eurofins	Always check on-line for validity. **Client Specific: Table 3 Compounds by Direct Injection Using LC/MS/MS**	Level: **Work Instruction**
Document number: **T-PFAS-WI20127**		
Old Reference:		
Version: **2**		Organisation level: **5-Sub-BU**
Approved by: **UKL3** Effective Date **12-APR-2019**	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**

LIMS ID
14675

Revision Log

Revision 2		**Effective Date:** This version
Section	**Justification**	**Changes**
Revision Log	Formatting requirement	Removed revision logs up to the previous version
Throughout document	Update requirements	Add additional compounds requested by the client throughout the document to reflect current practice.

Revision 1		**Effective Date:** **27-SEP-2018**
Section	**Justification**	**Changes**

Revision	1	Effective Date:	27-SEP-2018
		new sop	

Reference

1. Determination of Table 3 Compounds by LC/MS/MS, Chemours Fluoroproducts Analytical Method Draft, A. Petlick edit of J.Boyle, 1/8/2018.

2. *Chemical Hygiene Plan*, current version.

Cross Reference

Document	Document Title
T-PEST-WI9847	Common Equations Used During Chromatographic Analyses
QA-SOP11892	Determining Method Detection Limits and Limits of Quantitation

Scope

This method is applicable for the determination of selected perfluoroethercarboxylic acids (PFECA) in aqueous samples to include potable and non-potable waters. 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#
TAF n=4 $C_7HF_{13}O_7$ (aka PFO5DoA)	TAFN4	39492-91-6
PFESA Byproduct 1 $C_7HF_{13}O_5S$	PFESA-B1	29311-67-9
PFO4DA $C_6HF_{11}O_6$	PFO4DA	39492-90-5
PFO2HxA $C_4HF_7O_4$	PFO2HXA	39492-88-1
PFESA Byproduct 2 $C_7H_2F_{14}O_5S$	PFESA-B2	749836-20-2
PFECA-G $C_7HF_{13}O_3$	PFECA-G	801212-59-9
PFO3OA $C_5HF_9O_5$	PFO3OA	39492-89-2
PFMOAA $C_3HF_5O_3$	PFMOAA	674-13-5
FRD903 HFPODA $C_6HF_{11}O_3$	HFPODA	13252-13-6
PEPA	PEPA	267239-61-2
PMPA $C_4HF_7O_3$	PMPA	13140-29-9
DFSA $C_2H_2F_2O_5S$	DFSA	422-67-3
MMF $C_3H_2F_2O_4$	MMF	1514-85-8

MTP $C_4H_4F_4O_3$	MTP	93449-21-9
PPF Acid $C_3HF_5O_2$	PPF Acid	422-64-0
R-EVE $C_8H_2F_{12}O_5$	R-EVE	NA
Byproduct 4 $C_7H_2F_{12}O_6S$	Byproduct 4	NA
Byproduct 5 $C_7H_3F_{11}O_7S$	Byproduct 5	NA
NVHOS $C_4H_2F_8O_4S$	NVHOS	1132933-86-8
PFECA B $C_5HF_9O_4$	PFECA B	151772-58-6
PES $C_4HF_9O_4S$	PES	113507-82-7
Hydro-EVE Acid $C_8H_2F_{14}O_4$	Hydro-EVE Acid	773804-62-9
EVE Acid $C_8HF_{13}O_4$	EVE Acid	69087-46-3
Byproduct 6 $C_6H_2F_{12}O_4S$	Byproduct 6	NA

Basic Principles
A 5-mL aqueous sample is diluted 1:1 with methanol followed by analysis by LC/MS/MS operated in negative electrospray ionization (ESI) mode for detection and quantification of the analytes. Quantitative analysis is performed using external standard calibration.

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. A precolumn, Phenomenex Luna, 30 x 2 mm, 5 µm C18 column, is installed before the injection valve to separate PFAS in standards/samples from those from the LC system and mobile phases.

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.

Sample Collection, Preservation, and Handling

A. Sample Collection

The samples are collected in polypropylene bottles as per the client's sample collection protocols for submission to the laboratory for analysis.

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 analyzed within 14 days.

Apparatus and Equipment

A. Apparatus

1. 250ml HDPE bottles: Scientific Specialties; # 334008-blk-1, or equivalent.

