Document Information

Document Number:	**Revision:**
Document Title:	
Department(s):	

Date Information

Effective Date:

Notes

Document Notes:

All Dates and Times are listed in:

Signature Manifest

Document Number: ENV-SOP-MIN4-0178 **Revision:** 00

Title: Determination of Selected 36 Per- and Polyfluoroalkyl Substances (PFAS) by LC/MS/MS (Isotope Dilution)

All dates and times are in Central Time Zone.

ENV-SOP-MIN4-0178 - DoD 36

QM Approval

Name/Signature	Title	Date	Meaning/Reason
Janielle Ward (007319)	Manager - Quality	14 Dec 2020, 10:39:27 AM	Approved

Management Approval

Name/Signature	Title	Date	Meaning/Reason
Krista Carlson (004514)	Project Manager 1	11 Dec 2020, 02:46:59 PM	Approved
Adam Haugerud (005828)	General Manager 2	11 Dec 2020, 04:42:54 PM	Approved
Keith Sturgeon (003603)	Manager	14 Dec 2020, 12:11:36 PM	Approved

COPYRIGHT © 2020 Pace Analytical Services, LLC

1.0 SCOPE AND APPLICATION

The purpose of this Standard Operating Procedure (SOP) is to document the procedure used by Pace Analytical Services, LLC – Minnesota for the identification and simultaneous measurement of per- and polyfluoroalkyl substances (PFAS) in non-potable waters, leachate, solid (e.g. soil, sediment, and wipe), and tissue matrices using LC/MS/MS based on Department of Defense Quality Systems Manual (DOD QSM) Version 5.3, Table B-15, which is listed in Appendix F of this SOP, and Wisconsin PFAS Aqueous (Non-Potable Water) and Non-Aqueous Matrices Method Expectations.

1.1 Target Analyte List and Limit of Quantitation (LOQ)

The target analytes and the normal LOQ that can be achieved with this procedure are provided in Table 1, Appendix A.

LOQ are established in accordance with Pace policy and SOPs for method validation and for the determination of detection limits (DL) and quantitation limits (LOQ). DL and LOQ are routinely verified and updated when needed. The current LOQ for each target analyte that can be determined by this SOP as of the effective date of this SOP is provided in Table 1, Appendix A.

The reporting limit (RL) is the value to which analytes are reported as detected or not detected in the final report. When the RL is less than the lower limit of quantitation (LLOQ), all detects and non-detects at the RL are qualitative. The LLOQ is the lowest point of the calibration curve used for each target analyte.

DL, LOQ, and RL are always adjusted to account for actual amounts used and for dilution.

2.0 SUMMARY OF METHOD

A 250-mL water sample is fortified with a known quantity of isotope dilution extracted internal standards (EIS) and then passed through a solid phase extraction (SPE) cartridge (e.g., Strata™ PFAS, WAX/GCB sorbent, weak anion exchange mixed-mode) to extract the method analytes and EIS. The analytes and EIS are eluted from the cartridge with a small amount of ammonia/methanol solution. The method for the analysis of PFAS in solid materials extracts 5 g of material with a total of 9-mL aliquot of 0.2% ammonia/methanol. The extract is treated with 50 mg ENVI-Carb™ and filtered prior to nitrogen concentration. For tissue samples, 2 g of material is extracted with 7 mL of 1% ammonia acetonitrile for 16 hours. The extract is treated with ENVI-Carb™ and filtered prior to SPE cleanup. The water or solid sample extract is concentrated to ~0.8 mL while the tissue extract is concentrated to ~0.1 mL with nitrogen and spiked with Injection Internal Standards (IIS) and then brought to 1 mL with 96:4% (vol/vol) methanol:H_2O solution prior to LC/MS/MS analysis. A 3-μL injection is made into a Liquid Chromatography (LC) System equipped with a C18 column that is interfaced to a tandem mass spectrometer (MS/MS). The concentration of each analyte is determined by using the isotope dilution and internal standard techniques, depending on target analyte. EIS is added to all calibration standards, field samples, blanks and QC samples to monitor the extraction efficiency of the method analytes.

*Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies.
Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete.
Users of the SOP should verify the copy in possession is the current version of the SOP before use.*

COPYRIGHT © 2020 Pace Analytical Services, LLC

3.0 INTERFERENCES

3.1 All glassware must be meticulously cleaned. Wash glassware with non-phosphate alkaline detergent and deionized (DI) water, rinse with DI water and reagent water, followed by a methanol rinse. Non-volumetric glassware can be heated in a muffle furnace at 400 °C for 2 h or solvent rinsed. Volumetric glassware should be solvent rinsed and not be heated in an oven above 120 °C. Store clean glassware inverted or capped. **Do not cover with aluminum foil because PFAS can be potentially transferred from the aluminum foil to the glassware.**

> **NOTE:** PFAS standards, extracts and samples should not come in contact with any glass containers or pipettes as these analytes can potentially adsorb to glass surfaces. PFAS analytes, EIS and IIS commercially purchased in glass ampoules are acceptable; however, all subsequent transfers or dilutions performed by the analyst must be prepared and stored in polypropylene or equivalent containers

3.2 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 method 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, SPE sample transfer lines, etc. All items such as these must be routinely demonstrated to be free from interferences (less than 1/2 the RL) under the conditions of the analysis by analyzing method blanks. **Subtracting blank values from sample results is not permitted.**

3.3 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature of the water. Humic and/or fulvic material can be co-extracted during SPE and high levels can cause enhancement and/or suppression in the electrospray ionization source or low recoveries on the SPE sorbent. Total organic carbon (TOC) is a good indicator of humic content of the sample. Under the LC conditions used during method development, matrix effects due to TOC were not observed.

