent blanks must contain levels of target analyte less than 50% of the limit of quantitation (LOQ). The initial solvent blank in each of the sample run sequences for days 1 and 2 data had background levels of PFOS >50% of the LOQ. Day 1, run #19 exhibited a background level of PFOS>50% of the LOQ. Acceptable solvent blanks were analyzed before each calibration curve.
- **Specificity (according to OPPTS 830.7840):** The solubility determination as stated in the EPA Guidelines follows. This method should only be applied to:
  - Pure substance. This study shows a purity of 97.9%.
  - Substances that are stable in water. Hydrolytic studies have been conducted at the 3M Environmental Laboratory showing stability (Report # W1878).

DATA SUMMARY

Table 8 summarizes individual sample data. Representative chromatograms are presented in Attachment C. The table displays PFOS concentrations for individual injections. Also included are the average concentrations for each Centrifuge Tube replicate, standard deviation, and % coefficient of variation.

Table 8. PFOS TCR-00017-046 (PFOS) Data Summary

	Sample ID	TCR-00017-046 PFOS ug/mL Corrected for Purity	Average ug/mL Std Dev %Coefficient of variation
Day 1 Centrifuge Tube #1	Rep 1: 73100-wPFOS-1.1.1	594	596*
	Rep 2: 73100-wPFOS-1.1.2	579	14
	Rep 3: 73100-wPFOS-1.1.3	613	2%
	Rep 4: 73100-wPFOS-1.1.4	2593*	*Rep 4 excluded via Q-Test
Day 1 Centrifuge Tube #2	Rep 1: 73100-wPFOS-1.2.1	659	617
	Rep 2: 73100-wPFOS-1.2.2	653	42
	Rep 3: 73100-wPFOS-1.2.3	599	7%
	Rep 4: 73100-wPFOS-1.2.4	556	
Day 1 Centrifuge Tube #3	Rep 1: 73100-wPFOS-1.3.1	667	650
	Rep 2: 73100-wPFOS-1.3.2	659	33
	Rep 3: 73100-wPFOS-1.3.3	595	5%
	Rep 4: 73100-wPFOS-1.3.4	680	
Day 2 Centrifuge Tube #1	Rep 1: 73100-wPFOS-2.1.1	646	723
	Rep 2: 73100-wPFOS-2.1.2	718	115
	Rep 3: 73100-wPFOS-2.1.3	617	16%
	Rep 4: 73100-wPFOS-2.1.4	911	
Day 2 Centrifuge Tube #2	Rep 1: 73100-wPFOS-2.2.1	580	603*
	Rep 2: 73100-wPFOS-2.2.2	572	38
	Rep 3: 73100-wPFOS-2.2.3	656	6%
	Rep 4: 73100-wPFOS-2.2.4	1446*	*Rep 4 excluded via Q-Test
Day 2 Centrifuge Tube #3	Rep 1: 73100-wPFOS-2.3.1	851	826*
	Rep 2: 73100-wPFOS-2.3.2	932	98
	Rep 3: 73100-wPFOS-2.3.3	696	12%
	Rep 4: 73100-wPFOS-2.3.4	3702*	*Rep 4 excluded via Q-Test
Day 3 Centrifuge Tube #1	Rep 1: 73100-wPFOS-3.1.1	648	703
	Rep 2: 73100-wPFOS-3.1.2	723	32
	Rep 3: 73100-wPFOS-3.1.3	723	5%
	Rep 4: 73100-wPFOS-3.1.4	717	
Day 3 Centrifuge Tube #2	Rep 1: 73100-wPFOS-3.2.1	705	705
	Rep 2: 73100-wPFOS-3.2.2	702	4
	Rep 3: 73100-wPFOS-3.2.3	712	1%
	Rep 4: 73100-wPFOS-3.2.4	702	
Day 3 Centrifuge Tube #3	Rep 1: 73100-wPFOS-3.3.1	683	699
	Rep 2: 73100-wPFOS-3.3.2	685	15
	Rep 3: 73100-wPFOS-3.3.3	718	2%
	Rep 4: 73100-wPFOS-3.3.4	711	

The average PFOS concentration and standard deviation for each day, as well as the final overall average for all non-excluded replicates and time points were as follows:

Table 9. Summary Table of Solubility of PFOS TCR-00017-046 in Water.

Sampling Event	PFOS Solubility in Water (Corrected for Purity)	Standard Deviation	% CV
Day 1	621 µg/mL	23	4%
Day 2	717 µg/mL	91	13%
Day 3	702 µg/mL	2	0.3%
Average Value	**680 µg/mL**	43	6%

The solubility of PFOS TCR-00017-046 in water is 680 µg/mL at 24-25 °C.

Attachment B contains data summary tables.

Statistical Methods

Statistical methods were limited to calculating means and standard deviations. Data that did not meet acceptance criteria as described in the OPPTS and OECD guidelines was excluded using statistical justification provided by Dixon's Q-test. Refer to Attachment E for formulas and example calculations.

Statement of Conclusion

Under the conditions of the present study, the solubility of PFOS TCR-00017-046 in ASTM Type I water is 680 µg/mL at 24-25 °C.

References

1. Fate, Transport and Transformation Test Guidelines Office of Prevention, Pesticides and Toxic Substances (OPPTS) 830.7840 Water Solubility: Column Elution Method; Shake Flask Method. EPA 712-C-96-041, August 1996.

2. OECD 105: Water Solubility. Adopted 27July, 1995.

LIST OF ATTACHMENTS

- Attachment A: Extraction and Analytical Methods
- Attachment B: Data Summary Tables
- Attachment C: Sample Chromatograms
- Attachment D: Deviations from the Protocol
- Attachment E: Sample Calculations

SIGNATURE PAGE

We certify that this report is a true and complete representation of the data for this phase of the study:

Mark E. Ellefson — Date: 03/30/01
Principal Investigator

William K. Reagen — Date: 03/30/01
Laboratory Manager

ATTACHMENT A: EXTRACTION AND ANALYTICAL METHODS

3M ENVIRONMENTAL LABORATORY

SOLUBILITY SCREEN TEST: APPROXIMATE SOLUBILITY DETERMINATION OF A TEST SUBSTANCE IN VARIOUS SOLVENTS

Method Number: ETS-8-170.1

Adoption Date: 09/08/00

Effective Date: 03/14/01

Approved By:

William K. Reagen, Laboratory Manager — Date: 03/14/01

Mark E. Ellefson, Project Leader — Date: 03/14/01

1.0 SCOPE AND APPLICATION

1.1 Purpose. According to methods set forth by the United States Environmental Protection Agency (US EPA) and the Organization for the Economic Cooperation and Development (OECD) a preliminary study, which will be outlined in this method, serves as a prerequisite to performing solubility testing via the Column Elution Method and Shake Flask Methods. The EPA and OECD solubility testing guidelines state that if the preliminary solubility determination test indicates a solubility of $> 10^{-2}$ g/L (10 ppm), the Shake Flask Method (ETS-8-172.0) is to be used. If preliminary testing indicates a solubility of $< 10^{-2}$ g/L (10 ppm) the Column Elution Method (ETS-8-171.0) will be used to determine the solubility in the solvent of interest. Additionally, due to the tendency of certain compounds to be highly soluble in particular solvents, no further solubility determination will be necessary for compounds having solubility greater than 10% (mass/volume).

1.2 Compatible Analytes. Test substance and degradation products for solubility testing.

MH Initial — 30 Mar 01 Date

1.3 **Acceptable Matrices**. Aqueous (e.g. Milli-Q water, 0.01M $CaCl_2$), Acetone, Methanol, or other solvent(s) of interest.

2.0 SUMMARY OF METHOD

2.1 **Preliminary Solubility Determination in solvent.** Weigh approximately 10 mg of test substance into a 2.5 mL-4 mL glass screw-capped vial. Add test solvent in varying increments to the vial. After each solvent addition, vortex mix for approximately 15 sec., and sonicate 2-5 minutes. Make observations of the solution and visually confirm whether or not particles are present; it may be necessary to allow the solution to sit for up to 5 minutes. Add test solvent in a stepwise fashion up to 2 mL. If the substance fully dissolves after the first solvent addition of 100 µL, the test substance shall be considered highly soluble, and no further testing is required. If there are still undissolved particles in the vial, weigh 10 mg of test substance into a 100 mL glass stoppered volumetric flask or graduated cylinder. Add test solvent until visual confirmation of the substance dissolution has been achieved. Record all observations on a standardized preparation sheet or logbook. Once 100 mL solvent has been reached, the approximate concentration is at 100 µg/mL. If the solubility limit has not yet been achieved, weigh approximately 10 mg of test substance and transfer to a 1 L graduated cylinder/volumetric flask (concentration is approximately 10 ppm). The container should be sonicated and allowed to sit overnight to allow for maximal dissolution. If the undissolved particles are still observed after sitting > 12 hours, the column elution method will be utilized (ETS-8-171.0). If the test substance dissolves in 1 L or less of solvent, the shake flask method (ETS-8-172.0) will be used to determine the solubility.

3.0 DEFINITIONS

3.1 **Test substance.** A liquid or solid material for which the relative solubility in a specified solvent will be determined.

3.2 **Test solvent.** The matrix to which the test substance of interest is introduced. The test solvent may include but is not limited to aqueous matrices, including ASTM Type I Water and various salt matrices (e.g. 0.01M $CaCl_{2\,(aq)}$); and organic solvent matrices, including methanol, and acetone.

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

4.1.1 Wear the proper lab attire for all parts of these procedures. Wear gloves and eye protection at all times.

4.1.2 Handle all solvents in a hood for all parts of the described sample preparation procedure.

4.1.3 For potential hazards of each chemical used, refer to material safety data sheets, packing materials, and 3M Environmental Laboratory's Chemical Hazard Review.

4.1.4 No mouth pipetting is allowed.

4.2 Cautions:

4.2.1 Glassware in which standards are prepared should be triple rinsed with acetone and methanol to reduce the possibility of accidental contamination.

5.0 INTERFERENCES

5.1 Impurities may significantly affect the solubility of the test substance. The purity of the test substance should be known and documented prior to starting the screening procedure.

6.0 EQUIPMENT

6.1 Analytical balance sensitive to 0.1 mg.

6.2 Vortex mixer.

6.3 Sonicating device.

7.0 SUPPLIES AND MATERIALS

7.1 Disposable glass graduated pipettes, 1 mL to 100 mL.

7.2 Disposable glass Pasteur pipettes and rubber bulbs.

7.3 Glass beakers, various sizes.

7.4 2.5 mL-4 mL glass screw-top vial.

7.5 Glass volumetric flasks, 10 mL to 1000 mL.

7.6 10 µL-1000 µL Pipetteman™ manual pipettor and plastic pipette tips, or equivalent.

8.0 REAGENTS AND STANDARDS

8.1 **Methanol** (MeOH), HPLC/SPEC/GC grade from EM Science, or equivalent.

8.2 **Acetone**, HPLC/SPEC/GC grade from EM Science or equivalent.

8.3 **Water,** ASTM Type I, or equivalent.

8.4 **Calcium Chloride Dihydrate,** Approximately 99% or better, from Sigma or equivalent.

8.5 **0.01 M $CaCl_2$,** A 0.01 M $CaCl_2$ stock solution is prepared by weighing 1.5 g $CaCl_2$ Dihydrate in a weigh boat and transferring to a 1 L volumetric flask and diluting to the mark with Milli-Q™ water.

8.6 **Test substance of known purity.**

9.0 SAMPLE HANDLING

9.1 Record times of initial preparation and dilution on a sample preparation sheet or logbook.

9.2 Once the preliminary testing has been completed and the appropriate information is extracted, the screen test samples should be disposed into the proper waste stream.

10.0 QUALITY CONTROL

10.1 Not applicable.

11.0 CALIBRATION AND STANDARDIZATION

11.1 The compounds of interest must be of known characterization according to laboratory specifications.

11.2 All equipment used, such as the analytical balance, should be calibrated daily prior to use.

12.0 PROCEDURES

12.1 Solubility screen.

12.1.1 Weigh 10 mg ± 1 mg of test substance into a 4 mL glass screw-top vial or equivalent. Record the weight (refer to attachment A for an example of a standardized prep sheet).

12.1.2 Add solvent (e.g. water, methanol, acetone) according to the table below to the test substance.

4 mL vial or equivalent	Solvent addition 1	Solvent addition 2	Solvent addition 3	Solvent addition 4
Total volume solvent added (mL)…	0.1	0.5	1	2
Approximate Solubility (µg/mL)…	100000	20000	10000	5000

12.1.3 Following addition of solvent, vortex mix approximately 15 seconds, sonicate 2-5 minutes. Visually check for undissolved particles. If undissolved test substance remains, continue adding solvent according to the above chart. It may be necessary to allow the solution to settle for up to 5 minutes before making observations. Record observations on the standardized prep sheet.

12.1.4 If all particles dissolve, then estimate the approximate solubility and document the concentration. Because the concentration is >10 µg/mL, the shake flask method (ETS-8-172.0) will be used to determine the accurate solubility point. However, if the test substance dissolves after the *first* solvent addition of 100 µL the solubility is determined to be >10% (mass/volume). The test substance shall be regarded as "highly soluble," and no further testing is required.

12.1.5 If the test substance did not dissolve in the 2 mL of solvent, weigh 10 mg ± 1 mg of test substance into a 100 mL glass volumetric flask or equivalent. Record the weight (refer to attachment A for an example of a standardized prep sheet).

12.1.6 Add solvent according to the table below to the test substance to deliver the appropriate amount of solvent. As described in 12.1.3, vortex mix, sonicate, and allow the test solution to settle. Record all observations and procedures on the preparation sheet.

100 mL volumetric flask or equivalent	Solvent addition 1	Solvent addition 2	Solvent addition 3
Total volume solvent added (mL)...	10	50	100
Approximate Solubility (µg/mL)...	1000	200	100

12.1.7 If the solubility point is reached at or prior to the complete addition of 100 mL, the concentration estimation at which the substance is soluble should be documented and shall be used as a starting point for the shake flask method (ETS-8-172.0).

