Document number: **T-PFAS-WI39592**	Always check on-line for validity. **Client specific-Polyfluorinated Alkyl Substances (PFAS) in Aqueous Samples Using Isotope Dilution and LC/MS/MS**	Level: **Work Instruction**
Old Reference:		
Version: **1.2**		Organisation level: **5-Sub-BU**
Approved by: **XL3S** Effective Date **14-MAY-2021**	Document users: **5_EUUSLA_PFAS_Manager, 6_EUUSLA_PFAS_Analyst, 6_EUUSLA_PFAS_Data_Reviewers, 6_EUUSLA_PFAS_Management_Team, 6_EUUSLA_PFAS_Sample_Prep**	Responsible: **5_EUUSLA_PFAS_Manager**

Revision Log

Revision:	1	**Effective Date:**	This version
Section	**Justification**		**Changes**
	NEW		

Reference

1. US EPA Method 537, Determination of Selected Perfluorinated Alkyl Acids in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LCMSMS), Version 1.1, September 2009.

2. Standard Test Method for Determination of Perfluorinated Compounds in Soil by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS), ASTM Method D7968, 2014.

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3. ISO 25101:2009(E) Water quality, Determination of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA); Method for unfiltered samples using solid phase extraction and liquid chromatography/mass spectrometry, March 2009.

4. Test Methods for Evaluating Solid Wastes, SW-846 Method 8321B, Solvent Extractable Non-Volatile Compounds by High Performance Liquid Chromatography/Thermospray Mass Spectrometry or Ultraviolet Detection, Rev 2, January 1998.

5. Determinative Chromatographic Separations, SW-846, Method 8000D, July 2014.

6. Method for Trace Level Analysis of C8, C9, C10, C11, and C13 Perfluorocarbon Carboxylic Acids in Water. Karen Risha, John Flaherty, Roice Wille, Warren Buck, Francesco Morandi, and Tsuguhide Isemura. Anal. Chem. 2005, 77, 1503-1508.

7. *Chemical Hygiene Plan*, current version.

Cross Reference

Document	Document Title
T-PEST-WI9847	Common Equations Used During Chromatographic Analyses
T-PFAS-WI13881	Standards Management in the PFAS Laboratory
QA-SOP11178	Demonstrations of Capability
QA-SOP11892	Determining Method Detection Limits and Limits of Quantitation

Scope

This method is applicable for the determination of selected per- and polyfluorinated alkyl substances (PFAS) in aqueous samples to include non-potable waters and non-regulatory potable water when directed by the client. The compounds analyzed in this method are listed in the table below. The most current MDLs and LOQs are listed in the LIMS.

Analyte	Acronym	CAS#
Perfluorobutanesulfonic acid	PFBS	375-73-5
Perfluorodecanoic acid	PFDA	335-76-2
Perfluorododecanoic acid	PFDoDA	307-55-1
Perfluoroheptanoic acid	PFHpA	375-85-9
Perfluorohexanesulfonic acid	PFHxS	355-46-4
Perfluorohexanoic acid	PFHxA	307-24-4
Perfluorononanoic acid	PFNA	375-95-1

Analyte	Acronym	CAS#
Perfluorooctanesulfonic acid	PFOS	1763-23-1
Perfluorooctanoic acid	PFOA	335-67-1
Perfluorotetradecanoic acid	PFTeDA	376-06-7
Perfluorotridecanoic acid	PFTrDA	72629-94-8
Perfluoroundecanoic acid	PFUnDA	2058-94-8
Perfluoro-n-butanoic acid	PFBA	375-22-4
Perfluoro-n-pentanoic acid	PFPeA	2706-90-3
8:2 - Fluorotelomersulfonic acid	8:2FTS	39108-34-4
N-methylperfluoro-1-octanesulfonamidoacetic acid	NMeFOSAA	2355-31-9
N-ethylperfluoro-1-octanesulfonamidoacetic acid	NEtFOSAA	2991-50-6
4:2-Fluorotelomersulfonic acid	4:2-FTS	757124-72-4
Perfluoropentanesulfonic acid	PFPeS	2706-91-4
6:2-Fluorotelomersulfonic acid	6:2-FTS	27619-97-2
Perfluoroheptanesulfonic acid	PFHpS	375-92-8
Perfluorononanesulfonic acid	PFNS	68259-12-1
Perfluorodecanesulfonic acid	PFDS	335-77-3
10:2-Fluorotelomersulfonic acid	10:2-FTS	120226-60-0
Perfluorododecanesulfonic acid	PFDoDS	79780-39-5
Perfluorohexadecanoic acid	PFHxDA	67905-19-5
Perfluorooctadecanoic acid	PFODA	16517-11-6
Perfluorooctanesulfonamide	PFOSA	754-91-6
2-(N-methylperfluoro-1-octanesulfonamido)- ethanol	NMePFOSAE	24448-09-7
N-methylperfluoro-1-octanesulfonamide	NMePFOSA	31506-32-8
2-(N-ethylperfluoro-1-octanesulfonamido)- ethanol	NEtPFOSAE	1691-99-2
N-ethylperfluoro-1-octanesulfonamide	NEtPFOSA	4151-50-2
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-propanoic acid; (Hexafluoropropylene oxide dimer acid)	HFPODA	13252-13-6
Ammonium 4,8-dioxa-3H-perfluorononanoic acid	DONA **	919005-14-4 *
Potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS, F53B major	756426-58-1 *

Analyte	Acronym	CAS#
Potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS, F53B minor	763051-92-9 *
3-Perfluoropropylpropanoic acid	3:3 FTCA	356-02-5
3-Perfluoropentylpropanoic acid	5:3 FTCA	914637-49-3
3-Perfluoroheptylpropanoic acid	7:3 FTCA	812-70-4
2-Perfluorohexylethanoic acid	6:2 FTCA	53826-12-3
2H-Perfluoro-2-octenoic acid	6:2 FTUCA	70887-88-6
2-Perfluorooctylethanoic acid	8:2 FTCA	27854-31-5
2H-Perfluoro-2-decenoic acid/p>	8:2 FTUCA	70887-84-2
2-Perfluorodecylethanoic acid	10:2 FTCA	53826-13-4
2H-Perfluoro-2-dodecenoic acid	10:2 FTUCA	70887-94-4
Perfluoropropionic acid (PPF Acid)	PFPrA	422-64-0
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	PFECA B	151772-58-6
Perfluoro-4-methoxybutanoic acid (PFMBA)	PFECA A	863090-89-5
Perfluoro-3-methoxypropanoic acid (PFMPA)	PFECA F	377-73-1
Difluoro(perfluoromethoxy)acetic acid	PFMOAA	674-13-5
Perfluoro-4-isopropoxybutanoic acid	PFECA G	801212-59-9
Perfluoro-3,5,7,9-butaoxadecanoic acid	PFO4DA	39492-90-5
Perfluoro-3,5,7-trioxaoctanoic acid	PFO3OA	39492-89-2
Perfluoro-3,5-dioxahexanoic acid	PFO2HxA	39492-88-1
4-(2-carboxy-1,1,2,2-tetrafluoroethoxy)-2,2,3,3,4,5,5,5-octafluoro-pentanoic acid	R-EVE	2416366-22-6
1,1,2,2,4,5,5,5-heptafluoro-3-oxapentanesulfonic acid	NVHOS	801209-99-4
2,2,3,3-Tetrafluoro-3{[1,1,1,2,3,3-hexafluoro-3-(1,2,2,2-tetrafluoroethoxy)propan-2-yl]oxy}propanoic acid	Hydro-EVE Acid	773804-62-9
2,2,3,3-Tetrafluoro-3{[1,1,1,2,3,3-hexafluoro-3-(1,2,2-trifluoroethenoxy)propan-2-yl]oxy}propanoic acid	EVE Acid	69087-46-3
Perfluoro-3,5,7,9,11-pentaoxadodecanoic acid	PFO5DA	39492-91-6
Perfluoro-2-(perfluoromethoxy)propanoic acid	PMPA	13140-29-9
2,3,3,3-Tetrafluoro-2-(pentafluoroethoxy)propanoic acid	PEPA	267239-61-2
3-(Methoxy)tetrafluoroproprionic acid	MTP	93449-21-9

