eurofins	Always check on-line for validity. **Client Specific: Determination of Table 6 Compounds by LC/MS/MS**	Level: **Work Instruction**
Document number: **T-PFAS-WI32768**		
Old Reference:		
Version: **1**		Organisation level: **5-Sub-BU**
Approved by: **XL3S** Effective Date **05-JUN-2020**	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: 01	**Effective Date:** This version	
Section	**Justification**	**Changes**
		New

Reference

1. *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 compounds 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. Reporting limits (LOQs) are typically in the range of 2-4 ng/L.

Analyte	Acronym	CAS#
PEPA $C_5HF_9O_3$	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

Basic Principles

A 250-mL aqueous sample is fortified with an isotopically labeled standard and is passed through a solid phase extraction (SPE) cartridge to extract the analytes. The extract is concentrated to 1.5 mL with nitrogen on a heated water bath and reconstituted to 2 mL with [redacted] Internal standard is spiked and samples are brought to a final volume of 10 mL with [redacted] Samples are analyzed by LC/MS/MS operated in negative electrospray ionization (ESI) mode for detection and quantification of the analytes. Quantitative analysis is performed using an extracted internal standard calibration, consisting of a carbon-13 labeled analyte of similar chemistry.

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 3 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 for the LCS/LCSD. The criteria include the calculation of mean accuracy and standard deviation.

Sample Collection, Preservation, and Handling

A. Sample Collection

The samples are collected in 250-ml polypropylene (PP) or high density polyethylene (HDPE) 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 should be prepared within 28 days of collection.

Apparatus and Equipment

A. Apparatus

1. 250-mL HDPE bottles with HDPE screw caps

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 A – Fisher Scientific, Cat. No. S02288 or equivalent.

4. Polypropylene bottles for reagent storage: 1000-mL, 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. SPE Cartridges –

8. Large volume SPE Reservoir (25-mL) - Millipore-Sigma; Product# 54258-U

9. SPE Tube Adaptor - Millipore-Sigma; Product# 57020-U

10. SPE Vacuum extraction manifold - Resprep 24 port manifold; Restek Corp catalog# 26080, or equivalent

11. Polypropylene SPE delivery needles - Agilent Catalog# 12234511

12. Auto Pipettes – Eppendorf; capable of accurately dispensing 500µl – 10,000µl

13. Auto Pipettes – Eppendorf; capable of accurately dispensing 50µl – 1000µl.

14. Polypropylene pipette tips: 0-200µl. Fisher; Cat. No. 02-681-135

15. Polypropylene pipette tips: 101-1000µl. Fisher, Cat. No. 02-707-508

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

17. 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.

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

B. Equipment

1. AB Sciex 5500 Turbo V Ion Source Tandom Mass Spectrometer

   ExionLC Controller
   ExionLC AC Pump
   ExionLC AC Autosampler
   Exion AC Column Oven
   Data system –Analyst1.6.3

2. HPLC columns

   a. Analytical column-Gemini 3µm C18, 100 x 3 mm, 100 A Phenomenex Cat# 00D-4439-Y0 or equivalent

   b. Pre-column- Luna, 5 µm C18, 30 x 3 mm, Phenomenex Cat# 00A-4252-Y0, or equivalent

c. KrudKatcher ULTRA HPLC In-ine Filter, Part no. AFO-8497.

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. [redacted] – Honeywell Burdick and Jackson "Chromasolv LC-MS" grade or equivalent

2. [redacted] – HPLC grade or equivalent

3. [redacted] – Weigh 1.54 ± 0.01g [redacted] into a 1L glass bottle. Add 1L Milli Q water and mix well. The solution is prone to volatility losses and is replaced weekly. Store at room temperature.

4. [redacted]: Prepared by measuring 20 mL of concentrated [redacted].

B. Standards

1. All target analytes were provided by Chemours as certified solutions at 0.1 % by weight. Stocks are stored in a refrigerator at 0-6°C.

2. Internal standard (IS) solution and isotope dilution analyte (IDA) solution purchased from Wellington (or other reputable vendor) at 50,000 ppb.