12.1.8 If the 100 mL solvent level has been reached without complete dissolution of test substance, weigh out 10 mg ± 1 mg test substance and transfer to a 1 L volumetric flask, graduated cylinder, or equivalent. Dilute to 1 L with test solvent. The approximate concentration of test substance is at 10 µg/mL. The flask should be allowed to sit 12-24 hours to allow for maximal dissolution. If the undissolved particles are still observed, the column elution method will be utilized (ETS-8-171.0). If no visible particulates are observed, the shake flask method (ETS-8-172.0) shall be used to determine the solubility.

13.0 Data Analysis and Calculations

13.1 The solubility determination in this method is qualitative/semi-quantitative.

13.2 The point to which the substance dissolves in solvent is confirmed visually. The solubility point is therefore a qualitative determination.

13.3 The concentration estimation is semi-quantitative in that the approximate concentration is calculated by the following equation:

$c = a/b$

Where:

c= the semi-quantitative concentration µg/mL,

a= amount of substance weighed out (µg), and

b= the approximate amount of solvent added (mL).

14.0 Method Performance

14.1 Limitation of data. The accuracy to which the solubility is determined is subject to a large margin of error due to the way in which the solvent is added to the solute. Since large and varying increments of solvent are being added to the solute, this error margin must be considered when reporting the concentration estimation.

14.2 The data obtained through this study is an estimation only and should be treated as a qualitative estimation of the solubility of test substance in a given solvent.

14.3 For an accurate measurement of the substance solubility concentration, the shake flask method, or column elution method will be used.

15.0 Pollution Prevention and Waste Management

15.1 Dispose of sample waste by placing in high or low BTU containers as appropriate. Use broken glass containers to dispose of glass pipettes.

16.0 Records

16.1 Sign and date all observations and calculations.

17.0 Attachments

17.1 **Attachment A**-Example of a Standardized Sample Preparation Sheet: Solubility Screen Prep Sheet.

18.0 References

18.1 Organization for Economic Cooperation and Development. OECD Guideline for Testing of Chemicals. Water Solubility -OECD Guideline 105: pp. 1–7, Adopted 1995

18.2 United States Environmental Protection Agency. OPPTS 830.7860 Water Solubility (Generator Column Method). Prevention, Pesticides and Toxic Substances: Fate, Transport and Transformation Test Guidelines. EPA 712-C-96-042: pp. 1–17, 1996

18.3 United States Environmental Protection Agency. OPPTS 830.7840 Water Solubility: Column Elution Method; Shake Flask Method. Prevention, Pesticides and Toxic Substances: Fate, Transport and Transformation Test Guidelines. EPA 712-C-96-041: pp. 1–12, 1996

18.4 3M Environmental Laboratory Method ETS-8-171.0, "Column Elution Method: Solubility Determination of Test Substance in Various Solvents."

18.5 3M Environmental Laboratory Method ETS-8-172.0, "Shake Flask Method: Solubility Determination of Test Substance in Various Solvents."

19.0 Affected Documents

19.1 ETS-8-171.0, "Column Elution Method: Solubility Determination of Test Substance in Various Solvents."

19.2 ETS-8-172.0, "Shake Flask Method: Solubility Determination of Test Substance in Various Solvents."

20.0 REVISIONS

Revision Number	Reason For Revision	Revision Date
1	It was desirable to make the method more universally applicable by removing references to specific test substances.	03/13/01

Attachment A: Example Preparation Worksheet, page 1 of 2

3M Environmental Laboratory SOLUBILITY SCREEN

GLP Study Number: _____ ***Sample preparation worksheet***

Test Substance: _____ **ID#:** _____ **Date:** _____

Source: _____

Solvent: _____ **ID#:** _____

Source: _____

Step

1 *Weigh approximately 10 mg ± 1 mg of test substance into a 2.5 mL-4 mL glass screw-top vial.*
Weight of test substance: _____mg Balance ID:_____ Date/Initials:_____
*2nd replicate optional:*_____mg Thermometer ID:_____

2 *Add test solvent according to the table below. Following each addition of solvent, shake vigorously/vortex, and sonicate the mixture and visually check for undissolved particles.*

Solubility data	volume added (mL)/ approx conc. after addition (ug/mL)	Sample Prep	OBSERVATIONS/NOTES
Analyst Date/Time ___/___ ___ Total volume solvent added (mL) Approximate Solubility (ug/mL)	**Step 2-1** 0.1 100,000	Flask vortex-mixed? Yes/No Room Temp ___°C Flask sonicated? Yes/No Time ___min Flask allowed to settle? Yes/No Time ___min	Is there solute still present? ~~Yes~~ continue to step 2-2 **NO-** The solution is at 10%, and is considered "infinitely soluble." No further solubility testing is required.
Analyst Date/Time ___/___ ___ Total volume solvent added (mL) Approximate Solubility (ug/mL)	**Step 2-2** 0.5 20,000	Flask vortex-mixed? Yes/No Room Temp ___°C Flask sonicated? Yes/No Time ___min Flask allowed to settle? Yes/No Time ___min	Is there solute still present? Yes/No
Analyst Date/Time ___/___ ___ Total volume solvent added (mL) Approximate Solubility (ug/mL)	**Step 2-3** 1 10,000	Flask vortex-mixed? Yes/No Room Temp ___°C Flask sonicated? Yes/No Time ___min Flask allowed to settle? Yes/No Time ___min	Is there solute still present? Yes/No
Analyst Date/Time ___/___ ___ Total volume solvent added (mL) Approximate Solubility (ug/mL)	**Step 2-4** 2 5,000	Flask vortex-mixed? Yes/No Room Temp ___°C Flask sonicated? Yes/No Time ___min Flask allowed to settle? Yes/No Time ___min	Is there solute still present? Yes/No

Conclusions:

Did the substance dissolve in 2 mL or less of solvent?

Yes -Approximate concentration at which the test substance is soluble in solvent: _____ug/ml
The solubility of the test substance is to be determined via the shake flask method.

or **No** Continue to page two for further testing.

Notes/ additional comments:

Attachment A Cont.: Example Preparation Worksheet, page 2 of 2

3M Environmental Laboratory SOLUBILITY SCREEN

GLP Study Number: _____ ***Sample preparation worksheet***

Test Substance: _____ **ID#:** _____ **Date:** _____

Source: _____

Solvent: _____ **ID#:** _____

Source: _____

Step (continued from page 1)

*****Did the test substance dissolve in 2 mL? Yes/No If YES, the test is complete, and there is no need to continue with the screen test. If NO, continue to step 3 to determine the approximate solubility of the substance.**

3 *Weigh approximately 10 mg ± 1 mg of test substance into a 100 mL graduated stoppered cylinder/volumetric flask.*

Weight of test substance: _____ mg Balance ID: _____ Date/Initials: _____

2nd replicate optional: _____ mg Thermometer ID: _____

4 *Add test solvent according to the table below. Following each addition of solvent, shake vigorously/vortex, and sonicate the mixture and visually check for undissolved particles.*

Solubility data	volume added (mL)/ approx conc. after addition (ug/mL)	Sample Prep	OBSERVATIONS/NOTES
Analyst _____ Date/Time ____/____	**Step 4-1**	Flask vortex-mixed? Yes/No Room Temp ____ °C	Is there solute still present? Yes/No
Total volume solvent added (mL)...	10	Flask sonicated? Yes/No Time ____ min	
Approximate Solubility (ug/mL)...	1,000	Flask allowed to settle? Yes/No Time ____ min	
Analyst _____ Date/Time ____/____	**Step 4-2**	Flask vortex-mixed? Yes/No Room Temp ____ °C	Is there solute still present? Yes/No
Total volume solvent added (mL)...	50	Flask sonicated? Yes/No Time ____ min	
Approximate Solubility (ug/mL)...	200	Flask allowed to settle? Yes/No Time ____ min	
Analyst _____ Date/Time ____/____	**Step 4-3**	Flask vortex-mixed? Yes/No Room Temp ____ °C	Is there solute still present? Yes/No
Total volume solvent added (mL)...	100	Flask sonicated? Yes/No Time ____ min	
Approximate Solubility (ug/mL)...	100	Flask allowed to settle? Yes/No Time ____ min	

5 *Weigh approximately 10 mg ± 0.5 mg of test substance into a 1 L graduated stoppered cylinder/volumetric flask/or equivalent.*

Weight of test substance: _____ mg Balance ID: _____ Date/Initials: _____

Analyst _____ Date/Time ____/____	**Step 5-1**	Flask vortex-mixed? Yes/No Room Temp ____ °C	Is there solute still present? Yes/No
Total volume solvent added (mL)...	1000	Flask sonicated? Yes/No Time ____ min	
Approximate Solubility (ug/mL)...	10	Flask allowed to settle? Yes/No Time ____ min	

Conclusions:

Did the substance dissolve in 1000 mL or less of solvent?

Yes -Approximate concentration at which the test substance is soluble in solvent: _____ ug/ml

The solubility of the test substance is to be determined via the shake flask method.

or **No** -The solubility of the test substance is to be determined via the column elution method.

Notes/ additional comments:

3M ENVIRONMENTAL LABORATORY

SHAKE FLASK METHOD: SOLUBILITY DETERMINATION OF A TEST SUBSTANCE IN VARIOUS SOLVENTS

Method Number: ETS-8-172.1 **Adoption Date:** 09/08/00

Effective Date: 03/27/01

Authors: Kristin L. Terrell, Mark L. Anderson, and Mark E. Ellefson

Approved By:

William K. Reagen, Laboratory Manager — Date 03/27/01

Mark E. Ellefson, Project Lead — Date 03/27/01

1.0 Scope and Application

1.1 Purpose. According to the United States Environmental Protection Agency (U.S. EPA) and Organization for the Economic Cooperation and Development (OECD) guidelines, solubility determination of substances that have solubilities in a given test matrix (e.g. water/acetone/methanol) of greater than 100 µg/mL must be analyzed via the shake flask method (OECD Guideline 105, and OPPTS Guideline 830.7840). The prerequisite to this method is a preliminary screen of the test compound for its approximate solubility level. Refer to ETS-8-170.0 for the preliminary solubility screen test procedures.

MVA 30 Mar 01

1.2 **Compatible Analytes.** Test substance and degradation products for solubility testing.

1.3 **Acceptable Matrices.** Water, Acetone, and Methanol, or other solvents of interest.

2.0 SUMMARY OF METHOD

2.1 Using the qualitative/semi-quantitative data obtained from the preliminary solubility screen test, weigh out more than five times the estimated soluble concentration of test substance into each of twelve screw-capped vessels (three time points with four test vessels each-sample, duplicate, triplicate, and matrix blank). Add the appropriate amount of test solvent gravimetrically. Cap the tubes, seal the caps with tape, and place horizontally on an incubator/shaker set to 30 °C ± 2 °C, shaking for approximately 24 hours. At approximately 24 hours, the first time point will be pulled and equilibrated at room temperature (about 20 °C-26 °C) for approximately 24 hours. The equilibrated samples are centrifuged and aliquoted into autovials. Samples are then diluted 1:10 or higher with a suitable solvent for analysis via LC/MS. At approximately 48 hours and 72 hours, the second and third sets, respectively, will be pulled, equilibrated, centrifuged, aliquoted and diluted with a suitable analytical solvent for LC/MS analysis. Depending on the analytical range, dilutions may be made using a diluter or syringe to make successive serial dilutions using a suitable analytical solvent to reach the desired concentration (e.g. 1:10 or higher sample:methanol/acetone serial dilutions), and an appropriate internal standard (e.g. 1H,1H,2H,2H tetrahydroperfluorooctane sulphonic acid (THPFOS)) will be added to the final dilution prior to analysis. Samples are to be analyzed via LC/MS against a standard curve containing the test substance and an appropriate internal standard.

3.0 DEFINITIONS

3.1 **Method blank:** An analyte-free matrix (e.g. methanol, water, or acetone) to which all reagents agree are added in the same volumes or proportions as used in the sample processing. The method blank is used to document contamination resulting from the entire sample treatment and analytical process. The method blank is carried through the complete sample preparation, treatment, and analytical procedure.

3.2 **Solvent blank:** A sample of analyte-free medium (e.g. methanol/water/acetone solution) that is not taken through the sample treatment process. This blank is used to evaluate instrument and reagent contamination.

3.3 **Shake Flask Sample Triplicates:** Three test vessels taken from and representative of the same sample source carried through all steps of the treatment, extraction, and analytical procedures in an identical manner.

3.4 **Sample replicates:** Replicate samples (two or more) taken from and representative of the same sample source (e.g. test vessel containing test analyte and solvent) and separately carried through analytical procedures in an identical manner.

3.5 **Internal Standard (IS):** A known amount of a compound similar in analytical behavior to the compound(s) of interest, added to all samples and standards, and carried through the entire measurement process.

3.6 **Calibration Standard:** A dilution of various amounts of a stock, intermediate or purchased standard to achieve standard solutions in a concentration range of interest.

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

- 4.1.1 Wear the proper lab attire for all parts of these procedures. Wear gloves and eye protection at all times.
- 4.1.2 Handle all solvents in a hood for all parts of the described sample preparation procedure.
- 4.1.3 For potential hazards of each chemical used, refer to material safety data sheets, packing materials, and 3M Environmental Laboratory's Chemical Hazard Review.
- 4.1.4 No mouth pipetting is allowed.

4.2 **Cautions:**

- 4.2.1 Glassware in which standards are prepared are to be triple rinsed with acetone and methanol to reduce the possibility of accidental contamination.
- 4.2.2 All test vessels (e.g. centrifuge tubes) are to be sufficiently rinsed with solvent prior to their usage.

5.0 INTERFERENCES

5.1 Impurities may significantly affect the solubility of the test substance. The purity of the test substance should be known and documented prior to starting the preliminary solubility screening procedure.

5.2 Contaminants in solvents, reagents, glassware and other sample processing or analysis hardware may cause interference. The routine analysis of laboratory method blanks must be used to demonstrate that there is no interference under the conditions of the analysis.