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

3. 10-mL polypropylene volumetric flask, class B – BrandTech Scientific, Inc., Cat. No. V677941 or equivalent.

4. Polypropylene bottles for reagent storage: 1000ml, Fisher; Cat. No. 02896F.

5. Analytical Balance – Capable of weighing to 0.0001 g

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

7. Centrifuge – “Q-Sep 3000”; Restek Corp. Cat. No. 26230, or equivalent, capable of a minimum rotational speed of 3000 rpm.

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

9. Auto Pipettes – Eppendorf; capable of accurately dispensing 10ul – 1000ul.

10. Polypropylene pipette tips: 0-200ul. Fisher; Cat. No. 02-681-135

11. Polypropylene pipette tips: 101-1000ul. Fisher, Cat. No. 02-707-508

12. Pipettes – Disposable transfer. Fisher Scientific, Cat. No. 13-711-7M

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

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

15. Thermo Target PP Polyspring inserts, catalog number C4010-630P

16. Waters 9mm vial kit pack with cap and PTFE/Sil Septa, catalog number 16005660CV, or equivalent

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

18. Syringes- Hamilton #80400 - 25 ul, #80500 - 50 ul, #80600 - 100 ul, #80700 - 250 ul, #80800 - 500ul.

19. Stainless steel spatula/scoop set. Bel-Art SP Scienceware; Product # 11-865-130.

20. Syringe filter - Acrodisc, Syringe Filter, GHP, 13 mm, 0.2 µm, Aqueous, 100/pkg ,Part # WAT097962.

21. EMD Millipore MColorpHast pH test strips and indicator paper, Fisher Scientific, Cat# M1095350001.

B. Equipment

1. AB Sciex Triple Quad 4500 Turbo V Ion Source

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

2. AB Sciex API 4000 Turbo V Ion Source

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

3. HPLC columns

a. Analytical column-Gemini 3µm C18, 50 x 3 mm, Phenomenex Cat# 00B-4439-YO or equivalent

b. Analytical column-Gemini 3µm C18, 100 x 3 mm, Phenomenex Cat# 00D-4439-YO or equivalent

c. Pre-column- Luna, 5 um C18, 30 x 2 mm, Phenomenex Cat# 00A-4252-B0, or equivalent

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:

1. Methanol (MeOH) – Honeywell Burdick and Jackson "Chromasolv LC-MS" grade or equivalent

2. Acetonitrile (ACN) – Fisher Scientific, Optima or equivalent

3. Ammonium acetate – HPLC grade or equivalent

4. 20 mM ammonium acetate solution – Weigh 1.54 ± 0.01g ammonium acetate into a 1L glass bottle. Add 1 L Milli Q water and mix well. The solution is prone to volatility losses and is replaced weekly. Store at room temperature

5. 50:50 Methanol:water for preparing sample dilutions.

6. Sulfuric acid(H_2SO_4), 1N(0.5M), Acros Organics(part number 124240010

7. Potassium hydroxide(KOH) (8N): Acros Organics; 380625000

8. Working KOH solution for pH adjustment (0.1M): In a 100ml volumetric flask add approximately 75ml of MQ water. Add 1.25ml 8N KOH to the flask and bring to final volume of 100ml with MQ water. Stable 6 months. Store at room temp.

9. Working H_2SO_4 solution for pH adjustment (0.1M): In a 100ml volumetric flask add approximately 50ml of MQ water. Add 20ml 0.5M H_2SO_4 to the flask and bring to final volume 100ml with MQ water. Stable 6 months. Store at room temp.

B. CalibrationStandards:

Standards are stored in accordance with the manufacturer recommended storage conditions and expiration dates. Intermediate and calibration standards are stored at room temperature and expire after 3 months.

1. Calibration and spiking standards are prepared from standard solutions received from the client (0.1% by weight in water) with the exception of HFPODA (Wellington Catalogue # HFPO-DA) which is purchased as a 50,000 ppb stock solution.