Analyte	Acronym	CAS#
Perfluoro-3,6-dioxa-4-methyl-7-octene-1-sulfonic acid	PS Acid	29311-67-9
Perfluoro-2-{[perfluoro-3-(perfluoroethoxy)-2-propanyl]oxy}ethanesulfonic acid	Hydro-PS Acid	749836-20-2
Pentanoic acid, 2,2,3,3,4,5,5,5-octafluoro-4-(,1,2,2-tetrafluoro-s-sulfoethoxy)-	R-PSDA, Byproduct 4	2416366-18-0
Acetic acid, 2-fluoro-2-[1,1,2,3,3,3-hexafluoro-2-(1,1,2,2-tetrafluoro-2-sulfoethoxy)propoxy]-	Hydrolyzed PSDA	2416366-19-1
Ethanesulfonic acid, 1,1,2,2-tetrafluoro-2-[1,2,2,3,3-pentafluoro-1-(trifluoromethyl)propoxy]-	R-PSDCA, Byproduct 6	2416366-21-5
Perfluoro-4-ethylcyclohexanesulfonic acid	PFECHS	133201-07-7
Perfluoropropanesulfonic acid	PFPrS	423-41-6
Perfluoro(2-ethoxyethane) sulfonic acid (PFEESA)	PES	113507-82-7

*CAS# for the free acid form of the analyte

**Acronym for the free acid form of the analyte

Basic Principles

A 250-mL aqueous sample is fortified with isotopically-labeled extraction standards and is passed through a solid phase extraction (SPE) cartridge to extract the analytes. The compounds are eluted from the solid phase with a combination of solvents. The extract is concentrated to ~400-500µl with nitrogen in a heated water bath, and then reconstituted to 1 ml with methanol. Isotopically-labeled injection internal standards are added to the sample extract and it is analyzed by LC/MS/MS operated in negative electrospray ionization (ESI) mode for detection and quantification of the analytes. Quantitative analysis is performed using isotope dilution.

Interferences

Compounds which have similar structures to the compounds of interest and similar molecular weights would potentially interfere. Method interferences may be caused by contaminants in solvents, reagents (including reagent water), sample bottles and caps, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. The analytes in this method can also be found in many common laboratory supplies and equipment, such as PTFE (polytetrafluoroethylene) products, LC solvent lines, methanol, aluminum foil, etc. A laboratory blank is performed with each batch of samples to demonstrate that the extraction system is free of contaminants.

Precaution to Minimize Method Interference

1. LC system components contain many of the target analytes. To minimize the background PFAS peaks, PTFE solvent frits and tubing are replaced by PEEK™ solvent frits and tubing where possible.

2. PROPRIETY CONTENT

3. PFAS standards, extracts and samples should not come in contact with any glass containers as these analytes can potentially adsorb to glass surfaces. PFAS analytes and internal standards commercially purchased in glass ampules are acceptable; however, all subsequent transfers or dilutions performed by the analyst must be prepared and stored in polypropylene containers.

Safety Precautions and Waste Handling

See *Chemical Hygiene Plan* for general information regarding employee safety, waste management, and pollution prevention.

The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. PFOA has been described as "likely to be carcinogenic to humans". Each chemical should be treated as a potential health hazard and exposure to these chemicals should be minimized.

Exposure to these chemicals must be reduced to the lowest possible level by whatever means available, such as fume hoods, lab coats, safety glasses, and gloves. Gloves, lab coats, and safety glasses should be worn when preparing standards and handling samples. Avoid inhaling solvents and chemicals and getting them on the skin. Wear gloves when handling neat materials. When working with acids and bases, take care not to come in contact and to wipe any spills. Always add acid to water when preparing reagents containing concentrated acids.

All laboratory waste is accumulated, managed, and disposed of in accordance with all Federal, State, and local laws and regulations. All solvent waste and extracts are collected in approved solvent waste containers in the laboratory and subsequently emptied by personnel trained in hazardous waste disposal into the lab-wide disposal facility. HPLC vials are disposed of in the lab container for waste vials, and subsequently lab packed. Any solid waste material (disposable pipettes and broken glassware, etc.) may be disposed of in the normal solid waste collection containers.

Personnel Training and Qualifications

All personnel performing this procedure must have documentation of reading, understanding, and agreeing to follow the current version of this SOP and an annual documented Demonstration of Capability (DOC).

Each chemist performing the extraction must work with an experienced employee for a period of time until they can independently perform the extraction. Also, several batches of sample extractions must be performed under the direct observation of another experienced chemist to assure the trainee is capable of independent preparation. Proficiency is measured through a documented Initial Demonstration of Capability (IDOC).

Each LC/MS/MS analyst must work with an experienced employee for a period of time until they can independently calibrate the LC/MS/MS, review and process data, and perform maintenance procedures. Proficiency is measured through a documented Initial Demonstration of Capability (IDOC).

The IDOC and DOC consist of four laboratory control samples (or alternatively, one blind sample for the DOC) that is carried through all steps of the extraction and meets the defined acceptance criteria. The criteria include the calculation of mean accuracy and standard deviation.

See *QA-SOP11178* for additional information on IDOC and DOC.

Sample Collection, Preservation, and Handling

A. Sample Collection

The samples are collected in 250-mL polyethylene bottles. Keep the sample sealed from time of collection until extraction.

NOTE: PFAS contamination during sampling can occur from a number of common sources, such as food packaging and certain foods and beverages. Proper hand washing and wearing nitrile gloves will aid in minimizing this type of accidental contamination of the samples.

B. Sample Storage and Shipment

1. Samples must be chilled during shipment and must not exceed 10°C during the first 48 hours after collection. Sample temperature must be confirmed to be at or below 10°C when the samples are received at the laboratory.

2. Samples stored in the lab must be held at a temperature of 0° to 6°C, not frozen, until extraction.

3. Water samples must be extracted within 14 days. Extracts must be analyzed within 28 days after extraction. Extracts are stored at room temperature.

Apparatus and Equipment

A. Apparatus

1. 250-mL HDPE bottles: Scientific Specialties; Catalog No. 334008-blk-1, or equivalent.

2. Centrifuge tubes – 15-mL conical polypropylene with polypropylene screw caps; Fisher Scientific, Catalog No. 05-539-5 or equivalent

3. 10-mL polypropylene volumetric flask, Class A – Fisher Scientific, Catalog No. S02288 or equivalent.

4. HPDE bottles for extraction fluid storage: L; Environmental Sampling Supply, Catalog No. 1000-1902-PC.

5. Analytical Balance – Capable of weighing to 0.0001 g

6. Top-Loading Balance – Capable of weighing to 0.01 g

7. PROPRIETY CONTENT

8. PROPRIETY CONTENT

9. PROPRIETY CONTENT

10. SPE vacuum extraction manifold –"Resprep" 24-port manifold; Restek Corp Catalog No. 26080, or equivalent.

11. Polypropylene SPE delivery needles – Agilent; Catalog No. 12234511.

12.Centrifuge – "Q-Sep 3000"; Restek Corp. Catalog No. 26230, or equivalent, capable of a minimum rotational speed of 3000 rpm.

13. Disposable polyethylene pipette – Fisher Scientific, Cat. No. S30467-1 or equivalent.

14. Auto Pipettes – Eppendorf; capable of accurately dispensing 10- to 1000-µL. FisherScientific Catalog No. 14-287-150, or equivalent.

15. Polypropylene pipette tips: 0-200µL. Fisher; Catalog No. 02-681-135

16. Polypropylene pipette tips: 101-1000µL. Fisher, Catalog No. 02-707-508

17. Pipettes – Disposable transfer. Fisher Scientific, Catalog No. 13-711-7M

18. Vortex mixer, variable speed, Fisher Scientific or equivalent.

19. N-Evap sample extract concentrator with N_2 supply and water bath for temperature control. Organomation, Inc. Catalog No. 11250, or equivalent.

20. Reagent Water Purification System: Capable of producing ultrapure "Type 1/Milli-Q"-grade water from in-house deionized water system. Millipore SAS; Catalog No. FTPF08831.