6.0 EQUIPMENT

6.1 Analytical balance sensitive to 0.1 mg.
6.2 Centrifuge capable of holding 15 mL centrifuge tubes or equivalent.
6.3 Incubator with heating/cooling capabilities.
6.4 Diluter, Hamilton Microlab® 500 Series, or equivalent.
6.5 Vortex-mixer.

7.0 SUPPLIES AND MATERIALS

7.1 Thermometer capable of reading at least 15 °C-40 °C.
7.2 5-250 mL polypropylene centrifuge tubes, or equivalent.
7.3 Disposable glass graduated pipettes, 1 mL to 10 mL.
7.4 Disposable glass pasteur pipettes and rubber bulbs.
7.5 Glass beakers, various sizes.
7.6 Crimp cap autovials-1.5 mL, caps, crimper, and decapper.
7.7 Hamilton Gastight® syringes (precision ± 1% of the total volume), 5 µL to 1000 µL.
7.8 Pipette-man manual pipettor.

8.0 SUPPLIES AND MATERIALS

8.1 **Methanol** (MeOH), HPLC/SPEC/GC grade from EM Science, or equivalent.
8.2 **Acetone**, HPLC/SPEC/GC grade from EM Science or equivalent.
8.3 **Water,** ASTM Type 1.
8.4 **Test substance of known purity.**
8.5 **Calcium Chloride Dihydrate,** Approximately 99% or better, from Sigma.
8.6 **0.01 M $CaCl_2$ solution,** Example: A 0.01 M $CaCl_2$ stock solution is prepared by weighing 1.4 g $CaCl_2$ in a weigh boat and transferring to a 1 L volumetric flask and diluting to the mark with Milli-Q™ water.

9.0 SAMPLE HANDLING

9.1 Record times of initial preparation, set-up, and sample aliquoting/analysis on a sample preparation sheet or logbook.
9.2 Once the samples have been diluted, they may be analyzed via LC/MS. Alternatively, the diluted samples may be kept in cold storage (e.g. approximately 1-5 °C) in crimp-capped autovials until the time of analysis.

10.0 QUALITY CONTROL

10.1 **Shake Flask Sample Triplicates.** Set up each test vessel in triplicate to provide a measure of the precision on sample preparation.
10.2 **Sample Replicates.** Prepare and analyze all samples (from each test vessel for each time point) in multiple replicates (2 or more) to provide a measure of the precision on analysis.
10.3 **Quality Control Blank Samples.**
10.3.1 **Method blank.** Set up a fourth test vessel without test substance to measure any contamination accrued throughout the sample preparation process. The method blank is carried through the same sample preparation procedures as the three test vessels with test substance, only no test substance is added throughout the entire procedure.
10.3.2 **Solvent Blank.** An aliquot of the dilution solvent (i.e. methanol) directly analyzed for possible contaminants. The solvent blank is used to document any possible contamination of the solvent(s) used during the sample preparation process, and to detect any instrumental contamination or background interferences.

11.0 CALIBRATION AND STANDARIZATION

11.1 The compounds of interest must be standardized according to laboratory specifications.
11.2 All equipment used, such as the analytical balance and automated diluter, should be calibrated prior to use (daily, weekly, etc.) as specified in its standard operating procedure.

11.3 All samples analyzed will be run against a standard curve containing varying amounts of test substance, and a fixed amount of internal standard.

12.0 CALIBRATION AND STANDARIZATION

12.1 Preparation of Test Vessel. Record all data and observations on the example sample preparation worksheet (Attachment A) or equivalent.

12.1.1 Prepare a solution at least five times more concentrated than the concentration at which the substance was known to be dissolved at in the preliminary solubility screen test (ETS-8-170.0).

12.1.2 Weigh out test substance into a tared test vessel (e.g. 15 mL polypropylene centrifuge tube).

12.1.3 Add the test solvent to the test vessel gravimetrically until the desired volume has been obtained (*note: solvents have differing densities, therefore the weight needed to achieve a specific volume will be dependent on the density.).* Record all weights on a standardized preparation sheet or logbook.

12.1.4 Visually confirm that there are undissolved particles. If no particles are present, repeat 12.1.2 with more test substance or add more test substance and record the additional weight.

12.1.5 Prepare the solution described in 12.1.1-12.1.4 nine times and label (at minimum) the test vessels as Day 1, -Rep 1, -Rep 2, and -Rep 3; Day 2, -Rep 1, -Rep 2, and -Rep 3; and Day 3, -Rep 1, -Rep 2, and -Rep 3. Also include the day of initial sample prep, the person(s) responsible for the sample, the test compound, nature of the study (e.g. water solubility), the solvent utilized, and the study number.

12.1.6 Prepare three test vessels as described in 12.1.3 without the test substance and label (at minimum) as Day1,Rep4; Day2,Rep4; and Day3,Rep4 along with the information described under 12.1.5. These three test vessels are the "method blank" samples.

12.1.7 Seal the cap to the test vessel. Mix/shake test vessel to ensure contact between the test substance and the solvent. And wrap the cap with tape.

12.2 Equilibration of samples.

12.2.1 Place all of the test vessels on their side (horizontal) in an orbital incubator set to approximately 30 °C ± 2 °C and rotating at a considerable speed to ensure sufficient contact/mixing between the test substance and solvent.

12.2.2 At approximately 24 hours, four test vessels are removed: three containing test substance labeled "Day 1,Rep1", "Day1,Rep2", "Day1,Rep3", and "Day1,Rep4".

12.2.3 The samples are shaken to ensure no test substance is stuck to the side of the tube.

12.2.4 The tubes are then placed in a stable temperature environment of approximately 20-26 °C (record the actual temperature) for about a 24 hour period.

12.2.5 After about 24 hours of equilibration at 20 °C-26 °C, centrifuge the samples until the solution is visibly clear. If micelle formation is suspected, an additional high-speed centrifugation step may be added (20,000 RCF for approximately 1 hour). The test vessels are now ready for dilution. See section 12.3 for further sample preparation.

12.2.6 At approximately 48 hours of incubation time, the second set of test vessels may be pulled (labeled "Day2,Rep1", "Day2,Rep2", "Day2,Rep3", and "Day2,Rep4") and equilibrated as described in 12.2.3-12.2.5.

12.2.7 At approximately 72 hours, the final set of samples is pulled (labeled "Day3,Rep1", "Day3,Rep2", "Day3,Rep3", and "Day3,Rep4") and equilibrated as described in 12.2.3-12.2.5.

12.3 Sample preparation for analysis.

12.3.1 Observe the test solution. Due to the possibility of emulsions/or a concentrated layer of solute at the surface of the solution, it may be necessary to pipette off the top layer of solution (or an aliquot of sample may be centrifuged as stated in 12.2.5). The use of a pasteur pipette or equivalent is recommended.

12.3.2 Aliquot the test solution into each of four autovials (e.g. 1.5 mL polypropylene or glass crimp-cap vials).

12.3.2.1 Sample aliquots may be taken by submerging the pipette or syringe tip below the surface of the test solution. *Note: when selecting the type of pipette to be used, take into consideration the test substance's tendency to adsorb to certain materials (i.e. adsorption of test substance to glass or plastic).*

12.3.2.2 Prior to aliquoting sample to the autovial, the pipette tip must be equilibrated/saturated with the test solution by drawing and gently expelling the test solution in and out of the pipette tip, taking care not to disturb the solid particulate.

12.3.3 Label the four autovials and record the sample id.

12.3.4 Using a diluter, make a 1:10 or higher dilution of sample:extraction solvent (i.e. methanol, acetone) into a labeled autovial.

12.3.5 Using the solubility information obtained in the preliminary screen test, estimate the appropriate dilutions, if any, required to bring the concentration of sample into the appropriate analytical range (e.g. approximately 3 ng/mL to 3000 ng/mL test substance). Utilize the diluter to make serial dilutions of the sample using a suitable analytical solvent.

12.3.6 To the final analytical sample, add internal standard at a concentration suitable to the analytical method.

12.3.7 Samples will be analyzed via LC/MS against a standard curve of varied, known concentrations of test substance with a constant concentration of internal standard equal to the concentration in the samples.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Samples will be analyzed via a calibration curve. The amount of test substance in the sample will be quantified against a standard curve.

13.2 Means will be calculated by adding the individual entities and dividing the resultant sum by the number of individual entities.

13.3 Standard deviations will be calculated using either Microsoft Excel® or Microsoft Access® to calculate standard deviation. The built in function contains the following equation which is based on the individual entities (n) being less than 30:

$$\sqrt{\frac{n\Sigma x^2 - (\Sigma x)^2}{n(n-1)}}$$

13.4 Sample precision will be reported as %RSD (or %CV). Sample precision will be calculated using the following equation:

$$A/B \times 100 = \text{Sample \%RSD}$$

where: A= standard deviation of averaged samples
B= average of samples

Sample RPD will be calculated using the following equation:

$$|A-B|/((A+B)/2) = \text{Sample RPD}$$

where: A= concentration of first replicate
B= concentration of second replicate

13.5 Exclusion of an outlier data point may be performed by utilizing Dixon's Q-Test. The questionable data point may err to the high or low end of the data set. Calculate the variable "Qobserved." If Qobserved > Qtabulated, then the data point may be rejected with 90% confidence (see table 1 below for Qtabulated values).

$Q_{observed}$= gap/range

where: Gap= the difference between the questionable point and the nearest value.
Range= total spread of the data.

Table 1 Criteria for Rejection of Outlier Values:

Number of observations	$Q_{tabulated}$, 90% Confidence Range
3	0.886
4	0.679
5	0.557

14.0 DATA ANALYSIS AND CALCULATIONS

14.1 Precision of data. Sample data must have a percent relative standard deviation (%RSD) (or relative percent difference) of ≤ 15%. Non-compliant data must be evaluated for obvious outliers. The Q-Test may be applied to exclude questionable data points. If an outlier value exists, sample average and precision is re-calculated and reported without the questionable data point. Document the non-compliant data on data summary sheets, and include results of the statistical analysis with the final results.

14.1.1 Sample replicate %RSD's should be ≤15%. If the average of the sample triplicate data is >15% RSD, evaluate the three values for an outlier value. To questionable data points, apply the Dixon's Q-Test. If the outlier value is rejected with 90% confidence, exclude the sample from further data calculations and calculate the average and RPD for the remaining two values. If no data points can be excluded and the data does not meet the criteria, then the data set may not be used in the final reported data. The remaining shake flasks for the timepoint shall be used to report the data.

14.1.2 The %RSD between shake flasks should agree within 15%. If this criteria is not met, apply the Q-test to outlier datum. If the questionable data point is discarded

via the Q-Test, then the non-compliant shake flask data is not to be used for the timepoint. Include justification for dropping the shake flask (e.g. q-test results).

14.1.3 And finally, the %RSD between the three days should be ≤15 % for the shake flask method. If this criteria is not met, it may be necessary to re-evaluate the data for possible outliers. Three agreeable time intervals are required to report the final solubility. If three consecutive timepoints do not agree within 15%, it may be necessary to repeat the entire test, or re-dilute/re-shoot samples to check for error.

14.2 **Limit of Quantification**. For this study, the LOQ will be equal to the lowest calibration standard used in the calibration curve containing more than twice the area counts of the highest Quality Control Blank.

14.3 **Quality Control Blanks: Method and Solvent Blank samples.** The level of analyte (test analyte OR internal standard analyte) shall be less than 50% of the area counts of the LOQ. If background levels of analyte exist in the initial method blanks, but subsequent blanks prior to the calibration standard curve are clean, the data may be accepted (provided that there are no other indicators that either the samples or the instrument contain significant background levels of analyte).

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

15.1 Dispose of sample waste by placing in high or low BTU containers as appropriate. Use broken glass containers to dispose of glass pipettes.

16.0 RECORDS

16.1 Sign and date all observations and calculations.
16.2 Fill out all appropriate sample preparation worksheets.

17.0 ATTACHMENTS

17.1 Attachment A-Example Sample Preparation Worksheet.

18.0 REFERENCES

18.1 Organization for Economic Cooperation and Development. OECD Guideline for Testing of Chemicals. Water Solubility -OECD Guideline 105: pp. 1–7, Adopted 1995
18.2 United States Environmental Protection Agency. OPPTS 830.7860 Water Solubility (Generator Column Method). Prevention, Pesticides and Toxic Substances: Fate, Transport and Transformation Test Guidelines. EPA 712-C-96-042: pp. 1–17, 1996
18.3 United States Environmental Protection Agency. OPPTS 830.7840 Water Solubility: Column Elution Method; Shake Flask Method. Prevention, Pesticides and Toxic

Substances: Fate, Transport and Transformation Test Guidelines. EPA 712-C-96-041: pp. 1–12, 1996

18.4 ETS-8-170.0, Solubility Screen Test: Approximate Solubility Determination of a Test Substance in Various Solvents.

18.5 Harris, Daniel C. Statistics. *Quantitative Chemical Analysis.* 4th ed.; W.H. Freeman and Company: New York, 1982; p 70-71.

18.6 Natrella, Mary Gibbons. The Treatment of Outliers. *Experimental Statistics,* National Bureau of Standards Handbook 91; U.S. Government Printing Office: Washington, D.C., 1963; Pages 17-3, and T-27.

19.0 AFFECTED DOCUMENTS

19.1 ETS-8-170.0, "Solubility Screen Test: Approximate Solubility Determination of a Test Substance in Various Solvents."

20.0 REVISIONS

Revision Number	Reason For Revision	Revision Date
1	Section 1.2, references to specific test substances were removed. Section 3.6, removed "internal standard blank" from definition section. Section 12.2.5, an optional high-speed centrifugation step was added. Fixed the incorrect numbering of sections 13.5 through 14.3. Section 14.3, more clearly defined the acceptance criterion for method and solvent blanks.	03-27-01

ATTACHMENT A: Example Sample Preparation Sheet (page 1 of 2).