3.4 SPE cartridges can be a source of interferences. The analysis of field and method blanks can provide important information regarding the presence or absence of such interferences. Brands and lots of SPE devices should be tested to ensure that contamination does not preclude analyte identification and quantitation.

4.0 DEFINITIONS

Refer to the Laboratory Quality Manual for a glossary of common lab terms and definitions.

4.1 **Confirmation Ion** – One of the product ions used to help qualitatively confirm presence of the analytes. The product ion chosen is typically one of the remaining ions with high sensitivity and minimum interferences after the quantitation ion has been chosen. Not all precursor ions provide confirmation ions.

4.2 **Extraction Internal Dilution standards (EIS)** – Isotopically labeled internal standards that undergo the same extraction and analysis as the other analytes in the sample. The EIS are added to the sample at the beginning of the procedure before extraction, centrifugation, filtering, or phase separation. Ideally, there are exact isotopically labeled analogs of the native analytes

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

so that identical behavior can be assumed. The recoveries of these standards are used to adjust the native analyte results.

4.3 **Internal Standard Quantitation** – measurement of native analytes using an alternate analog isotope (one that has the same chemical behavior and is close in retention time to the native analyte), thus providing a close approximation of matrix effects and losses that can occur during the preparation and analysis. The native analyte concentration is adjusted for the recovery of the alternate analog isotope. An alternate analog isotope is typically used when an exact analog isotope is not available.

4.4 **Isotope Dilution Quantification** – measurement of native analytes using an exact analog isotope of the native analyte. The native analyte concentration is adjusted for the recovery of the exact analog isotope that has been included in the preparatory and analytical procedure.

4.5 **Precursor Ion** – For the purpose of this method, the precursor ion is the deprotonated molecule ($[M-H]^-$) of the method analyte. In MS/MS, the precursor ion is mass selected and fragmented by collisionally activated dissociation to produce distinctive product ions of smaller *m/z*.

4.6 **Product Ion** – For the purpose of this method, a product ion is one of the fragment ions produced in MS/MS by collisional activated dissociation of the precursor ion.

4.7 **Primary Dilution Standard (PDS) solution** – A solution containing the analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

4.8 **Preparation Batch** – A group of up to 20 field samples (not including QC samples) extracted together by the same person(s) during a work day (24 hours) using the same lot of SPE devices, solvents, surrogate, internal standard and fortifying solutions. Required QC samples include MB, LCS, MS, and MSD.

4.9 **Quantitation Ion** – One of the product ions used to quantitate analyte concentrations. The product ion chosen is typically one of high sensitivity and minimum interference

5.0 HEALTH AND SAFETY

The toxicity or carcinogenicity of each chemical material used in the laboratory has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable.

The laboratory maintains documentation of hazard assessments and OSHA regulations regarding the safe handling of the chemicals specified in each method. Safety data sheets for all hazardous chemicals are available to all personnel. Employees must abide by the health, safety and environmental (HSE) policies and procedures specified in this SOP and in the Pace Chemical Hygiene / Safety Manual.

Personal protective equipment (PPE) such as safety glasses, gloves, and a laboratory coat must be worn in designated areas and while handling samples and chemical materials to protect against physical contact with samples that contain potentially hazardous chemicals and exposure to chemical materials used in the procedure.

Concentrated corrosives present additional hazards and are damaging to skin and mucus membranes. Use these acids in a fume hood whenever possible with additional PPE designed

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies.
Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete.
Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

for handing these materials. If eye or skin contact occurs, flush with large volumes of water. When working with acids, always add acid to water to prevent violent reactions. Any processes that emit large volumes of solvents (evaporation/concentration processes) must be in a hood or apparatus that prevents employee exposure.

Contact your supervisor or local HSE coordinator with questions or concerns regarding safety protocol or safe handling procedures for this procedure.

6.0 SAMPLE COLLECTION, PRESERVATION, HOLDING TIME, AND STORAGE

Samples should be collected in accordance with a sampling plan and procedures appropriate to achieve the regulatory, scientific, and data quality objectives for the project.

The laboratory does not perform sample collection or field measurements for this test method. To assure sample collection and field checks and treatment are performed in accordance with applicable regulations. Pace project managers will inform the client of these requirements at the time of request for analytical services when the request for testing is received prior to sample collection. If samples were already collected, the laboratory will record any nonconformance to these requirements in the laboratory's sample receipt record when sufficient information about sample collection is provided with the samples.

The laboratory will provide containers for the collection of samples upon client request for analytical services. Bottle kits are prepared in accordance with the Method.