21. Thermo Target PP Polyspring inserts, Catalog No. C4010-630P

22. Agilent 9mm vial kit pack, Catalog No. 5190-2278, or equivalent

23. Centrifuge tubes – 50-mL conical polypropylene with polypropylene screw caps; Fisher Scientific, Catalog No.06-443-21 or equivalent

24. Polypropylene bottles for standard storage - 4 mL; Fisher Scientific, Catalog No. 2006-9125

25. Stainless steel spatula/scoop set. Bel-Art SP Scienceware; Product No. 11-865-130.

B. Equipment

1. AB Sciex Triple Quad 4500/5500/5500 Plus Turbo V Ion Source

ExionLC Controller
ExionLC AC Pump
ExionLC AC Autosampler
Exion AC Column Oven
Data system –Analyst 1.6.3

2. HPLC columns

a. PROPRIETY CONTENT
b. PROPRIETY CONTENT

Reagents and Standards

All solvents, acids, and bases are stored in glass bottles in flammable proof cabinets or pressure resistant steel drums. Solvents, acids, and bases are stored at ambient temperature for up to 1 year. All non-solvents are stored according to manufacturer's storage conditions.

A. Reagents: PROPRIETY CONTENT

B. Standards: See SOP *T-PFAS-WI13881*.

Calibration

A. Initial Calibration

1. A minimum of five calibration standards are required. In general, Cal1, Cal2, Cal3, Cal4, Cal5, Cal6, and Cal 7 are included in the initial calibration. The calibration standards contain the branched

isomers for PFHxS, PFOS, NMeFOSAA, and NEtFOSAA. S/N ratio must be greater than or equal to 10:1 for all ions used for quantification.

2. Analyze a Cal3 level standard that contains linear and branch chained isomers of PFOA. The analysis of this standard is used to demonstrate where the branch chained isomers elute and not included in the calibration curve. This will assist the chemist in identifying and properly integrating this compound in samples.

3. Isotopically-labeled compounds are not available for some compounds. See below for compounds and their referenced extraction standards. See *Attachment 2* for additional information about compound relationships.

Compound	Extraction Standard
PFECA F	13C4-PFBA
PFPrS	
EVE Acid	
Hydro-EVE Acid	
MTP	
PEPA	
PFECA G	
PFMOAA	
PFO2HxA	
PFO3OA	
PFO4DA	
PFO5DA	
PFPrA	
PMPA	
R-EVE	
3:3 FTCA	13C5-PFPeA
PFPeS	13C3-PFBS
Hydrolyzed PSDA	
Hydro-PS Acid	
NVHOS	
PES	

Compound	Extraction Standard
PFECA A	
PFECA B	
PS Acid	
R-PSDA	
R-PSDCA	
PFHpS	13C3-PFHxS
PFECHS	
DONA	13C4-PFHpA
5:3 FTCA	
PFNS	13C8-PFOS
PFDS	
9Cl-PF3ONS	
11CL-PF3OUdS	
PFDoS	
10:2-FTS	13C2-8:2-FTS
PFTrDA	13C2-PFDoDA
PFHxDA	13C2-PFTeDA
PFODA	
7:3 FTCA	13C2-6:2-FTCA

4. Fit the curve

a. If the % RSD for the response factors is less than or equal to 20%, the average response factor (Ave RRF) can be used to quantitate the data.

b. If the %RSD is greater than 20%, then a linear regression with a concentration weighing factor of 1/x forced through zero is tried for the compounds not meeting the criteria in 4.a. R^2 for each analyte using the linear regression must be greater than or equal to 0.99.

c. If the linear regression curve fails, then a quadratic regression with a concentration weighing factor $1/x^2$ is tried for the compounds not meeting 4.a or 4.b. R^2 for each analyte using the quadratic regression must be greater than or equal to 0.99. A minimum or six standards must be analyzed to use a quadratic fit.

d. For all curve fits, each calibration point is calculated back against the curve. The back calculated concentration should be within ±30% of its true value.

e. If the criteria are not met, the source of the problem must be determined and corrected. Situations may exist where the initial calibration can be used. In those cases, the data will be reported with a qualifying comment.

NOTE: The concentrations referenced for the sulfonate salts, (for example PFBS, PFHxS and PFOS) have already been corrected to the acid form by the standards supplier as noted in the example Certificate of Analysis (CofA). See *Attachment 4*.

5. Initial Calibration Verification (ICV)

A check standard prepared from a second source (ICV) is injected to confirm the validity of the calibration curve/standard. If a second source is not available, a separate preparation from the same stock may be used. The calculated amount for each analyte must be within ±30% of the true value. If this criteria is not met, re-inject or remake the standard. If the criteria is still not met, recalibration is necessary. Instrument maintenance may be needed prior to recalibrating.

B. Continuing calibration

1. Once the calibration curve has been established, the continuing accuracy must be verified by analysis of a continuing calibration verification (CCV) standard every ten samples and at the end of the analysis sequence.

a. The CCV run after the initial calibration must be at the CAL3 level.

b. Subsequent CCV standards should alternate between the CAL3, CAL4, and CAL5 levels of the calibration curve.

2. Acceptance criteria

a. The calculated amount for each compound (native and extraction standard) in the CCV standard must be within ±30% of the true value. Samples that are not bracketed by acceptable CCV analyses must be reanalyzed. The exception to this would be if the CCV recoveries are high, indicating increased sensitivity, and there are no positive detections in the associated samples, the data may be reported with a qualifying comment. If two consecutive CCVs fail criteria for target analytes, two passing CCVs must be analyzed or the source of the problem determined and the system recalibrated before continuing sample analysis.

b. The absolute areas of the injection internal standards should be within 50-150% of the average areas measured during the initial calibration.

Procedure

A. Sample Preparation

1. Weigh sample container with contents on a calibrated top loading balance, and record the first reading in the automated prep entry system.

a. For all samples, the full bottle must be extracted. The sample must remain in the original sample container until after spiking solutions have been added. If limited sample is submitted, spike sample in original container, then add Milli-Q water to bring to final volume of 250 mL prior to SPE extraction (see B.5 for spiking details).

b. If the sample matrix is such that SPE extraction cannot be performed using the full volume, see Procedure C.

c. If the sample has dissolved and/or settleable solid content (i.e; is cloudy or has a layer of sediment/solids at the bottom of the bottle), the sample must be centrifuged in order to minimize the difficulty of passing through the SPE sorbent bed. In order to preserve the integrity of the sample and ensure the full volume of the container is used, see Procedure D.

2. Use a 250-mL HDPE bottle for the method blank and the laboratory control sample (LCS) and LCSD if needed. Fill each bottle with 250 mL of Milli-Q water. Record 250 mL as the volume for the batch QC samples on the batchlog.

B. Solid Phase Extraction (SPE)

1. PROPRIETY CONTENT
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15. PROPRIETY CONTENT

16. PROPRIETY CONTENT

17. Reconstitute to 1.0 mL with 100% methanol. Vortex to mix. Centrifuge 15 mL collection tubes for one full cycle.

18. Place each empty sample bottle on the top-loading balance and weigh. Record the second reading in the automated prep entry system. The prep entry system will calculate the sample weight.Record the calculated weight as the sample volume on the batchlog.

Note: The instrument lab chemist performs the next steps.

19. Transfer 400 µL of the final extract to the corresponding labeled auto-sampler vial. Add 20 µL of labeled internal standard spike to each labeled auto-sampler vial. Cap and vortex the auto-sampler vial.Samples are now ready for analysis.

20. Cap the centrifuge tube. Store the remaining centrifuged extracts at room temperature for dilution or reinjection if needed.

C. Reduced Sample Volume due to matrix interference

1. Determine the aliquot to be used for extraction (i.e.; 50 mL, 100 mL).

2. Label a clean 250 mL HDPE bottle with the associated ELLE sample number.

3. Label the appropriate number of 50 mL centrifuge tubes with the associated ELLE sample number. The number required will be determined by the volume to be used for extraction.

4. Shake/invert the sample bottle to thoroughly mix the sample before pouring aliquot(s).

5. Pour sample from original bottle into centrifuge tubes. Cap tubes and centrifuge for 5 minutes at full speed (one full cycle).

6. On a calibrated, top-loading balance, place labeled empty 250 mL PP wide-mouthed bottle.

7. Decant centrifuged sample aliquot(s) from centrifuge tube(s) to the 250 mL bottle until desired volume (weight in grams) is reached. 100 g = 100 mL, 50 g = 50 mL, etc. If the weight is exceeded, remove excess volume with a disposable pipette and discard to a waste container.

