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Title: Analysis of Perfluorooctanoic Acid (PFOA) and other Perfluorinated Hydrocarbons (PFCs) and Perfluorinated Hydrocarbon Sulfonates (PFSs) in Water and Soil by LC/MS/MS

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1.0 Scope and Application

1.1 This procedure describes the analysis of water and soil samples for perfluorooctanoic acid (PFOA, CAS# 335-67-1), and other perfluorinated hydrocarbons (PFCs) and perfluorinated hydrocarbon sulfonates (PFSs) or perfluorinated hydrocarbon sulfonic acids using liquid chromatography / tandem mass spectrometry (LC/MS/MS). See Table 6 for the list of compounds and their common names. Basic structure for the perfluorinated hydrocarbon carboxylic acids is $C_nF_{(2n+1)}COOH$; basic structure for the perfluorinated hydrocarbon sulfonates is $C_nF_{(2n+1)}SO_3^-$ in the anion form or $C_nF_{(2n+1)}SO_3H$ in the acid form.

1.2 Throughout this procedure the term perfluorinated hydrocarbon sulfonates (PFSs) is used to describe a sub-set of the compounds listed in Table 6. Namely, these compounds are PFOS, PFBS, PFHxS, and PFDS. The term PFC is used to describe all of the compounds listed in Table 6, including the PFSs.

1.3 This procedure has also been adapted for the analysis of Perfluoro(2-propoxy propanoic) acid (HFPO).

1.4 This procedure does not include the sample extraction. See SOP DV-OP-0019 *Extraction of Perfluorooctanoic Acid (PFOA) and Perfluorooctanoic Sulfonate (PFOS) and other Perfluorinated Hydrocarbons (PFCs) in Water and Soil*.

1.5 The method has been validated by TestAmerica Denver for reagent water, drinking water, groundwater, and soil. Analytes and reporting limits in water and soil are provided in Table 1.

2.0 Summary of Method

2.1 The extracts are analyzed by LC/MS/MS. The PFCs are separated from other components on an HPLC C_{18} column with a mobile phase of 0.010 M ammonium acetate in 90:10 water:methanol. The mass spectrometer detector is operated in the electrospray (ESI) negative ion mode. Ion transitions monitored for quantitation are listed in Tables 2 and 3. Sample results are quantitated using the isotope dilution (internal standard) method.

2.2 Labeled PFCs are used in this analysis as an isotope dilution standard. The isotope dilution technique allows correction for analytical bias encountered when analyzing more chemically complex environmental samples. Known quantities of the labeled PFC compounds are added to every sample and to the batch quality control samples prior to extraction. Because the isotopically labeled compounds are chemically similar to the compounds of concern, they are affected by any interfering substances in the sample to the same extent as the compound of concern. The recoveries of the labeled PFCs are then used to mathematically correct the final results for the PFCs.

2.3 The perfluorinated hydrocarbon sulfonates are received from the vendor as sodium or potassium salts. These compounds are treated as anions throughout

the method, including the calibration concentrations, and the results are reported as the anions or the acids depending upon client requests. The concentration of the anions and the acids are the same to two significant figures, therefore, the sample concentration will be the same whether analyzes as the anion or the acid for these compounds.

2.4 There are four separate methods that are described in this SOP:

2.4.1 **PFC** – for the analysis of the full spectrum of PFC compounds, except FOSA in water samples

2.4.2 **PFC_FOSA** – for the analysis of FOSA in water samples

2.4.3 **LCMS_PFOA** – for the analysis of only PFOA, APFO and PFOS in both water and soil samples

2.4.4 **LCMS_HFPO** – for the analysis of HFPO in water samples

3.0 Definitions

Refer to the Glossary of the TestAmerica Denver Quality Assurance Manual (QAM) and policy DV-QA-003P, "Quality Assurance Program," for definitions of general analytical and QA/QC terms.

4.0 Interferences

4.1 Perfluorinated compounds have been used in a wide variety of manufacturing processes, and laboratory supplies should be considered potentially contaminated until they have been tested and shown to be otherwise. The materials and supplies used during the method validation process have been tested and shown to be clean. These items are listed below in Section 6.

4.2 To avoid contamination of samples, standards are prepared in a ventilation hood in an area separate from where samples are extracted.

4.3 PTFE products can be a source of PFOA contamination. The use of PTFE in the procedure should be avoided.

4.4 Volumetric glassware and syringes are difficult to clean after being used for solutions containing high levels of PFCs and PFSs. These items should be labeled for use only with similarly concentrated solutions or verified clean prior to re-use. To the extent possible, disposable labware is used.

4.5 Aluminum foil and foilware has been identified as a possible source of PFOA contamination. These materials should be avoided during the extraction process.

4.6 PFC compounds can adhere to glass surfaces to varying degrees. Glass containers should be avoided as much as possible during the extraction and

analysis of the samples.

4.7 Solvents used on the HPLC instrumentation can also be a source of contamination. Highest purity solvents are used to minimize the presence of contaminants. Also, the solvents are passed through an analytical column (Section 6.1.1.3) prior to interaction with the sample, to chromatographically separate any contaminants from the target compounds in the sample.

5.0 Safety

5.1 Employees must abide by the policies and procedures in the Environmental Health and Safety Manual, Radiation Safety Manual and this document.

5.2 This procedure may involve hazardous material, operations and equipment. This SOP does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of the method to follow appropriate safety, waste disposal and health practices under the assumption that all samples and reagents are potentially hazardous. Safety glasses, gloves, lab coats and closed-toe, nonabsorbent shoes are a minimum.

5.3 Specific Safety Concerns or Requirements

5.3.1 Preliminary toxicity studies indicate that PFC compounds could have significant toxic effects. In the interest of keeping exposure levels as low as reasonably achievable, PFCs must be handled in the laboratory as if they are hazardous and toxic chemicals.

5.3.2 Eye protection that satisfies ANSI Z87.1 (as per the Environmental Health and Safety Manual), laboratory coat, and nitrile gloves must be worn while handling samples, standards, solvents, and reagents. Disposable gloves that have been contaminated will be removed and discarded; other gloves will be cleaned immediately.

5.3.3 PFCs are acids and are not compatible with strong bases.

5.3.4 PFCs are not known to be highly flammable. However, methanol is highly flammable and is used throughout this procedure. Methanol should be stored away from any ignition sources and kept in closed containers with secondary containment measures or within a fume hood.

5.4 Primary Materials Used

The following is a list of the materials used in this method, which have a serious or significant hazard rating. **Note: This list does not include all materials used in the method. The table contains a summary of the primary hazards listed in the SDS for each of the materials listed in the table.** A complete list of materials used in the method can be found in the reagents and materials section. Employees must review the information in the SDS for each material before using it for the first time or when there are major changes to the SDS.

Material (1)	Hazards	Exposure Limit (2)	Signs and symptoms of exposure
Hydrochloric Acid	Corrosive Poison	5 ppm (Ceiling)	Can cause pain and severe burns upon inhalation, ingestion, eye or skin contact. Exposure to concentrated solutions may cause deep ulcerations to skin, permanent eye damage, circulatory failure and swallowing may be fatal.
Sodium Hydroxide	Corrosive Poison	2 mg/cm^3 (Ceiling)	Severe irritant. Can cause severe burns upon inhalation, ingestion, eye or skin contact. Exposure to concentrated solutions may cause severe scarring of tissue, blindness, and swallowing may be fatal.
Formic Acid	Combustible Corrosive Flammable	5 ppm (TWA)	Causes severe burns, toxic by inhalation, harmful if swallowed, possible sensitizer.
Methanol	Flammable Poison Irritant	200 ppm (TWA)	A slight irritant to the mucous membranes. Toxic effects exerted upon nervous system, particularly the optic nerve. Symptoms of overexposure may include headache, drowsiness and dizziness. Methyl alcohol is a defatting agent and may cause skin to become dry and cracked. Skin absorption can occur; symptoms may parallel inhalation exposure. Irritant to the eyes.
Ammonium hydroxide	Corrosive Toxic	25 ppm (TWA)	Causes severe burns and possible irreversible eye damage. May cause severe and permanent damage to the digestive tract.
1 – Always add acid to water to prevent violent reactions. 2 – Exposure limit refers to the OSHA regulatory exposure limit.			

6.0 Equipment and Supplies

6.1 Instrumentation

6.1.1 HPLC system connected to a Waters Quattro Micro MS/MS detector (LCMS-4), Waters XEVO MS/MS detector (LCMS-7) or an Agilent 6460 MS/MS detector (LCMS-5).

6.1.1.1 (LCMS_4 LCMS-7) Phenomenex Synergi 4u Hydro RP 75x3.0mm and security Guard cartridge or equivalent

6.1.1.2 (LCMS_5) Phenomenex Gemini NX analytical column and Security Guard cartridge, or equivalent.

6.1.1.3 (LCMS_5): ZORBAX Eclipse Plus C_{18} (Agilent) column, 4.6x30mm, 3.5-micron, installed after solvent mixer, prior to injector.

6.2 Supplies

6.2.1 Volumetric Flasks (Class A): 100 mL; 200 mL; 500 mL; 1000 mL.

6.2.2 2.0-mL autosampler vials, Waters PN 186000307, or equivalent.

6.2.3 Miscellaneous laboratory apparatus (beakers, test tubes, volumetric flasks, pipettes etc.). These should be disposable where possible, or marked and segregated for high-level versus low-level use.

6.3 Computer Software and Hardware

Please refer to the master list of documents and software located on R:\QA\Read\Master List of Documents\Master List of Documents, Software and Hardware.xls or current revision for the current software and hardware to be used for data processing.

7.0 Reagents and Standards

7.1 The solvents used for this procedure must be HPLC grade or better. LCMS-grade methanol is used during sample preparation, standards preparation and instrument analysis to minimize potential contamination due to solvent impurities.

7.2 Mobile Phase

0.010 M Ammonium Acetate in 90:10 water : methanol – Weigh 0.72 - 0.88 g of ammonium acetate. Place in a 1 L class A volumetric flask and dilute to 1 L with a 90:10 solution of water: methanol.

7.3 Stock Standard Materials

PFCs and labeled PFCs are purchased at certified concentrations in solution. Standard materials are verified compared to a second source material at the time of initial calibration. The Compounds Perfluorohexanesulfonate, Perfluorooctane Sulfonate and Techinical Ammonium Perfluorooctanate are obtained as the branched isomers from Wellington Laboratories (product code br-PFHxSK, br-PFOSK and T-PFOA respectively)

7.3.1 The stock standard solutions are stored at ≤6°C. Stock standard solutions should be brought to room temperature before using. Standards are monitored for signs of degradation or evaporation. Standard solutions must be replaced at least annually from the date of opening or earlier if the vendor indicates an earlier expiration date.

7.3.2 All standards are stored in HDPE bottles and vials at ≤6°C and are assigned an expiration date of one year or earlier if the parent stock materials have an earlier expiration date. Dilutions from stocks may not

be assigned expiration dates that exceed the expiration of the stock materials. Glass containers are avoided.

7.4 The perfluorinated hydrocarbon sulfonates are received from the vendor as sodium or potassium salts. These compounds are treated as anions throughout the method, including the calibration concentrations, and the results are reported as the anions or the acids depending upon client requests. The concentration of the anions and the acids are the same to two significant figures, therefore, the sample concentration will be the same whether analyzes as the anion or the acid for these compounds.

7.4.1 The primary vendor lists both the concentration of the salt and the anion on the certificate of analysis. The concentration of the anion is determined by multiplying the concentration of the salt by the ratio of the molecular weights of the anion to the salt, example:

$$[\text{Anion}] = \frac{MW_{anion}}{MW_{salt}} \times [Salt] \qquad \textit{Equation 1}$$

$$[\text{Acid}] = \frac{MW_{acid}}{MW_{salt}} \times [Salt] \qquad \textit{Equation 2}$$

Example calculations:

	MW Ratio (Anion / Na salt)	**MW Ratio (Anion / K salt)**
PFBS	0.929	0.885
PFHxS*	0.945	0.911
PFOS*	0.956	0.928
PFDS	0.963	0.939

*****Both the branched and linear isomers are used to evaluate these compounds

7.5 LCS Standards (Spike Solutions)

The LCS stock solutions are prepared by diluting the appropriate amounts of PFC stock solutions in LCMS-grade methanol. These standards are at a concentration of 0.5 µg/mL. These standards are stored in HDPE bottles at ≤6°C and given a 1 year expiration date. See DV-OP-0019 for details. These standards are also used to spike the MS and MSD extracts.

7.5.1 PFC Method LCS:

A spike solution is prepared containing all PFC compounds at 0.5 µg/mL by

diluting 2.5 mL PFAC-MXB-Stk (2.0 μg/mL) and 0.1 mL FOSA-Stock (50.0 μg/mL) to 10 mL in LCMS-grade methanol.