Parent Solution	Analyte
Client Provided/ Commercial	DFSA
	MMF
	MTP
	PPF Acid
	PFMOAA
	BP4
	R-EVE
	BP5
	PMPA
	NVHOS
	PFO2HXA
	PEPA
	PES

Parent Solution	Analyte
	PFECA-B
	PFO3OA
	Hydro-EVE Acid
	BP6
	PFESA-BP2
	PFECA-G
	PFO4DA
	PFESA-BP1
	EVE Acid
	TAFN4 (PFO5DoA)
	HFPODA

2. Prepare 10,000 ppb intermediate A in water:

Compound	Conc. (ppb)	Init. Vol. (mL)	Final Vol. (mL)	Intermediate A Conc. (ppb)
DFSA	1 000 000	0.1	10	10 000
MMF	1 000 000	0.1	10	10 000
MTP	1 000 000	0.1	10	10 000
PPF Acid	1 000 000	0.1	10	10 000
PFMOAA	1 000 000	0.1	10	10 000
BP4	1 000 000	0.1	10	10 000
R-EVE	1 000 000	0.1	10	10 000
BP5	1 000 000	0.1	10	10 000
PMPA	1 000 000	0.1	10	10 000
NVHOS	1 000 000	0.1	10	10 000
PFO2HXA	1 000 000	0.1	10	10 000
PEPA	1 000 000	0.1	10	10 000
PES	1 000 000	0.1	10	10 000
PFECA-B	1 000 000	0.1	10	10 000
PFO3OA	1 000 000	0.1	10	10 000
Hydro-EVE Acid	1 000 000	0.1	10	10 000
BP6	1 000 000	0.1	10	10 000
PFESA-BP2	1 000 000	0.1	10	10 000
PFECA-G	1 000 000	0.1	10	10 000
PFO4DA	1 000 000	0.1	10	10 000
PFESA-BP1	1 000 000	0.1	10	10 000
EVE Acid	1 000 000	0.1	10	10 000
TAFN4 (PFO5DoA)	1 000 000	0.1	10	10 000

3. Prepare 100 ppb Intermediate B in water:

Compound	Intermediate A/HFPODA stock Conc. (ppb)	Init. Vol. (mL)	Final Vol. (mL)	Intermediate B Conc. (ppb)
DFSA	10 000	0.1	10	100
MMF	10 000	0.1	10	100
MTP	10 000	0.1	10	100
PPF Acid	10 000	0.1	10	100
PFMOAA	10 000	0.1	10	100
BP4	10 000	0.1	10	100
R-EVE	10 000	0.1	10	100
BP5	10 000	0.1	10	100
PMPA	10 000	0.1	10	100
NVHOS	10 000	0.1	10	100
PFO2HXA	10 000	0.1	10	100
PEPA	10 000	0.1	10	100
PES	10 000	0.1	10	100
PFECA-B	10 000	0.1	10	100
PFO3OA	10 000	0.1	10	100
HFPODA	50 000	0.02	10	100
Hydro-EVE Acid	10 000	0.1	10	100
BP6	10 000	0.1	10	100
PFESA-BP2	10 000	0.1	10	100
PFECA-G	10 000	0.1	10	100
PFO4DA	10 000	0.1	10	100
PFESA-BP1	10 000	0.1	10	100
EVE Acid	10 000	0.1	10	100
TAFN4 (PFO5DoA)	10 000	0.1	10	100

4. Prepare 1 ppb Intermediate C in methanol:water(1:1):

Compound	Intermediate B Conc. (ppb)	Init. Vol. (mL)	Final Vol. (mL)	Intermediate C Conc. (ppb)
DFSA	100	0.1	10	1
MMF	100	0.1	10	1
MTP	100	0.1	10	1
PPF Acid	100	0.1	10	1
PFMOAA	100	0.1	10	1
BP4	100	0.1	10	1
R-EVE	100	0.1	10	1

Compound	Intermediate B Conc. (ppb)	Init. Vol. (mL)	Final Vol. (mL)	Intermediate C Conc. (ppb)
BP5	100	0.1	10	1
PMPA	100	0.1	10	1
NVHOS	100	0.1	10	1
PFO2HXA	100	0.1	10	1
PEPA	100	0.1	10	1
PES	100	0.1	10	1
PFECA-B	100	0.1	10	1
PFO3OA	100	0.1	10	1
HFPODA	100	0.1	10	1
Hydro-EVE Acid	100	0.1	10	1
BP6	100	0.1	10	1
PFESA-BP2	100	0.1	10	1
PFECA-G	100	0.1	10	1
PFO4DA	100	0.1	10	1
PFESA-BP1	100	0.1	10	1
EVE Acid	100	0.1	10	1
TAFN4 (PFO5DoA)	100	0.1	10	1