3. Standard solutions form vendors may be purchased in glass ampules, but all subsequent transfers, dilutions, and storage vessels should be polypropylene (PP) or high density polyethylene (HDPE).

C. Calibration Standards:

Stock standards and solutions are stored in accordance with the manufacturer recommended storage conditions and expiration dates. Intermediate and calibration standards are stored in refrigerator and expire after 6 months.

1. IDA Standard
   a. IDA Intermediate Solution (13C4-PFBA) - dilute 0.1 mL of IDA stock solution from Wellington (50,000 ng/mL) to 10 mL with [redacted] for a 500 ng/mL solution. Store in refrigerator at 0-6°C for up to 6 months.

b. IDA Working Solution (13C4-PFBA) - dilute 0.5 mL of IDA intermediate solution to 10 mL [redacted] for a 25 ng/mL solution. Other dilutions schemes may be used based on the volume of IDA working solution required. Store in refrigerator at 0-6°C for up to 6 months.

2. Internal Standard (IS)

a. IS Intermediate solution (13C3-PFOA) - dilute 0.1 mL of IS stock solution from Wellington (50,000 ng/mL) to 10 mL with [redacted] for a 500 ng/mL solution. Store in a refrigerator at 0-6°C for up to 6 months.

b. IS working solution (13C3-PFOA) - dilute 0.5 mL of IS intermediate solution to 10 mL [redacted] for a 25 ng/mL solution. Other dilution schemes may be used based on the volume of IS working solution required. Store in refrigerator at 0-6°C for up to 6 months.

3. Calibration Standards

a. Calibration and spiking standards are prepared from standard solutions received from Chemours (0.1% by weight in water).

Parent Solution	Analyte
Client supplied individual Stocks at 1,000,000 ppb	DFSA
	MMF
	MTP
	PPF Acid
	PEPA
	PMPA

b. Prepare 10,000 ng/mL intermediate A in [redacted]:

Compound	Conc. (ng/mL)	Init. Vol. (mL)	Final Vol. (mL)	Intermediate A Conc. (ng/mL)
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
PMPA	1 000 000	0.1	10	10 000
PEPA	1 000 000	0.1	10	10 000

c. Prepare 100 ng/mL Intermediate B in methanol:

Compound	Intermediate A Conc. (ng/mL)	Init. Vol. (mL)	Final Vol. (mL)	Intermediate B Conc. (ng/mL)

Compound	Intermediate A Conc. (ng/mL)	Init. Vol. (mL)	Final Vol. (mL)	Intermediate B Conc. (ng/mL)
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
PMPA	10 000	0.1	10	100
PEPA	10 000	0.1	10	100

d. Prepare 10 ng/mL Intermediate C in [redacted]:

Compound	Intermediate B Conc. (ng/mL)	Init. Vol. (mL)	Final Vol. (mL)	Intermediate C Conc. (ng/mL)
DFSA	100	1.0	10	10
MMF	100	1.0	10	10
MTP	100	1.0	10	10
PPF Acid	100	1.0	10	10
PMPA	100	1.0	10	10
PEPA	100	1.0	10	10

e. Prepare Calibration Standards.

All calibration standards are extracted in the same way as samples. Add the volumes listed below of the appropriate intermediate solution to achieve the listed concentration. Units are ng/mL for each calibration level. Not all calibration points are used for all analytes.

Table 6 Compound		CAL1*	CAL2	CAL3*	CAL4*	CAL5#	CAL6#	CAL7#	CAL8^	CAL9^	CAL10^
DFSA		0.025	0.05	0.25	0.5	1.0	2.5	5.0	10	20	50
MMF		0.025	0.05	0.25	0.5	1.0	2.5	5.0	10	20	50
MtP		0.025	0.05	0.25	0.5	1.0	2.5	5.0	10	20	50
PPF Acid		0.025	0.05	0.25	0.5	1.0	2.5	5.0	10	20	50
PMPA		0.025	0.05	0.25	0.5	1.0	2.5	5.0	10	20	50
PEPA		0.025	0.05	0.25	0.5	1.0	2.5	5.0	10	20	50
IDA	13C4-PFBA	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
IS	13C2-PFOA	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25

* - Use listed value in mL of Intermediate Solution C
# - Use listed value in mL of Intermediate Solution B
^ - Use listed value in mL of Intermediate Solution A

4. Native spiking solution (LCS/Matrix Spike)

Working Native Spike Solution is prepared by placing 0.1 mL of Intermediate A from standard preparation into a 10-mL volumetric flask and bringing to volume with [redacted].