8. Add Milli-Q water to the bottle until a weight of 250 g (total of 250 mL) is reached.

9. Shake/invert several times to mix thoroughly.

10. Record the aliquot taken from the original bottle (50 mL, 100 mL) as the sample volume.

11. Extract sample beginning with Procedure B.

D. Samples Containing Dissolved and/or Settleable Solids

1. Spike sample with appropriate spikes as in Procedure B.5

2. Centrifuge the full bottle.

3. DO NOT SHAKE BOTTLE FOLLOWING THE CENTRIFUGE STEP.

4. Follow steps in Procedure B, 1 through 3.

5. Attach a 25-mL SPE reservoir to each cartridge. Decant centrifuged sample onto its respective SPE cartridge. Allow full volume to pass through each cartridge by gravity, if possible. Apply light vacuum if necessary. The drip rate should be approximately 1-2 drops per second.

6. Rinse the sample bottle with 5 mL of Milli-Q water, add the rinseate to the cartridge, and repeat.

7. Continue extraction process with Procedure B.7.

E. Preparation of non-aqueous liquid samples for analysis.

1. Determine if sample is miscible in MeOH.

   a. PROPRIETY CONTENT

   b. PROPRIETY CONTENT

   c. PROPRIETY CONTENT

d. PROPRIETY CONTENT

e. PROPRIETY CONTENT

2. PROPRIETY CONTENT

3. PROPRIETY CONTENT

4. PROPRIETY CONTENT

5. Add 25 µL of surrogate solution (ex.: SSMODX_) to the 4 mL microcentrifuge tube. Vortex to mix thoroughly.

6. Transfer a 400 µL aliquot to a 9 mm LC analysis vial.

7. Add 20 µL of internal standard (ex: IS1927633A) to the LC vial. Vortex to mix thoroughly. Sample is ready for LC/MS/MS analysis.

F. Prepare the batch QC for non-aqueous liquid samples.

1. Method Blank Preparation:

a. Pipette 975 µL of MeOH into a 4 mL microcentrifuge tube.

b. Add 25 µL of surrogate solution(ex: SSMODX_) to the 4 mL microcentrifuge tube. Vortex to mix thoroughly.

c.Transfer a 400 µL aliquot to a 9 mm LC analysis vial.

d.Add 20 µL of internal standard (ex: IS1927633A) to the LC vial. Vortex to mix thoroughly. Method Blank is ready for LC/MS/MS analysis.

2. LCS/LCSD Preparation:

a. Pipette 935 µL of MeOH into a 4 mL microcentrifuge tube.

b. Add 25 µL of surrogate solution(ex: SSMODX_) to the 4 mL microcentrifuge tube.

c. Add 40 µL of MS spiking solution (ex: MSMODWX_) to the 4 mL microcentrifuge tube.

d. Vortex to mix thoroughly.

e. Transfer a 400 µL aliquot to a 9 mm LC analysis vial.

f. Add 20µL of internal standard (ex: IS1927633A) to the LC vial.

g. Vortex to mix thoroughly. LCS is ready for LC/MS/MS analysis.

G. LC/MS/MS Analysis

1. Mass Calibration and Tuning

a. At instrument set up and installation and after the performance of major maintenance, calibrate the mass scale of the MS with calibration compounds and procedures described by the manufacturer. The entire mass range must be calibrated.

b. When masses fall outside of the ±0.5 amu of the true value, the instrument must be retuned using PPG according to the manufacturer's specifications. Mass assignments of the tuning standard must be within 0.5 amu of the true value. Refer to the instrument manufacturer's instructions for tuning and conditions. These values are stored in the tune file for future reference.

2. The mass spectral acquisition rate must include a minimum of 10 spectra scans across each chromatographic peak. See the AB Sciex (4500/5500/5500 Plus) Acquisition, Quantitation, Gradient, and detector condition files for the most up to date chromatographic conditions. Modifications to these conditions can be made at the discretion of the analyst to improve resolution or the chromatographic process.

3. Acquisition method: See PROPRIETY. Mass Transitions: See *Attachment 1*.

4. Load sample vials containing standards, quality control samples, and sample extracts into autosampler tray. Allow the instrument adequate time to equilibrate to ensure the mass spec and LC have reached operating conditions (approximately 5 minutes) before the first injection. Analyze several solvent blanks clean the instrument prior to sample acquisition.

5. After the initial calibration and when analyzing samples within the same tune, inject an instrument blank, followed by the ICV, Linear branched (L/B) standard, closing Cal3 level CCV, extraction batch QC, and samples. Bracket each set of ten samples with a CCV standard, alternating between the CAL3, CAL4, and CAL5 levels.

6. After injections are completed, check all CCV recoveries and absolute areas to make sure they are within method control limits. See Calibration section B.2 for acceptance criteria. Process each chromatogram and closely evaluate all integrations, baseline anomalies, and retention time differences. If manual integrations are performed, they must be documented and a reason given for the change in integrations. The manual integrations are documented during data processing and all original integrations are reported at the end of the sample PDF file with the reason for manual integration clearly listed.

7. Quantitate results for the extraction blank. No target analytes at or above the reporting limit may be found in the extraction blank for acceptable batch results. If a target analyte is detected in the extraction blank but not detected in the sample, the data is reported. If a target analyte is detected in the method blank at a concentration greater than the reporting limit and also in the sample, the sample must be reextracted. If the target analyte in the sample is detected at a concentration greater than 10 times the amount detected in the method blank, the data is reported.

8. Calculate the recoveries of spiked analytes for the LCS, matrix spike and matrix spike duplicate (MS/MSD) by comparing concentrations observed to the true values.

a. LCS, MS, extraction standard recoveries and RPDs are calculated and compared to the limits stored on the LIMS.

b. If LCS and/or LCSD recoveries are acceptable, proceed to sample quantitation.

c. If the LCS recoveries are above QC acceptance criteria and there are no detections for the compound(s) in the associated sample(s), the data can be reported with a qualifying comment. In all other cases,cvthe samples associated with the LCS must be reextracted.

d. If MS/MSD recoveries are outside QC acceptance criteria, the associated data will be flagged or noted in the comments section of the report.

9. Isotopically-labeled extraction standards are added to all samples, extraction blank, LCS/LCSD, and MS/MSD prior to extraction. The recovery of the extraction standards should be within QC acceptance criteria. If the extraction standard recovery(ies) is(are) outside the QC limit(s), consult a supervisor to determine the appropriate course of action based on batch and sample results.

10. Isotopically-labeled injection standards are added to each QC and field sample extract prior to analysis. The absolute areas of the injection standards should be within 50-150% of the average areas measured during the initial calibration. If the internal standards are recovered outside 50-150%, consult a supervisor to determine the appropriate course of action based on batch and sample results.

11. Compare the retention times of all of the analytes, surrogates, and internals standards to the retention time from the initial calibration. The retention times should not vary from the expected retention time by more than

a. 0.4 minutes for isotopically-labeled compounds

b. 0.1 minutes from their analog for native compounds with an exact isotopically-labeled compound

c. 0.4 minutes from their assigned analog for native compounds without an exact isotopically-labeled compound.

If the retention time is outside of the criteria, the compound is considered a false positive unless it is a compound with branched isomers. Compounds with branched isomers can vary in intensity of the individual isomers that are used for reporting and must be reviewed and compared to the preceding CCV to determine if it should be reported.

12. Two ion transitions and the ion transition ratio per analyte shall be monitored and documented with the exception those listed in the table below. The expected ion ratio for each compound is calculated by using the average of ion ratios of each compound from initial calibration standards. When an ion ratio for a compound differs from the expected ion ratio by more than 50%, a qualifier is placed on the raw data and on the sample report. No corrective action is required.

Native compounds without two ion transitions monitored.

PFO5DA

Compound
PFBA
PFPeA
PFOSA
NMePFOSAE
NMEPFOSA

Compound
NEtPFOSAE
NEtPFOSA
PFDoS
6:2 FTUCA
6:2 FTCA
8:2 FTUCA
8:2 FTCA
10:2 FTCA
10:2 FTUCA
PPFA (PFPrA)
3:3 FTCA
L-PFPrS
PFEESA
7:3 FTCA
Eve Acid
Hydro-EVE Acid
MTP
PEPA
PFECA G
PFMOAA
PFO2HxA
PFO3OA
PFO4DA
PMPA
R-EVE
Hydrolyzed PSDA
Hydro-PS acid
NVHOS
PS ACID

Compound
R-PSDA

13. The linear/branch chain standard is used when assessing the correctness of the computer generated peak integrations for PFOA.