7.5.2 PFC_FOSA Method LCS:

A spike solution is prepared containing FOSA at 0.05 μg/mL by diluting 0.1 mL FOSA-Stock (50.0 μg/mL) to 100 mL in LCMS-grade methanol.

7.5.3 LCMS_PFOA Method LCS

A spike solution is prepared containing PFOA and PFOS at 0.5 μg/mL by diluting 1.0 mL PFOA-stock (50.0 μg/mL) and 1.0 mL PFOS-Stock (50.0 μg/mL) to 100 mL in LCMS-grade methanol.

7.5.4 HFPO Method LCS

A spike solution is prepared containing HFPO at 0.5 µg/mL by diluting 1 mL of HFPO-DA stock (50.0 µg/mL) to 100 mL in LCMS grade methanol.

7.5.5 Table of LCS spike volumes and final sample concentrations:

Method	Matrix	Volume of Spike Added	Concentration in Sample
PFC	Water	0.1 mL	0.2 μg/L
	Soil	0.4 mL	20 μg/kg
PFC_FOSA	Water	1.0 mL	0.2 μg/L
	Soil	N/A	N/A
LCMS_PFOA	Water	0.1 mL	0.2 μg/L
	Soil	0.4 mL	20 μg/kg
LCMS_HFPO	Water	0.1 mL	0.2 µg/L
	Soil	N/A	N/A

7.6 Surrogate Standard Solutions, 0.5 μg/mL (Instrument)

A surrogate spike solution is prepared containing the labeled PFC isotopes, $^{13}C_8$ PFOA and $^{13}C_8$ PFOS, at 0.5 μg/mL by diluting 0.1 mL $^{13}C_8$PFOA-stk (50.0 μg/mL) and 0.1 mL $^{13}C_8$PFOS-stk (50.0 μg/mL) to 10 mL in LCMS-grade methanol. This surrogate solution is utilized for the PFC method and LCMS_PFOA method, the PFC_FOSA method does not utilize a surrogate. Standards are stored in HDPE bottles at ≤6°C and given an expiration date of one year or earlier if the parent stock materials have an earlier expiration date.

7.7 Internal Standard Solutions, 0.5 μg/mL (Instrument)

All individual internal standards and surrogates are received from the vendor at a concentration of 50.0 μg/mL. Internal standard solutions are prepared for each method by combining 0.1 mL of each compound listed below and diluting to 10 mL in LCMS-grade methanol. Standards are stored in HDPE bottles at ≤6°C and given an expiration date of one year or earlier if the parent stock materials have an earlier expiration date.

PFC Method:	$^{13}C_4$ PFBA	$^{13}C_2$ PFHxA	$^{13}C_4$ PFOA
	$^{13}C_4$ PFNA	$^{13}C_2$ PFDA	$^{13}C_2$ PFUnA
	$^{13}C_4$ PFDoA	$^{18}O_2$ PFHxS	$^{13}C_4$ PFOS
	$^{13}C_8$ FOSA		
LCMS_PFOA Method:	$^{13}C_4$ PFOA	$^{13}C_4$ PFOS	
LCMS_HFPO Method	$^{13}C_3$ HFPO		

7.8 Internal Standards / Surrogates Spiking Solutions (Prep)

All individual internal standards and surrogates are received from the vendor at a concentration of 50.0 μg/mL. An internal standard / surrogate solution is prepared by diluting 1.0 mL of each compound to 100 mL in LCMS-grade methanol. Standards are stored in HDPE bottles at ≤6°C and given an expiration date of one year or earlier if the parent stock materials have an earlier expiration date.

PFC Method:

Internal Standards:	$^{13}C_4$ PFBA	$^{13}C_2$ PFHxA	
	$^{13}C_4$ PFOA	$^{13}C_4$ PFNA	$^{13}C_2$ PFDA
	$^{13}C_2$ PFUnA	$^{13}C_4$ PFDoA	$^{18}O_2$ PFHxS
	$^{13}C_4$ PFOS	$^{13}C_8$ FOSA	
Surrogates:	$^{13}C_8$ PFOA	$^{13}C_8$ PFOS	

PFC_FOSA Method:

Internal Standard: $^{13}C_8$ FOSA

LCMS_PFOA Method:

Internal Standards:	$^{13}C_4$ PFOA	$^{13}C_4$ PFOS
Surrogates:	$^{13}C_8$ PFOA	$^{13}C_8$ PFOS

LCMS_HFPO Method:

Internal Standard: $^{13}C_3$ HFPO

Surrogate: $^{13}C_3$ HFPO

7.9 Calibration Stock Standard (Instrument)

The calibration stock solution is prepared by diluting the appropriate amounts of PFC LCS solution and surrogate solution in 80% methanol in water. This standard is at a concentration of 20 µg/L and is diluted with 80% methanol in water to produce other initial calibration standards. For the HFPO calibration a 50% methanol in water solution is used for preparation of the calibration standards. For the PFC_FOSA method, the PFC standards are used to calibrate the instrument. Standards are stored in HDPE bottles at ≤6°C and given an expiration date of one year or earlier if the parent stock materials have an earlier expiration date.

7.9.1 For the PFC method, take 0.4 mL of the PFC LCS Spike Solution and 0.4 mL of the Surrogate Standard Solution (Instrument) and dilute to 10 mL with 80% methanol in water.

7.9.2 For the LCMS_PFOA method, take 0.4 mL of the LCMS_PFOA LCS Spike Solution and 0.4 mL of the Surrogate Standard Solution (Instrument) and dilute to 10 mL with 80% methanol in water.

7.10 Initial Calibration (ICAL) Levels (Instrument)

7.10.1 The calibration levels in the table below are provided as an example. Final volume of each calibration level standard is 1 mL and standards are brought to volume with 80% methanol in water. To each calibration standard, 20 µL of Internal Standard solution (Section 7.7) must be added.

7.10.2 Note that the PFS compounds are purchased in solutions at certified concentrations as salts. They are reported as anions or acids depending upon client request. The concentration is adjusted to be expressed as the anion or acid concentration. If a different salt is used (e.g. potassium versus sodium), these concentrations would be different.

ICAL Level	Volume Stk Soln to Add (µL)	On Column Conc. of PFCs (µg/L)	Equiv. Sample Conc Water (ng/L)	Equiv. Sample Conc Soil (µg/kg)	On Column Conc. of PFBS as the anion or acid (µg/L)	On Column Conc. of PFHxS as the anion or acid (µg/L)	On Column Conc. of PFOS as the anion or acid (µg/L)	On Column Conc. of PFDS as the anion or acid (µg/L)
1	10 µL CALSTK	0.2	4	0.4	0.177	0.189	0.191	0.193
2	25 µL CALSTK	0.5	10	1.0	0.4425	0.4725	0.4775	0.4825
3	50 µL CALSTK	1.0	20	2.0	0.885	0.945	0.955	0.965
4	100 µL CALSTK	2.0	40	4.0	1.77	1.89	1.91	1.93
5	250 µL CALSTK	5.0	100	10.0	4.425	4.725	4.775	4.825

ICAL Level	Volume Stk Soln to Add (µL)	On Column Conc. of PFCs (µg/L)	Equiv. Sample Conc Water (ng/L)	Equiv. Sample Conc Soil (µg/kg)	On Column Conc. of PFBS as the anion or acid (µg/L)	On Column Conc. of PFHxS as the anion or acid (µg/L)	On Column Conc. of PFOS as the anion or acid (µg/L)	On Column Conc. of PFDS as the anion or acid (µg/L)
6	500 µL CALSTK	10	200	20.0	8.85	9.45	9.55	9.65
7	40 µL LCS & 40 µL Surrogate	20	400	40.0	17.7	18.9	19.1	19.3
8	100 µL LCS & 100 µL Surrogate	50	1000	100	44.25	47.25	47.75	48.25
9	250 µL LCS & 250 µL Surrogate	125	2500	250	110.625	118.125	119.375	120.625

The calibration range for HFPO varies slightly from the concentrations listed in the table above, beginning with an on column concentration of 0.25 µg/L and ending with an on column concentration of 100 µg/L for a range of equivalent sample concentration of 5 ng/L to 2000 ng/L. Actual HFPO on-column concentrations for the entire nine-point curve are: 0.25, 0.50, 1.0, 2.0, 5.0, 10, 25, 50 and 100 µg/L.

7.11 Initial Calibration Verification Standard (ICV), 2.0 µg/L

7.11.1 The second source standard must be obtained from a different source than the standards used for initial calibration. This standard is used to verify the accuracy of the calibration standards.

7.11.2 In the case of the PFC and PFC_FOSA methods, a custom standard is purchased from Supelco with most PFC compounds at 1 µg/mL; as necessary, the concentrations of the sulfonate salts are adjusted to the anion concentration. Second source standards for PFDS and FOSA are not currently available, so these compounds are added from separate vials purchased from the primary vendor. If a second lot is not available, the standard is prepared by a different analyst. These standards are combined and diluted to a final concentration of 2.0 µg/L for analysis at the instrument.

7.11.3 In the case of the LCMS_PFOA method, the second sources of PFOA and PFOS are purchased from Accustandard and diluted to a final concentration of 2.0 µg/L for analysis at the instrument.

7.11.4 In the case of HFPO, the second source standards are not currently available. These compounds are added from separate vials purchased from the primary vendor. The standard is diluted to a final concentration of 2.0 µg/L for analysis at the instrument.

7.12 Continuing Calibration Verification Standards (CCV), 5.0 μg/L and 10.0 μg/L

The continuing calibration verification (CCV) standards alternate between the 5.0 μg/L solution (L5) and the 10.0 μg/L solution (L6) of PFCs in 80% methanol in water made from the same source as the initial calibration standards.

8.0 Sample Collection, Preservation, Shipment and Storage

Sample container, preservation techniques and holding times may vary and are dependent on sample matrix, method of choice, regulatory compliance, and/or specific contract or client requests. Listed below are the holding times and the references that include preservation requirements.

Matrix	Sample Container	Min. Sample Size	Preservation	Extraction Holding Time[1]	Analytical Holding Time	Reference
Water	HDPE[2]	250 mL (x2)[3]	Cool, $\leq 6^{o}C$	14 Days	40 Days	N/A[4]
Soil	HDPE	10 g	Cool, $\leq 6^{o}C$	14 Days	40 Days	N/A[4]

[1] Extraction holding times were determined based on stability studies performed during the development of this method. TestAmerica Denver has conducted stability studies indicating that medium- and low-level standard solutions of PFOA are stable for at least two months in glass, polystyrene, and polypropylene plastics at 0-6 °C.

[2] PFCs have been shown to adhere to glass. Glass containers are not an appropriate sample collection vessel.

[3] If FOSA is a target analyte for a specific client's samples in addition to other PFCs, duplicate water samples are required because FOSA is extracted separately and therefore 2-250 mL bottles are required.

[4] This method was developed and validated by TestAmerica Denver.

9.0 Quality Control

9.1 The minimum quality controls (QC), acceptance criteria, and corrective actions are described in this section. When processing samples in the laboratory, use the LIMS Method Comments to determine specific QC requirements that apply.

9.1.1 The laboratory's standard QC requirements, the process of establishing control limits, and the use of control charts are described more completely in TestAmerica Denver policy DV-QA-003P, Quality Assurance Program.

9.1.2 Specific QC requirements for Federal programs, e.g., Department of Defense (DoD) and Department of Energy (DOE), are described in

TestAmerica Denver policy DV-QA-024P, Requirements for Federal Programs. This procedure meets all criteria for DoD QSM 5.0 unless otherwise stated. Any deviation or exceptions from QSM 5.0 requirements must have prior approval of the client in the project requirements. See Table 5 for a summary of the DoD QSM 5.0 requirements for this method and possible client approved technical specifications.

9.1.3 Project-specific requirements can override the requirements presented in this section when there is a written agreement between the laboratory and the client, and the source of those requirements should be described in the project documents. Project-specific requirements are communicated to the analyst via Method Comments in the LIMS and the Quality Assurance Summaries (QAS) in the public folders.

9.1.4 Any QC result that fails to meet control criteria must be documented in a Nonconformance Memo (NCM). The NCM is automatically sent to the laboratory Project Manager by e-mail so that the client can be notified as appropriate. The QA group periodically reviews NCMs for potential trends. The NCM process is described in more detail in SOP DV-QA-0031. This is in addition to the corrective actions described in the following sections.

9.2 Batch Definition

9.2.1 Batches are defined at the sample preparation stage. The batch is a set of up to 20 samples of the same matrix, plus required QC samples, processed using the same procedures and reagents within the same time period. Batches should be kept together through the whole analytical process as far as possible. See Policy DV-QA-003P for further details.

9.2.2 Since the extraction method for waters has a separate FOSA method, each job of water samples submitted for PFC testing will be separated into at least two batches, one for FOSA and one for all other PFCs. There will also be two separate runs on the instrument.