3M Environmental Laboratory Solubility Test: Shake Flask Method

Study #_____

Temperature of Study:_____°C — *Sample preparation worksheet* — Analyst(s): _____

Test Substance: _____ ID#: _____ Source: _____ Date: _____

Test Solvent: _____ ID#: _____ Source: _____ *Test Vessel:* _____ 15ml centrifuge tube/Other(specify): _____

solvent density: _____ g/ml

Was the solubility screen test performed prior to start? Y/N If so, what is the approximate concentration? _____ *ug/ml*

Balance ID:_____ Thermometer ID:_____ Room Temp: _____°C Date/Time/Initials:_____

SFM vials ID#: "sampleID" =_____	Sample Description	weight test substance added:	Total mass (solute + solvent) added:	[1]Internal Standard addition _____uL/____mls soln IS added to dilution factor:	Observations/Comments:
sampleID-1.1.1 thru -1.1.4	Day1, Smp1			1:_____	
sampleID-1.2.1 thru -1.2.4	Day1, Smp2			1:_____	
sampleID-1.3.1 thru -1.3.4	Day1, Smp3			1:_____	
sampleID-1.4.1 thru -1.4.4	Day1, Smp4 blank	none added		1:_____	
sampleID-2.1.1 thru -2.1.4	Day2, Smp1			1:_____	
sampleID-2.2.1 thru -2.2.4	Day2, Smp2			1:_____	
sampleID-2.3.1 thru -2.3.4	Day2, Smp3			1:_____	
sampleID-2.4.1 thru -2.4.4	Day2, Smp4 blank	none added		1:_____	
sampleID-3.1.1 thru -3.1.4	Day3, Smp1			1:_____	
sampleID-3.2.1 thru -3.2.4	Day3, Smp2			1:_____	
sampleID-3.3.1 thru -3.3.4	Day3, Smp3			1:_____	
sampleID-3.4.1 thru -3.4.4	Day3, Smp4 blank	none added		1:_____	
	Date/Time:				
	Initials:				

Test Vessels sealed w/ tape? Y/N Test Vessels shaken/vortex-mixed? Y/N

Test vessels placed on orbital incubator? Y/N Incubator ID:_____ Speed:_____rpm Initial Temperature: _____°C

[1]Internal Standard:_____ ID#: _____

Int. Std. Conc.: _____ug/ml

Day 1:

Four "Day 1" samples are pulled and allowed to be placed in a temperature controlled environment for approx. 24 hours with no agitation. Date/Time/Initials:_____

Location of samples:_____ Temperature of environment:_____°C Thermometer ID:_____

Visual observations:

ATTACHMENT A CONTINUED (page 2 of 2).

Day 2:

Four "Day 2" samples are pulled and allowed to be placed in a temperature controlled environment for approx. 24 hours with no agitation. Date/Time/Initials: _____

Location of samples:_____ Temperature of environment:_____ °C Thermometer ID:_____.

Visual observations:

"Day 1" Test Vessels Centrifuged: Cent. ID:_____ RPM or RCF readout: _____ duration:_____ min. Date/Time/Initials: _____

Visual observations:

-Four aliquots from each of the four samples are to be made into glass/plastic autovials (also, check autovials for correct labeling). Yes/No

-Immediately dilute the solution 1:_____ with methanol. Methanol TN-A:_____. Date/Time/Initials: _____

Additional dilutions: Dilution factor (test solution:methanol): 1:_____ X's how many dilutions: _____ =final dilution factor: 1:_____.

Diluter ID used:_____ Amounts used: _____uL methanol, _____ul samples

-Store samples and extracts in a cooler at until time of analysis. Cooler ID: _____ Cooler Temp: _____ °C Date/Time/Initials: _____

Day 3:

Four "Day 3" samples are pulled and allowed to be placed in a temperature controlled environment for approx. 24 hours with no agitation. Date/Time/Initials: _____

Location of samples:_____ Temperature of environment:_____ °C Thermometer ID:_____.

Visual observations:

"Day 2" Test Vessels Centrifuged: Cent. ID:_____ RPM or RCF readout: _____ duration:_____ min. Date/Time/Initials: _____

Visual observations:

-Four aliquots from each of the four replicates are to be made into glass/plastic autovials (also, check autovials for correct labeling). Yes/No

-Immediately dilute the solution 1:_____ with methanol. Methanol TN-A:_____. Date/Time/Initials: _____

Additional dilutions: Dilution factor (test solution:methanol): 1:_____ X's how many dilutions: _____ =final dilution factor: 1:_____.

Diluter ID used:_____ Amounts used: _____uL methanol, _____ul samples

-Store samples and extracts in a cooler at until time of analysis. Cooler ID: _____ Cooler Temp: _____ °C Date/Time/Initials: _____

Day 4:

At approx. 24 hours of equilibration, the **"Day 3"** samples are to be aliquoted/extracted. Environment Temp.:_____ °C Therm. ID:_____.

Test Vessels Centrifuged: Cent. ID:_____ RPM or RCF readout: _____ duration:_____ min. Date/Time/Initials: _____

Visual observations:

-Four aliquots from each of the four samples are to be made into glass/plastic autovials (also, check autovials for correct labeling). Yes/No

-Immediately dilute the solution 1:_____ with methanol. Methanol TN-A:_____. Date/Time/Initials: _____

Additional dilutions: Dilution factor (test solution:methanol): 1:_____ X's how many dilutions: _____ =final dilution factor: 1:_____.

Diluter ID used:_____ Amounts used: _____uL methanol, _____ul samples

-Store samples and extracts in a cooler at until time of analysis. Cooler ID: _____ Cooler Temp: _____ °C Date/Time/Initials: _____

3M ENVIRONMENTAL LABORATORY

METHOD

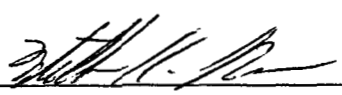
ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROCHEMICALS IN WASTE STREAM OR WATER EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: ETS-8-155.0 **Adoption Date:** 11/10/00

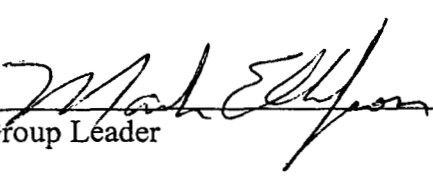
Revision Date:

Author: Mark L. Anderson, Mark E. Ellefson

Approved By:



11/10/00
Laboratory Manager Date



11/10/00
Group Leader Date

1.0 SCOPE AND APPLICATION

1.1 Scope: This method describes the analysis of waste stream or water extracts using HPLC-electrospray/mass spectrometry.

1.2 Applicable Compounds: Fluorochemicals or other electrospray ionizable compounds.

1.3 Matrices: Tap water, ground water, wastewater and other aqueous solutions.

2.0 SUMMARY OF METHOD

2.1 This method describes the analysis of fluorochemicals or other electrospray ionizable compounds extracted from water, using HPLC-electrospray mass spectrometry (HPLC-ES/MS). The analysis is performed by the mass selection of a single ion characteristic of a particular compound, such as the perfluorooctanesulfonate (PFOS) anion, m/z = 499 or perfluorooctanoate (PFOA), m/z = 413.

3.0 DEFINITIONS

3.1 Atmospheric Pressure Ionization (API): The Micromass Platform LCZ single quadrupole system and other commercially available LC/MS systems allow for various methods of ionization by utilizing a variety of sources, probes, and interfaces. These include but are not limited to: Electrospray Ionization (ESI), Atmospheric Pressure chemical Ionization (APcI), Thermospray, etc. The ionization in these processes 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 droplets are produced by the application of a strong electrical field.

3.3 Mass Spectrometer (MS): The Platform LCZ and other commercially manufactured ES/MS systems are equipped with a single quadrupole mass selective detector. Ions are selectively discriminated by mass to charge ratio (m/z) and subsequently detected.

3.4 Conventional vs. Z-spray probe interface: The Micromass Platform LCZ system utilizes a "Z-spray" conformation. The spray emitted from the 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.). Other commercially manufactured ES/MS systems may have similar features.

3.5 Mass Lynx Software: System software designed for the specific operation of Micromass LCZ Mass spectrometer. Currently MassLynx has Windows 95 and WindowsNT 4.0 versions. All versions are similar. For more details see the manual specific to the instrument (MassLynx NT User's Guide or Micromass Platform LCZ 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 clothing, gloves, and eyewear.

4.2 Cautions:

4.2.1 Operate solvent pumps below a backpressure of 400 bar (5800 psi). If the backpressure 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 Platform LCZ 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 (or house air system.).

7.1.2 HPLC analytical column, such as a Betasil C18 column (50x2mm, 5 µm particle size) or equivalent.

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 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.

8.1.3.1 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 L volumetric flask, add the appropriate volume of Milli-Q water, mix until all solids are dissolved. Store at room temperature.

8.2 Calibration Standards

8.2.1 Typically two method blanks (Milli-Q water), two matrix blanks, and solvent standards are prepared during the sample extraction procedure.

9.0 Sample Handling

9.1 Standards and sample extracts are stored in capped autovials or capped 15 mL centrifuge tubes until analysis.

9.2 If analysis will be delayed, standards and sample extracts may be refrigerated at approximately 4° C until analyses 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 sample set 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 for each sample set and analyzed to determine the matrix effect on the recovery efficiency.

10.2.2 Matrix spike duplicates are prepared periodically to measure the precision associated with the analysis.

10.2.3 Analyze the matrix spike and matrix spike duplicate (if prepared) in the same run as the original sample.

10.2.4 Matrix spike and matrix spike duplicate concentrations should fall in the mid-range of the initial calibration curve or should be prepared at 1.5-5 times the endogenous

concentration of the analyte. Spike concentrations should fall in the low-range of the initial calibration curve if extremely low levels are expected.

10.3 Continuing Calibration Verifications

10.3.1 Continuing calibration verifications (CCV) 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 sample set.

10.4 Internal Standard/Surrogate Standard

10.4.1 An internal standard (IS) may be used to quantify the target analytes by establishing a relationship between the ratio of analyte response to IS response and a known concentration of the analyte of interest. The IS should be spiked at an amount that will fall within the mid-range of the calibration curve. The IS should be added after the extraction process and before analysis.

10.4.2 A surrogate standard may be used for quality control. The surrogate is used to quantitatively evaluate the entire analytical procedure including sample preparation and analysis. The surrogate should be spiked to fall within the low to mid-range of the calibration curve.

11.0 Calibration and Standardization

11.1 Analyze the standard curves prior to and following each set of extracts. The average of two standard curves may be plotted by linear regression ($y = mx + b$) weighted 1/x, or quadratic fit ($y = ax^2 + bx + c$) using MassLynx or other suitable software. The calibration curves should not be forced through zero.

11.2 If the calibration curve does not meet acceptance criteria perform routine maintenance or prepare a new 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. 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 the first letter of the name of the instrument (T for Tucker), the year (00 for 2000), the month (04 for April), and the day (T001012 for October 12, 2000). If more than one list is made on the same day, use increasing letters of the alphabet starting with A at the end of the list.

12.1.1.2 Assign a method (MS) file.
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. Set ionization mode as appropriate and mass to 499 or other appropriate masses. A full scan is usually collected in addition to the SIRs. Save acquisition method. See the Micromass MassLynx GUIDE TO DATA ACQUISITION for additional information.

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

12.1.4 Samples are analyzed with a continuing calibration verification (CCV) injected after every tenth sample. Solvent blanks should be analyzed periodically to monitor for possible analyte carryover.

12.2 Using the Autosampler/Column Heater

12.2.1 Place sample vials into the sample tray according to the sample list prepared in Section **12.1.1.**

12.2.2 Attach the proper analytical column in the column heater compartment. If using the switching valve, make sure that the tubing is run to the appropriate ports.

12.3 Using the Inlet Editor

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

12.3.1.1 Sample size = 10 µL injection

12.3.1.2 Flow rate = 300 µL/min.

12.3.1.3 Cycle time = 10.0 minutes

12.3.1.4 Mobile phase components:
Solvent A: 2.0 mM Ammonium Acetate
Solvent B: Methanol (MeOH)

Solvent Gradient:	Time (min.)	% B
	0.00	5.00 %
	1.00	5.00 %
	4.50	95.0 %
	8.00	95.0 %
	8.50	5.00 %
	10.0	Stop

12.4 Instrument Set-up

12.4.1 Refer to the Platform LCZ User's Guide, the MassLynx NT User's Guide or **ETS-9-36,** "Operation and Maintenance of the Micromass Platform LCZ Electrospray/Mass Spectrometer".

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

12.4.3 Check the tip of the stainless steel capillary at the end of the probe with an eyepiece. 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.

12.4.4 Turn on the nebulizing gas.

12.4.5 Open the tune page. Click on 'Operate' to initiate the desolvation heaters.

12.4.6 Open the Inlet Editor.

12.4.5.1 Set HPLC pump to "On".
12.4.5.2 Set the solvent flow to the desired flow rate.
12.4.5.3 Observe droplets coming out of the tip of the probe. A fine mist should be expelled with no nebulizing gas leaking around the tip of the probe. Readjust the tip of the probe if no mist is observed.
12.4.5.4 Allow to equilibrate for at least 10 minutes.

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

12.4.6.1 Drying gas 250-425 liters/hour

12.4.6.2 ESI nebulizing gas 10-15 liters/hour

12.4.6.3 HPLC constant flow mode, flow rate 10 – 500 µL/min

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

12.4.6.5 Source Block temperature 150°.

12.4.6.6 Desolvation temperature 250°.

12.4.7 Print the tune page with its parameters, the Inlet page, sample list, mass spec information, and all other applicable information and store it in the study binder with copies taped into the instrument run logbook.

12.4.7.1 All copies must be initialed and dated.

12.4.8 Click on start button on the MassLynx toolbar. Ensure start and end sample numbers include all samples to be analyzed.

13.0 Data Analysis and Calculations

13.1 Calculations:

13.1.1 Calculate matrix spike percent recoveries using the following equation:

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

13.1.2 Calculate percent difference using the following equation:

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

13.1.3 Calculate actual concentration of analyte in matrix (µg/mL):

On-Column Concentration (µg/mL) x Dilution Factors = Calculated Concentration

14.0 METHOD PERFORMANCE

14.1 The Limit of Quantitation (LOQ) is method, analyte, and matrix specific. For many analytes, the LOQ concentration is selected as the lowest acceptable non-zero standard in the calibration curve.