14. If the calculated concentration exceeds the calibration range of the system, determine the appropriate dilution required and dilute the extract with MeOH. If the sample dilution required exceeds 100 fold, the client must be contacted to determine if the data can be reported with result(s) that exceed the calibration range or if the sample should be re-prepped at a reduced volume.

Dilution Example 1/10: Mix 900 µL of MeOH with 100 µL of sample extract. Vortex to mix. Using an auto-pipette, transfer 400 µL of the mixed solution into a labeled auto-sampler vial containing a plastic insert. Using an auto-pipette, add 20 µL of labeled injection standard to the 400 µL aliquot. Cap and vortex thoroughly to mix.

Calculations

A. Peak Area Ratio

$$\text{Peak Area Ratio} = \frac{\text{Analyte Response}}{\text{Labeled Analyte Response}}$$

B. On-Column Analyte Concentration using average RRF

On-column Concentration = (peak area ratio x Internal Standard concentration) ÷ AVE RRF

C. On-Column Analyte Concentration using linear through zero curves

On-column Concentration = (peak area ratio x Internal Standard concentration) ÷ slope

D. Sample Concentration

Sample concentration (ng/l) = (On-column concentration x Final Sample volume x DF) ÷ Sample weight

E. Ion Ratio

ion ration = (peak area or height of quantifier)/(peak area or height of qualifier)

F. See *T-PEST-WI9847* for additional calculations used to evaluate the calibrations and quality control samples.

Statistical Information/Method Performance

The LCS should contain all compounds of interest. LCS, MS, extraction standard recoveries and RPD are compared to the limits stored on the LIMS. These limits are statistically derived when sufficient data points are available. If sufficient data points are not available to generate statistical windows, an advisory window of 70% to 130% will be used. Historical data for MS/Ds, LCS/Ds, measurement of uncertainty, is reviewed at least annually. Reporting limits including method detection limits (MDLs) and limits of quantitation (LOQs) are set according to EPA method requirements and are evaluated annually. Refer to *QA-SOP11892* for specific guidelines and procedures. Updates to the LIMS are made as needed by the QA Department and only as directed by the supervisor.

Quality Assurance/Quality Control

For each batch of samples extracted, a method blank and an LCS/LCSD (Milli-Q water spiked with all compounds to be determined carried through the entire procedure) must be extracted and analyzed. If an MS/MSD is submitted then an LCSD would not be extracted. A batch is defined as the samples to be extracted on any given day, but not to exceed 20 field samples. If more than 20 samples are prepared in a day, an additional batch must be prepared. If any client, state, or agency has more stringent QC or batching requirements, these must be followed.

Attachment:
Attachment 1 - Mass Transitions (.doc)
Attachment 2 - Standard relationships (.docx)
Attachment 3 – PROPRIETY CONTENT
Attachment 4 - Example Certificate of Analysis (.pdf)

QA-SOP11178 Demonstrations of Capability
QA-SOP11892 Determining Method Detection Limits and Limits of Quantitation
T-PEST-WI9847 Common Equations Used During Chromatographic Analyses
T-PFAS-WI13881 Standards Management in the PFAS Laboratory
Attachment: Attachment 1 - Mass Transitions (doc)
Attachment: Attachment 2 - Standard relationships (docx)
Attachment: Attachment 3 – PROPRIETY CONTENT
Attachment: Attachment 4 - Example Certificate of Analysis (pdf)

End of document

Version history

Version	Approval	Revision information	
1	26.MAR.2021		
1.1	03.MAY.2021	Ver 1.1 fixes typo in calculations section	
1.2	14.MAY.2021	Ver 1.2 corrects CAS# for NVHOS	

Attachment 1

Mass Transitions AB Sciex 4500

Compound	Parent Ion	Daughter Ion
13C3-PFBA	216	172
13C4-PFBA	217	172
PFBA	213	169
13C5-PFPeA	268	223
PFPeA	263	219
13C3-PFBS	302	80
PFBS	299	80
PFBS (2)	299	99
13C2-4:2-FTS	329	81
4:2-FTS	327	307
4:2-FTS (2)	327	81
13C5-PFHxA	318	273
PFHxA	313	269
PFHxA (2)	313	119
PFPeS	349	80
PFPeS (2)	349	99
13C3-PFHxS	402	80
PFHxS	399	80
PFHxS (2)	399	99
13C4-PFHpA	367	322
PFHpA	363	319
PFHpA (2)	363	169
13C2-6:2-FTS	429	81
6:2-FTS	427	407
6:2-FTS (2)	427	81
PFHpS	449	80
PFHpS (2)	449	99
13C2-PFOA	415	370
13C8-PFOA	421	376
PFOA	413	369
PFOA (2)	413	169
13C4-PFOS	503	80
13C8-PFOS	507	80
PFOS	499	80
PFOS (2)	499	99
13C9-PFNA	472	427
PFNA	463	419

Attachment 1

Compound	Parent Ion	Daughter Ion
PFNA (2)	463	169
13C8-PFOSA	506	78
PFOSA	498	78
PFNS	549	80
PFNS (2)	549	99
13C2-PFDA	515	470
13C6-PFDA	519	474
PFDA	513	469
PFDA (2)	513	169
13C2-8:2-FTS	529	81
8:2-FTS	527	507
8:2-FTS (2)	527	81
d7-NMePFOSAE	623	59
NMePFOSAE	616	59
d3-NMePFOSA	515	169
NMEPFOSA	512	169
d3-NMeFOSAA	573	419
NMeFOSAA	570	419
NMeFOSAA (2)	570	483
d9-NEtPFOSAE	639	59
NEtPFOSAE	630	59
d5-NETPFOSA	531	169
NEtPFOSA	526	169
PFDS	599	80
PFDS (2)	599	99
13C7-PFUnDA	570	525
PFUnDA	563	519
PFUnDA (2)	563	169
d5-NEtFOSAA	589	419
NEtFOSAA	584	419
NEtFOSAA (2)	584	526
13C2-PFDoDA	615	570
PFDoDA	613	569
PFDoDA (2)	613	169
10:2-FTS	627	607
10:2-FTS (2)	627	81
PFDoS	699	80
PFTrDA	663	619
PFTrDA (2)	663	169
13C2-PFTeDA	715	670

Attachment 1

Compound	Parent Ion	Daughter Ion
PFTeDA	713	669
PFTeDA (2)	713	169
PFHxDA	813	769
PFHxDA (2)	813	169
PFODA	913	869
PFODA (2)	913	169
13C3-HFPODA	332	287
HFPODA	329	285
HFPODA (2)	285	169
DONA	377	251
DONA (2)	377	251
9Cl-PF3ONS	531	351
9Cl-PF3ONS (2)	531	351
11Cl-PF3OUdS	631	451
11Cl-PF3OUdS (2)	631	451
13C2-6:2 FTUCA	359	294
6:2 FTUCA	357	293
13C2-6:2 FTCA	379	294
6:2 FTCA	377	293
13C2-8:2 FTUCA	459	394
8:2 FTUCA	457	393
13C2-8:2 FTCA	479	394
8:2 FTCA	477	393
13C2-10:2 FTCA	579	494
10:2 FTCA	577	493
13C2-10:2 FTUCA	559	494
10:2 FTUCA	557	493
PPFA (PFPrA)	163	119
PFECAB	201	85
PFECAB (2)	295	201
PFECAF	229	85
PFECAF (2)	229	185
3:3 FTCA	241	177
L-PFPrS	249	99
PFECCA (PES)	279	85
PFECAA (2)	279	235
PFEESA	315	135
5:3 FTCA	341	237
5:3 FTCA (2)	339	295
7:3 FTCA	441	337