9.3 QC Samples

9.3.1 Method Blank

9.3.1.1 A method blank is prepared with each batch of samples. The method blank demonstrates freedom from contamination in the laboratory. The standard matrix used for method blanks consists of reagent water (for aqueous sample batches) and reagent sand (for soil sample batches) plus the isotopically labeled internal standard / surrogate spike (Section 7.8).

9.3.1.2 For the FOSA extraction from water samples, surrogates are not added to any sample or QC, recovery of the internal standard is used to evaluate extraction performance.

Acceptance Criteria: The method blank result must be less than ½ the reporting limit (RL) for all target compounds.

Corrective Action: If the method blank exceeds ½ the RL, the associated samples must be evaluated and any samples with hits less than 10x the concentration in the MB must be re-extracted and reanalyzed. If the associated samples are reported as ND for the compounds that are found in the MB the data may be reported if the program or client requirements allow. If results are reported, they must be qualified and an NCM generated.

9.3.2 Laboratory Control Standard (LCS)

Each batch of samples is prepared with an LCS, spiked as described in Section 0. This QC sample serves to verify within-batch accuracy and long-term precision at mid-level concentrations. The LCS matrix for aqueous samples consists of reagent water and the LCS matrix for soil samples consists of reagent sand (Ottawa sand).

Acceptance Criteria: The percent recovery must fall within control limits, which are set at ± 3 standard deviations around the historical mean. Historical limits are stored in the LIMS.

Corrective Action: If the recovery falls outside of the control limits, corrective action must be taken. All associated samples must be re-extracted and reanalyzed. If the recovery is biased high and the analyte of interest is not detected in the samples, results can be reported with a comment in the final report case narrative. Flag the data and document the decision in an NCM. This is an exception to DoD QSM 5.0 criteria and must be approved by the client as documented in the project records.

9.3.3 Matrix Spike Samples

The matrix spike (MS) is an aliquot of a selected field sample that is spiked with the analytes of interest. The matrix spike duplicate (MSD) is a second

aliquot of the selected sample that is spiked in the same manner as the MS. At a minimum, the laboratory must spike one sample in every batch. The MS/MSD must be analyzed at the same dilution level as the un-spiked sample, unless the matrix spike components would then be above the calibration range. The matrix spike sample is prepared as described in Section 0. The matrix spike demonstrates acceptable accuracy for individual samples.

Acceptance Criteria: The MS and MSD recoveries and the relative percent difference (RPD) between the MS and MSD results must be within the established control limits. Percent recovery control limits are set at ± 3 standard deviations around the historical mean of the LCS recovery data, unless otherwise dictated by the client or project. The RPD control limit is set at 3 standard deviations above the mean of the historical data.

NOTE: DOD QSM 5 limits apply to projects performed under this program.

Corrective Actions: The information obtained from MS data are sample/matrix specific and are not normally used to determine the validity of the entire batch. If the MS and/or MSD recovery falls outside of the established control limits, the bracketing CCV and batch LCS recoveries must be within control limits in order to accept results for the associated samples. The following corrective actions are required for MS/MSD recovery failures to rule out lab error:

- Check calculation and instrument performance;
- Verify, if possible, that the MS and MSD were spiked correctly (e.g., very low or very high recoveries);
- Consider objective evidence of matrix interference (e.g., heterogeneous sample, interfering peaks seen on chromatograms, or interference demonstrated by prior analyses);
- Flag the data for any results outside of acceptance limits.
- For any single RPD failure, check calculations; verify, if possible, that the MS and MSD were spiked correctly; check instrument performance; consider objective

evidence of matrix interference or sample inhomogeneity; and flag the data.

- If both the parent sample and associated matrix spike results are over range the parent and the spikes shall be diluted by the same amount and the results from the reanalysis reported for both. If the analyte concentration in the parent sample is greater than four times the concentration of spike added, then spike recovery results are not compared to control limits, and the recovery is either reported as "NC" (not calculated) or with a qualifier flag to indicate that the spike was less than four times the analyte concentration in the sample. If the dilution will cause the spike to be less than two times the reporting limit, the MS/MSD do not need to be diluted and the recovery reported as "NC" (not calculated).

- For MS/MSD that serve as batch QC, if the parent sample result is within the calibration range and the MS/MSD results are above the calibration range, the results are reported with the MS/MSD result being flagged as an over-range measurement (e.g., the E-flag qualifier).

- If the MS/MSD are client requested, the parent sample result is within calibration range and the MS/MSD results are above the calibration range, the sample and spike should be diluted, keeping in mind that we need to assess whether or not the dilution will best serve the client's needs. Consult with the PM as needed. Both the parent sample and MS/MSD samples must have the same dilution factor. Some EDDs do not accept data that are at different dilution factors.

- If the native analyte concentration in the MS/MSD sample exceeds 4 times the spike level for that analyte, the recovery data are reported as NC (i.e., not calculated) and the appropriate qualifier flags are added.

NOTE: See Denver Policy Memorandum P16-001 and Corporate Policy Memorandum CA-Q-QM-013 for more detail.

NOTE: Some client programs require reanalysis to confirm matrix interferences. Check special project requirements for this corrective action.

NOTE: If the recovery is outside the control limits for both the matrix spikes and the LCS, then the entire batch should be re-prepared and reanalyzed.

9.3.4 Sample Duplicate and/or Matrix Spike Duplicate (MSD)

Depending on client requirements, a sample duplicate and/or a matrix spike duplicate (MSD) may be prepared and analyzed. Comparison of duplicate results provides an assessment of the precision of the method in actual sample matrices.

Acceptance Criteria: Acceptance criteria for the relative percent difference (RPD) between the duplicate sample results are specified by client or project requirements. Control limits for the RPD may be based on historical precision of the method or may be project-specific limits. If the duplicate is an MSD, then the recovery for the analyte(s) of interest must also meet the established control limits as described in Section 9.3.3 above. For DoD QSM 5.0, the acceptance limits for the RPD for all analytes is $\leq$ 30%.

Corrective Action: Corrective actions or data reporting based on duplicate results are specified in client or project requirements, which are provided outside of the scope of this SOP. The analyst must ensure that MS/MSD recoveries or RPD limits outside of acceptance criteria are not due to lab error.

9.3.5 Isotopically Labeled Surrogate Standard

The surrogate spike solution is added to each field and QC sample as described in Sections 7.6 and 7.8. For the FOSA extraction from water samples, surrogates are not added to any sample or QC, recovery of the internal standard is used to evaluate extraction performance.

Acceptance Criteria: The recovery of the labeled surrogate standards are based on historical data, and are 3 standard deviations around the mean. Historical limits are stored in the LIMS.

Corrective Action: If the recoveries are not within these ranges, the sample should be re-extracted to determine if this

is an extreme sample matrix effect.

9.3.6 Isotopically Labeled (Internal) Standard (IS)

The isotope dilution spike solution is added to each field and QC sample as described in Section 7.7. This is also the internal standard.

Acceptance Criteria: The recoveries of the labeled internal standards must be within 50% -150% of the true value or of the expected concentration for a dilution up to x50.

Corrective Action: If the recoveries are not within these ranges in samples, the recovery for the batch QC (MB, LCS) must be checked. If these recoveries are within limits and the labeled standard(s) are outside of control limits in the sample, then the sample is considered to have an extreme matrix effect and an NCM must be generated.

If the batch QC (MB, LCS) recoveries are also out of control, the samples must be re-extracted and reanalyzed.

9.4 Instrument QC

9.4.1 Initial Calibration Blank (ICB)

Immediately following the ICAL, a calibration blank is analyzed that consists of an injection of 80% methanol in water containing the PFC internal standards.

Acceptance Criteria: The result for the calibration blank must be less than two times the method detection limit, or less than ½ the RL, which ever is smaller.

Corrective Action: If the ICB does not meet the acceptance criteria, then the source of contamination must be identified and any necessary cleaning completed, and then the instrument should be recalibrated.

9.4.2 Initial Calibration Verification (ICV)

Following the ICAL and the ICB, the ICV standard described in Section 7.11 is analyzed.

Acceptance Criteria: The recovery for the ICV must be within 70-130%. DoD QSM 5.0 requires ICV limits of 75-125%.

Corrective Action: If results are outside these limits, the standards should be

verified and/or remade, and then the instrument should be recalibrated.

9.4.3 Detection Limit Calibration Verification (DLCK)

Following the ICAL, ICB and ICV, the 0.5 µg/L calibration standard (L2) is re-analyzed to verify that detections at the reporting limit are accurately quantified by the calibration curve.

Acceptance Criteria: The recovery for the DLCK must be within 70-130%. Some programs (like West Sacramento) require a L1 standard to be analyzed at the start of the sequence. The recovery for the L1 standard must be within 50-150%.

Corrective Action: If results are outside these limits, the standards should be verified and/or remade, and then the instrument should be recalibrated.

9.4.4 Continuing Calibration Verification (CCV)

The 5.0 µg/L (L5) and 10.0 µg/L (L6) CCV standards described in Section 7.12 are alternately analyzed after every 10 injections and at the end of the analytical sequence.

Acceptance Criteria: The recovery for the CCV standards must be 70-130%. DoD QSM 5.0 requires CCV limits of 75-125%.

Corrective Action: If this is not achieved, the instrument has drifted outside the calibration limits. The instrument must be recalibrated or two CCVs must be analyzed consecutively and pass. All samples analyzed since the last passing CCV must be reanalyzed. If CCV recovery is >130% (125% for DoD QSM 5.0) and the samples are ND, the samples can be reported without reanalysis. Flag the data and document the decision in an NCM. This is an exception to DoD QSM 5.0 criteria and must be approved by the client as documented in the project records.

10.0 Procedure

10.1 One-time procedural variations are allowed only if deemed necessary in the professional judgment of supervision to accommodate variation in sample matrix, radioactivity, chemistry, sample size, or other parameters. Any variation in procedure shall be completely documented using an NCM. The NCM is automatically sent to the laboratory Project Manager by e-mail so that the client can be notified as appropriate. The QA group periodically reviews NCMs for potential trends. The NCM process is described in more detail in SOP DV-QA-

0031. The NCM shall be filed in the project file and addressed in the case narrative.

10.2 Any deviations from this procedure identified after the work has been completed must be documented in an NCM, with a cause and corrective action described.

10.3 Sample Extraction

Refer to SOP DV-OP-0019 *"Extraction of Perfluorooctanoic Acid (PFOA) and Perfluorooctanoic Sulfonate (PFOS) and other Perfluorinated Hydrocarbons (PFCs) in Water and Soil".*

10.4 Run Sequence

Analyze calibration standards, samples and quality control samples following the analysis sequence shown in Table 4.

10.5 Sample Analysis

10.5.1 If results are to be reported as ammonium perfluorooctanoate (APFO), instead of PFOA, a multiplier of 1.0406 is applied to the sample results to correct for the molecular weight differences between PFOA and APFO. This adjustment is made during the preparation of the standards used for calibration. APFO is calibrated using the same peak as PFOA.

10.5.2 Dilutions

10.5.2.1 Samples that exceed the working range must be diluted with 80:20 methanol:water (for HFPO use 50:50 methanol:water) to concentrations within the working range and reanalyzed. Dilutions are done without fortifying the internal standard concentrations as this is an isotope dilution method. Therefore, maximum dilution factors will be dependent on the recovery of the internal standards for each sample matrix. The maximum dilution performed on an extract will be 50x. Beyond the maximum dilution, the data will be E-flagged and reported as a greater than concentration.

10.5.2.2 If samples are known to be of high concentration the dilution may be performed at the preparation step. Reporting limits will be adjusted based on the dilution and further dilution of the sample extract may be performed if needed as described in Section 10.5.2.1. This prep dilution allows the laboratory to extend the calibration range beyond that provided by extract dilution alone.

10.5.3 Raw data, calibration summaries, QC data, and sample results are reviewed by the analyst. These must also be thoroughly reviewed by a second qualified person. These reviews are documented on the LC/MS

Data Review Checklist. The data review process is described in DV-QA-0020.

10.6 Troubleshooting and Maintenance

For instrument maintenance guidelines see the Denver Quality Assurance Manual.

11.0 Calibration

11.1 Instrument Tuning

11.1.1 Prior to analyzing samples, evaluate the lowest standard for the ability to see compounds. If sensitivity and/or resolution are compromised, infuse a solution of each target compound into the mobile phase at a point just before the entrance to the electrospray probe. The responses for the precursor ions and product ions are observed and optimized for sensitivity and resolution.

11.1.2 Instrument mass calibration is performed if the analyst notices mass assignment errors, see Table 7 for solutions and masses to use.

11.1.3 DoD QSM 5.0 requires a tune check containing all analytes of interest be analyzed prior to an initial calibration and after any mass calibration or maintenance is performed. The ICV may be used for this tune.