14.2 Solvent and method blank values must be < ½ that of the lowest standard used in the calibration curve.

14.3 The coefficient of determination (r^2) value for the calibration curve must be greater than or equal to 0.980.

14.4 Continuing Calibration Verification (CCV) percent recoveries must be ± 30% of the standard concentration.

14.5 Internal Standard recoveries should be within ± 50% of the spiked concentration.

14.6 If criteria listed in this 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 raw data.

14.7 If data is 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 and other applicable information to include in the appropriate study folder. Copy these pages and tape into the instrument runlog.

16.3 Plot the calibration curve 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 and store in the study folder.

16.6 Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

17.0 ATTACHMENTS

17.1 None

18.0 REFERENCES

18.1 Platform LCZ User's Guide, Micromass UK Limited, Tudor Road, Altrincham, WA14 5RZ; or Floats Road, Wythenshawe M23 9LZ; United Kingdom.

18.2 MassLynx NT User's Guide, Micromass UK Limited, Tudor Road, Altrincham, WA14 5RZ; or Floats Road, Wythenshawe M23 9LZ; United Kingdom.

18.3 MassLynx NT Guide To Data Acquisition, Micromass UK Limited, Tudor Road, Altrincham, WA14 5RZ; or Floats Road, Wythenshawe M23 9LZ; United Kingdom.

18.4 ETS-9-36.0, "Operation and Maintenance of the Micromass Platform LCZ Electrospray/Mass Sprectrometer".

19.0 AFFECTED DOCUMENTS

19.1 None

20.0 REVISIONS

Revision Number.	Reason For Revision	Revision Date

ATTACHMENT B: DATA SUMMARY TABLES

H082700.s Samples acquired on Hillary 08-27-00, Reprocessed by KLT on 08-29-00. (Day 1)

Sample ID	Sample Desc	Run #	Mtd	Data File	Vial#	THPFOS ppb	THPFOS RT	THPFOS Area	PFOS RT	PFOS Area	PFOS ppb	
Standards Data/Performance												
00027-43-11		2	H82700B.M	HILL0002.D	12	250.0	6.146	2822194.3	6.543	35185920.0	0.0	
00027-43-11		3	H82700B.M	HILL0003.D	12	250.0	6.139	2762785.5	6.541	34447728.0	0.0	
00027-43-00		6	H82700B.M	HILL0006.D	1	250.0	6.141	2735708.0	6.542	88942.6	1.2	
00027-43-01	System Suitability Stds	7	H82700B.M	HILL0007.D	2	250.0	6.143	2820521.3	6.543	268916.0	4.8	
00027-43-02	System Suitability Stds	8	H82700B.M	HILL0008.D	3	250.0	6.140	2804231.5	6.541	339346.9	6.3	
00027-43-03	System Suitability Stds	9	H82700B.M	HILL0009.D	4	250.0	6.147	2783125.8	6.544	511958.0	10.0	
00027-43-04	System Suitability Stds	10	H82700B.M	HILL0010.D	5	250.0	6.138	2816558.0	6.541	1271388.6	25.5	
00027-43-05	System Suitability Stds	11	H82700B.M	HILL0011.D	6	250.0	6.139	2815918.5	6.541	2052451.9	41.9	
00027-43-06	System Suitability Stds	12	H82700B.M	HILL0012.D	7	250.0	6.142	2863963.3	6.541	2591958.0	52.4	
00027-43-07	System Suitability Stds	13	H82700B.M	HILL0013.D	8	250.0	6.146	2809055.3	6.542	3750688.3	76.5	
00027-43-08	System Suitability Stds	14	H82700B.M	HILL0014.D	9	250.0	6.140	2812877.5	6.541	5002986.0	106.0	
00027-43-09	System Suitability Stds	15	H82700B.M	HILL0015.D	10	250.0	6.143	2825108.3	6.544	11418789.0	256.9	
00027-43-10	System Suitability Stds	16	H82700B.M	HILL0016.D	11	250.0	6.146	2805414.5	6.541	19901130.0	516.4	
00027-43-11	System Suitability Stds	17	H82700B.M	HILL0017.D	12	250.0	6.143	2843693.3	6.542	33450226.0	0.0	
00027-43-00	0 Std	21	H82700B.M	HILL0021.D	1	250.0	6.137	2774394.0	6.544	63275.9	0.7	
00027-43-01	Std 1 (LOQ-2.5 ppb)	22	H82700B.M	HILL0022.D	2	250.0	6.139	2850030.0	6.541	185661.4	3.1	124.3%
00027-43-02	Std 2	23	H82700B.M	HILL0023.D	3	250.0	6.144	2837082.3	6.543	299971.8	5.4	108.7%
00027-43-03	Std 3	24	H82700B.M	HILL0024.D	4	250.0	6.147	2830421.8	6.541	494519.7	9.4	94.1%
00027-43-04	Std 4	25	H82700B.M	HILL0025.D	5	250.0	6.148	2812000.3	6.541	1249220.6	25.1	100.3%
00027-43-05	Std 5	26	H82700B.M	HILL0026.D	6	250.0	6.142	2806759.3	6.543	1996569.8	40.9	102.0%
00027-43-06	Std 6	27	H82700B.M	HILL0027.D	7	250.0	6.142	2851692.8	6.541	2495294.5	50.8	101.1%
00027-43-07	Std 7	28	H82700B.M	HILL0028.D	8	250.0	6.153	2798432.0	6.541	3844372.5	76.5	101.8%
00027-43-08	Std 8	29	H82700B.M	HILL0029.D	9	250.0	6.148	2798668.8	6.540	4826860.0	102.7	102.5%
00027-43-09	Std 9	30	H82700B.M	HILL0030.D	10	250.0	6.143	2802453.3	6.543	11151017.0	254.3	101.6%
00027-43-10	Std 10	31	H82700B.M	HILL0031.D	11	250.0	6.145	2823974.3	6.542	19737746.0	506.0	101.0%
00027-43-11	Std 11 (Excluded to better fit data)	32	H82700B.M	HILL0032.D	12	250.0	6.140	2813255.0	6.543	33120708.0	0.0	
00027-43-06	CCV	44	H82700B.M	HILL0044.D	7	250.0	6.139	2845795.3	6.542	2410067.3	48.9	97.7%
00027-43-06	CCV	56	H82700B.M	HILL0056.D	7	250.0	6.144	2861852.0	6.544	2454911.8	49.6	99.0%
00027-43-00	0 Std	59	H82700B.M	HILL0059.D	1	250.0	6.136	2772065.0	6.544	58645.5	0.5	
00027-43-01	Std 1 (LOQ-2.5 ppb)	60	H82700B.M	HILL0060.D	2	250.0	6.148	2875237.0	6.543	161235.6	2.6	103.4%
00027-43-02	Std 2	61	H82700B.M	HILL0061.D	3	250.0	6.140	2812862.5	6.542	282366.2	5.1	102.5%
00027-43-03	Std 3	62	H82700B.M	HILL0062.D	4	250.0	6.137	2774225.3	6.544	470515.5	9.1	91.1%
00027-43-04	Std 4	63	H82700B.M	HILL0063.D	5	250.0	6.147	2792990.3	6.544	1215011.4	24.6	98.1%
00027-43-05	Std 5	64	H82700B.M	HILL0064.D	6	250.0	6.146	2817281.0	6.541	1949906.5	39.7	99.1%
00027-43-06	Std 6	65	H82700B.M	HILL0065.D	7	250.0	6.141	2841104.8	6.541	2417009.5	49.2	98.2%
00027-43-07	Std 7	66	H82700B.M	HILL0066.D	8	250.0	6.139	2815518.5	6.541	3580460.0	74.2	98.7%
00027-43-08	Std 8	67	H82700B.M	HILL0067.D	9	250.0	6.142	2828082.3	6.541	4677907.5	98.2	98.0%
00027-43-09	Std 9	68	H82700B.M	HILL0068.D	10	250.0	6.139	2806456.5	6.543	10850736.0	246.1	98.3%
00027-43-10	Std 10	69	H82700B.M	HILL0069.D	11	250.0	6.140	2788683.3	6.544	19181934.0	495.1	98.9%
00027-43-11	Std 11 (Excluded to better fit data)	70	H82700B.M	HILL0070.D	12	250.0	6.138	2777985.3	6.541	32261558.0	0.0	

Sample Data/Performance

Sample ID	Sample Desc	Run #	Mtd	Data File	Vial#	ppb	RT	Area	RT	Area	ppb ppb	Undiluted ppm	Average PPM Std Dev %CV
	TCR-00017-46 TCR-001 PFOS Solubility in water												
73100-wPFOS1.1.1	Day 1 Samples MLA 8-2-00	35	H82700B.M	HILL0035.D	21	250.0	6.145	2870339.3	6.544	2993830.5	60.7	607.1	608.3 –(excludes rep 4)
	TCR-00017-46 TCR-001 PFOS Solubility in water												
73100-wPFOS1.1.2	Day 1 Samples MLA 8-2-00	36	H82700B.M	HILL0036.D	22	250.0	6.144	2893204.5	6.544	2943648.8	59.2	591.7	14.1
	TCR-00017-46 TCR-001 PFOS Solubility in water												
73100-wPFOS1.1.3	Day 1 Samples MLA 8-2-00	37	H82700B.M	HILL0037.D	23	250.0	6.148	2862875.8	6.541	3076239.0	62.6	626.1	2.3%
	TCR-00017-46 TCR-001 PFOS Solubility in water												
~~73100-wPFOS1.1.4~~	~~Day 1 Samples MLA 8-2-00~~	~~38~~	~~H82700B.M~~	~~HILL0038.D~~	~~24~~	~~250.0~~	~~6.144~~	~~2841280.8~~	~~6.542~~	~~11717878.0~~	~~264.9~~	~~2648.8~~	
	TCR-00017-46 TCR-001 PFOS Solubility in water												
73100-wPFOS1.2.1	Day 1 Samples MLA 8-2-00	40	H82700B.M	HILL0040.D	25	250.0	6.146	2861874.0	6.542	3295552.3	67.3	672.8	630.0
	TCR-00017-46 TCR-001 PFOS Solubility in water												
73100-wPFOS1.2.2	Day 1 Samples MLA 8-2-00	41	H82700B.M	HILL0041.D	26	250.0	6.145	2842725.5	6.541	3248576.0	66.7	667.5	43.0
	TCR-00017-46 TCR-001 PFOS Solubility in water												
73100-wPFOS1.2.3	Day 1 Samples MLA 8-2-00	42	H82700B.M	HILL0042.D	27	250.0	6.146	2891975.3	6.543	3038282.0	61.2	611.7	6.8%
	TCR-00017-46 TCR-001 PFOS Solubility in water												
73100-wPFOS1.2.4	Day 1 Samples MLA 8-2-00	43	H82700B.M	HILL0043.D	28	250.0	6.140	2862364.8	6.543	2800103.5	56.8	568.0	
	TCR-00017-46 TCR-001 PFOS Solubility in water												
73100-wPFOS1.3.1	Day 1 Samples MLA 8-2-00	47	H82700B.M	HILL0047.D	29	250.0	6.143	2865592.0	6.540	3339678.3	68.1	681.2	664.0
	TCR-00017-46 TCR-001 PFOS Solubility in water												
73100-wPFOS1.3.2	Day 1 Samples MLA 8-2-00	48	H82700B.M	HILL0048.D	30	250.0	6.140	2898872.3	6.543	3339718.3	67.3	673.1	33.6
	TCR-00017-46 TCR-001 PFOS Solubility in water												
73100-wPFOS1.3.3	Day 1 Samples MLA 8-2-00	49	H82700B.M	HILL0049.D	31	250.0	6.146	2887931.3	6.541	3013332.0	60.7	607.3	5.1%
	TCR-00017-46 TCR-001 PFOS Solubility in water												
73100-wPFOS1.3.4	Day 1 Samples MLA 8-2-00	50	H82700B.M	HILL0050.D	32	250.0	6.145	2879242.8	6.540	3418030.3	69.4	694.4	
	TCR-00017-46 TCR-001 PFOS Solubility in water												
73100-wPFOS1.4.1	Day 1 Samples MLA 8-2-00	52	H82700B.M	HILL0052.D	33	250.0	6.149	2901136.3	6.544	41786.1	0.2		
	TCR-00017-46 TCR-001 PFOS Solubility in water												
73100-wPFOS1.4.2	Day 1 Samples MLA 8-2-00	53	H82700B.M	HILL0053.D	34	250.0	6.151	2887013.5	6.542	47069.1	0.3		
	TCR-00017-46 TCR-001 PFOS Solubility in water												
73100-wPFOS1.4.3	Day 1 Samples MLA 8-2-00	54	H82700B.M	HILL0054.D	35	250.0	6.146	2898727.8	6.542	34471.0	0.0		
	TCR-00017-46 TCR-001 PFOS Solubility in water												
73100-wPFOS1.4.4	Day 1 Samples MLA 8-2-00	55	H82700B.M	HILL0055.D	36	250.0	6.142	2873969.3	6.542	40063.7	0.2		