General Requirements

Matrix	Routine Container	Minimum Sample Amount	Preservation	Holding Time
Aqueous	250 mL HDPE bottle fitted with polyethylene screw-cap lid	250 mL	Thermal: <6 °C but >0 °C Chemical: NA	Collection to Prep: 28 Days Prep to Analysis: 28 Days Extract stored at 0-6ºC
Solid	250 mL HDPE bottle fitted with polyethylene screw-cap lid	5 g	Thermal: <6 °C but >0 °C Chemical: NA	Collection to Prep: 28 Days Prep to Analysis: 28 Days Extract stored at 0-6ºC
Tissue	250 mL HDPE bottle fitted with polyethylene screw-cap lid	2 g	Thermal: Frozen Chemical: NA	Collection to Prep: 1 year Prep to Analysis: 30 Days Extract stored at 0-6ºC

6.1 Sample Collection

6.1.1 Laboratory samples are collected, preserved, shipped, and stored in accordance with ENV-SOP-MIN4-0008 *Sample Management* and ENV-SOP-MIN4-0009 *Bottle Preparation* Guidelines for Sampling Container Kits, Sample Preservation, and Holding Times or equivalent replacements.

6.1.2 The sample handler must wash their hands before sampling and wear nitrile gloves while filling and sealing the sample bottles. 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.

6.1.3 Fill sample bottles. Do not fill aqueous sample containers completely.

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

6.1.4 Matrix spike (MS) and matrix spike duplicate (MSD) sample. Analysis of a MS is requested in each extraction batch and is used to determine that the sample matrix does not adversely affect method accuracy.

6.1.5 Field Duplicates. Collect one per sampling event for each sampling site

6.2 Sample shipment and storage – Aqueous and solid samples must be chilled during shipment and must not exceed 6 °C after collection. Ship tissue samples frozen. Aqueous and solid sample temperature must be confirmed to be at or below 6 °C when the samples are received at the laboratory. Samples stored in the lab must be held at or below 6 °C until extraction, but should not be frozen. Tissue samples received frozen can be documented as "frozen" at sample receipt. Store tissue samples at less than or equal to -10ºC at the laboratory.

7.0 Equipment And Supplies

7.1 Equipment

7.1.1 Brand names and catalogue numbers represent materials in use at the time of this revision. Due to potential adsorption of analytes onto glass, polypropylene containers were used for all standard, sample and extraction preparations. Other materials which meet the QC requirements may be substituted.

Table 7.1.1 - Equipment and Supplies (Including Computer Software)

Supply	Description	Vendor/Item #/Description
SPE cartridge	Phenomenex Strata™ PFAS, WAX/GCB, 200 mg/50 mg 6 mL	Phenomenex, Cat# CS0-9207 or equivalent
Extraction manifold	An automatic/robotic sample preparation system designed for use with SPE cartridges	Supelco Cat# 57030 and 57275 or equivalent
Analytical column	Gemini® 100 × 3 mm 3 µm C18 reverse phase LC column	Phenomenex Cat# 00D-4439-Y0 or equivalent
HPLC	1100/1290 infinity series/NexeraXR	Agilent/Shimadzu
MS	API 4000/5500 quadrupole	Sciex
Analyst®	Data acquisition software	Version 1.6.3
Multiquant™	Data processing software	Version 3.0.2
Avalon	Data reporting software	See master list for current version
Nitrogen evaporator	N-EVAP™ 112 nitrogen evaporator equivalent nitrogen evaporator/heated waterbath capable of heating 25-60ºC	Oasys Heating system (Berlin, MA, USA)
Balance	Electronic, capable of weighing to 0.001 g or equivalent.	NA
Syringe pump	Model # NE-300 or equivalent system capable of delivering variable flow rates.	New Era Pump Systems, Inc
Ultrasonicator	Branson ultrasonicator	Branson Model 8510
Sample container	High density polyethylene (HDPE) or polypropylene, 250 mL, wide mouth, with screw top	C&C Container, Cat# 183277

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

Supply	Description	Vendor/Item #/Description
Centrifuge tube and cap	15-mL and 50-mL conical polypropylene tubes with polypropylene screw caps for collection and storage of the extracts	BD Falcon, P/N 352096 and P/N352070
Polypropylene bottles	4-mL narrow-mouth polypropylene bottles	Thermo Cat# 2006-9125
Polypropylene bottles	15-mLnarrow-mouth polypropylene bottles	Thermo Cat# 2002-9050
Autosampler vials	Polypropylene 0.3-mL autosampler vials with polypropylene caps	Phenomenex Cat# AR0-9995-12-C
Adjustable auto-pipettors	Ranges 10-100 µL, 100-1000 µL, and 1000-5000 µL. Laboratory or aspirator vacuum system	NA
ENVI-Carb	Supelclean™ ENVI-Carb™ SPE Bulk Packing	Sigma Aldrich, Cat# 57210-U.
Vacuum extraction manifold	A manual vacuum manifold with Visiprep volume sampler (Supelco Cat# 57030 and 57275 or equivalent) for extraction, or an automatic/robotic sample preparation system designed for use with SPE cartridges, may be used if all QC requirements are met.	Supelco Cat# 57250-U and 57275 or equivalent

7.1.2 **Liquid chromatography (LC)/ Tandem Mass Spectrometer (MS/MS)**

7.1.2.1 **LC system** – Liquid chromatography (LC) system with binary pump, autosampler, column heater. All solvent lines were replaced with (polyether ether ketone) PEEK tubing. PFAS isolator column (Phenomenex Luna ® 30 × 3 mm 5 µm C18 reverse phase LC column, Cat# 00A-4252-Y0) and stainless steel tubing installed between the mixing chamber and injection port. Other equivalent automated LC system capable of reproducibly injecting up to 5-µL aliquots, and performing binary linear gradients at a constant flow rate near the flow rate used for development of this method (0.5 mL/min) may be used.