Acceptance Criteria: Mass assignments of tuning standard must be within 0.5 amu of true value.

Corrective Action: Retune instrument. If the tuning does not meet acceptance criteria, an instrument mass calibration must be performed and the tuning redone. No samples shall be analyzed without acceptable tuning.

11.2 Initial Calibration (ICAL)

11.2.1 Detailed information regarding calibration models and calculations can be found in Section 11 and Corporate SOP, CA-Q-P-003, *Calibration Curves and the Selection of Calibration Points.*

11.2.2 Routine instrument operating conditions are listed in Tables 2 & 3.

11.2.3 Target compound recommended internal standard associations are listed in Table 6.

11.2.4 The instrument is calibrated using nine concentration levels (see Section 7.9 and 7.10 for details). These concentrations define the working range for analysis.

11.3 Rejection of Calibration Points

11.3.1 If the analyst believes that a point on the curve is inaccurate, the preferred practice is to rerun the ICAL. Generally it is NOT acceptable to remove points from a calibration for the purposes of meeting calibration criteria, unless the points are the highest or lowest on the curve AND the reporting limit and/or linear range is adjusted accordingly. A "Huge Error Test" or other statistical test should be used to determine that a point does not fit the calibration model.

11.3.2 The only exception is that a level may be removed from the calibration if the reason is clearly documented, for example a broken vial. A minimum of five levels must remain for linear fits, and there must be a minimum of six levels for a quadratic fit. All raw data, including data from rejected calibration points, will be included in the study package.

11.4 Linear Calibration Using Average Response Factors

For each target analyte, calculate the response factor of each calibration level as follows:

$$RF_i = \frac{R_x C_{is}}{R_{is}} \qquad \textit{Equation 3}$$

Where:
- RF_i = Response factor for the i^{th} level
- R_x = Response for analyte
- C_{is} = Concentration for internal standard
- R_{is} = Response for internal standard

For each target analyte, calculate the average response factor as follows:

$$\text{Average Response Factor} = \overline{RF} = \frac{\sum_{i=1}^{n} RF_i}{n} \qquad \textit{Equation 4}$$

Where:
- n = Number of calibration levels
- RF_i = Response factor for the i^{th} level

The relative standard deviation (RSD) is calculated as follows:

$$\%RSD = \frac{SD}{\overline{RF}} \times 100\% \qquad \textit{Equation 5}$$

Where SD is the standard deviation of the average RF, which is calculated as follows:

$$\text{SD} = \sqrt{\frac{\sum_{i=1}^{N} \left(RF_i - \overline{RF}\right)^2}{N-1}} \qquad \textit{Equation 6}$$

11.5 Average Calibration Factor Evaluation

The calibration relationship can be graphically represented as a line through the origin with a slope equal to the average calibration factor.

Acceptance Criteria: The RSD of the average response factor must be <20%. Also examine the residuals, especially for the high points versus the fitted function. If the residual values are large, a linear regression should be considered.

Corrective Action: If the RSD is > 20%, average response factor cannot be used and least-squares linear regression should be attempted.

11.6 Linear Fit

Calibration using least-squares linear regression produces a straight line that does not pass through the origin. The calibration relationship is constructed by performing a linear regression of the instrument response (peak area) versus the concentration of the standards. The instrument response is treated as the dependent variable (y) and the concentration as the independent variable (x). The weighting used is the reciprocal of the concentration or the reciprocal of the square of the concentration. The regression produces the slope and intercept terms for a linear equation in the following form for an internal standard calibration:

$$\frac{R_x C_{is}}{R_{is}} = m_1(C_s) + b \qquad \textit{Equation 7}$$

Where:

C_s	=	Analyte concentration in calibration standard, µg/L
R_x	=	Response for analyte
R_{is}	=	Response for internal standard
C_{is}	=	Concentration of internal standard
b	=	y - Intercept
m_1	=	Slope

To calculate the concentration in an unknown sample extract, the regression equation is solved for concentration, resulting in the following equation:

$$C_{ex} = \frac{\left[\frac{R_x C_{is}}{R_{is}} - b\right]}{m_1} \qquad \textit{Equation 8}$$

Where:

C_{ex}	=	Extract analyte concentration, µg/L
R_x	=	Response for analyte
R_{is}	=	Response for internal standard
C_{is}	=	Concentration of internal standard
b	=	y - Intercept

11.7 Evaluation of the Linear Least-Squares Regression Calibration Function:

11.7.1 With an unweighted linear regression, points at the lower end of the calibration curve have less weight in determining the curve than points at the high concentration end of the curve. For this reason, inverse weighting of the linear function is recommended to optimize the accuracy at low concentrations.

11.7.2 Note that the August 7, 1998 EPA memorandum "Clarification Regarding Use of SW-846 Methods", Attachment 2, Page 9, includes the statement "The Agency further recommends the use of this for weighted regression over the use of an unweighted regression."

Acceptance Criteria: The linear regression must have a correlation coefficient (r) ≥ 0.99 (r^2 ≥ 0.98). In order to meet support work for the West Sacramento laboratory the linear regression must have a correlation coefficient (r) ≥ 0.995 (r^2 ≥ 0.990).

11.8 Quadratic Fit

When the instrument response does not follow a linear model over a sufficiently wide working range, or when the previously described calibration approaches fail acceptance criteria, a non-linear, second-order calibration model may be employed. The second-order calibration uses the following equation for an internal standard calibration:

$$\frac{R_x C_{is}}{R_{is}} = m_2(C_s)^2 + m_1(C_s) + b \qquad \textit{Equation 9}$$

Where:

C_s	=	Analyte concentration in calibration standard, µg/L
R_x	=	Response for analyte
R_{is}	=	Response for internal standard
C_{is}	=	Concentration of internal standard
m_2	=	Curvature
m_1	=	Slope
b	=	y - Intercept

To calculate the concentration in an unknown sample extract, the roots of the quadratic equation are solved for:

$$C_{ex} = \frac{-m_1 \pm \sqrt{(m_1)^2 - 4(m_2)\left(b - \frac{R_x C_{is}}{R_{is}}\right)}}{2m_2} \qquad \textit{Equation 10}$$

Where:

C_{ex}	=	Extract analyte concentration, µg/L
R_x	=	Response for analyte

R_{is}	=	Response for internal standard
C_{is}	=	Concentration of internal standard
m_2	=	Curvature
m_1	=	Slope
b	=	y – Intercept

11.9 Evaluation of Second-Order Regression Calibration:

A minimum of six points must be used for a second-order regression fit.

Acceptance Criteria:

11.9.1 Second-order regressions should be the last option, and note that some programs (e.g., South Carolina) do not allow the use of second-order regressions.

11.9.2 The coefficient of determination (COD, r^2) must be ≥ 0.99.

11.9.3 The response increases significantly with increasing standard concentration (i.e., the instrument response does not plateau at high concentrations).

11.9.4 The distribution of concentrations is adequate to characterize the curvature.

11.9.5 Calibrations are modeled as calibration curves.

The following requirements must be met for any calibration to be used. If these criteria are not met, instrument conditions and standards will be checked, and the ICAL successfully repeated before continuing.

11.9.6 Response must increase with increasing concentration.

11.9.7 The absolute value of the intercept of the curve at zero response must be less than the concentration that corresponds to ½ the Reporting Limit.

11.9.8 Per Minnesota QAS for sample results from sites in Minnesota, the recovery (accuracy) for each point in the calibration curve must be 75-125% except for the lowest point in the curve which must be 70-130%.

12.0 Calculations/ Data Reduction

12.1 Qualitative Identification

12.1.1 An analyte is identified by retention time and ion mass (see Tables 2 and 3). The sample component retention time must compare to within ± 0.5 minute of the average retention time of the standard component in the initial calibration. If a compound cannot be verified by all of the above

criteria, but in the technical judgment of the analyst the identification is correct, the analyst shall report that identification and proceed with quantitation. This decision is documented in an NCM.

12.1.2 The perfluorinated hydrocarbon acids and sulfonates may have both linear and branched isomers due to the manufacturing process. These isomers have been studied and identified for PFOA and PFOS, however, the other compounds are also likely to have isomers. The linear and branched isomers have the same MS/MS transitions; however the retention time of the branched isomers is generally earlier and the ratio of the quant and qual ions may be different. The calibration standards are composed primarily of the linear isomers. For samples, all isomers should be integrated and quantified; if there is a double peak due to the difference in retention time, the entire area of both peaks should be integrated.

12.1.3 For all compounds except PFBA and PFPA, two product ions are measured, a quant ion and a qual ion. The ratio of the peak areas of the two PFC ions is calculated and generally within ± 30% of the ion ratios for the mid-point ICAL.

12.1.4 While the linear and branched isomers described in Section 12.1.2 produce the same product ions, the ratio is not necessarily the same for both types of isomers. If the ion ratio is outside of this 30% window, but in the analyst's judgment, there are still PFCs present due to retention time and peak presence, the results will still be reported as valid detections.

12.2 Manual Integrations

Upon completion of the analytical sequence, transfer the raw instrument data to Chrom for further processing. Review the chromatograms to ensure correct assigning of peaks and correct integration of each peak. If manual data manipulations are necessary, they must be justified and documented. See DV-QA-011P requirements for manual integration.

12.3 Calculations for Calibration Standards and Sample Extracts

The concentration of each identified analyte and surrogate in the extract is calculated from the linear or quadratic curve fitted to the initial calibration points, or from the average response factor (RF) of the initial calibration.

12.4 Calculations for reporting original samples

12.4.1 Calculating concentration in an aqueous / soil samples:

Note: Perfluorinated hydrocarbon sulfonate compounds are calibrated as the anion and reported as either the anion concentration or acid concentration based on client request. (See Section 7.4)

The concentration in a water sample is calculated as follows:

$$\text{Concentration, } \mu\text{g/L} = \frac{C_{ex}V_t}{V_o} xDF \qquad \textit{Equation 11}$$

Where:
- C_{ex} = Extract analyte concentration, µg/L
- V_t = Volume of total extract in liters
- V_o = Volume of water extracted in liters
- DF = Dilution factor

The concentration in a sediment/soil, sludge (on a dry-weight basis) and waste (normally on a wet-weight basis) is calculated as follows:

$$\text{Concentration, } \mu\text{g/kg} = \frac{C_{ex}V_t}{W_s D} \times DF \qquad \textit{Equation 12}$$

Where:
- C_{ex} = Extract analyte concentration, µg/L
- V_t = Volume of total extract in liters
- W_s = Weight of sample extracted or diluted in kilograms
- D = (100 - % moisture in sample)/100, for dry weight basis or 1 for wet-weight basis
- DF = Dilution factor

12.4.2 LCS and CCV Percent Recovery

$$\text{ControlSpike Recovery} = \frac{S_{SR}}{S_A} \times 100\% \qquad \textit{Equation 13}$$

Where (in µg/L):
- S_{SR} = Calculated analyte concentration of spiked sample
- S_A = Concentration of standard added

12.4.3 MS / MSD Percent Recovery Calculation

$$\text{Matrix Spike Recovery} = \frac{S_{SR} - S_R}{S_A} \times 100\% \qquad \textit{Equation 14}$$

Where (in µg/L):
- S_{SR} = Calculated analyte concentration of spiked sample
- S_R = Calculated analyte concentration of parent sample
- S_A = Concentration of standard added

12.4.4 Relative Percent Difference Calculation for the MS/MSD

$$RPD = \frac{\left|MS_R - MSD_R\right|}{1/2(MS_R + MSD_R)} \times 100 \qquad \textit{Equation 15}$$

Where:

RPD	=	Relative percent difference
MS_R	=	Matrix spike result of analyte
MSD_R	=	Matrix spike duplicate result of analyte

13.0 Method Performance

13.1 Method Detection Limits (MDL)

An initial method detection limit study is performed for each analyte and each sample matrix type in accordance with the method validation protocol. MDLs are stored in the LIMS. The procedure for determining detection limits is defined in Policy CA-Q-S-006.

13.2 Method Detection Limit Verification (MDLV)

13.2.1 Calculated MDLs from the annual studies are subject to verification by analyzing an MDLV standard prepared at 1-4 times the calculated MDL concentration. An MDLV standard is analyzed immediately after each MDL study and annually thereafter to satisfy the NELAC requirements. For DoD and DOE projects, the MDL is verified quarterly. DoD QSM 5.0 requires that the MDLV (LODV) be spiked at 2-4 times the MDL concentration. This standard is subject to the entire preparation and analysis process. The calculated MDL is verified if the MDLV standard is detected under routine instrument conditions.

13.2.2 If the first MDLV is not detected, the MDLV standard will be re-prepared and analyzed at twice the original concentration. The lowest concentration that produces a detectable signal will then be reported as the MDL.