(1) study # should read TCR-002
(RE) KLT 11-20-00

Rep 4 excluded via Dixon's Q-test KLT 01-19-01

Average:	2831206.4	(Stds AND Smpls Included)
Std Dev:	38740.1	
% CV:	1.4%	

Total Day 1 ppm	Std Dev	%CV
636.5	40.6	6%

TCR-001 002 (VE) 10/11/2020

Blank Data/Performance

Sample ID	Sample Desc	Run #	Mtd	Data File	Vial#	ppb	RT	Area	RT	Area	ppb	
MeOH TNA4464	Aliquoted 25 Jul 00	1	H82700B.M	HILL0001.D	91	0.0	0.000	0.0	6.543	459292.2	0.0	>50% LOQ
MeOH TNA4464	Aliquoted 25 Jul 00	4	H82700B.M	HILL0004.D	91	0.0	0.000	0.0	6.542	66765.3	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	5	H82700B.M	HILL0005.D	91	0.0	0.000	0.0	6.542	45585.9	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	18	H82700B.M	HILL0018.D	91	0.0	0.000	0.0	6.541	62874.8	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	19	H82700B.M	HILL0019.D	92	0.0	0.000	0.0	6.542	106202.0	0.0	>50% LOQ
MeOH TNA4464	Aliquoted 25 Jul 00	20	H82700B.M	HILL0020.D	92	0.0	0.000	0.0	6.543	65638.9	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	33	H82700B.M	HILL0033.D	92	0.0	0.000	0.0	6.542	69268.1	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	34	H82700B.M	HILL0034.D	92	0.0	0.000	0.0	6.544	53477.6	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	39	H82700B.M	HILL0039.D	93	0.0	0.000	0.0	6.543	74133.6	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	45	H82700B.M	HILL0045.D	93	0.0	0.000	0.0	6.542	54522.9	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	46	H82700B.M	HILL0046.D	93	0.0	0.000	0.0	6.542	47828.8	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	51	H82700B.M	HILL0051.D	93	0.0	0.000	0.0	6.541	48818.3	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	57	H82700B.M	HILL0057.D	94	0.0	0.000	0.0	6.540	78030.4	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	58	H82700B.M	HILL0058.D	94	0.0	0.000	0.0	6.543	46175.2	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	71	H82700B.M	HILL0071.D	94	0.0	0.000	0.0	6.543	54563.8	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	72	H82700B.M	HILL0072.D	94	0.0	0.000	0.0	6.542	40344.1	0.0	
										50% Average LOQ Area 86724.3		

Day 2 Sample Data Acquired on Hillary 8-29-00 Sequence: H082900.s

Standards Data/Performance

Sample ID	Sample Desc	Run #	Mtd	Data File	Vial#	THPFOS ppb	THPFOS RT	THPFOS Area	PFOS RT	PFOS Area	PFOS ppb	
00027-43-11	System Suitability Samples	2	H082900A.M	HILL0002.D	12	250	6.15	1068757.1	6.54	25785074.0	0.0	
00027-43-11	System Suitability Samples	3	H082900A.M	HILL0003.D	12	250	6.15	1153389.8	6.54	26263012.0	0.0	
00027-43-00	System Suitability Samples	6	H082900A.M	HILL0006.D	1	250	6.14	1388342.3	6.54	69273.1	1.2	
00027-43-01	System Suitability Samples	7	H082900A.M	HILL0007.D	2	250	6.15	1484845.6	6.54	431029.4	14.2	
00027-43-02	System Suitability Samples	8	H082900A.M	HILL0008.D	3	250	6.14	1549846.9	6.54	316248.5	9.5	
00027-43-03	System Suitability Samples	9	H082900A.M	HILL0009.D	4	250	6.15	1618214.9	6.54	458319.6	13.8	
00027-43-04	System Suitability Samples	10	H082900A.M	HILL0010.D	5	250	6.15	1672729.0	6.54	1121996.5	35.0	
00027-43-05	System Suitability Samples	11	H082900A.M	HILL0011.D	6	250	6.14	1728931.1	6.54	1784189.6	55.1	
00027-43-06	System Suitability Samples	12	H082900A.M	HILL0012.D	7	250	6.14	1824887.9	6.54	2219681.3	65.5	
00027-43-07	System Suitability Samples	13	H082900A.M	HILL0013.D	8	250	6.14	1820424.8	6.54	3163340.6	96.0	
00027-43-08	System Suitability Samples	14	H082900A.M	HILL0014.D	9	250	6.15	1856518.4	6.54	4244997.0	127.8	
00027-43-09	System Suitability Samples	15	H082900A.M	HILL0015.D	10	250	6.15	1899933.8	6.54	9618981.0	309.6	
00027-43-10	System Suitability Samples	16	H082900A.M	HILL0016.D	11	250	6.15	1940971.8	6.54	18949810.0	634.1	
00027-43-11	System Suitability Samples	17	H082900A.M	HILL0017.D	12	250	6.15	1972672.0	6.54	28705196.0	0.0	
00027-43-00	0 Std	21	H082900A.M	HILL0021.D	1	250	6.14	2048297.4	6.54	60752.7	0.1	
00027-43-01	Std 1 (Excluded to better fit data)	22	H082900A.M	HILL0022.D	2	250	6.14	2111404.0	6.54	248047.4	X	Excluded
00027-43-02	Std 2 (LOQ-5 ppb)	23	H082900A.M	HILL0023.D	3	250	6.14	2098961.8	6.54	270481.7	5.5	109.1%
00027-43-03	Std 3	24	H082900A.M	HILL0024.D	4	250	6.14	2132470.0	6.54	451537.4	9.9	99.1%
00027-43-04	Std 4	25	H082900A.M	HILL0025.D	5	250	6.15	2144905.3	6.54	1104108.5	26.4	105.4%
00027-43-05	Std 5	26	H082900A.M	HILL0026.D	6	250	6.15	2171599.8	6.54	1771716.9	43.0	107.3%
00027-43-06	Std 6	27	H082900A.M	HILL0027.D	7	250	6.15	2205455.0	6.54	2195559.5	53.0	105.9%
00027-43-07	Std 7	28	H082900A.M	HILL0028.D	8	250	6.15	2171843.3	6.54	3203310.3	80.2	106.8%
00027-43-08	Std 8	29	H082900A.M	HILL0029.D	9	250	6.15	2190710.0	6.54	4271387.5	107.8	107.8%
00027-43-09	Std 9	30	H082900A.M	HILL0030.D	10	250	6.15	2209004.8	6.54	9714680.0	262.9	105.0%
00027-43-10	Std 10	31	H082900A.M	HILL0031.D	11	250	6.15	2175422.0	6.54	18905772.0	533.6	106.5%
00027-43-11	Std 11 (Excluded to better fit data)	32	H082900A.M	HILL0032.D	12	250	6.14	2224192.3	6.54	28831362.0	X	Excluded
00027-43-06	CCV	44	H082900A.M	HILL0044.D	7	250	6.14	2341912.3	6.54	2213222.8	50.2	100.2%
00027-43-06	CCV	56	H082900A.M	HILL0056.D	7	250	6.14	2375128.5	6.54	2157711.5	48.2	96.2%
00027-43-00	0 Std	59	H082900A.M	HILL0059.D	1	250	6.14	2358025.3	6.54	55850.0	X	
00027-43-01	Std 1 (Excluded to better fit data)	60	H082900A.M	HILL0060.D	2	250	6.14	2355983.5	6.54	206739.0	X	Excluded
00027-43-02	Std 2 (LOQ-5 ppb)	61	H082900A.M	HILL0061.D	3	250	6.14	2369146.8	6.54	249832.4	4.2	84.0%
00027-43-03	Std 3	62	H082900A.M	HILL0062.D	4	250	6.15	2354475.5	6.54	417615.9	8.1	80.6%
00027-43-04	Std 4	63	H082900A.M	HILL0063.D	5	250	6.15	2380583.0	6.54	1075203.6	23.2	92.5%
00027-43-05	Std 5	64	H082900A.M	HILL0064.D	6	250	6.15	2378348.5	6.54	1705371.0	37.5	93.7%
00027-43-06	Std 6	65	H082900A.M	HILL0065.D	7	250	6.15	2422405.0	6.54	2167708.8	47.4	94.6%
00027-43-07	Std 7	66	H082900A.M	HILL0066.D	8	250	6.15	2380072.0	6.54	3116888.8	70.8	94.2%
00027-43-08	Std 8	67	H082900A.M	HILL0067.D	9	250	6.14	2373409.8	6.54	4177286.0	96.7	96.5%
00027-43-09	Std 9	68	H082900A.M	HILL0068.D	10	250	6.15	2401039.3	6.54	9586870.0	234.9	93.8%
00027-43-10	Std 10	69	H082900A.M	HILL0069.D	11	250	6.14	2356475.3	6.54	16577574.0	469.6	93.8%
00027-43-11	Std 11 (Excluded to better fit data)	70	H082900A.M	HILL0070.D	12	250	6.15	2390706.0	6.54	28617368.0	X	Excluded

Points Excluded to better fit data.

page 1 of 3 KLT 8/30/00

Exact Copy of Original

KLT Initial — 08/09/01 Date p. 1 of 3

TCR-001 PFOS Solubility

Day 2/Water/PFOS TCR-00017-46

Sample Data/Performance

Sample ID	Sample Desc	Run #	Mtd	Data File	Vial#	THPFOS ppb	THPFOS RT	THPFOS Area	Dilution Factor	PFOS RT	PFOS Area	PFOS ppb	Undiluted ppm	Average PPM Std Dev %CV
	TCR-00017-46 TCR-001 PFOS Solubility in water													
73100-wPFOS2.1.1	Day 2 Samples MLA 8-3-00 Diluted 10,000X by KLT 8-27-00	35	H082900A.M	HILL0035.D	21	250	6.14	2267283.8	10,000X	6.54	2779207.8	66.0	659.9	738.5
	TCR-00017-46 TCR-001 PFOS Solubility in water													
73100-wPFOS2.1.2	Day 2 Samples MLA 8-3-00 Diluted 10,000X by KLT 8-27-00	36	H082900A.M	HILL0036.D	22	250	6.15	2383389.5	10,000X	6.54	3230355.8	73.4	733.6	117.1
	TCR-00017-46 TCR-001 PFOS Solubility in water													
73100-wPFOS2.1.3	Day 2 Samples MLA 8-3-00 Diluted 10,000X by KLT 8-27-00	37	H082900A.M	HILL0037.D	23	250	6.14	2359026.8	10,000X	6.54	2766552.0	63.0	629.9	15.9%
	TCR-00017-46 TCR-001 PFOS Solubility in water													
73100-wPFOS2.1.4	Day 2 Samples MLA 8-3-00 Diluted 10,000X by KLT 8-27-00	38	H082900A.M	HILL0038.D	24	250	6.14	2364024.8	10,000X	6.54	4013110.0	93.0	930.5	
	TCR-00017-46 TCR-001 PFOS Solubility in water													
73100-wPFOS2.2.1	Day 2 Samples MLA 8-3-00 Diluted 10,000X by KLT 8-27-00	40	H082900A.M	HILL0040.D	25	250	6.14	2377500.8	10,000X	6.54	2629857.8	59.2	592.4	615.8
	TCR-00017-46 TCR-001 PFOS Solubility in water													
73100-wPFOS2.2.2	Day 2 Samples MLA 8-3-00 Diluted 10,000X by KLT 8-27-00	41	H082900A.M	HILL0041.D	26	250	6.14	2396023.0	10,000X	6.54	2617008.3	58.5	584.5	38.8
	TCR-00017-46 TCR-001 PFOS Solubility in water													
73100-wPFOS2.2.3	Day 2 Samples MLA 8-3-00 Diluted 10,000X by KLT 8-27-00	42	H082900A.M	HILL0042.D	27	250	6.14	2415598.0	10,000X	6.54	3005818.8	67.0	670.5	6.3%
	TCR-00017-46 TCR-001 PFOS Solubility in water													
~~73100-wPFOS2.2.4~~	~~Day 2 Samples MLA 8-3-00 Diluted 10,000X by KLT 8-27-00~~	~~43~~	~~H082900A.M~~	~~HILL0043.D~~	~~28~~	~~250~~	~~6.14~~	~~2350015.0~~	~~10,000X~~	~~6.54~~	~~6147075.0~~	~~147.7~~	~~1477.0~~	
	TCR-00017-46 TCR-001 PFOS Solubility in water													
73100-wPFOS2.3.1	Day 2 Samples MLA 8-3-00 Diluted 10,000X by KLT 8-27-00	47	H082900A.M	HILL0047.D	29	250	6.15	2414468.3	10,000X	6.54	3844820.0	87.0	869.6	844.2
	TCR-00017-46 TCR-001 PFOS Solubility in water													
73100-wPFOS2.3.2	Day 2 Samples MLA 8-3-00 Diluted 10,000X by KLT 8-27-00	48	H082900A.M	HILL0048.D	30	250	6.15	2410272.8	10,000X	6.54	4179820.3	95.2	951.8	99.9
	TCR-00017-46 TCR-001 PFOS Solubility in water													
73100-wPFOS2.3.3	Day 2 Samples MLA 8-3-00 Diluted 10,000X by KLT 8-27-00	49	H082900A.M	HILL0049.D	31	250	6.14	2408211.5	10,000X	6.54	3169192.8	71.1	711.2	11.8%
	TCR-00017-46 TCR-001 PFOS Solubility in water													
~~73100-wPFOS2.3.4~~	~~Day 2 Samples MLA 8-3-00 Diluted 10,000X by KLT 8-27-00~~	~~50~~	~~H082900A.M~~	~~HILL0050.D~~	~~32~~	~~250~~	~~6.14~~	~~2410886.8~~	~~10,000X~~	~~6.54~~	~~14402318.0~~	~~378.1~~	~~3781.2~~	
	TCR-00017-46 TCR-001 PFOS Solubility in water													
73100-wPFOS2.4.1	Day 2 Samples MLA 8-3-00 Diluted 10,000X by KLT 8-27-00	52	H082900A.M	HILL0052.D	33	250	6.14	2408902.5	10,000X	6.54	35990.8	0.0		
	TCR-00017-46 TCR-001 PFOS Solubility in water													
73100-wPFOS2.4.2	Day 2 Samples MLA 8-3-00 Diluted 10,000X by KLT 8-27-00	53	H082900A.M	HILL0053.D	34	250	6.14	2406550.3	10,000X	6.54	80307.8	0.3		
	TCR-00017-46 TCR-001 PFOS Solubility in water													
73100-wPFOS2.4.3	Day 2 Samples MLA 8-3-00 Diluted 10,000X by KLT 8-27-00	54	H082900A.M	HILL0054.D	35	250	6.14	2409505.5	10,000X	6.54	49571.0	0.0		
	TCR-00017-46 TCR-001 PFOS Solubility in water													
73100-wPFOS2.4.4	Day 2 Samples MLA 8-3-00 Diluted 10,000X by KLT 8-27-00	55	H082900A.M	HILL0055.D	36	250	6.14	2415089.0	10,000X	6.54	46530.8	0.0		
							Average:	2147802.7						
							Std Dev:	336071.7						
							% CV:	15.6%						