7.1.2.2 **LC/MS/MS** – The LC/MS/MS must be capable of negative ion electrospray ionization (ESI) near the suggested LC flow rate of 0.6 mL/min. The system must be capable of performing MS/MS to produce unique product ions for the method analytes within specified retention time segments. A minimum of 10 scans across the chromatographic peak is required to ensure adequate precision.

8.0 Reagents And Standards

8.1 Gases, Reagents, and Solvents – LC/MS grade or equivalent is used (Fisher equivalent is Optima). Other grades may be used, provided it is first determined that the reagent is of sufficiently high purity to permit its use without lessening the quality of the determination. Fisher solvents are preferred for mobile phases (water, methanol, acetonitrile) as the one liter bottles can be directly loaded on the instrument removing a transfer step with the inherent low level contamination.

COPYRIGHT © 2020 Pace Analytical Services, LLC

Reagent/Standard	Concentration/ Description	Requirements/Vendor/Item #
Ammonium Acetate ($NH_4C_2H_3O_2$, CAS# 631-61-8)	Optima LC/MS grade, demonstrated to be free of analytes and interferences	Fisher or equivalent
Acetonitrile (CH_3CN, CAS# 75-05-8)	Optima HPLC grade	Fisher or equivalent Cat# A955-4
Ammonium Hydroxide (NH_4OH, 28-30% in water)	Certified ACS Plus grade demonstrated to be free of analytes and interferences	Fisher or equivalent
Ammonia/Methanol Solution	Optima HPLC grade, w = 0.2% mass fraction. Mix 0.72 mL of 28-30% ammonia solution with 99.28 mL of methanol	Fisher or equivalent
Glacial Acetic Acid ($C_2H_3CO_2H$, CAS# 64-19-7)	HPLC grade. Demonstrated to be free of analytes and interferences	VWR Analytical Cat# BDH20108
Sodium hydroxide (NaOH, 1310-73-2)	Certified ACS. High purity demonstrated to be free of analytes and interferences	Fisher, or equivalent
Methanol (CH_3OH, CAS# 67-56-1)	Optima HPLC grade, demonstrated to be free of analytes and interferences	Fisher, or equivalent
Reagent water (H_2O, CAS# 7732-18-5)	Optima HPLC grade, demonstrated to be free of analytes and interferences	Fisher or equivalent Cat# W7-4
Aqueous Mobile Phase (20 mM Ammonium acetate)	To prepare 1 L, add 1.54 g ammonium acetate to 1 L of reagent water.	Fisher or equivalent
Acetate Buffer (25 mM, pH 4)	Mix 0.5 mL of acetic acid with 349.5 mL of water. Dissolve 0.116 g of ammonium acetate in 60 mL of water. Mix 200 mL of the diluted acetic acid with 50 mL of the ammonium acetate solution	Fisher or Equivalent
PPG Tuning Solutions	Instrument tuning compound. Using standards chemical kit with low/high concentration is recommend, however solution can be prepared from a neat material.	Sciex P/N 4406127
Ottawa Sand	To prepare method blank, LCSs, for the extraction of soil samples	EMD or equivalent Cat# SX0075-3
Nitrogen (N_2)	Nitrogen aids in aerosol generation of the ESI liquid spray and is used as collision gas in some MS/MS instruments. The nitrogen (Ultra High Purity or equivalent) used should meet or exceed instrument manufacturer's specifications	Ultra High Purity or equivalent
Canola Oil	Canola oil, or equivalent, for Oil quality control sample matrix,	Local grocery store
Lake Michigan Fish Tissue	Standard reference materials (SRM) for tissue analysis	NIST, 1947

8.2 **Stock Standards** – non-neat standards purchased from vendors that are used for the preparation of working standards. Standards containing both branched and linear isomers must be used when commercially available. If not available, the total response of the analyte must be integrated, (i.e. accounting for peaks that are identified as linear and branched isomers) and quantitated using a calibration curve which includes the linear isomer only for that analyte, i.e. PFOA.

If no expiration date is assigned by the vendor, expiration date is 1 year from the date of receipt. For open stock standards, the expiration is date is 1 year for the open date.

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, 4:2FTS, 6:2FTS, 8:2FTS, DONA, 9Cl-PF3ONS and 11Cl-PF3OUdS are not available as the acid form, but rather as their corresponding salts, such as Na^+ and K^+. These salts are acceptable for use as stock standards as long as the weight is corrected for the salt content according to the equation below.

$$Mass_{acid} = Measured\ Mass_{salt} \times \frac{MW_{acid}}{MW_{salt}}$$

Where: $MW_{(acid)}$ = the molecular weight of PFAS

$MW_{(salt)}$ = the molecular weight of purchased salt

NOTE: All standards purchased are greater than or equal to 98% purity, therefore the weight can be used without correction to calculate the concentration of stock standards. Primary stock standards are stored at ≤ 4 ± 2 ºC. Stock solution is brought to room temperature before using. PFAS may be purchased in glass ampoules however all further solutions and storage is in polypropylene or equivalent containers.