13.2.3 DoD also requires a quarterly LOQV sample that is spiked at 1-2x the LOQ (RL) and must meet laboratory determined control limits for precision and accuracy at the spike level.

13.3 Demonstration of Capabilities

All personnel are required to perform an initial demonstration of proficiency (IDOC) on an instrument they will be using for analysis prior to testing samples. On-going proficiency must be demonstrated annually. IDOCs and on-going proficiency demonstrations are conducted as follows.

13.3.1 Four aliquots of the QC check sample are analyzed using the same procedures used to analyze samples, including sample preparation. The concentration of the QC check sample should be equivalent to a mid- level calibration.

13.3.2 Calculate the average recovery and standard deviation of the recovery for each analyte of interest.

13.3.3 If any analyte does not meet the acceptance criteria, the test must be repeated. Only those analytes that did not meet criteria in the first test need to be evaluated. TNI 2009 requires consecutive passing results. Repeated failure for any analyte indicates the need for the laboratory to evaluate the analytical procedure and take corrective action.

13.3.4 Until the IDOC is approved by the QA Manager (or designee); the trainer and trainee must be identified in the batch record.

13.3.5 Further details concerning demonstrations of proficiency are described in SOP DV-QA-0024.

13.4 Training Requirements

The Group Leader is responsible for ensuring that this procedure is performed by an associate who has been properly trained in its use and has the required experience. A new analyst must be working under documented supervision prior to approval of the IDOC. Documentation that a new analyst is performing under supervision must be entered into the batch record (View Batch Information) until that analyst's IDOC has been approved by the QA Manager (or designee). See requirements for demonstration of analyst proficiency in SOP DV-QA-0024.

14.0 Pollution Control

Standards and reagents are prepared in volumes consistent with laboratory use to minimize the volume of expired standards and reagents requiring disposal.

15.0 Waste Management

15.1 All waste will be disposed of in accordance with Federal, State, and local regulations. Where reasonably feasible, technological changes have been implemented to minimize the potential for pollution of the environment. Employees will abide by this procedure, the policies in section 13, "Waste Management and Pollution Prevention", of the Corporate Safety Manual, and HS-001, "Waste Management Program."

15.2 The following waste streams are produced when this method is carried out:

15.2.1 Vial waste – Collect in PFC waste containers

15.2.2 Instrument process waste – Flammable Solvent (C)

15.2.3 Expired Chemicals/Reagents – Contact Waste Coordinator

15.2.4 Soil Samples, post extraction, standards, and any solid waste contacting solutions which contained PFCs, and all other solid waste generated by this procedure, such as disposable pipette tips and extraction bottles are collected in special PFC waste containers and turned into the Waste Coordinator for incineration.

NOTE: Radioactive and potentially radioactive or mixed waste must be segregated from non-radioactive waste as appropriate. Contact the Waste Coordinator for proper management of radioactive or potentially radioactive waste generated by this procedure.

16.0 References / Cross-References

16.1 Cheryl Moody, Wai Chi Kwan, Johnathan W. Martin, Derek C. G. Muir, Scott A. Mabury, "Determination of Perfluorinated Surfactants in Surface Water Samples by Two Independent Analytical Techniques: Liquid Chromatography/Tandem Mass Spectrometry and 19FNMR," *Analytical Chemistry,* 2001, 73, 2200-2206.

16.2 John Giesy et al., "Accumulation of Perfluorooctane Sulfonate in Marine Mammals", *Environmental Science & Technology*, 2001 Vol. 35, No. 8, pages 1593-1598.

16.3 U.S. EPA, "Residue Chemistry Test Guidelines, OPPTS 860.1340, Residue Analytical Method", EPA 712-C-95-174, August 1995.

16.4 STL Denver White Paper DEN-W-LC-002, "Method Validation Study for Analysis of Ammonium Perfluorooctanoate in Soil Matrices by High Performance Liquid Chromatography/Mass Spectrometry (HPLC/MS/MS)", Mark Dymerski, September 5, 2003.

16.5 STL Denver White Paper DEN-W-LC-003, "Addendum A to Method Validation Study for Analysis of Ammonium Perfluorooctanoate in Soil Matrices by High Performance Liquid Chromatography/Mass Spectrometry (HPLC/MS/MS)", Mark Dymerski, August 6, 2003.

16.6 STL Denver White Paper DEN-W-LC-004, "Method Validation Study for Analysis of Perfluorooctanoic Acid in Waters by High Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC/MS/MS)", Mark Dymerski, January 26, 2005.

16.7 SOP No. DV-OP-0019 *"Extraction of Perfluorooctanoic Acid (PFOA) and Perfluorooctanoic Sulfonate (PFOS) and other Perfluorinated Hydrocarbons (PFCs) in Water and Soil"*.

16.8 SW-846, Test Methods for Evaluating Solid Waste, Physical/Chemical Methods,

Third Edition and all promulgated updates, EPA Office of Solid Waste, January 2005.

16.8.1 Method 8321A, Solvent Extractable Nonvolatile Compounds by High Performance Liquid Chromatography/Thermospray/Mass Spectrometry (HPLC/TS/MS) or Ultraviolet (UV) Detection, Revision 1, December, 1996,

16.8.2 Method 8321B, Solvent Extractable Nonvolatile Compounds by High Performance Liquid Chromatography/Thermospray/Mass Spectrometry (HPLC/TS/MS) or Ultraviolet (UV) Detection, Revision 2, February, 2007.

16.8.3 Method 8000B, Determinative Chromatographic Separations, Revision 2, December 1996.

16.8.4 Method 8000C, Determinative Chromatographic Separations, Revision 3, March 2003.

16.8.5 Method 3535A, Solid-Phase Extraction (SPE), Revision 1, February 2007

16.9 J. A. Shoemaker, P.E. Grimmett, B. K. Boutin, "Method 537: Determination of Selected Perfluorinated Alkyl Acids in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)," EPA/600/R-08/092, Version 1.1, September 2009.

17.0 Method Modifications:

17.1 This SOP was developed during an extensive method development and evaluation process. This SOP presents the best practices that were demonstrated during the development process for the full suite of target compounds.

17.2 As of this writing, Method 537 provides for 14 day holding time for water samples preserved with Trizma buffer. The scientific literature indicates the perfluorinated substances are highly persistent in the environment. TestAmerica Sacramento has conducted holding time studies that support 14 day holding time for aqueous samples with and without Trizma preservation. TestAmerica Denver has conducted stability studies indicating that medium and lower-level solutions of PFOA are stable for at least three months in polystyrene and polypropylene plastics at 0-6 degrees C.

18.0 Tables / Figures / Attachments

Table 1	Standard Reporting Limits for Waters and Soils
Table 2	Recommended Agilent (LCMS_5) Instrument Operating Conditions
Table 3	Recommended Waters (LCMS_4) Instrument Operating Conditions for analysis of HFPO
Table 4	Analytical Sequence and Summary of QC Criteria
Table 5	DoD QSM 5.0 Requirements

19.0 Revision History

Revision 15, dated 7 May 2018

- Annual Review

Revision 14, dated 31 January 2017

- Added language to Section 7 regarding standard expiration dates
- Revised Section 9.3.3 to reflect current MS/MSD policy
- Moved instrument tuning, initial calibration, ICV and CCV criteria to Section 11 and renumbered remainder of document accordingly.
- Updated gradient in Table 3 to reflect current practice

Revision 13, dated 6 January 2016

- Removed Confidentiality Restriction Note boxes
- Added procedure and requirements for analysis of HFPO in water throughout
- Added description of quantitation of perfluorinated sulfonates as either the anion or the acid (throughout)
- Revised Sections 3, 9, and 12 to reflect current practice
- Moved Instrument maintenance statement from Section 9.4.1 to Section 10.6
- Revised Section 10.5.2.2 to describe use of dilution at the preparation step if expected concentration of sample is known and removed Section 10.5.3.
- Revised Equations 11 & 12 in Section 11.4.1 to account for dilution factor of extract.
- Removed all references to WI-DV-0049. Work instruction is being permanently archived as the process for calculation of dilution factors is now handled by Chrom.
- Added surrogate associations to Table 6
- Formatting and editorial changes throughout

Revision 12, dated 13 October 2014

- Revised holding time for water samples to 14 days, consistent with stability studies performed during method development.
- Added statement to Section 9.1.2 regarding DoD QSM 5.0 compliance.
- Added new Section 11.4.2 for reference to WI-DV-0049, *PFC Method Data Review* which addresses calculations of dilutions.
- Added requirements for compliance with DoD QSM 5.0 in text when different from laboratory requirements and as new Table 5
- Renumbered existing Tables 5 and 6 to accommodate addition of new table.
- Removed Appendix I (client-specific information)
- Formatting and editorial changes throughout

Revision 11, dated Dec 04, 2013

- Confidential information included

Revision 10, dated July 12, 2013

- Annual review
- Added Figure 1, Figure 2 and Figure 3 of chromatograms
- Removed Appendix 2
- Updated sections 9.1 and 10.1 and inserted new section 10.2

Revision 9.2 dated July 13, 2012

- Updated solvent grade requirements (Section 7.1)

Revision 9.1 dated April 4, 2012

- Annual review

Revision 9 dated February 28, 2011

- Added Table 1 for the standard reporting limits for waters and soils.
- Moved Table 1 to Table 6 for the mass calibrations.
- Added $^{13}C_8$ PFOA and $^{13}C_8$ PFOS as surrogates.
- Expanded dilution section to reflect isotope dilution method.
- Updated standards section to explicitly list all standards used in this method.
- Updated Table 2 with Waters Instrument method conditions.
- Added Table 3 to include Agilent Instrument (LCMS_5) method conditions.
- Changed numbering of Attachments to incorporate the addition of Table 3 for the Agilent Instrument.
- Removed references to client-specific requirements.
- Added Appendices 1 and 2 for specific client requirements.
- Updated references

Earlier revision histories have been archived and are available upon request.

Table 1: Standard Reporting Limits

Compound	PFC Method			PFC_FOSA Method		LCMS_PFOA Method	
	Water 5 mL FV (µg/L)	Water 1 mL FV (µg/L)	Soil (µg/kg)	Water 5 mL FV (µg/L)	Water 1 mL FV (µg/L)	Water (µg/L)	Soil (µg/kg)
Perfluorobutyric Acid	0.02		0.8	--		--	--
Perfluoropentanoic Acid	0.03		0.8	--		--	--
Perfluorohexanoic Acid	0.02		0.8	--		--	--
Perfluoroheptanoic Acid	0.03		0.8	--		--	--
Brch-Perfluorooctanoic Acid	0.02		0.8	--		0.02	0.8
Perfluorononanoic Acid	0.04		0.8	--		--	--
Perfluorodecanoic Acid	0.02		0.8	--		--	--
Perfluoroundecanoic Acid	0.02		0.8	--		--	--
Perfluorododecanoic Acid	0.03		2.0	--		--	--
Perfluorotridecanoic Acid	0.04		0.8	--		--	--
Perfluorotetradecanoic Acid	0.03		2.0	--		--	--
Perfluorobutane Sulfonate	0.02		0.8	--		--	--
Perfluorobutanesulfonic Acid	0.02		0.8	--		--	--
Brch-Perfluorohexane Sulfononic Acid	0.03		0.8	--		--	--
Perfluorohexanesulfonic Acid	0.03		0.8	--		--	--
Brch-Perfluorooctane Sulfonic Acid	0.03		0.8	--		0.03	0.8
Perfluorooctanesulfonic Acid	0.03		0.8	--		--	--
Perfluorodecane Sulfonate	0.02		0.8	--		--	--
Perfluorodecanesulfonic Acid	0.02		0.8	--		--	--
Perfluorooctanesulfonamide	--		0.8		0.05	--	--

Brch= quantitation using standards containing branched isomers

Compound	HFPO Method	
	Water (µg/L)	Soil (µg/kg)
Perfluoro(2-propoxypropanoic) acid	0.01	--

Table 2: Agilent (LCMS_5) Recommended Instrument Operating Conditions

NOTE: The conditions listed below for HPLC conditions and MS interface gas flows, temperatures, probe voltage, and collision energy are subject to final fine adjustments to maximize sensitivity. Changes to the below conditions will be documented in the project data file. Argon or nitrogen collision gas pressure is set according to the instrument manufacturer.