5. Prepare Calibration Standards in methanol:water (1:1)

		CAL8		CAL7	CAL6	CAL5	CAL4	CAL3	CAL2	CAL1
	Initial Volume Intermediate B (mL)	0.5	Initial Volume Intermediate C (mL)	0.1	0.05	0.025	0.01	0.005	0.002	0.001
	Final Volume (mL)	10	Final Volume (mL)	10	10	10	10	10	10	10
Compound	Intermediate B Conc. (ppb)	CAL8	Intermediate C Conc. (ppb)	CAL7	CAL6	CAL5	CAL4	CAL3	CAL2	CAL1
DFSA	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001
MMF	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001
MTP	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001
PPF Acid	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001
PFMOAA	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001
BP4	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001
R-EVE	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001
BP5	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001

PMPA	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001
NVHOS	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001
PFO2HXA	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001
PEPA	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001
PES	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001
PFECA-B	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001
PFO3OA	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001
HFPODA	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001
Hydro-EVE Acid	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001
BP6	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001
PFESA-B2	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001
PFECA-G	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001
PFO4DA	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001
PFESA-B1	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001
EVE Acid	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001
TAFN4 (PFO5DoA)	100	0.5	1	0.1	0.05	0.025	0.01	0.005	0.002	0.001

C. Native spiking solution

Spiking standards are stored at room temperature and expire after 3 months.

Working Native Spike Solution is prepared by placing 0.25 mL of Intermediate B from standard preparation into a 10 mL volumetric flask and bringing to volume with Milli-Q water.

Analyte	**Native Spike Solution Concentration (ppb)**
DFSA	2.5
MMF	2.5
MTP	2.5
PPF Acid	2.5
PFMOAA	2.5
BP4	2.5
R-EVE	2.5
BP5	2.5
PMPA	2.5
NVHOS	2.5
PFO2HXA	2.5
PEPA	2.5
PES	2.5
PFECA-B	2.5
PFO3OA	2.5
Hydro-EVE Acid	2.5

BP6	2.5
PFESA-BP2	2.5
PFECA-G	2.5
PFO4DA	2.5
PFESA-BP1	2.5
EVE Acid	2.5
TAFN4 (PFO5DoA)	2.5

Calibration

A. Initial Calibration

1. Calibration standards are prepared at 0.001 ppb, 0.002 ppb, 0.005 ppb, 0.01 ppb, 0.025 ppb, 0.05 ppb, 0.1 ppb, and 0.5 ppb.

2. Fit the curve with a linear through zero or linear with a concentration weighing factor of 1/x or quadratic regression with a concentration weighing factor $1/x^2$.

3. Initial calibration acceptance criteria

The R^2 value for each calibration curve must be ≥ 0.99 for each analyte.

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.

4. Initial Calibration Verification (ICV)

2nd source standards are not available. A separately prepared standard at a final concentration of 0.05 ppb is analyzed as the ICV. The calculated amount for each analyte should be ± 30% of the true value.

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 0.01 ppb level.

2. Acceptance criteria

The calculated amount for each compound in the CCV standard must be within ±30% of the true value. Samples that are not bracketed by acceptable CCV analyses must be reanalyzed.If CCV fails, a new initial calibration will be analyzed.

Procedure

A. Sample Preparation

1. Check pH of the sample with pH paper to verify sample pH is 6-8. If necessary, use dilute KOH or H_2SO_4 to adjust pH in the sample container.

2. Using auto pipette, pipet 5 ml of MQ water(blank) or water sample into 15 ml centrifuge tube.

3. Using auto pipette, add 5 ml of methanol to sample tube. Use vortex to mix. The resulting solution is a 2X dilution of the sample.

4. Load 3 ml plastic syringe with about 2 ml of diluted sample. Attach GHP syringe filter. Place filter over opening of vial insert and filter about 0.200 ml into insert.

5. Cap autosampler vial and submit for LC/MS/MS analysis.

Dilution of target analytes is required when the calculated concentration exceeds the calibration range of the system. See example below.

100X dilution example:

a) Using auto pipette, add 9.8 ml of 50:50 methanol water to a 15 ml centrifuge tube.

b) Add 0.200 ml of the 2X sample dilution.

c) Use vortex to mix. T

d) The resulting solution is a 100X dilution of the sample. Complete sample preparation with steps 4 and 5 above.