Table 8.2 Description and concentration of each analyte in stock solution

Analyte/Concentration		Used to prepare
Wellington Laboratories PFAC-30PAR (µg/mL)		Cal curve and spiking solution.
PFBA	1	
PFPeA	1	
PFHxA	1	
PFHpA	1	
PFOA	1	
PFNA	1	
PFDA	1	
PFUdA	1	
PFDoA	1	
PFTrDA	1	
PFTeDA	1	
PFOSA	1	
N-EtFOSAA*	1	
N-MeFOSAA*	1	
HFPO-DA	1	
DONA	0.945	
PFBS	0.887	
PFPeS	0.941	
PFHxS*	0.914	
PFHpS	0.953	
PFOS*	0.928	
PFNS	0.962	
PFDS	0.965	
4:2FTS	0.937	
6:2FTS	0.951	
8:2FTS	0.960	
9Cl-PF3ONS	0.933	
11Cl-PF3OUdS	0.943	
Wellington Laboratories PFAC-8Native (µg/mL)		
10:2FTS	48.2	
N-MeFOSA	50	
N-EtFOSA	50	
N-MeFOSE	50	
N-EtFOSE	50	

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

Table 8.2 Description and concentration of each analyte in stock solution

Analyte/Concentration		Used to prepare
PFDoS	48.4	
PFHxDA	50	
PFODA	50	
Wellington Laboratories PFAC-24PAR (µg/mL)		Use for the ICV
PFBA	2	
PFPeA	2	
PFHxA	2	
PFHpA	2	
PFOA	2	
PFNA	2	
PFDA	2	
PFUdA	2	
PFDoA	2	
PFTrDA	2	
PFTeDA	2	
PFOSA	2	
N-EtFOSAA	2	
N-MeFOSAA	2	
PFBS	1.77	
PFPeS	1.88	
PFHxS*	1.82	
PFHpS	1.90	
PFOS*	1.86	
PFNS	1.92	
PFDS	1.93	
4:2FTS	1.87	
6:2FTS	1.90	
8:2FTS	1.92	
Wellington Laboratories PFAC-12Native (µg/mL)		Use for the ICV
10:2FTS	48.2	
HFPO-DA	50	
DONA	47.25	
N-MeFOSA	50	
N-EtFOSA	50	
N-MeFOSE	50	
N-EtFOSE	50	
9Cl-PF3ONS	46.6	
11Cl-PF3OUdS	47.1	
PFDoS	48.4	
PFHxDA	50	
PFODA	50	
Wellington Laboratories MPFAC-24ES (µg/mL)		Isotopically labelled Extracted Internal Standards
13C4_PFBA	1	
13C5_PFPeA	1	
13C3_PFBS	0.929	
13C2_4:2FTS	0.935	
13C5_PFHxA	1	
13C4_PFHpA	1	
13C3_PFHxS	0.946	
13C2_6:2FTS	0.949	
13C8_PFOA	1	
13C9_PFNA	1	
13C8_PFOS	0.957	
13C2_8:2FTS	0.958	
13C6_PFDA	1	
d3-MeFOSAA	1	

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

Table 8.2 Description and concentration of each analyte in stock solution

Analyte/Concentration		Used to prepare
13C8_FOSA	1	
d5-EtFOSAA	1	
13C7_PFUdA	1	
13C2_PFDoA	1	
13C2_PFTeDA	1	
Wellington Laboratories MPFAC-6ES (µg/mL)		
13C3_HFPO-DA	50	
13C2_PFHxDA	50	
d7-N-MeFOSE	50	
d9-N-EtFOSE	50	
d3-N-MeFOSA	50	
d5-N-EtFOSA	50	
Wellington Laboratories MPFAC-Injection Internal Standards (µg/mL)		Isotopically labelled Injection Internal Standards (IIS)
13C2_PFHxA	50	
13C4_PFOA	50	
13C2_PFDA	50	
13C4_PFOS	50	
Wellington Laboratories T-PFOA (µg/mL)		Qualitative Standard for PFOA (branch isomer of PFOA)
T-PFOA*	50	

* Indicates the present of both linear and branch isomers
See Appendix A for additional information about acronyms

9.0 PROCEDURE

9.1 Equipment Preparation

9.1.1 Instrument

9.1.1.1 Routine Instrument Operating Conditions

Table 9.1.1.1 LC-MS/MS Operating Conditions

Injector	Syringe Size	100 µL		
	Sample Loop vol.	40 µL		
	Injection Volume:	3 µL		
	Needle Wash 1	100% Methanol		
Pump	Flow rate	400 µL/min		
	Flow method	Gradient		
	Mobile Phase 1	20 mM Ammonium Acetate H2O		
	Mobile Phase 2	LCMS Acetonitrile		
	Gradient Program	Time	% Mobile Phase 1	% Mobile Phase 2
		Initial	90	10
		0.5	90	10
		8.0	20	80
		10.0	20	80
		10.1	5	95

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

		12.0	5	95
		12.1	90	10
		15.0	90	10
Column	Type:	Phenomenex Gemini® (or equivalents)		
	Part Number:	00D-4439-Y0		
	Running temp	40 °C		
	Length:	100 mm		
	Diameter:	3 mm		
	Particle Size	3.0 µm		
Nominal Tune Values	Collision Gas	10 psi		
	Curtain Gas	25 psi		
	Ion Source Gas 1	40 psi		
	Ion Source Gas 2	50 psi		
	IonSpray Voltage	-4500 v		
	Temperature	450 °C		
	ESI polarity	Negative		
	Declustering Potential	Optimized for each analyte (See Appendix C for reference)		
	Collision Energy			
	Collision Cell Exit Potential			

9.1.1 **Routine Instrument Maintenance**

9.1.1.1 Routine instrument maintenance is critical to achieve optimum method sensitivity. All laboratory materials must be determined to be free of contamination to ensure potential background interferences are minimized.