Mass Spectrometer Interface Settings (QQQ)	
MS Interface Mode	ESI + Agilent Jet Stream Negative Ion
Gas Temp (^{o}C)	320
Gas Flow (L/min)	4
Nebulizer (psi)	45
Sheath Gas Temp (^{o}C)	350
Sheath Gas Flow (L/min)	12
Negative Capillary (V)	2500
Nozzle Voltage (V)	500

Water PFC_FOSA Method:

HPLC Conditions (Agilent 1200)	
Column (equivalent may be used)	Phenomenex Gemini NX plus Gemini guard cartridge
Column Temp (^{o}C)	25
Flow Rate	0.5 mL/min
Mobile Phase Composition	A=0.005 M NH_4OAc (aq, 10% MeOH), B=LCMS grade methanol
Gradient for FOSA only (waters)	Time Flow %B 0 0.4 10 0.5 0.4 10 2.5 0.4 50 6 0.4 95 8.5 0.4 95 9 0.8 10 9.5 0.4 10 Hold for 2.5 min. before next injection.
Injection Size	25 µL, with Needle Wash
FOSA run time	12 minutes

QQQ Mass Spectrometer Scan Settings					
Analyte	**Parent Ion (Da)**	**Product Ions (Da)**	**Dwell (s)**	**Fragment (V)**	**Collision Energy (V)**
13C8 FOSA	505.9	78	0.100	127	36
FOSA	497.9	78	0.100	127	36

Table 2 (continued)

PFOA / PFOS Only Method (LCMS_PFOA):

HPLC Conditions (Agilent 1200)	
Column (equivalent may be used)	Phenomenex Gemini NX plus Gemini guard cartridge
Column Temp (^{o}C)	40
Flow Rate	0.4 mL/min
Mobile Phase Composition	A=0.005 M NH_4OAc (aq, 10% MeOH), B=LCMS grade methanol
Gradient for FOSA only (waters)	30% B → 100% B over 3 minutes. Hold for 1 minute. Back to initial conditions over 0.5 minute. Hold for 1 min. before next injection.
Injection Size	25 μL, with Needle Wash
PFOA/PFOS run time	5 minutes

QQQ Mass Spectrometer Scan Settings					
Analyte	**Parent Ion (Da)**	**Product Ions (Da)**	**Dwell (s)**	**Fragment (V)**	**Collision Energy (V)**
PFOA	412.9	169	0.05	70	12
PFOA	412.9	219	0.05	70	6
PFOA	412.9	368.9	0.05	70	0
$^{13}C_4$ PFOA	417.0	371.9	0.05	70	0
$^{13}C_8$ PFOA	421.0	375.9	0.05	70	0
PFOS	498.8	79.9	0.05	159	72
PFOS	498.8	98.9	0.05	159	48
PFOS	502.9	130	0.05	159	96
$^{13}C_4$ PFOS	502.9	80	0.05	159	72
$^{13}C_8$ PFOS	502.9	80	0.05	159	72

Table 2 Continued.

PFC Method:

HPLC Conditions (Agilent 1200)	
Column (equivalent may be used)	Phenomenex Gemini NX plus Gemini guard cartridge
Column Temp (^{o}C)	25
Flow Rate	0.5 mL/min
Mobile Phase Composition	A=0.005 M NH_4OAc (aq, 10% MeOH), B=LCMS grade methanol
Gradient for PFCs	Time Flow %B 0 0.4 10 0.5 0.4 10 2.5 0.4 50 6 0.4 95 8.5 0.4 95 9 0.8 10 9.5 0.4 10 Hold for 2.5 min. before next injection.
Injection Size	25 µL, with Needle Wash
Time Segments	0 – 1 min. – MS2 Scan – to Waste 1 – 9 min. – MRM – to MS 9 – 9.5 min. – MS2 Scan – to Waste
PFC run time	12 minutes

QQQ Mass Spectrometer Scan Settings					
Analyte	**Parent Ion (Da)**	**Product Ions (Da)**	**Dwell (s)**	**Fragment (V)**	**Collision Energy (V)**
PFBA	213	168.9	0.008	42	4
$^{13}C_4$ PFBA	216.7	172	0.008	42	4
PFPA	263	218.9	0.008	42	0
PFBS	298.9	79,9	0.008	121	32
PFBS	298.9	98.9	0.008	121	24
$^{18}O_2$ PFBS	303	84	0.008	121	32
PFHxA	313	118.6	0.008	62	8
PFHxA	313	169	0.008	62	8
PFHxA	313	268.9	0.008	62	0
$^{13}C_2$ PFHxA	314.9	269.9	0.008	62	0
PFHpA	362.9	318.9	0.008	68	0
PFHpA	362.9	168.9	0.008	68	8
Br-PFHxS	398.9	80	0.008	130	48
Br-PFHxS	398.9	98.9	0.008	130	32
$^{18}O_2$ PFHxS	402.9	84	0.008	130	32
$^{18}O_2$ PFHxS	402.9	103	0.008	130	32
Br-PFOA	412.9	169	0.008	70	12
Br-PFOA	412.9	219	0.008	70	6

Table 2 continued

QQQ Mass Spectrometer Scan Settings					
Analyte	**Parent Ion (Da)**	**Product Ions (Da)**	**Dwell (s)**	**Fragment (V)**	**Collision Energy (V)**
Br-PFOA	412.9	368.9	0.008	70	0
$^{13}C_4$ PFOA	417.0	371.9	0.008	70	0
$^{13}C_8$ PFOA	421	375.9	0.008	70	0
PFHpS	449.9	80	0.008	153	44
PFHpS	449.9	98.9	0.008	153	48
PFNA	463	218.9	0.008	71	8
PFNA	463	418.9	0.008	71	4
$^{13}C_5$ PFNA	467.9	423.0	0.008	71	4
FOSA	497.9	78	0.008	127	36
Br-PFOS	498.8	79.9	0.008	159	72
Br-PFOS	498.8	98.9	0.008	159	48
Br-PFOS	498.8	130	0.008	159	96
$^{13}C_4$ PFOS	502.9	80	0.008	159	72
$^{13}C_8$ FOSA	505.9	78	0.008	127	36
$^{13}C_8$ PFOS	506.9	80	0.008	159	72
PFDA	513	219	0.008	77	8
PFDA	513	269	0.008	77	12
$^{13}C_2$ PFDA	514.9	470	0.008	77	4
PFUnA	563	219	0.008	83	8
PFUnA	563	268.9	0.008	83	12
PFUnA	563	518.9	0.008	83	0
$^{13}C_2$ PFUnA	564.9	520	0.008	83	0
PFDS	598.9	79.9	0.008	174	84
PFDS	598.9	99	0.008	174	56
PFDoA	613	168.9	0.008	77	24
PFDoA	613	568.9	0.008	77	4
$^{13}C_2$ PFDoA	614.9	569.9	0.008	77	4
PFTriA	663	618.9	0.008	86	8
PFTeA	712.9	169	0.008	86	28

Table 3: Waters (LCMS_4, LCMS-7) Recommended Instrument Operating Conditions

for Analysis of HFPO

NOTE: The conditions listed below for HPLC conditions and MS interface gas flows, temperatures, probe voltage, and cone voltage are subject to final fine adjustments to maximize sensitivity. Changes to the below conditions will be documented in the project data file. Argon or nitrogen collision gas pressure is set according to the instrument manufacturer.

HPLC Conditions (Waters Alliance/Acquity)	
Column (equivalent may be used)	Phenomenex Synergi Hydro RP plus C18 guard cartridge 35 ^{o}C
Flow Rate	0.5mL/min
Mobile Phase Composition	A=LCMS grade methanol B=0.01 M NH_4OAc (aq),
Gradient for HFPO	70% A and 30% B isocratic.
Injection Size	40uL (Alliance) 20 μL (Acquity)
HFPO run time	2 minutes
Mass Spectrometer Interface Settings (Quattro Premier/Xevo)	
MS Interface Mode	ESI Negative Ion
Capillary (kV)	1.0
Source Temp	100 ^{o}C
Desolvation Temp	300 ^{o}C
Cone Gas (nitrogen) Flow	100 L/Hr
Desolvation Gas (nitrogen) Flow	500 L/Hr

Waters Acquity Mass Spectrometer Scan Settings								
Function	**Channel**	**Analyte**	**Parent Ions (Da)**	**Product Ions (Da)**	**Dwell (s)**	**Cone (V)**	**Collision (eV)**	**Delay (s)**
1	1	HFPO	328.8	284.8	0.6	9	6	0.1
	2 (IS)	$^{13}C_3$ HFPO	331.8	286.8	0.6	9	6	0.1

Table 4: Analytical Sequence and Summary of QC Criteria for Commercial Work and DoD QSM 4.2

	Parameter	Description	Acceptance Criteria	Corrective Action
1	Tune solution	Injection of the Level 1 calibration standard.	Acceptable peak shape and recovery.	Perform instrument maintenance and/or clean system.
2	ICAL	Calibration standards 0.2 – 125 µg/L.	r > 0.990 or %RSD≤20%	Recalibrate.
3	ICB	Analyze HPLC-grade methanol spiked with IS.	< 2x MDL or < ½RL whichever is smaller	Clean system & recalibrate.
4	ICV	Second-source, 2.0 µg/L standard.	70-130% recovery for PFCs	Check standards & recalibrate.
5	DLCK	Detection Limit Check, 0.5 µg/L standard.	70-130% recovery for PFCs	Check standards & recalibrate.
6	Method Blank	Extracted reagent water.	< ½RL	Identify & fix source of contamination, re-extract batch.
7	LCS	Extracted 0.2 µg/L or 20.0 µg/kg spiked blank.	±3 standard deviations from the historical mean	Re-extract & reanalyze batch, unless biased high and samples ND.
8	Sample	---	Positive results must be within working range	Dilute to within working range.
9	Matrix Spike and Matrix Spike Duplicate	Spiked second & third portions of sample.	±3 standard deviations from the historical mean	Verify results by reanalysis. Re-extract & rerun if LCS also outside limits.
10	Samples	---	Positive results must be within working range	Dilute to within working range
11	CCV	Alternate 5.0 µg/L and 10.0 µg/L standards, analyze after every 10 injections.	70-130% recovery	Recalibrate & reanalyze samples.
12	Samples	---	Positive results must be within working range	Dilute to within working range
13	Closing CCV	5.0 µg/L or 10.0 µg/L standard	70 - 130% Recovery	Recalibrate & reanalyze samples.
14	Internal Standards		50-150% Recovery	If out in QC, re-extract, reanalyze If out in samples and QC is acceptable, sample considered to have matrix effect (NCM)

Table 5: Summary of QC Criteria for DoD QSM 5.0

QSM 5.0 Table 15. Perfluorinated Compounds by Liquid Chromatography/Mass Spectrometry	
Requirement	DoD QSM 5.0 and DoE QSAS 3.0
Tune Check	Prior to ICAL and after any mass calibration or maintenance is performed. Tuning standards must contain the analytes of interest or appropriate substitute. Mass assignments of tuning standard must be within 0.5 amu of true value. Retune instrument and verify. If the tune check will not meet acceptance criteria, an instrument mass calibration must be performed and the tuning redone. No samples shall be analyzed without an acceptable tune check.
Initial Calibration (ICAL)	At instrument setup and after major maintenance. Minimum 5 points for linear or 6 points for quadratic; weighting is allowed. Each calibration point for each analyte must calculate to be within 75-125%, except the lowest cal point which must calculate to within 70-130%. Any problems must be corrected and ICAL repeated. No sample shall be run until ICAL is successful.
Initial Calibration Verification (ICV)	Measure a second-source standard with concentration at the midpoint of the calibration once after each ICAL, prior to sample analysis. All reported analytes and surrogates must be within ± 25% of the true value. Any problems must be corrected. No samples shall be analyzed until the second-source calibration verification is successful.
Continuing calibration Verification (CCV)	Analysis of mid-level standard after every 10 field samples and the end of the analytical sequence. On days an ICAL is not performed, at the beginning of the sequence, after every 10 field samples and at the end of the analytical sequence. All reported analytes must be within ± 25% of the true value. If the CCV is above the project acceptance limits and there are no detections in the samples, TestAmerica will report the non-detect results with a case narrative comment in addition to applying any data qualifier flags required by the project (3HR). Correct any problems then rerun CCV. If that fails, then repeat ICAL. Reanalyze all samples since last successful CCV. Results cannot be reported without a valid CCV. Or Immediately (within one hour) analyze two additional consecutive CCVs. If both pass, samples may be reported without reanalysis. If either fails, take corrective action(s) and re-calibrate; then reanalyze all affected samples since the last acceptable CCV. If reanalysis cannot be performed, data must be qualified and explained in the case narrative. Apply Q-flag to all results for the specific analytes(s) in all samples since the last acceptable CCV. Flagging is only appropriate in cases where the samples cannot be reanalyzed.