Analyte	Native Spike Solution Concentration (ng/mL)
DFSA	100
MMF	100
MTP	100
PPF Acid	100
PMPA	100
PEPA	100

An LCS or matrix spike is prepared by adding 0.5 mL of the Working native spiking solution to 250 mL of laboratory water or a field sample designated as QC. This will result in a sample extract concentration of 5 ng/mL. The spiking solution is stored in a refrigerator at 0-6°C for up to 6 months.

5. Initial Calibration Verification (ICV)

The ICV spiking solution is prepared the same as the native spiking solution except that the spiking solution is prepared by a second analyst as a second source standard. The expected in sample concentration is 5 ng/mL. The spiking solution is stored in a refrigerator at 0-6°C for up to 6 months.

Calibration

A. Initial Calibration

1. Mass calibration is performed by the instrument manufacturer at the time of installation and annually thereafter.

2. Calibration standards are prepared at the concentrations for the calibration standards, listed previously in this SOP, in the Reagents and Standards section, Subsection C.3.e. Standards are extracted in the same way samples are. Not all points may be used for all compounds. A minimum of 5 points are required for a linear curve fit. If quadratic curve fit is used, a minimum of 6 points are required.

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

4. Initial calibration acceptance criteria

a. The R value for each calibration curve must be ≥0.995 for each analyte.

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

c. The IS response (peak area) must not deviate from by more the 50% from the average response (peak area) of the initial calibration. If the criteria are not met, the source of the problem must be determined and corrected and a new calibration started.

5. Initial Calibration Blank (ICB)

Immediately following the initial calibration, a calibration blank is analyzed, which consists o[redacted] with both IDA and IS added to the ICB. The results of the analysis of the ICB should be all compounds below the reporting limit. Exceptions to this require corrections to the system and recalibration, reanalysis of the ICB.

6. Initial Calibration Verification (ICV)

The analysis of the ICV, described in Reagents and Standards, subsection C.5., should follow immediately after the initial calibrations and ICB and before any batches associated with field samples are analyzed. Acceptance criteria are:

a. Native analyte recovery 50-150% of expected concentration

b. IDA recovery must be within 50-150% of expected.

B. Continuing calibration

1. Once a valid calibration curve has been established, the accuracy must be verified by analysis of a continuing calibration verification (CCV) standard prior to sample analysis, after every ten field samples, and at the end of the analysis sequence. The exception to analysis of a CCV prior to field samples would be analysis of field samples right after the initial calibration and ICV.

a. CCVs should be varied throughout the run between low (LOQ, CAL2), mid (CAL4), and high (CAL8).

b. The CCV run after the initial calibration must be at the low CCV level.

c. Each analytical sequence, if the instrument is not run essentially continuously, should start with the analysis of a CCV.

2. Acceptance criteria

a. The calculated amount for each native compound in the CCV standard must be within ±40% of the true value, with the exception of the low CCV which must be within ±50% 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.

b. The recovery for the IDA must be 50-150%.

c. The IS response must be within ±50% from the midpoint of the calibration curve.

Procedure

A. Sample Preparation

This method/procedure allows for one-time unique modifications to the procedure to accommodate unique sample matrices, special compound reporting limits, sample size, and other unique chemistry aspects that the sample or sample analysis conditions may present. All such modifications will be appropriately document through a non-conformance memo (NCM) process.