Total Day 1 ppm	Std Dev	%CV
733.4	129.5	18%

-Data excludes 2.2.4 and 2.3.4

excluded via Dixon's Q test 01-19-01 KLT

excluded via Dixon's Q-test 01-19-01 KLT

KLT 8/30/00

(10)
0211-2000 KLT

Blank Data/Performance

Sample ID	Sample Desc	Run #	Mtd	Data File	Vial#	THPFOS ppb	THPFOS RT	THPFOS Area	PFOS RT	PFOS Area	PFOS ppb	
MeOH TNA4464	Aliquoted 25 Jul 00	1	H082900A.M	HILL0001.D	91	0	0.00	0.0	6.54	258396.3	0.0	>50% LOQ
MeOH TNA4464	Aliquoted 25 Jul 00	4	H082900A.M	HILL0004.D	91	0	0.00	0.0	6.54	69085.1	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	5	H082900A.M	HILL0005.D	91	0	0.00	0.0	6.54	42172.9	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	18	H082900A.M	HILL0018.D	91	0	0.00	0.0	6.54	56934.7	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	19	H082900A.M	HILL0019.D	92	0	0.00	0.0	6.54	42770.3	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	20	H082900A.M	HILL0020.D	92	0	0.00	0.0	6.54	40742.9	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	33	H082900A.M	HILL0033.D	92	0	0.00	0.0	6.54	46384.4	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	34	H082900A.M	HILL0034.D	92	0	0.00	0.0	6.54	39377.8	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	39	H082900A.M	HILL0039.D	93	0	0.00	0.0	6.54	34297.9	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	45	H082900A.M	HILL0045.D	93	0	0.00	0.0	6.54	34747.0	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	46	H082900A.M	HILL0046.D	93	0	0.00	0.0	6.54	37159.8	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	51	H082900A.M	HILL0051.D	93	0	0.00	0.0	6.54	34000.8	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	57	H082900A.M	HILL0057.D	94	0	0.00	0.0	6.54	40326.7	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	58	H082900A.M	HILL0058.D	94	0	0.00	0.0	6.54	35091.9	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	71	H082900A.M	HILL0071.D	94	0	0.00	0.0	6.54	40584.3	0.0	
MeOH TNA4464	Aliquoted 25 Jul 00	72	H082900A.M	HILL0072.D	94	0	0.00	0.0	6.54	40223.0	0.0	
									LOQ-5ppb Std	50% Average LOQ Area 130078.5		

Initial BK, PFOS was "washed out" in subsequent runs to a level that met criteria. KLT 8/30/00

KLT 8/30/00

Day 3

Standards Data/Performance

Sample ID	Sample Desc	Run #	Mtd	Data File	Vial#	ppb	RT	Area	RT	Area	ppb	
00027-43-00	0 Std	3	H82500A4.M	HILL0097.D	1	250	6.15	2827919.5	6.54	53350.4	1.5	
00027-43-01	Std 1 (Excluded to better fit data)	4	H82500A4.M	HILL0098.D	2	250	6.15	2887205.8	6.54	166313.7	3.7	Excluded
00027-43-02	Std 2 (LOQ-5 ppb)	5	H82500A4.M	HILL0099.D	3	250	6.15	2856399.5	6.54	298617.5	6.3	126.1%
00027-43-03	Std 3	6	H82500A4.M	HILL0100.D	4	250	6.14	2873539.5	6.54	507393.3	10.4	103.7%
00027-43-04	Std 4	7	H82500A4.M	HILL0101.D	5	250	6.15	2871680.0	6.54	1323633.9	26.6	106.3%
00027-43-05	Std 5	8	H82500A4.M	HILL0102.D	6	250	6.15	3161595.5	6.54	2040881.1	37.3	93.0%
00027-43-06	Std 6	9	H82500A4.M	HILL0103.D	7	250	6.15	2915268.8	6.54	2634008.8	52.3	104.4%
00027-43-07	Std 7	10	H82500A4.M	HILL0104.D	8	250	6.15	2875385.0	6.54	3827296.5	77.7	103.4%
00027-43-08	Std 8	11	H82500A4.M	HILL0105.D	9	250	6.14	2920716.5	6.54	5067724.5	102.2	102.0%
00027-43-09	Std 9	12	H82500A4.M	HILL0106.D	10	250	6.15	2889778.3	6.54	11650840.0	253.2	101.1%
00027-43-10	Std 10	13	H82500A4.M	HILL0107.D	11	250	6.15	2901318.3	6.54	20554404.0	501.8	100.2%
00027-43-11	Std 11 (Excluded to better fit data)	14	H82500A4.M	HILL0108.D	12	250	6.15	2911203.5	6.54	34501388.0	0.0	Excluded
00027-43-06	CCV	26	H82500A4.M	HILL0120.D	7	250	6.15	2903577.8	6.54	2595877.8	51.7	103.3%
00027-43-06	CCV	38	H82500A4.M	HILL0132.D	7	250	6.15	2930110.3	6.54	2589949.8	51.2	102.1%
00027-43-00	0 Std	41	H82500A4.M	HILL0135.D	1	250	6.15	2850464.3	6.54	52082.5	1.5	
00027-43-01	Std 1 (Excluded to better fit data)	42	H82500A4.M	HILL0136.D	2	250	6.15	2869825.5	6.54	158504.7	3.5	Excluded
00027-43-02	Std 2 (LOQ-5 ppb)	43	H82500A4.M	HILL0137.D	3	250	6.14	2855512.3	6.54	297578.5	6.3	125.7%
00027-43-03	Std 3	44	H82500A4.M	HILL0138.D	4	250	6.15	2842810.8	6.54	506642.3	10.5	104.6%
00027-43-04	Std 4	45	H82500A4.M	HILL0139.D	5	250	6.14	3141249.8	6.54	1229335.4	22.6	90.3%
00027-43-05	Std 5	46	H82500A4.M	HILL0140.D	6	250	6.15	2889577.3	6.54	2049667.8	41.0	102.2%
00027-43-06	Std 6	47	H82500A4.M	HILL0141.D	7	250	6.15	3207130.3	6.54	2538722.5	45.8	91.4%
00027-43-07	Std 7	48	H82500A4.M	HILL0142.D	8	250	6.15	2852557.5	6.54	3766329.8	77.0	102.5%
00027-43-08	Std 8	49	H82500A4.M	HILL0143.D	9	250	6.15	3112386.0	6.54	4901655.0	92.4	92.2%
00027-43-09	Std 9	50	H82500A4.M	HILL0144.D	10	250	6.14	2871272.3	6.54	11477140.0	250.8	100.2%
00027-43-10	Std 10	51	H82500A4.M	HILL0145.D	11	250	6.14	2871562.3	6.54	20257486.0	499.0	99.6%
00027-43-11	Std 11 (Excluded to better fit data)	52	H82500A4.M	HILL0146.D	12	250	6.15	3065178.3	6.54	35672188.0	0.0	Excluded

Points Excluded to better fit data.

Exact Copy of Original

KLT Initial

02/04/11 Date

p. 1083

KLT 8/30/13 p. 1083

Sample Data/Performance Sample ID	Sample Desc	Run #	Mtd	Data File	Vial#	ppb	RT	Area	Dilution Factor	RT	Area	ppb	Undiluted ppm	Average PPM Std Dev %CV
73100-wPFOS3.1.1	TCR-00017-46 TCR-001 PFOS Solubility in water	17	H82500A4.M	HILL0111.D	53	250	6.15	3144450.3	10,000X	6.54	3580306.8	66.2	661.8	718.0
73100-wPFOS3.1.2	TCR-00017-46 TCR-001 PFOS Solubility in water	18	H82500A4.M	HILL0112.D	54	250	6.15	2934449.5	10,000X	6.54	3720808.0	73.9	738.9	32.5
73100-wPFOS3.1.3	TCR-00017-46 TCR-001 PFOS Solubility in water	19	H82500A4.M	HILL0113.D	55	250	6.14	2880555.5	10,000X	6.54	3650296.3	73.8	738.5	4.5%
73100-wPFOS3.1.4	TCR-00017-46 TCR-001 PFOS Solubility in water	20	H82500A4.M	HILL0114.D	56	250	6.15	2917426.5	10,000X	6.54	3668545.0	73.3	732.7	
73100-wPFOS3.2.1	TCR-00017-46 TCR-001 PFOS Solubility in water	22	H82500A4.M	HILL0116.D	57	250	6.15	2929888.8	10,000X	6.54	3622661.0	72.0	720.1	720.2
73100-wPFOS3.2.2	TCR-00017-46 TCR-001 PFOS Solubility in water	23	H82500A4.M	HILL0117.D	58	250	6.14	2916985.3	10,000X	6.54	3590292.5	71.7	716.7	4.0
73100-wPFOS3.2.3	TCR-00017-46 TCR-001 PFOS Solubility in water	24	H82500A4.M	HILL0118.D	59	250	6.15	2871665.8	10,000X	6.54	3582872.0	72.7	726.8	0.6%
73100-wPFOS3.2.4	TCR-00017-46 TCR-001 PFOS Solubility in water	25	H82500A4.M	HILL0119.D	60	250	6.15	2853369.8	10,000X	6.54	3514435.0	71.7	717.3	
73100-wPFOS3.3.1	TCR-00017-46 TCR-001 PFOS Solubility in water	29	H82500A4.M	HILL0123.D	61	250	6.15	3012129.0	10,000X	6.54	3612695.0	69.8	698.0	714.2
73100-wPFOS3.3.2	TCR-00017-46 TCR-001 PFOS Solubility in water	30	H82500A4.M	HILL0124.D	62	250	6.14	2928784.8	10,000X	6.54	3522437.8	70.0	700.0	15.4
73100-wPFOS3.3.3	TCR-00017-46 TCR-001 PFOS Solubility in water	31	H82500A4.M	HILL0125.D	63	250	6.15	2890032.0	10,000X	6.54	3635725.0	73.3	733.0	2.2%
73100-wPFOS3.3.4	TCR-00017-46 TCR-001 PFOS Solubility in water	32	H82500A4.M	HILL0126.D	64	250	6.14	2879217.8	10,000X	6.54	3587451.5	72.6	725.8	
73100-wPFOS3.4.1	TCR-00017-46 TCR-001 PFOS Solubility in water	34	H82500A4.M	HILL0128.D	65	250	6.15	2951372.0	10,000X	6.54	63286.7	1.6		
73100-wPFOS3.4.2	TCR-00017-46 TCR-001 PFOS Solubility in water	35	H82500A4.M	HILL0129.D	66	250	6.15	3136930.0	10,000X	6.54	62025.0	1.5		
73100-wPFOS3.4.3	TCR-00017-46 TCR-001 PFOS Solubility in water	36	H82500A4.M	HILL0130.D	67	250	6.15	2956127.0	10,000X	6.54	173373.4	3.7	>50% LOQ	
73100-wPFOS3.4.4	TCR-00017-46 TCR-001 PFOS Solubility in water	37	H82500A4.M	HILL0131.D	68	250	6.15	2888791.0	10,000X	6.54	94691.5	2.3		
					Average:			2934461.9						
					Std Dev:			98685.5						
					% CV:			3.4%						

(1) (KE) KLT 11-20-00 Study# should read TCR-002

Total Day 1 ppm	Std Dev	%CV
717.5	21.1	3%

Blank Data/Performance

Sample ID	Sample Desc	Run #	Mtd	Data File	Vial#	ppb	RT	Area	RT	Area	ppb
MeOH TNA4464	Aliquoted 25 Jul 00	1	H82500A4.M	HILL0095.D	96	0	0.00	0.0	6.54	55538.7	0.4
MeOH TNA4464	Aliquoted 25 Jul 00	2	H82500A4.M	HILL0096.D	96	0	0.00	0.0	6.54	48190.1	0.4
MeOH TNA4464	Aliquoted 25 Jul 00	15	H82500A4.M	HILL0109.D	96	0	0.00	0.0	6.54	42932.7	0.4
MeOH TNA4464	Aliquoted 25 Jul 00	16	H82500A4.M	HILL0110.D	96	0	0.00	0.0	6.54	41835.0	0.4
MeOH TNA4464	Aliquoted 25 Jul 00	21	H82500A4.M	HILL0115.D	97	0	0.00	0.0	6.54	77874.9	0.4
MeOH TNA4464	Aliquoted 25 Jul 00	27	H82500A4.M	HILL0121.D	97	0	0.00	0.0	6.54	54579.8	0.4
MeOH TNA4464	Aliquoted 25 Jul 00	28	H82500A4.M	HILL0122.D	97	0	0.00	0.0	6.54	50519.9	0.4
MeOH TNA4464	Aliquoted 25 Jul 00	33	H82500A4.M	HILL0127.D	97	0	0.00	0.0	6.54	50779.9	0.4
MeOH TNA4464	Aliquoted 25 Jul 00	39	H82500A4.M	HILL0133.D	98	0	0.00	0.0	6.54	81693.0	0.4
MeOH TNA4464	Aliquoted 25 Jul 00	40	H82500A4.M	HILL0134.D	98	0	0.00	0.0	6.54	61039.4	0.4
MeOH TNA4464	Aliquoted 25 Jul 00	53	H82500A4.M	HILL0147.D	98	0	0.00	0.0	6.54	51639.5	0.4
MeOH TNA4464	Aliquoted 25 Jul 00	54	H82500A4.M	HILL0148.D	98	0	0.00	0.0	6.54	45487.9	0.4
MeOH TNA4464	Aliquoted 25 Jul 00	55	H82500A4.M	HILL0149.D	98	0	0.00	0.0	6.54	40381.1	0.4