9.1.1.2 Please refer to the instrument manual for maintenance procedures performed by the lab.

9.1.1.3 All maintenance activities are listed in maintenance logs that are assigned to each separate instrument.

9.1.1.4 **LC maintenance** – LC system components, as well as the mobile phase constituents, contain many of the method analytes in this method. Thus, these PFAS will build up on the head of the LC column during mobile phase equilibration.

9.1.1.5 **Column equilibrate** – To minimize the background PFAS peaks and to keep background levels constant, the time the LC column sits at initial conditions must be kept constant and as short as possible (while ensuring reproducible retention times).

9.1.1.6 **Column flush** – In addition, prior to daily use, flush the column with 95% methanol for at least 15 min before initiating a sequence. It may be necessary on

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

some systems to flush other LC components such as wash syringes, sample needles or any other system components before daily use.

9.1.2 **MS maintenance** – Please refer to the instrument manual for maintenance procedures performed by the lab. Common maintenance procedures are listed below

9.1.2.1 **Source cleaning** – Clean the ion source parts which include curtain plate, orifice plate or skimmer, Q0, and etc. with reagent water and methanol. Tuning or optimizing the instrument followed the instrument manual. Refer to the Operating Instruction – Tune and Calibrate, the ion source operator guide, or the Analyst® software Help system.

9.1.2.2 **Pump oil** – Check pump oil level and color periodically. Add or change pump oil when necessary followed the manual instruction.

9.1.3 **Trouble Shooting**

9.1.3.1 Any deviations from the norm encountered while conducting this analysis must be noted and brought to the attention of the section supervisor. This section contains basic information for troubleshooting basic system issues. Certain activities may be carried out by the Agilent and AB SCIEX trained Qualified Maintenance Person (QMP) in the laboratory. For advanced troubleshooting, contact field service agents of the instruments.

9.1.3.2 **LC troubleshooting** – Please refer to the instrument manual for troubleshooting procedures performed by the lab. Common LC issues are listed below.

9.1.3.2.1 Pressure issue – Large pressure variation could cause by the presence of air bubble in the system, blockage of the system, column contamination, system leaking, and etc. High pressure issue could be solved through system solvent purge, column rinse, clean or change of column inlet frit, injection valve, needle seat, and etc. Low pressure issue could usually be fixed by tighten or replace the capillary connection or other parts such as pump seals.

9.1.3.2.2 Peak shape issue –Split peaks, peak tailing, poor efficiency, and inconsistent response are usually associated with issues like column contamination, partially plugged frit, column void, injection solvent effects, or sample overload effects. Rinsing or changing the column, preparing fresh mobile solvent, reducing sample injection volume could usually

9.1.3.2.3 Retention time – Deviation of retention time from originally values could cause by column aging or contamination, insufficient system equilibration, mobile phase variation, change in column temperature, or other instrument issues. Cleaning the HPLC system and column could Ammonium acetate is volatile and may cause the shift of retention time over certain period of time. Prepare fresh mobile phase solvent when necessary.

9.1.3.2.4 Background contamination – After multiple injections or long period of operation, background interference may accumulate at the

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

gradient proportional valve, needle seat, or other instrument parts. Rinsing and cleaning corresponding parts with reagent water, methanol or stronger solvents to remove the interference.

9.1.3.2.5 MS troubleshooting – Please refer to the instrument manual for troubleshooting procedures performed by the lab. Common MS issues are listed below:

9.1.3.2.5.1 Sensitivity loss – The possible causes for intensity decrease could be contamination of TurboV ion spray, or the instrument requires tuning and optimization. Clean the ion source including curtain plate, orifice plate or skimmer, Q0. Tune or optimize the instrument following the instrument manual. Refer to the Operating Instruction – Tune and Calibrate, the ion source operator guide, or the Analyst® software Help system.

9.1.3.2.5.2 Low vacuum pressure – Low pump oil level could cause the vacuum pressure issue. Check the pump oil level and add oil if necessary.

9.2 Initial Calibration

9.2.1 Calibration Design

9.2.1.1 Demonstration and documentation of acceptable initial calibration is required before any samples are analyzed. After the initial calibration is successful, a continuing calibration verification (CCV) is required at the beginning and end of each period in which analyses are performed, and after every tenth field sample. Samples must be bracketed with CCV's passing for all criteria, or the samples should be re-analyzed (with the single exception mentioned in Appendix B).

9.2.1.2 ESI-MS/MS Tune. Tuning should occur at least every six months using PPGs for tuning. Tune the system when ICAL won't pass, when the peak shape is significantly off (indicating an MS problem), when major maintenance is performed, or instrument is moved.

9.2.1.3 The instrument tuning with PPGs has its own manufacturing criteria- see the documentation. After PPGs passes, calibration must be verified to be +/- 0.5 amu of true values by acquiring a full scan continuum mass spectrum of a PFAS stock standard.

9.2.1.4 Mass calibration range must bracket the ion masses of interest.

9.2.1.5 When done, run the Compound Optimization or Manual Tuning under the Tune and Calibrate tab to optimize response and peak shape.

9.2.1.6 Prepare a set of six CAL standards (Table 9.2.1.20A as an example). Analyze each standard level with the same acquisition method used to analyze samples, changes to retention times or other analytical parameters are saved as part of the local method generated with each analytical sequence, these parameters can be adjusted mid-sequence so long as they are applied to all data.