Table 5: Summary of QC Criteria for DoD QSM 5.0 (continued)

QSM 5.0 Table 15. Perfluorinated Compounds by Liquid Chromatography/Mass Spectrometry	
Requirement	DoD QSM 5.0 and DoE QSAS 3.0
Internal Standards (IS)	Isotopically-labeled analytes must be added to all field samples, QC samples (batch and instrument) and standards. Absolute areas of the quantitation ions of the IS(s) must be within 50-150% from the average areas measured during ICAL. If recoveries are acceptable for QC samples but not field samples, the field samples may be considered to suffer from a matrix effect. Apply Q-flag and explain in the case narrative. For failed QC samples, correct problem and rerun all associated failed field samples. Failing internal standard should be thoroughly documented in the case narrative.
Method Blank (MB)	One per prep batch. No analytes detected > ½ LOQ (RL) or >1/10 the amount measured in any sample or 1/10 the regulatory limit, whichever is greater. If criteria not met, correct problem. If required, reprep and reanalyze MB and all samples processed with the contaminated blank. If reanalysis is not possible, apply B-flag to all results for the specific analyte(s) in all samples processed with the contaminated blank. Must be explained in the case narrative. Flagging is only appropriate when samples cannot be reanalyzed.
LCS	One per prep batch. As DoD limits are not available, use in-house LCS limits if project limits are not specified. If in-house limits do not exist, use 70-130% until limits are established. If the LCS recovery is above the project acceptance limits and there are no detections in the samples, TestAmerica will report the non-detect results with a case narrative comment in addition to applying any data qualifier flags required by the project (3HR). Correct any problems then re-prep and reanalyze the LCS and all associated samples for failed analytes. If insufficient sample or corrective action fails, then apply Q-flag to specific analyte(s) in all samples in the associated prep batch. Must be explained in the case narrative.
Matrix Spike	One per prep batch. As DoD limits are not available, use in-house LCS limits if project limits are not specified. For failures, consult project-specific DQOs and contact client for additional measures to be taken. For specific analyte(s) in parent sample, apply J-flag if acceptance criteria are not met. If MS falls outside LCS limits, evaluate data to determine the source of the difference, i.e., matrix effect or analytical error.

Table 5: Summary of QC Criteria for DoD QSM 5.0 (continued)

QSM 5.0 Table 15. Perfluorinated Compounds by Liquid Chromatography/Mass Spectrometry	
Requirement	DoD QSM 5.0 and DoE QSAS 3.0
MSD or Sample Duplicate	One per prep batch. Analyze MS/MSD for low concentration samples and Sample/MD for high concentration samples. Use DoD-specific criteria for LCS. If DoD limits are not available, use in-house LCS limits if project limits are not specified. RPD for duplicates ≤ 30%. For failures, consult project-specific DQOs and contact client for additional measures to be taken. For specific analyte(s) in parent sample, apply J-flag if acceptance criteria are not met. If MS falls outside LCS limits, evaluate data to determine the source of the difference, i.e., matrix effect or analytical error.
Surrogate Spike	All field and QC samples. As DoD limits are not available, use in-house LCS limits if project limits are not specified. If in-house limits do not exist, use 70-130% until limits are established. If recoveries are acceptable for QC samples, but not field samples, the field samples may be considered to suffer from a matrix effect. For failed QC samples, correct problem and rerun all failed samples. If samples cannot be reanalyzed, apply Q-flag and discuss in the case narrative.

Table 6: PFC Names, Abbreviations and Recommended Internal Standard and Surrogate Associations

PFC Name	PFC Abbreviation	Internal Standard	Surrogate
Perfluorobutyric Acid	PFBA	$^{13}C_4$ PFBA	$^{13}C_4$ PFBA
Perfluoropentanoic Acid	PFPA	$^{13}C_2$ PFHxA	$^{13}C_8$ PFOA
Perfluorohexanoic Acid	PFHxA	$^{13}C_2$ PFHxA	$^{13}C_8$ PFOA
Perfluoroheptanoic Acid	PFHpA	$^{13}C_4$ PFOA	$^{13}C_8$ PFOA
Perfluorooctanoic Acid	PFOA	$^{13}C_4$ PFOA	$^{13}C_8$ PFOA
Perfluorononanoic Acid	PFNA	$^{13}C_5$ PFNA	$^{13}C_8$ PFOA
Perfluorodecanoic Acid	PFDA	$^{13}C_2$ PFDA	$^{13}C_8$ PFOA
Perfluoroundecanoic Acid	PFUnA	$^{13}C_2$ PFUnA	$^{13}C_8$ PFOA
Perfluorododecanoic Acid	PFDoA	$^{13}C_2$ PFDoA	$^{13}C_8$ PFOA
Perfluorotridecanoic Acid	PFTriA	$^{13}C_2$ PFDoA	$^{13}C_8$ PFOA
Perfluorotetradecanoic Acid	PFTeA	$^{13}C_2$ PFDoA	$^{13}C_8$ PFOA
Perfluorobutane Sulfonate	PFBS	$^{18}O_2$ PFHxS	$^{13}C_8$ PFOS
Perfluorobutanesulfonic Acid	PFBS	$^{18}O_2$ PFHxS	$^{13}C_8$ PFOS
Perfluorohexane Sulfonate	PFHxS	$^{18}O_2$ PFHxS	$^{13}C_8$ PFOS
Perfluorohexanesulfonic Acid	PFHxS	$^{18}O_2$ PFHxS	$^{13}C_8$ PFOS
Perfluorooctane Sulfonate	PFOS	$^{13}C_4$ PFOS	$^{13}C_8$ PFOS
Perfluorooctanesulfonic Acid	PFOS	$^{13}C_4$ PFOS	$^{13}C_8$ PFOS
Perfluorodecane Sulfonate	PFDS	$^{13}C_4$ PFOS	$^{13}C_8$ PFOS
Perfluorodecanesulfonic Acid	PFDS	$^{13}C_4$ PFOS	$^{13}C_8$ PFOS
Perfluorooctanesulfonamide	FOSA	$^{13}C_8$ FOSA	$^{13}C_8$ PFOA
$^{13}C_8$ Perfluorooctanoic Acid (Surrogate)	$^{13}C_8$ PFOA	$^{13}C_4$ PFOA	--
$^{13}C_8$ Perfluorooctane Sulfonate (Surrogate)	$^{13}C_8$ PFOS	$^{13}C_4$ PFOS	--
Perfluoro(2-propoxypropanoic) Acid	HFPO	$^{13}C_8$ HFPO	$^{13}C_8$ HFPO

Table 7: Mass Calibrations

PEG	NaCsI	Na Formate
89.06	22.99	22.99
133.09	132.91	90.98
177.11	172.88	105.04
221.14	322.78	158.96
239.15	472.67	226.95
283.18	622.57	294.94
327.20	772.46	362.93
371.23	922.36	430.91
415.25	1072.25	498.90
459.28		566.89
503.31		634.88
564.36		702.86
608.39		770.85
652.41		838.84
696.44		906.83
740.46		974.81
784.49		1042.80
828.52		
872.54		
916.57		
960.60		
1004.62		