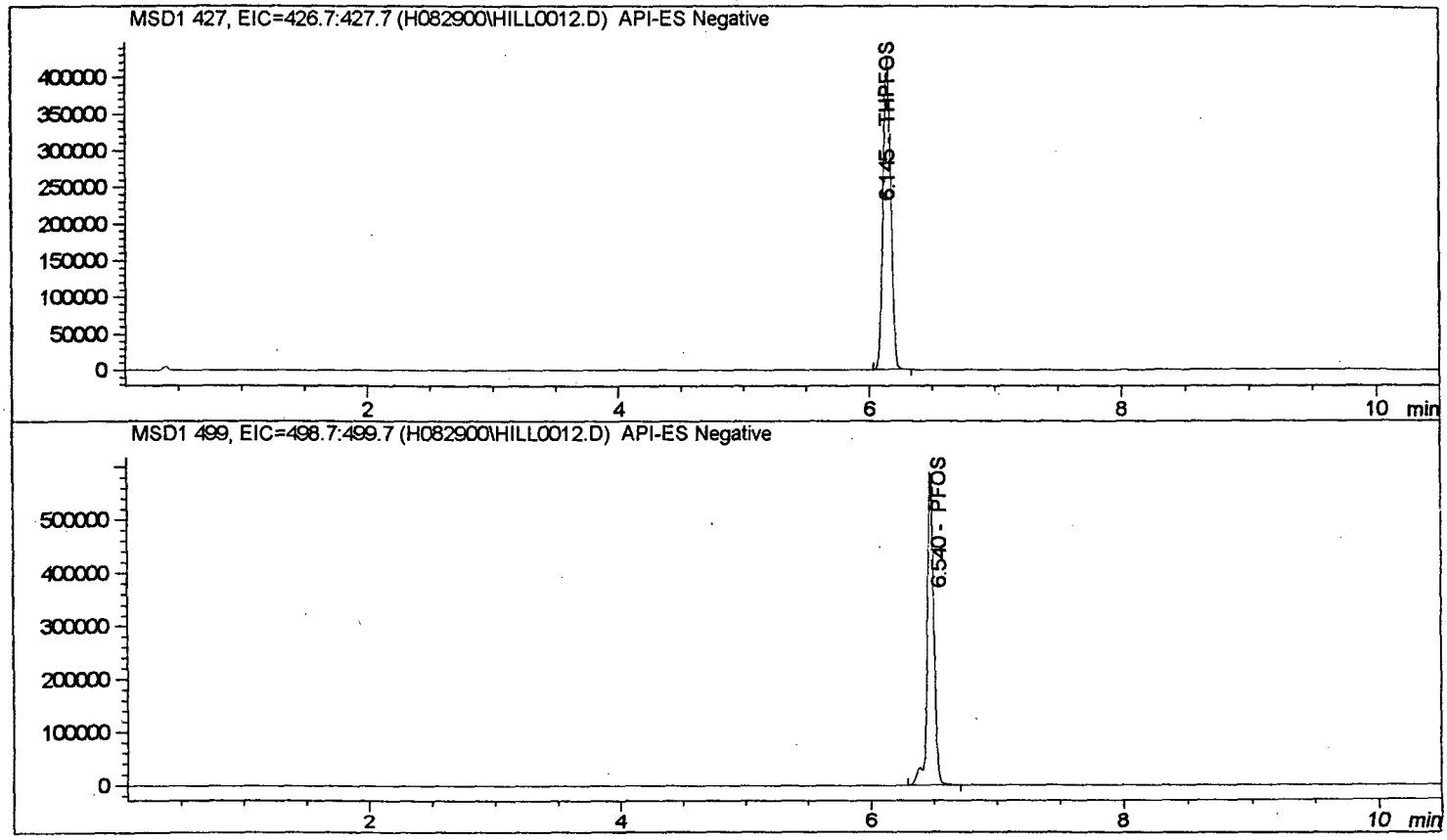
LOQ-5ppb Std

50% Average LOQ Area
149049.0

ATTACHMENT C: SAMPLE CHROMATOGRAMS

Data File C:\HPCHEM\1\DATA\H082900\HILL0012.D Sample Nam


```
Injection Date  : 8/29/00 7:09:50 PM          Seq. Line :  12
Sample Name     : 00027-43-06                      Vial :   7
Acq. Operator   : KLT on "Hillary"                  Inj :   1
                                             Inj Volume : 5 µl
Acq. Method     : C:\HPCHEM\1\METHODS\PFOS825.M
Last changed    : 8/29/00 4:33:39 PM by KLT
Analysis Method : C:\HPCHEM\1\METHODS\H082900A.M
Last changed    : 8/30/00 11:31:37 AM
                  (modified after loading)
Data Reprocessing on Workstation "Lefty"
PFOS Quant using internal Std THPFOS
KLT 8-30-00
```



Internal Standard Report

```
Sorted By             :      Signal
Calib. Data Modified  :      Wednesday, August 30, 2000 11:17:18 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample ISTD Information:
```

ISTD #	ISTD Amount [ppb]	Name
1	250.00000	THPFOS

Exact Copy of Original

KLT 02/09/01

Initial Date

Data File C:\HPCHEM\1\DATA\H082900\HILL0012.D Sample Na

Injection Date : 8/29/00 7:09:50 PM Seq. Line : 12
Sample Name : 00027-43-06 Vial : 7
Acq. Operator : KLT on "Hillary" Inj : 1
Inj Volume : 5 µl

Acq. Method : C:\HPCHEM\1\METHODS\PFOS825.M
Last changed : 8/29/00 4:33:39 PM by KLT
Analysis Method : C:\HPCHEM\1\METHODS\H082900A.M
Last changed : 8/30/00 11:31:37 AM
(modified after loading)
Data Reprocessing on Workstation "Lefty"
PFOS Quant using internal Std THPFOS
KLT 8-30-00

Signal 1: MSD1 427, EIC=426.7:427.7

RetTime [min]	Type	Area	Amt/Area ratio	Amount [ppb]	Grp	Name
6.145	BB I	1.82489e6	1.00000	250.00000		THPFOS

Totals without ISTD(s) : 0.00000

Signal 2: MSD1 499, EIC=498.7:499.7

RetTime [min]	Type	Area	Amt/Area ratio	Amount [ppb]	Grp	Name
6.540	VBA+	2.21968e6	2.15268e-1	65.45964		PFOS

Totals without ISTD(s) : 65.45964

*** End of Report ***

Exact Copy of Original
KLT Initial 02/09/01 Date

ATTACHMENT D: DEVIATIONS FROM THE PROTOCOL

Centre Analytical Laboratories, Inc.
3048 Research Drive State College, PA 16801 www.centrelab.com
Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

PROTOCOL DEVIATION

Deviation Number: 001
Date of Occurrence: Entire Study

Centre Study Number: 023-021
Sponsor Study Number: FACT-TCR002

DESCRIPTION OF DEVIATION

Solubility studies of PFOS Primary Standard, Test Control Reference # TCR-00017-46, were not conducted in methanol or acetone due to limited quantities of test substance.

ACTIONS TAKEN

i.e., amendment issued, SOP revision, etc...

Protocol Deviation issued.

Recorded By/Date: 2/8/01

IMPACT ON THE STUDY

Results in limited solubility data for the test substance.

John M Haberl 2/8/01
Study Director Signature Date

2/8/01
Sponsor Management Signature Date

Centre QAU Review 2/8/01

January 5, 2001/3

Exact Copy of Original

KLT 02/09/01
Initial Date

Record of Deviation

I. Identification

Study / Project No.

TCR-002

Deviation Type: ☐ SOP ☐ Method ☐ Equipment Procedure
(Check one) ☒ Protocol ☐ Other:

Document Number: ETS-8-155.0 Date(s) of occurrence: 13 Jul 00 to 29 Aug 00

II. Description:

Required Procedure/process: ETS-8-155.0 section 10.1.2 states, "Analyze a method blank and a matrix blank prior to each calibration curve.

Actual Procedure/process: In this experiment, the method and matrix blanks are essentially the same. The method/matrix blanks were not ran before the calibration curves. The method blanks were ran after the first calibration curve.

III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

The ETS-8-155.0 method has been revised so that the method and matrix blanks are to be ran after the first calibration curve.

Recorded By: Date: 01/F/01

IV. Impact on Study / Project

The impact of this deviation on the study is minimal. The placement of the blanks have no bearing on the results. See Corrective Action.

Authorized By: *(Study Director / Project Lead)* Date: 02/01/01

3M Environmental Laboratory
Form ETS-4-8.0

Exact Copy of Original

Deviation No. 02
(assigned by Study Director or Project Lead at the end of study or project)

KA 02/09/01
Initial Date

Record of Deviation

I. Identification

Study / Project No.

TCR-002

Deviation Type: ☐ SOP ☐ Method ☐ Equipment Procedure
(Check one) ☒ Protocol ☐ Other:

Document Number: ETS-8-155.0 Date(s) of occurrence: 13 Jul 00 to 29 Aug 00

II. Description:

Required Procedure/process: ETS-8-155.0 section10.2.1 states, " Matrix spikes are prepared for each sample set and analyzed to determine the matrix effect on the recovery efficiency." Section 10.2.3 states, "analyze the matrix spike and the matrix spike duplicate (if prepared) in the same run as the original sample."

Actual Procedure/process: The matrix spikes were not prepared, therefore, they were not analyzed. See Corrective Action.

III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

The ETS-8-155.0 method has been revised so that matrix spikes do not need to be prepared when there is no need. See Corrective Action.

Recorded By: [signature] Date: 01 Feb 01

IV. Impact on Study / Project

The impact of this deviation on the study is minimal. The lack of a matrix spike has no bearing on the final results. The matrix is well defined and characterized. The matrix spike is actually not possible in this study since the matrix is saturated with the analyte of interest.

See Corrective Action.

Authorized By: *(Study Director / Project Lead)* [signature] Date: 02/01/01

Exact Copy of Original 02/09/01 Initial

3M Environmental Laboratory
Form ETS-4-8.0

Deviation No. 03
(assigned by Study Director or Project Lead at the end of study or project)

Record of Deviation

I. Identification

Study / Project No.

TCR-002

Deviation Type: ☐ SOP ☐ Method ☐ Equipment Procedure
(Check one) ☒ Protocol ☐ Other:

Document Number: ETS-8-155.0 Date(s) of occurrence: 13 Jul 00 to 29 Aug 00

II. Description:

Required Procedure/process: In dealing with the standard curve, the curve must have a coefficient of determination (r^2) value greater than or equal to 0.980 and individual curve points must have 'Percent Deviations' equal to or less than +/- 30%.

Actual Procedure/process: In some cases, high and/or low curve points were eliminated so that the r^2 value would meet the criteria. In other cases, high and/or low curve points were eliminated, as they were not within the +/-30% accuracy requirements. When this was done, the curve point(s) affected and the action(s) taken were annotated on the 'Data Summary Sheet'.
The following is a list of all curve points affected and the reasons why.
Day 1, (H082700), points Hill0032 and Hill0070.
Day 2, (H082900), points Hill0022, 0032, 0060 and 0070.
Day 3, (H082500a), points Hill0098, 0108, 0136 and 0146.
All of these points were excluded to provide a better fit over the range appropriate to the data.

III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

The 3M Environmental Method ETS-8-155.0 was amended to take into account the elimination of curve points. Curve points may be omitted for the following reasons:
(Comments are bulleted as they would appear on the summary sheets.)
(1) "High/low curve points were excluded to provide a better fit over the range appropriate to the data."
(2) "Low level point(s) were not 2x higher than the extraction blank; these points were excluded from the curve to disqualify a data range that may have been significantly affected by background levels of the analyte."
(3) "High/low curve point(s) were excluded as they were not within the +/- 30% accuracy requirements of the method when the curves were evaluated over a range appropriate to the data."

Exact Copy of Original 02/09/01 Date
KLF Initial

3M Environmental Laboratory
Form ETS-4-8.0

Deviation No. 04 page 1 of 2
(assigned by Study Director or Project Lead at the end of study or project)

Record of Deviation

Recorded By:	Date:
	01 Feb 01

IV. Impact on Study / Project

The elimination of curve points had a positive impact on this study. If curve points were not eliminated, than the curves would not be as accurate.

Authorized By: *(Study Director / Project Lead)*	Date:
	02/01/01

Exact Copy of Original
KLT Initial
02/09/01 Date

3M Environmental Laboratory
Form ETS-4-8.0

Deviation No. 04 (continued) page 2 of 2
(assigned by Study Director or Project Lead at the end of study or project)

ATTACHMENT E: SAMPLE CALCULATIONS

Mean and standard deviation were calculated using functions provided in Microsoft Excel® software.

Standard deviation population was used to measure the scatter about the mean of a data set; thus, it can be used to estimate the precision of a method. Relative standard deviation presents a measure of the magnitude of the standard deviation and is calculated by dividing the standard deviation population by the mean. Means are calculated by adding individual entities and dividing the resultant sum by the number of individual entities. Standard deviation population was calculated using the following equation:

$$\sqrt{\frac{n\Sigma x^2 - (\Sigma x)^2}{n^2}}$$

-Where *n* is the number of observations, and x is sample concentration.

Average/Standard Deviation Population/%RSD example calculations:

Sample ID	Concentration µg/mL	Calculations
Rep1	647.9	Average (647.9+723.4+723.0+717.3)/4= 702.9 Std Dev Pop (see above equation)= 31.8 %RSD (31.8/702.9)= 4.5%
Rep 2	723.4	
Rep 3	723.0	
Rep 4	717.3	

Data that did not meet acceptance criteria as described in the OPPTS and OECD guidelines was excluded using statistical justification provided by Dixon's Q-test. A data point may be excluded if "$Q_{observed}$" is greater than "$Q_{tabulated}$" with 90% confidence. $Q_{observed}$ = gap/range where "gap" is the difference between the questionable data point and the closest value of the data set, and "range" is the difference between the highest and lowest value of the data set. Q-tabulated (90% confidence) for 4 observations (or data points) is 0.679 and for 3 observations is 0.886.

Q-Test Example *(Shaded number represents the suspected outlier)*:

Description	µg/mL
Sample Conc., Rep1	607.1
Sample Conc., Rep2	591.7
Sample Conc., Rep3	626.1
Sample Conc., Rep4	
Range (Rep4-Rep2):	2057.14
Gap (Rep4-Rep3):	2022.66
$Q_{observed}$ (Range/Gap):	0.983
No. observations:	4
Retain?	***no***

Q-Test example conclusion: Since $Q_{observed}$, 0.983, was greater than $Q_{tabulated}$, 0.679, rep 4 may be excluded.