Attachment 1

Compound	Parent Ion	Daughter Ion
PFECHS	461	381
PFECHS (2)	461	99
Eve Acid	407	263
Hydro-EVE Acid	427	283
MTP	175	97
PEPA	279	235
PFECA G	379	185
PFMOAA	179	85
PFO2HxA	245	85
PFO3OA	311	85
PFO4DA	377	85
PFO5DA	443	85
PMPA	229	185
R-EVE	405	217
Hydrolyzed PSDA	439	343
Hydro-PS acid	463	263
NVHOS	297	135
PS ACID	443	147
R-PSDA	441	241

Attachment 2

PFAS Injection Standards/Extraction Standards/Native Compounds

Injection Standards

Inj Std	Internal Standard/Injection Standard
I13C3-PFBA	13C3-PFBA
I13C2-PFOA	13C2-PFOA
I13C4-PFOS	13C4-PFOS
I13C2-PFDA	13C2-PFDA

Extraction Standards

Extraction Standard	Internal Standard
E13C4-PFBA	13C3-PFBA
E13C5-PFPeA	
E13C3-PFBS	
E13C2-4:2-FTS	13C2-PFOA
E13C5-PFHxA	
E13C3-PFHxS	
E13C4-PFHpA	
E13C2-6:2-FTS	
E13C8-PFOA	
13C3-HFPODA	
13C2-6:2-FTCA	
13C2-6:2-FTUCA	
E13C8-PFOS	13C4-PFOS
E13C9-PFNA	

Extraction Standard	Internal Standard
E13C8-PFOSA	13C2-PFDA
E13C6-PFDA	
E13C2-8:2-FTS	
Ed7-NMePFOSAE	
Ed3-NMePFOSA	
Ed3-NMeFOSAA	
Ed9-NEtPFOSAE	
Ed5-NEtPFOSA	
E13C7-PFUnDA	
Ed5-NEtFOSAA	
E13C2-PFDoDA	
E13C2-PFTeDA	
13C2-10:2-FTCA	
13C2-10:2-FTUCA	
13C2-8:2-FTCA	
13C2-8:2-FTUCA	

Native PFAS Compounds

Native	Extraction Standard
PFBA	13C4-PFBA
PFECA F	
PFPrS	
EVE Acid	
Hydro-EVE Acid	
MTP	
PEPA	
PFECA G	
PFMOAA	
PFO2HxA	
PFO3OA	
PFO4DA	
PFO5DA	
PFPrA	
PMPA	
R-EVE	
PFPeA	13C5-PFPeA

Native	Extraction Standard
3:3 FTCA	
PFBS	13C3-PFBS
PFPeS	
Hydrolyzed PSDA	
Hydro-PS Acid	
NVHOS	
PES	
PFECA A	
PFECA B	
PS Acid	
R-PSDA	
R-PSDCA	
4:2-FTS	13C2-4:2-FTS
PFHxA	13C5-PFHxA
PFHxS	13C3-PFHxS
PFHpS	
PFECHS	
PFHpA	13C4-PFHpA
DONA	
5:3 FTCA	
6:2-FTS	13C2-6:2-FTS
PFOA	13C8-PFOA
PFOS	13C8-PFOS
PFNS	
PFDS	
9Cl-PF3ONS	
11CL-PF3OUdS	
PFDoS	
PFNA	13C9-PFNA
PFOSA	13C8-PFOSA
PFDA	13C6-PFDA
8:2-FTS	13C2-8:2-FTS
10:2-FTS	
NMePFOSAE	d7-NMePFOSAE
NMePFOSA	d3-NMePFOSA
NMeFOSAA	d3-NMeFOSAA
NEtPFOSAE	d9-NEtPFOSAE

Native	Extraction Standard
NEtPFOSA	d5-NEtPFOSA
PFUnDA	13C7-PFUnDA
NEtFOSAA	d5-NEtFOSAA
PFDoDA	13C2-PFDoDA
PFTrDA	
PFTeDA	13C2-PFTeDA
PFHxDA	
PFODA	
HFPODA	13C3-HFPODA
10:2 FTCA	13C2-10:2-FTCA
10:2 FTUCA	13C2-10:2-FTUCA
6:2 FTCA	13C2-6:2-FTCA
7:3 FTCA	
6:2 FTUCA	13C2-6:2-FTUCA
8:2 FTCA	13C2-8:2-FTCA
8:2 FTUCA	13C2-8:2-FTUCA

ST1927333A

WELLINGTON LABORATORIES

CERTIFICATE OF ANALYSIS
DOCUMENTATION

PFAC-MXC

Native Perfluorinated Compound Solution/Mixture

PRODUCT CODE:	PFAC-MXC
LOT NUMBER:	PFACMXC0617
SOLVENT(S):	Methanol / Water (<1%)
DATE PREPARED: (mm/ddlyyyy)	06/14/2017
LAST TESTED: (mm/dd/yyyy)	03/19/2019
EXPIRY DATE: (mmldd/yyyy)	03/19/2024
RECOMMENDED STORAGE:	Store ampoule in a cool, dark place

DESCRIPTION:

PFAC-MXC is a solution/mixture of thirteen native perfluoroalkylcarboxylic acids (C_4-C_{14}, C_6 and $C_{\ }$) and eight native perfluoroalkylsulfonates (C_4-$C_{\ }$ and C,J The full name, abbreviation and concentration for each of the components are given in Table A.

The individual perfluoroalkylcarboxylic acids and perfluoroalkylsulfonates all have chemical purities of >98%.

DOCUMENTATION/ DATA ATTACHED:

Table A: Components and Concentrations of the Solution/Mixture
Figure 1: LC/MS Data (SIR)
Figure 2: LC/MS/MS Data (Selected MRM Transitions)

ADDITIONAL INFORMATION:

See page 2 for further details.
Contains 4 mole eq. of NaOH to prevent conversion of the carboxylic acids to their respective methyl esters.

FOR LABORATORY USE ONLY: NOT FOR HUMAN OR DRUG USE

INTENDED USE:

The products prepared by Wellington Laboratories Inc. are for laboratory use only. This certified reference material (CRM) was designed to be used as a standard for the identification and/or quantification of the specific chemical compounds it contains.

HANDLING:

This product should only be used by qualified personnel familiar with its potential hazards and trained in the handling of hazardous chemicals. Due care should be exercised to prevent unnecessary human contact or ingestion. All procedures should be carried out in a well-functioning fume hood and suitable gloves, eye protection, and clothing should be worn at all times. Waste should be disposed of according to national and regional regulations. Safety Data Sheets (SDSs) are available upon request.

SYNTHESIS / CHARACTERIZATION:

Our products are synthesized using single-product unambiguous routes whenever possible. They are then characterized, and their structures and purities confirmed, using a combination of the most relevant techniques, such as NMR, GC/MS, LC/MS/MS, SFC/UV/MS/MS, x-ray crystallography, and melting point. Isotopic purities of mass-labelled compounds are also confirmed using HRGC/HRMS and/or LC/MS/MS.

HOMOGENEITY:

Prior to solution preparation, crystalline material is tested for homogeneity using a variety of techniques (as stated above) and its solubility in a given diluent is taken into consideration. Duplicate solutions of a new product are prepared from the same crystalline lot and, after the addition of an appropriate internal standard, they are compared by GC/MS, LC/MS/MS, and/or SFC/UV/MS/MS. The relative response factors of the analyte of interest in each solution are required to be <5% RSD. New solution lots of existing products, as well as mixtures and calibration solutions, are compared to older lots in a similar manner. This further confirms the homogeneity of the crystalline material as well as the stability and homogeneity of the solutions in the storage containers. In order to maintain the integrity of the assigned value(s), and associated uncertainty, the dilution or injection of a subsample of this product should be performed using calibrated measuring equipment.

UNCERTAINTY:

The maximum combined relative standard uncertainty of our reference standard solutions is calculated using the following equation:

The combined relative standard uncertainty, uc(y), of a value y and the uncertainty of the independent parameters $x_1, x_2 \ldots x_n$ on which it depends is:

$$ur(y(x_1,x_2,\ldots x_n)) = \sqrt{\sum_{i=1} u(y,x_i)^2}$$

where x is expressed as a relative standard uncertainty of the individual parameter.

The individual uncertainties taken into account include those associated with weights (calibration of the balance) and volumes (calibration of the volumetric glassware). An expanded maximum combined percent relative uncertainty of ±5% (calculated with a coverage factor of 2 and a level of confidence of 95%) is stated on the Certificate of Analysis for all of our products.