Figure 1: Example Chromatograms for the PFC Method

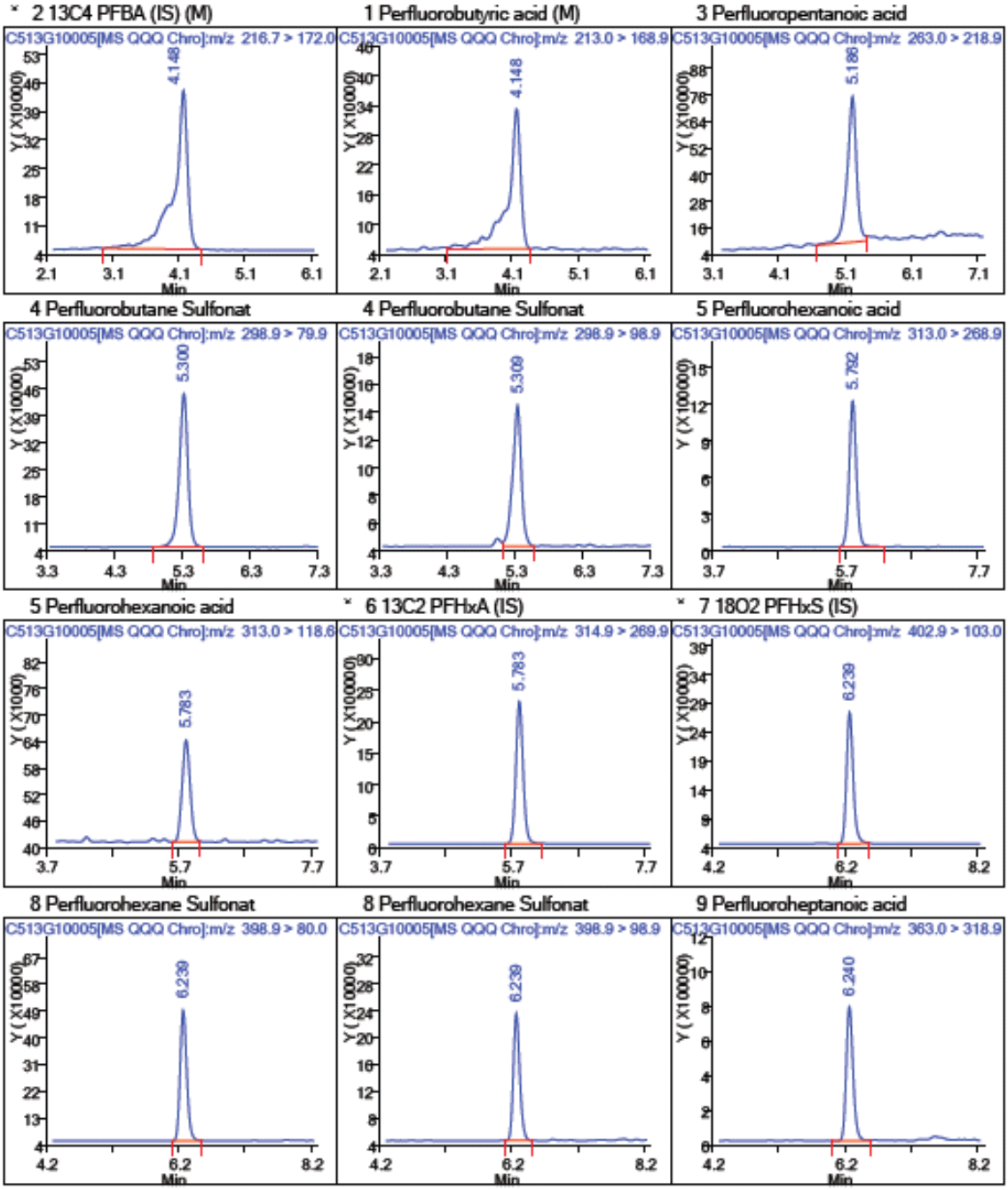

Figure 1 (cont.): Example Chromatograms for the PFC Method

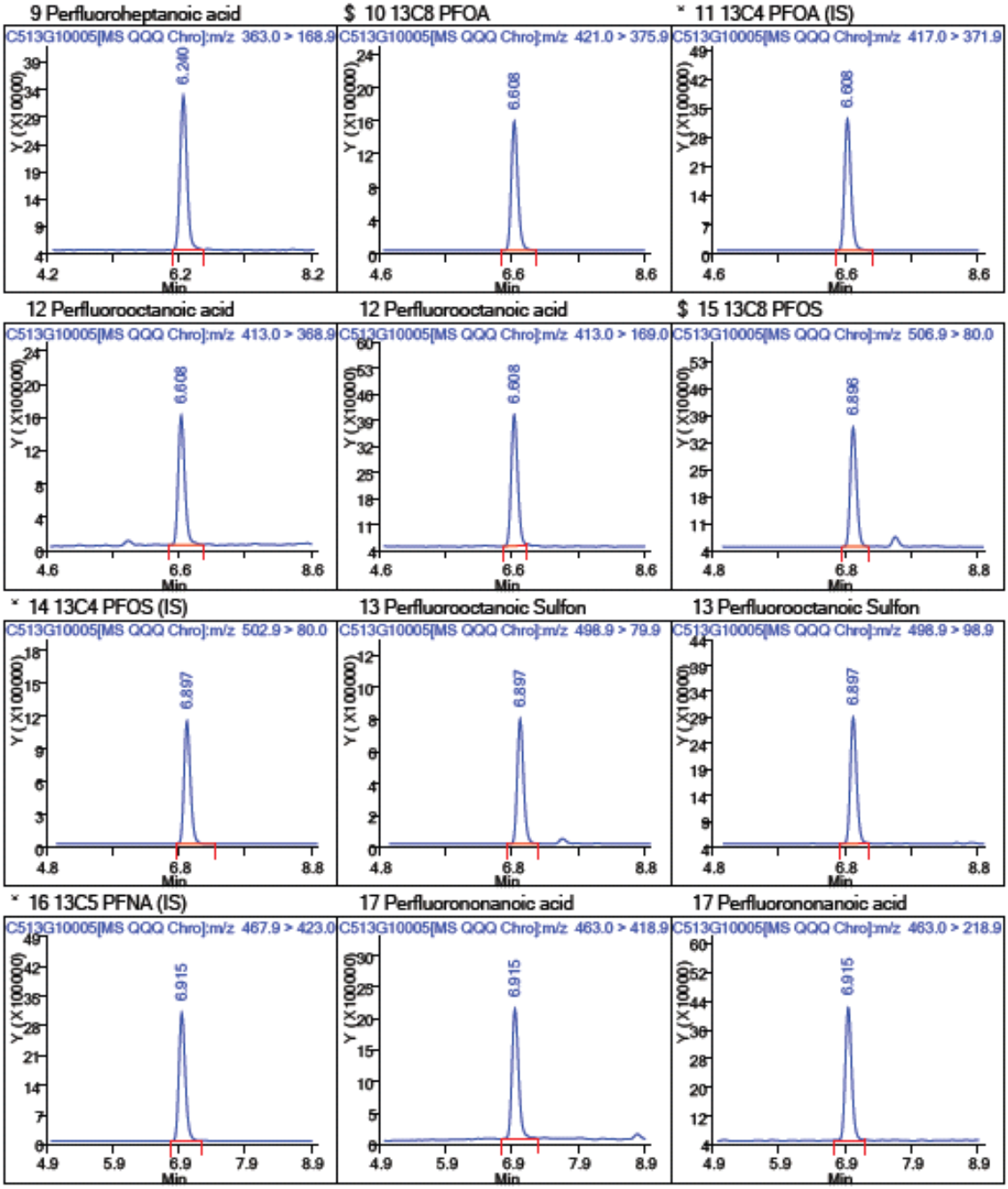

Figure 1 (cont.): Example Chromatograms for the PFC Method

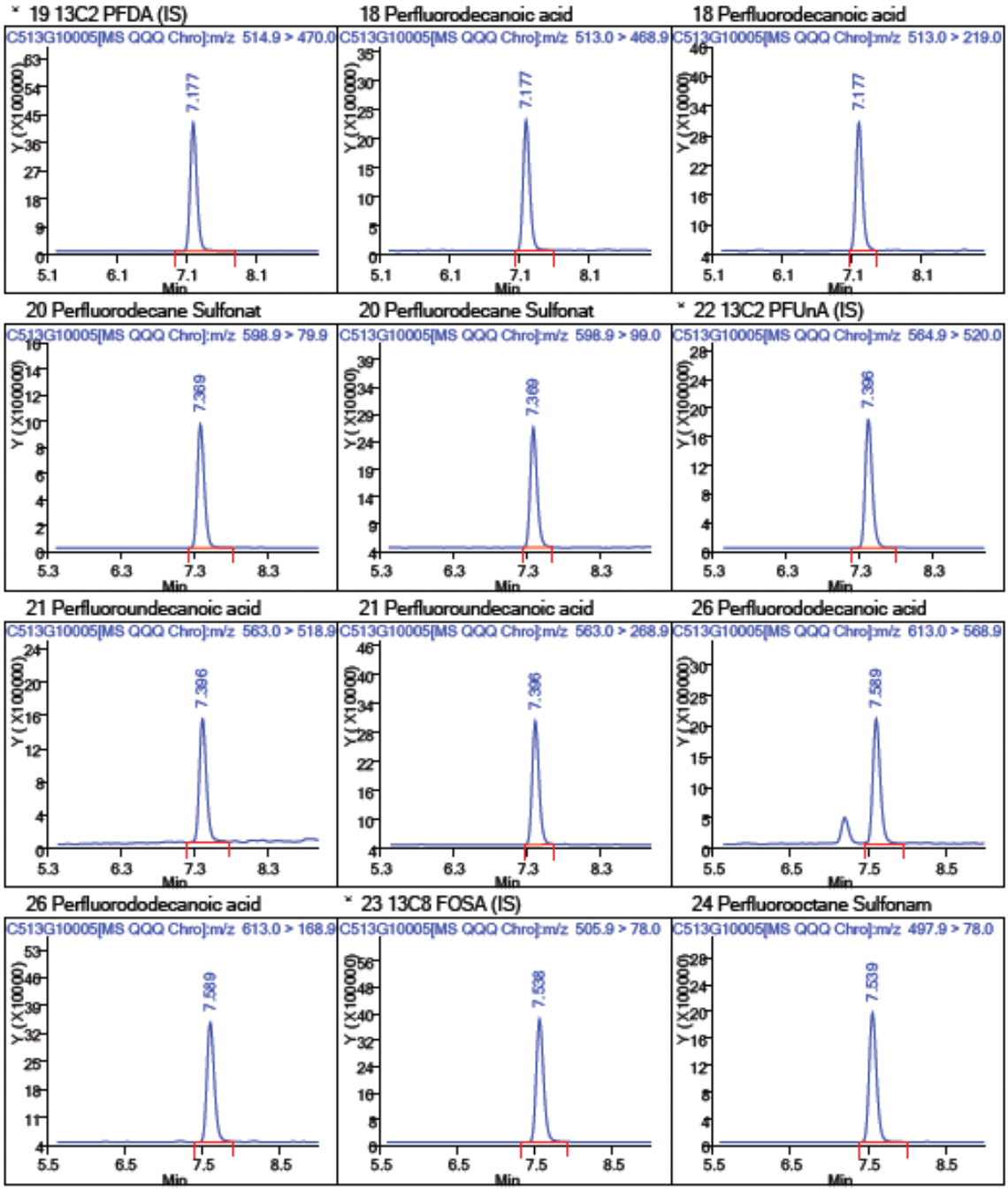

Figure 1 (cont.): Example Chromatograms for the PFC Method

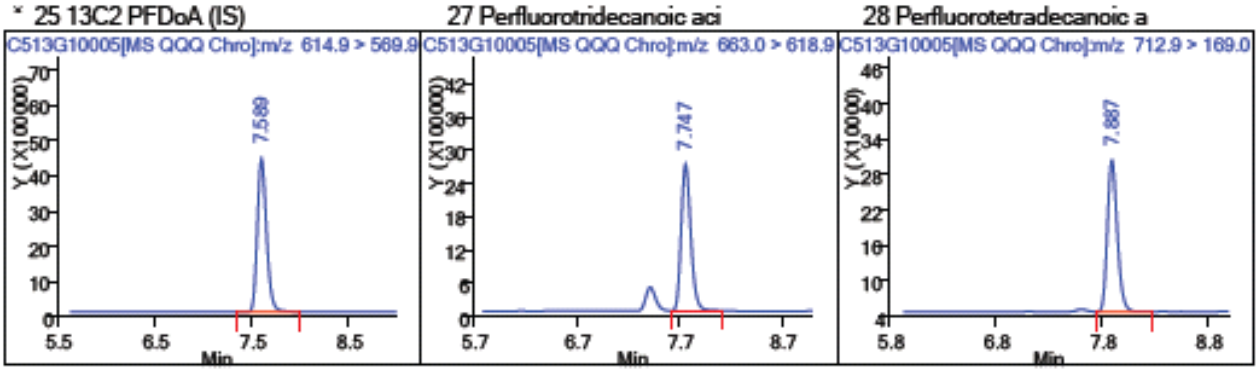

Figure 2: Example Chromatograms for the PFC_FOSA Method

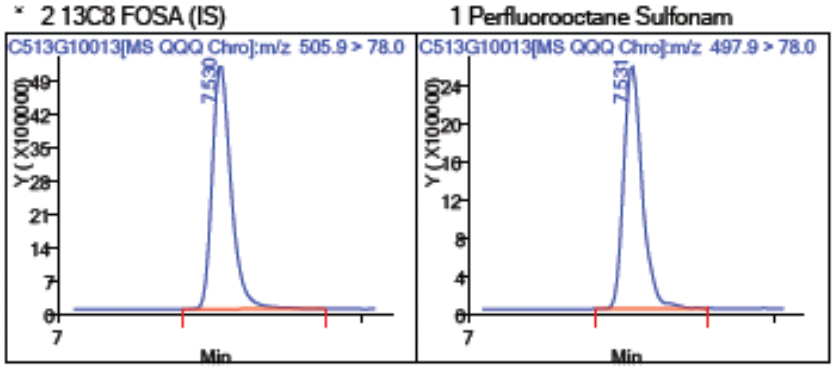

Figure 3: Example Chromatograms for the LCMS_PFOA Method

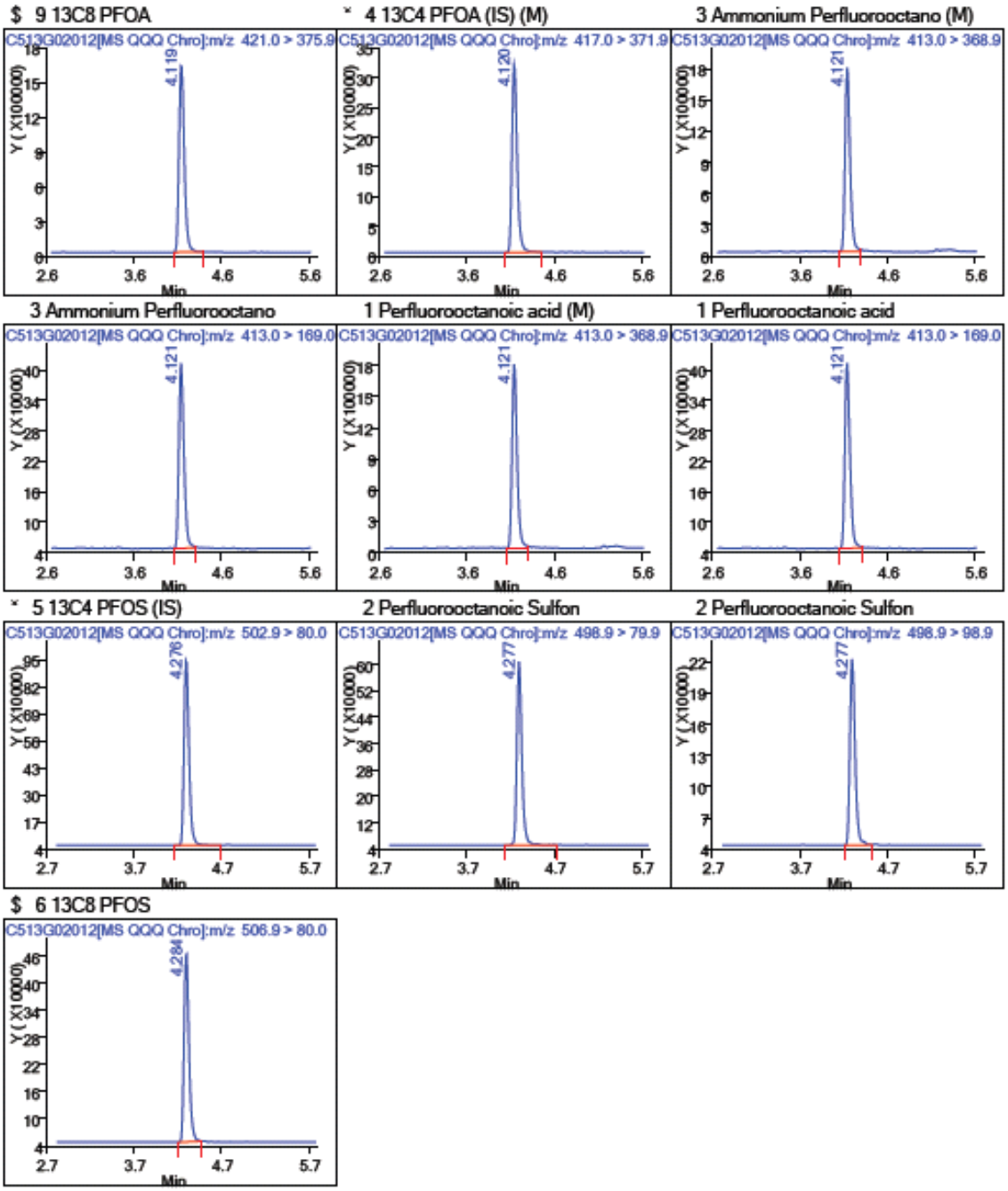

Figure 4: Example Chromatograms for LCMS - HPFO

Report Date: 17-Jun-2015 14:35:48 Chrom Revision: 2.2 05-May-2015 11:39:10

TestAmerica Denver

Data File: \\denchrom\ChromData\LC_LCMS4\20150617-36132.b\hfp45F17013.d

Injection Date: 17-Jun-2015 09:47:34 Instrument ID: LC_LCMS4

Lims ID: ICV

Client ID:

Operator ID: acm ALS Bottle#: 82 Worklist Smp#: 13

Injection Vol: 100.0 ul Dil. Factor: 1.0000

Method: 8321HFPO Limit Group: LC - 8321A_HFPO_Du

* 2 13C3 HFPO-DA (IS) $ 3 13C3 HFPO-DA 1 Perfluoro(2-propoxypropanoic) acid