1. Solvent Dilution/Direct Injection (SDI) Procedure

The following should be used for the analysis of samples known or suspected of have high concentrations of target analytes to determine the appropriate aliquot to be used towards the SPE procedure (i.e. reduction in initial sample amount from 250 mL to 2.5 mL). Samples of unknown history/concentration should also be screened by this technique.

a. Mix the sample thoroughly in its original container and withdraw a 0.5 mL aliquot.

b. Add 0.5 mL of IDA solution and 0.5 mL IS solution to the 0.5 mL of sample.

c. Prepare a method blank (MB) and LCS/LCSD aliquots in a similar fashion using 0.5 mL [redacted]

d. Dilute each of the prepared aliquots to 10 mL by adding 1.5 mL of [redacted] and 7 mL of [redacted]

e. Prepare TB6 calibration standards at the concentrations listed in Reagents and Standards section, subsection C.3.e. in [redacted]. These standards that are to be used for the SDI screening procedure do not need to go through any extraction.

f. Proceed to instrument calibration and sample analysis.

2. Water Sample Extraction and Preparation

a. Visually inspect the samples for the presence of settled or suspended solids. If present or if the sample is biphasic, add IDA to the sample prior to any centrifugation or decanting.

b. Centrifuge and/or decant the water/supernatent from the solids into a separate, clean PP or HDPE container.

c. Weigh the sample while in the container, prior to extraction and then weigh the empty sample container after extraction to determine the initial volume used. Unless directed otherwise by the client or dues to the results of the SDI screening, use the entire contents of the container.

d. Prepare additional aliquots of field samples for the MS/MSD, if provided.

e. Prepare a 250 mL aliquot of PFAS free laboratory water for the LCS and one for the method blank. If sample was not provide for an MS/MSD as indicated in d., then prepare a third aliquot of PFAS free laboratory water for an LCSD.

f. Spike the LCS (LCSD) and MS/MSD with 0.5 mL of the native spike working solution described previously.

g. Add 0.5 mL of the IDA spiking solution described previously into each sample and QC for a final extract concentration of 1.25 ng/mL.

3. Solid Phase Extraction (SPE) of Aqueous Samples

Note: All conditioning, sample addition, and certain elution steps assume that the column is not allowed to go dry. A residual meniscus of approximately 200 µl of solvent/sample should reside on the top of the cartridge at all times.

a. Condition the SPE cartridge (Phenomenex, [redacted]) by adding 15 mL of [redacted] solution and drawing through the column. Eluent can be discarded.

b. Wash with 10 mL of laboratory water and close valve after passing through. Eluent can be discarded.

c. Label each SPE cartridge, attach reservoirs and add sample/QC to each cartridge.

d. Draw the entire sample through each cartridge, using vacuum to maintain a drip rate of approximately 2-5 drops per second.

e. If any cartridge should plug (flow stops or is greatly reduced to <1 drop/second), then stop the elution and return any remaining sample to its container, to determine the total volume of sample actually used.

f. Alternatively, after consulting with group leader/manager, use of a second cartridge may be considered for increasing the volume of sample extracted. In this case, the final eluted sample extracts from each cartridge should be combined prior to instrumental analysis.

g. After the sample completely passes through the cartridge, allow the cartridge to thoroughly dry by drawing vacuum through for approximately 10 minutes.

h. Place 15 mL polypropylene centrifuge tubes in the SPE manifold as receiving tubes for each sample/QC.

i. Rinse the sample bottles with 4 mL of [redacted] and transfer to the column reservoir on the cartridge. Allow to soak for 5 minutes, then elute into the 15 mL centrifuge tube at a collection rate of 2-5 drops/second.

j. Repeat the rinse of the sample container with another 4 mL of [redacted]. add to the cartridge and elute into the 15 mL centrifuge tube. Total collection volume should be approximately 8 mL.

k. Add 2 mL of laboratory [redacted] to each centrifuge tube, cap and vortex to mix the contents.

l. Concentrate the extract volume to approximately 1.5 mL under a gentle stream of nitrogen, while in a water bath at 55°C.

m. Bring the sample extract up to 2 mL by addition of [redacted].

n. Add 0.5 mL of IS working solution to each sample

o. Bring sample extract up to final volume of 10 mL by addition of [redacted].

p. Bottle a portion of the extract for instrumental analysis. Archive the remaining extract for dilutions and/or reanalysis.

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. Mass calibration is performed by the instrument manufacturer at the time of installation and annually thereafter.

2. Instrument acquisition

The instrument acquisition parameters are detailed in *Attachment I*. These parameters are suggested operating conditions for an AB Sciex 5500 LC/MS/MS system. These parameters could be modified to accommodate other manufacturers LC/MS/MS instrumentation as long as comparable sensitivity, selectivity and chromatographic performance are achieved.