B. Matrix Spike/LCS Preparation.

1. Check pH of the sample with pH paper to verify sample pH is 6-8. If necessary, use dilute KOH or H_2SO_4 to adjust pH in the sample container.

2. Using auto pipette, pipet 5 ml of MQ water(LCS) or water sample(MS) into 15 ml centrifuge tube.

3. Add 0.05 ml of the native spiking solution.

4. Using auto pipette, add 5 ml of methanol to sample tube. Use vortex to mix. The resulting solution is a 2X dilution of the sample.

5. Load 3 ml plastic syringe with about 2 ml of diluted sample. Attach GHP syringe filter. Place filter over opening of vial insert and filter about 0.200 ml into insert.

6. Cap autosampler vial and submit for LC/MS/MS analysis.

B. LC/MS/MS Analysis

1. Tuning and Chromatographic conditions for LC/MS/MS Analysis

Refer to the instrument manufacturer's instructions for tuning and conditions. These values are stored in the tune file for future reference and may not need to be changed unless loss of response is noted.

See the AB Sciex (4000 / 4500) 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.

2. Acquisition method: See attachment I

3. 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 and allow it to stabilize.

4. After the initial calibration, inject a solvent blank, followed by the CCV at 0.1 ppb, and samples. Bracket each set of ten samples with a CCV standard. CAL3, CAL4 and CAL5 are alternated.

5. 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.

6. Quantitate results for the method blank. No target analytes at or above the reporting limit may be found in the method blank for acceptable batch results. If a target analyte is detected in the method 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 reprepared. 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.

7. Calculate the recoveries of spiked analytes for the LCS, and matrix spike (MS) by comparing concentrations observed to the true values. The QC acceptance limits for LCS and MS recovery are 70 to 130% for each analyte. The QC acceptance limit for the relative percent difference (%RPD) between unspiked sample and the duplicate sample is ≤25% at mid and high range concentrations and ≤50% at the minimum reporting limit . If LCS recoveries are acceptable, proceed to sample quantitation. If the LCS recoveries are unacceptable, the samples associated with the LCS may need to be reanalyzed. If LCS recoveries are above the QC acceptance limits, and there are no positive detections in the sample, the data may be reported. A comment must be added to the analytical report.

8. Compare the retention times of all of the analytes to the retention times of the calibration standards. The relative retention times should not vary by more than 0.2 retention time units.

9. The CAL1 standard is used when assessing the correctness of the computer generated peak integrations. For results that have responses at or near the CAL1, the analysts will calculate 1/2 of the area ratio of that compound in the CAL1 standard. If the area ratio for the compound in the sample exceeds that 1/2 the area ratio from the CAL1 standard, the peak is reported as a positive detection.

10. If the calculated concentration exceeds the calibration range of the system, dilute the extract with 50:50 MeOH:water as described in the Sample Preparation section and reanalyze the diluted sample.

Calculations

A. Analyte Concentration using linear through zero curves (MQ Data processing system)

Concentration = (area ÷ slope) x Dilution Factor

B. Sample Concentration (used only for aqueous samples using the MultiQuant data processing system on the AB Sciex LC/MS/MS)

Sample concentration (ug/l) = Calc conc x (Sample volume ÷ Sample weight) x DF

C. 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. The limits for LCS and MS are defined by the method. LCS, MS, and RPD are compared to the limits stored on the LIMS. Historical data for MSs, LCSs, 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, an LCS (Milli Q water spiked with all compounds to be determined carried through the entire procedure) must be extracted. For each sample an MS and a DUP must 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 instead.

The QC acceptance criteria are specified in the method and are as follows:

1. Blank – Value less than the limit of quantitation

2. LCS – 70% to 130% recovery

3. MS– 70% to 130% recovery

4. Sample Duplicate RPD (relative percent difference)

    a. ≤25% at mid and high range concentrations

    b. ≤50% at the minimum reporting limit

QA-SOP11892 Determining Method Detection Limits and Limits of Quantitation
T-PEST-WI9847 Common Equations Used During Chromatographic Analyses

Attachment:
Attachment 1 - Acquisition Parameters

End of document

Version history

Version	Approval	Revision information
1	27.SEP.2018	
2	12.APR.2019	