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

9.2.1.7 The LC/MS/MS system is calibrated using the isotope dilution and internal standard technique. Use the LC/MS/MS data system software to generate a linear regression calibration curve for each of the relevant analytes. This curve may be concentration weighted, if necessary.

9.2.1.8 A calibration meets criteria when the recovery for each calibration point reads back at ±30% for all calibration points.

9.2.1.9 For Wisconsin compliance analysis, re-quantitated concentrations for all target compounds at all concentration levels must be within the range 70-130% of their actual concentrations, except for the lowest calibration concentration level, which must be within the range of 50-150% of actual concentration.

9.2.1.10 Provided a minimum of five calibration points are still being used, a point at the top or bottom of the calibration curve may be dropped to achieve recovery requirements across the remaining points. Dropping high concentration points lowers the PQL of the calibration and may require that more dilutions are performed. Dropping low calibration points can potentially elevate the RL for this sequence.

9.2.1.11 An ICV (prepared from a second source standard or by different analyst) is run with every initial calibration curve (ICAL). The acceptance criteria are ±30% of the true value.

9.2.1.12 Additional calibration procedures (where applicable) can be found in ENV-POL-CORQ-0005 *Acceptable Calibration Practices for Instrument Testing* (or equivalent replacement).

9.2.1.13 **537 Mix** – Mix 40 mL of Optima grade Water with 960 mL of Optima grade Methanol. Expires 1 year from prep.

9.2.1.14 **PFAC_EIS – PFAC (Extracted Internal Standards) (0.05 µg/mL, 25 EIS)**

9.2.1.14.1 Dissolve 40 µL of each MPFAC-6ES standard in 537 Mix. Dilute to 2 mL.

9.2.1.14.2 Dissolve each 1 mL of MPFAC-24ES and 10.3.2.1 mix solution in 537 Mix. Dilute to 20 mL. Added 100 µL to each field sample, standard, blanks and QC samples prior to extraction.

9.2.1.14.3 Used for curve and sample prep. Store at room temperature. Expires 180 days from prep.

9.2.1.15 **PFAC_IIS – PFAC (Injection Internal Standards) (0.05 µg/mL)**

9.2.1.15.1 Dissolve 50 µL of each MPFAC-Injection Internal Standards in 537 Mix. Dilute to 50 mL. Added 100 µL to each field sample, standard, blanks and QC samples just prior to analysis.

9.2.1.15.2 Used for curve prep and sample extraction. Store at room temperature. Expires 180 days from prep.

9.2.1.16 **PFAC_Native Spike Solution**

COPYRIGHT © 2020 Pace Analytical Services, LLC

9.2.1.16.1 Dissolve 100 µL of each PFAC-8Native in 537 Mix. Dilute to 5 mL.

9.2.1.16.2 PFAC_Native Spike 1 (~0.2 µg/mL) – Dissolve 1 mL of PFAC-30PAR and 1 mL of 9.2.1.16.1 mix solution to 537 Mix. Dilute to 5 mL.

9.2.1.16.3 PFAC_Native Spike 2 (~0.050 µg/mL) – Dissolve 2.5 mL of PFAC_Native Spike 1 to 537 Mix. Dilute to 10 mL.

9.2.1.16.4 Used for curve prep and sample extraction. Store at room temperature. Expires 180 days from prep.

9.2.1.17 **PFAC_ICV Spike Solution**

9.2.1.17.1 Dissolve each of 100 µL PFAC-12Native in 537 Mix. Dilute to 2.5 mL.

9.2.1.17.2 PFAC_ICV Spike 1 (~0.2 µg/mL) – Dissolve 1 mL of PFAC-24PAR and 1 mL of 9.2.1.17.1 mix solution to 537 Mix. Dilute to 10 mL.

9.2.1.17.3 PFAC_ICV Spike 2 (~0.050 µg/mL) – Dissolve 2.5 mL of PFAC_ICV Spike 1 to 537 Mix. Dilute to 10 mL.

9.2.1.17.4 Used for ICV prep. Store at room temperature. Expires 180 days from prep.

9.2.1.18 **Isomer Check PDS – Isomer check Qualitative primary standard Spike**

9.2.1.18.1 Dissolve 40 µL of T-PFOA stock solution in 537 Mix, dilute to 1 mL. Expires 180 days from prep.

9.2.1.18.2 PFOA qualitative dilution standard spike – Dissolve 50 µL PFOA qualitative primary standard spike in 537 Mix, dilute to 2 mL. Expires 180 days from prep.

9.2.1.19 **Calibration Curve** – Different volumes of PFAC_Native Spike solutions at various concentrations are added to 1 mL 537 Mix (Table 9.2.1.20A). A known amount of EIS is added into each calibration point. The corresponding concentration in 1 mL final solvent is shown in Table 9.2.1.20B.