3. Sample Analysis

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 10-15 minutes) before the first injection. A typical analytical sequence might be as follows

a. Analyze several solvent blanks to allow the instrument to stabilize as well as check the system for background that might impact the analysis.

b. Start ICAL with CAL1

c. Run sequentially up through CAL10

d. Inject the ICB

e. Inject the ICV

f. Assuming injections b. through e. pass criteria, inject a CCB followed by up to 10 field samples

g. Inject CCV

i. CCV

j. etc.

4. After injections are completed, check all CCV recoveries to make sure they are within method control limits. See Calibration section B.2 for acceptance criteria. Assure that field samples and QC are bracketed by passing CCVs. 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, with the reason for manual integration clearly listed.

5. 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 re-extracted and re-analyzed. 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.

6. 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 50 to 150% for each analyte. The QC acceptance limit for the relative percent difference (%RPD) between an unspiked sample and a duplicate sample or between an MS and MSD is ≤25%. 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 re-extracted and re-analyzed. 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.

7. 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.

8. If the calculated concentration of a target analyte exceeds the calibration range of the system, dilute the extract with [redacted] and reconstitute with appropriate volume of IS. IDA is not to be added into sample extracts. If the dilution required is beyond the dilutable range for the IDA added at the point of extraction, re-extraction with a reduced aliquot will be necessary to report the over calibration range target analytes.

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 – 50% to 150% for all analytes.
3. MS/MSD – 50% to 150% recovery for all analytes.
4. Sample Duplicate or QC duplicate (LCS/LCSD; MS/MSD) RPD (relative percent difference) - ≤ 25%

QA-SOP11892 Determining Method Detection Limits and Limits of Quantitation
T-PEST-WI9847 Common Equations Used During Chromatographic Analyses
Attachment: Attachment 1 - Instrument Operating Conditions (doc)

Attachment:
Attachment 1 - Instrument Operating Conditions

End of document

Version history

Version	Approval	Revision information
1	05.JUN.2020	

Attachment 1

Recommended Instrument Operating Conditions

A. HPLC Conditions (Exion LC)

Column – Phenomenex Gemini 3um C18 110A, 100 X 3.0 mm
Column Temperature = [redacted]
Injection Size – [redacted]

Mobile Phase Composition	Time	A = [redacted] % A	B = [redacted] % B	Flow Rate (ml/min)
Gradient Program	0	90	10	0.3
	0.10	90	10	0.3
	2.00	80	20	0.3
	2.50	45	55	0.3
	5.00	5	95	0.3
	9.45	1	99	0.3
	9.50	90	10	0.3
	10.5	90	10	0.3

B. Mass Spectrometer Settings (AB Sciex 5500)

1. Mass Spectrometer Interface Settings

MS Interface Mode – ESI Negative Ion, minimum of 10 scans/peak
Ion Spray Voltage (kV) – 4.5
Entrance Potential (V) – 5
Declustering Potential (V) – 25
Desolvation Temp - 550°C
Curtain Gas – 35 psi
Collison Gas – 8 psi

Attachment 1

Mass Spectrometer Settings (AB Sciex 5500) – cont'd.

2. Mass Spectrometer Scan Settings

Compound	Reaction (MRM)	Dwell (msec)	Ent. Pot (V)	Col. Energy (V)	Decluster Pot. (V)	Cell Exit Pot.	Typical RT (Min)
PMPA	299 > 185	3-250	-10	- 12	-15	-5	6.52
PEPA	278.9 > 234.9	3-250	-10	-10	-20	-5	7.12
DFSA	174.9 > 81	3-250	-10	-32	-25	-7	1.60
MMF	139 >51	3-250	-10	-22	-20	-9	1.60
MTP	175 >97	3-250	-10	-16	-45	-9	3.50
PPF Acid	163 >118.9	3-250	-10	-12	-35	-13	4.70
13C4 PFBA	217 > 172	3-250	-5	-14	-25	-31	6.37
13C2 PFOA	415 > 370	3-250	-6	-14	-25	-44	8.59