TRACEABILITY:

All reference standard solutions are traceable to specific crystalline lots. The microbalances used for solution preparation are regularly calibrated by an external ISO/IEC 17025 accredited laboratory. In addition, their calibration is verified prior to each weighing using calibrated external weights traceable to an ISO/IEC 17025 accredited laboratory. All volumetric glassware used is calibrated, of Class **A** tolerance, and traceable to an ISO/IEC 17025 accredited laboratory. For certain products, traceability to international interlaboratory studies has also been established.

EXPIRY DATE / PERIOD OF VALIDITY:

Ongoing stability studies of this product have demonstrated stability in its composition and concentration, until the specified expiry date, in the unopened ampoule. Monitoring for any degradation or change in concentration of the listed analyte(s) is performed on a routine basis.

I.JMITED WARRANTY:

At the time of shipment, all products are warranted to be free of defects in material and workmanship and to conform to the stated technical and purity specifications.

QUALITY MANAGEMENT:

This product was produced using a Quality Management System registered to the latest versions of ISO 9001 by SAi Global, ISO/IEC 17025 by the Canadian Association for Laboratory Accreditation Inc. (CALA; A 1226), and ISO 17034 by ANSI-ASQ National Accreditation Board **(ANAB;** AR-1523).

CALA

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ACCREDITED

REFERENCE MATERIAL PRODUCER

**For additional information or assistance concerning this or any other products from Wellington Laboratories Inc.,

Table A: **PFAC-MXC; Components and Concentrations (ng/ml, ± 5% in Methanol/ Water (<1%))**

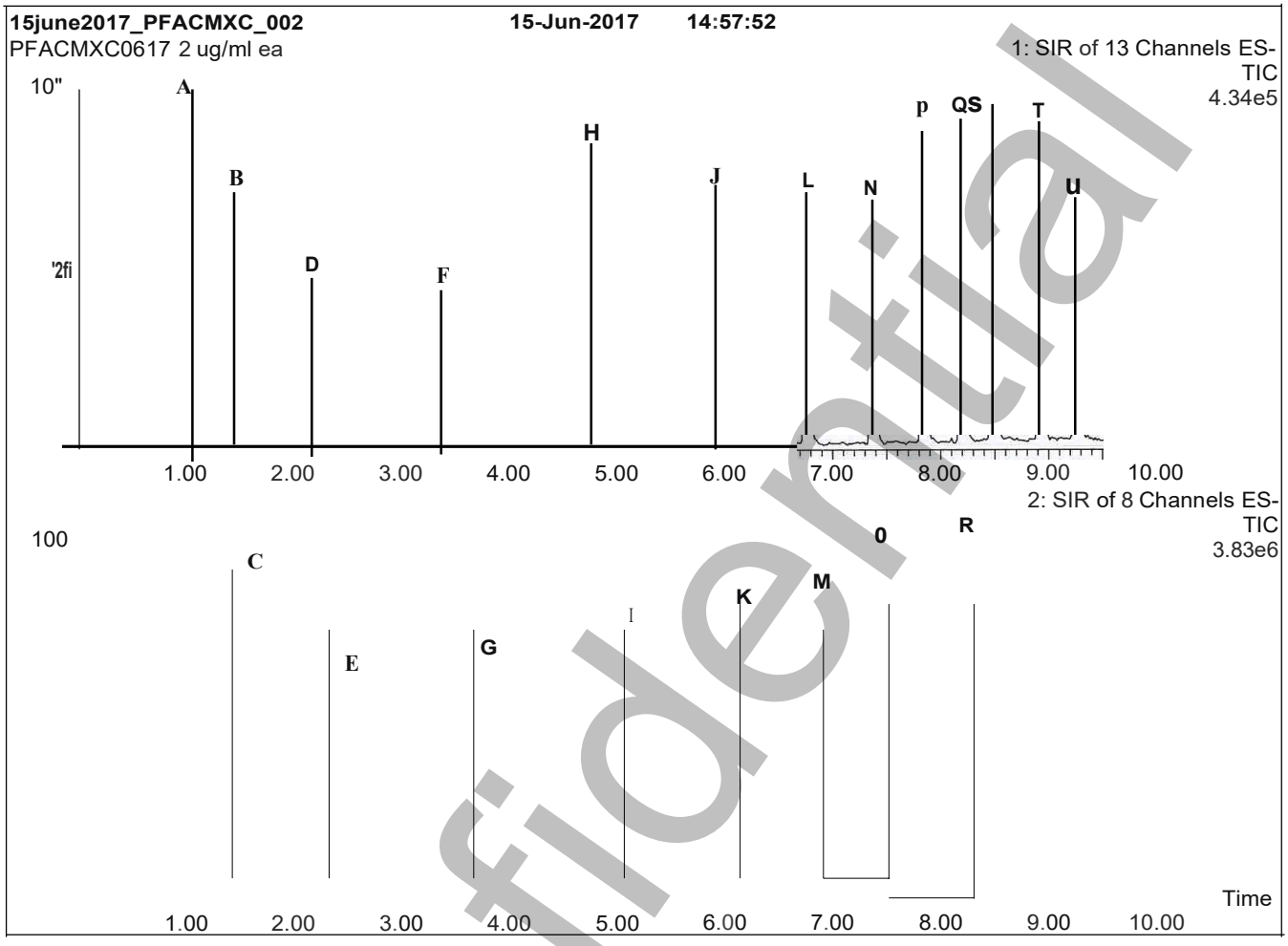
Compound	Abbreviation	Concentration (ng/ml)*		Peak Assignment in Figure 1
Perfluoro-n-butanoic acid	PFBA	2000		A
Perfluoro-n-pentanoic acid	PFPeA	2000		B
Perfluoro-n-hexanoic acid	PFHxA	2000		D
Perfluoro-n-heptanoic acid	PFHpA	2000		F
Perfluoro-n-octanoic acid	PFOA	2000		**H**
Perfluoro-n-nonanoic acid	PFNA	2000		J
Perfluoro-n-decanoic acid	PFDA	2000		L
Perfluoro-n-undecanoic acid	PFUdA	2000		**N**
Perfluoro-n-dodecanoic acid	PFDoA	2000		P
Perfluoro-n-tridecanoic acid	PFTrDA	2000		Q
Perfluoro-n-tetradecanoic acid	PFTeDA	2000		**S**
Perfluoro-n-hexadecanoic acid	PFHxDA	2000		**T**
Perfluoro-n-octadecanoic acid	PFODA	2000		**U**
Compound	**Abbreviation**	**Concentration (ng/ml)***		**Peak Assignment in Figure 1**
		As the salt	**As the anion**	
Potassium perfluoro-1-butanesulfonate	L-PFBS	2000	1770	C
Sodium perfluoro-1-pentanesulfonate	L-PFPeS	2000	1880	E
Sodium perfluoro-1-hexanesulfonate	L-PFHxS	2000	1890	G
Sodium perfluoro-1-heptanesulfonate	L-PFHpS	2000	1900	I
Sodium perfluoro-1-octanesulfonate	L-PFOS	2000	1910	K
Sodium perfluoro-1-nonanesulfonate	L-PFNS	2000	1920	**M**
Sodium perfluoro-1-decanesulfonate	L-PFDS	2000	1930	0
Sodium perfluoro-1-dodecanesulfonate	L-PFDoS	2000	1940	R

* Concentrations have been rounded to three significant figures.

Certified By: _____ **Date:** 06/06/2019
(mm/dd/yyyy)

B.G. Chittim, General Manager

Fi.9yr!L1 **PFAC-MXC; LC/MS Data (Total Ion Current Chromatogram; SIR)**



Conditions for Figure 1:

LC: **Waters Acquity Ultra Performance LC**
MS: **Micromass Quattro *micro* API MS**

Chromatographic Conditions

Column: Acquity UPLC BEH Shield RP.,
1.7 µm, 2.1 x 100 mm

Mobile phase: Gradient
Start: 50% H,O / 50% (80:20 MeOH:ACN)
(both with 10 mM NH,OAc buffer)
Ramp to 90% organic over 8 min and hold for 2 min
before returning to initial conditions in 1 min.

Time: 12 min

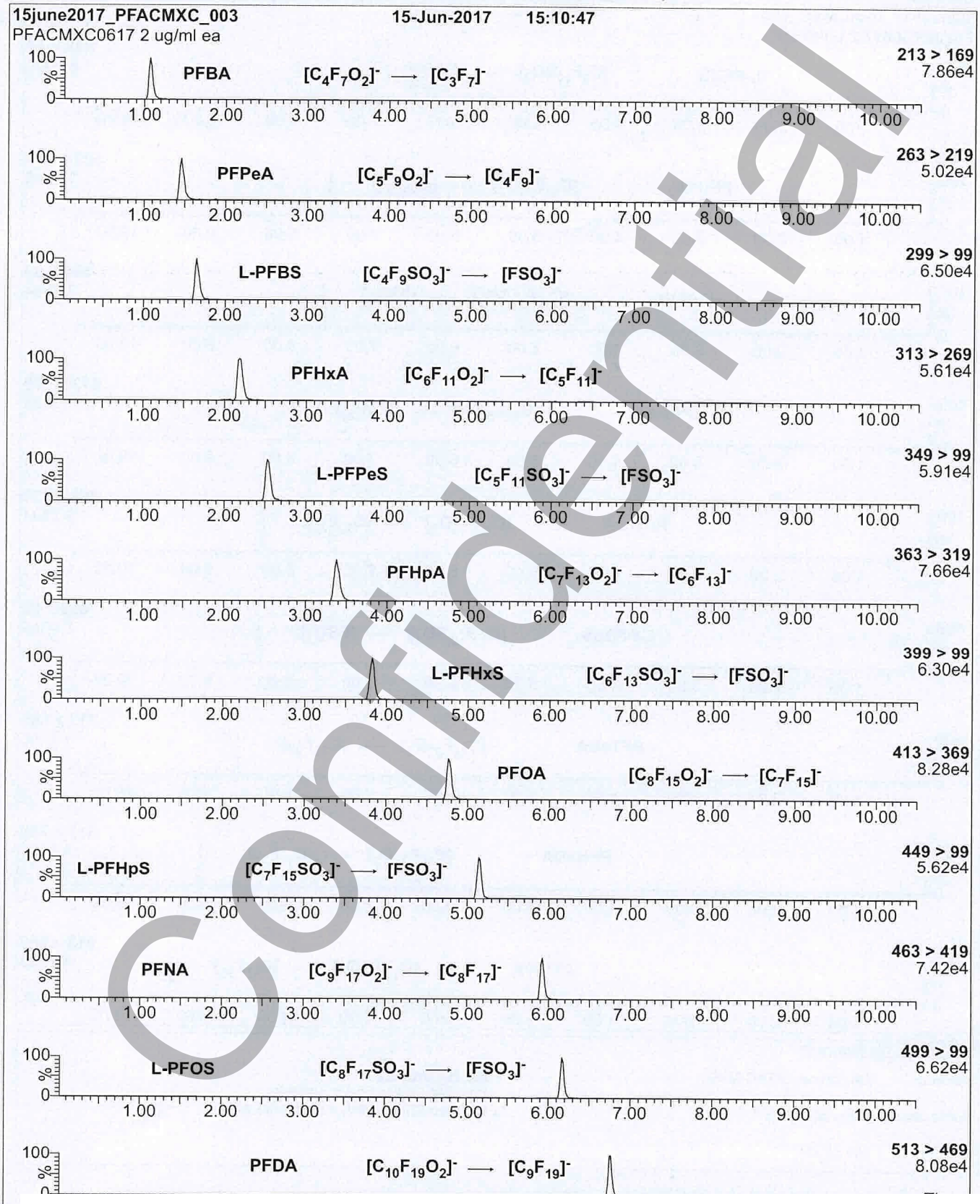
Flow: 300 µl/min

MS Parameters

Experiment: SIR of 21 Channels

Source: Electrospray (negative)
Capillary Voltage (kV) = 3.00
Cone Voltage (V) = variable (10-80)
Cone Gas Flow (l/hr) = 50
Desolvation Gas Flow (l/hr) = 750

Figure 2: **PFAC-MXC; LC/MS/MS Data (Selected MRM Transitions)**



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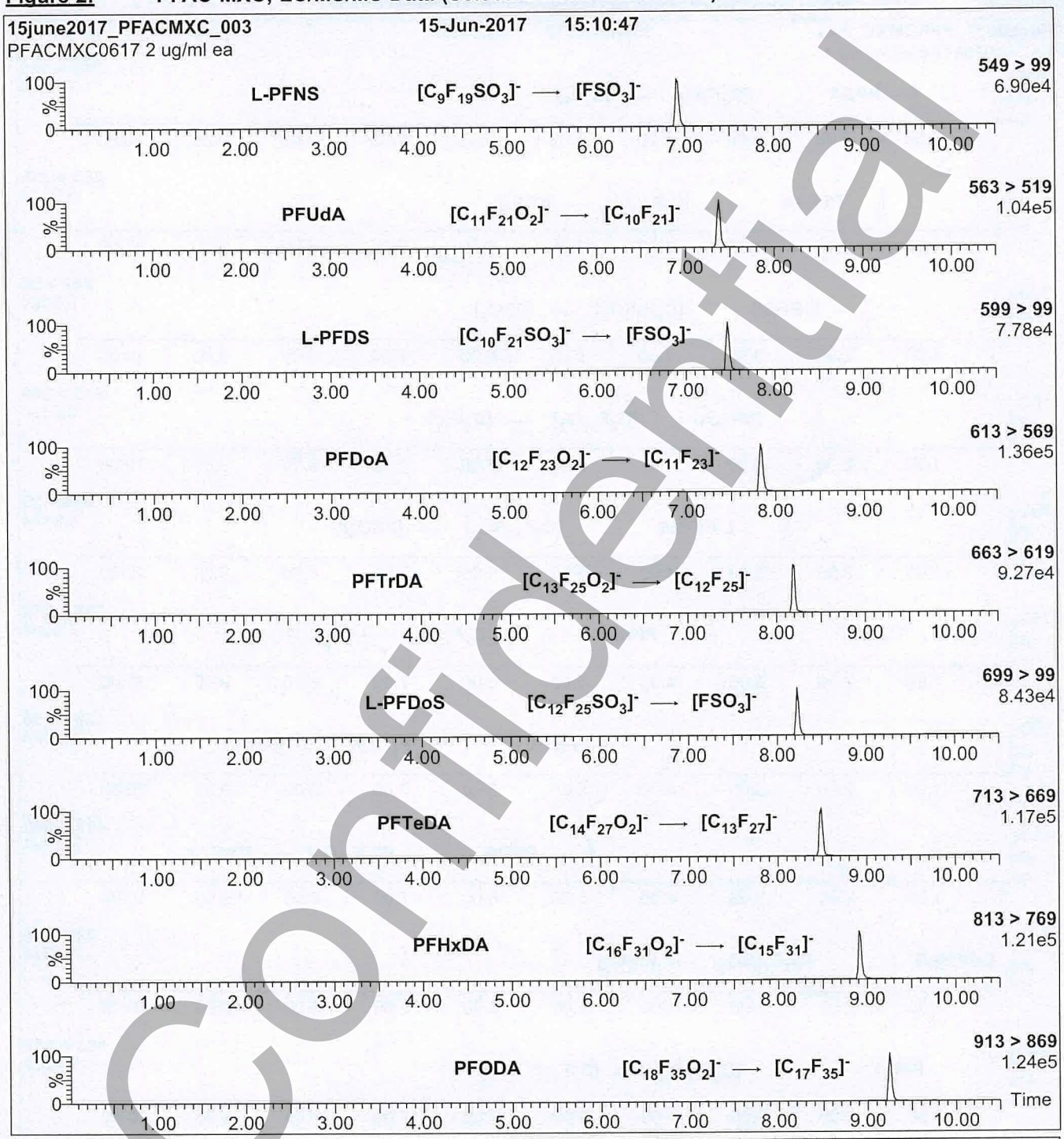
Re

Figure 2: **PFAC-MXC; LC/MS/MS Data (Selected MRM Transitions)**



Conditions for Figure 2:

Injection: On-column (PFAC-MXC)

Mobile phase: Same as Figure 1

Flow: 300 µl/min

MS Parameters

Collision Gas (mbar) = 3.46e-3

Collision Energy (eV) = 8-50 (variable)