Table 9.2.1.20A Example Calibration Curve

Calibration Standard Point	Native std. Soln added (µL)	Native std. Soln conc. (µg/mL)	Extracted IS Soln added (µL)	Extracted IS Soln conc. (µg/mL)	Injection IS Soln added (µL)	Injection IS Soln conc. (µg/mL)
CS-1	10	0.05	100	0.05	100	0.05
CS-2	20	0.05	100	0.05	100	0.05
CS-3	40	0.05	100	0.05	100	0.05
CS-4	100	0.05	100	0.05	100	0.05
CS-5	200	0.05	100	0.05	100	0.05
CS-6	100	0.20	100	0.05	100	0.05
CS-7	250	0.20	100	0.05	100	0.05
CS-8	500	0.20	100	0.05	100	0.05
ICV	100	0.05	100	0.05	100	0.05
T-PFOA (Qualitative Calibration)	100	0.05	100	0.05	100	0.05

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

Table 9.2.1.20B Concentrations of each analyte in 1 mL final solvent (ng/L)

Analyte	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6	CS-7	CS-8	ICV
PFBA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFPeA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFHxA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFHpA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFOA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFNA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFDA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFUdA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFDoA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFTrDA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFTeDA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFOSA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
N-EtFOSAA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
N-MeFOSAA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFBS	0.44	0.89	1.77	4.43	8.85	17.70	44.25	88.50	4.43
PFPeS	0.47	0.94	1.88	4.70	9.40	18.80	47.00	94.00	4.70
PFHxS*	0.46	0.91	1.82	4.55	9.10	18.20	45.50	91.00	4.55
PFHpS	0.48	0.95	1.90	4.75	9.50	19.00	47.50	95.00	4.75
PFOS*	0.47	0.93	1.86	4.65	9.30	18.60	46.50	93.00	4.65
PFNS	0.48	0.96	1.92	4.80	9.60	19.20	48.00	96.00	4.80
PFDS	0.48	0.97	1.93	4.83	9.65	19.30	48.25	96.50	4.83
4:2FTS	0.47	0.94	1.87	4.68	9.35	18.70	46.75	93.50	4.68
6:2FTS	0.48	0.95	1.90	4.75	9.50	19.00	47.50	95.00	4.75
8:2FTS	0.48	0.96	1.92	4.80	9.60	19.20	48.00	96.00	4.80
10:2FTS	0.48	0.97	1.93	4.83	9.65	19.30	48.25	96.50	4.83
HFPO-DA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
DONA	0.47	0.95	1.89	4.73	9.45	18.90	47.25	94.50	4.73
N-MeFOSA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
N-EtFOSA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
N-MeFOSE	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
N-EtFOSE	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
9Cl-PF3ONS	0.47	0.93	1.86	4.66	9.32	18.64	46.60	93.20	4.66
11Cl-PF3OUdS	0.47	0.94	1.88	4.71	9.42	18.84	47.10	94.20	4.71
PFDoS	0.48	0.97	1.94	4.84	9.68	19.36	48.40	96.80	4.84
PFHxDA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFODA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00

9.2.1.20 Store at room temperature. Expires 180 days from prep.

NOTE: Stock standards (Section 8.2) were stored at ≤ 4± 2 °C. Primary dilution standards were stored at room temperature to prevent adsorption of the method analytes onto the container surfaces that may occur when refrigerated. Storing the standards at room temperature will also minimize daily imprecision due to the potential of inadequate room temperature stabilization.

9.2.2 Calibration Sequence

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

9.2.2.1 ESI-MS/MS Tune – Tuning should occur at least every six months using PPGs for tuning. Tune the system when ICAL won't pass, when the peak shape is significantly off (indicating an MS problem), when major maintenance is performed, or instrument is moved.

9.2.2.1.1 Load a 500 µL syringe filled with PPGs tuning solution in the syringe pump and connect it directly to the probe. Hold syringe pump power button for a few seconds to purge the line. Use the SCIEX Analyst® 1.6.3 software to adjust the parameters under the Tune and Calibrate tab to a relative signal maxima for peaks 44.998, 585.385, 933.636, 1223.845, 1572.097, 1863.306, 2037.431, 2211.557 in negative mode and 59.050, 175.133, 616.464, 906.673, 1254.925, 1545.134, 2010.469, 2242.637 in positive mode.

9.2.2.1.2 Mass assignment of tuning standard within 0.5 amu of true value.

9.2.2.1.3 When done, run the Compound Optimization or Manual Tuning under the Tune and Calibrate tab to optimize response and peak shape.

9.2.2.2 Optimize the precursor ion and product ion for each target analyte by infusing a standard mix from calibration curve to MS. The MS parameters (voltages, temperatures, gas flows, etc.) and the MS/MS parameters (collision energy, declustering potential, collision cell exit potential, etc.) are determined to achieve optimal analyte responses.

NOTE: There have been reports that not all product ions in the linear PFOS are produced in all branched PFOS isomers. (This phenomenon may exist for many of the PFAS.) Thus, to reduce PFOS, PFBS and PFHxS bias, it is required that the precursor m/z → m/z 80 transition be used as the quantitation transition. Some MS/MS instruments may not be able to scan a product ion with such a wide mass difference from the precursor ion; therefore, if the MS/MS cannot measure the precursor m/z → m/z 80 transition they may not be used for this method if PFOS, PFBS, or PFHxS analysis is to be conducted.

9.2.2.3 Establish LC operating parameters that optimize resolution and peak shape.

9.2.2.4 Inject a mid-level CAL standard under optimized LC/MS/MS conditions to ensure that each target analyte is observed in its retention time window and that there are at least 10 scans across the peak for optimum precision.

9.2.2.5 Prepare a set of at least five calibration point standards for linear fit (Table 9.2.1.20A as an example). Analyze each standard level with the same acquisition method used to analyze samples, changes to retention times or other analytical parameters are saved as part of the local method generated with each analytical sequence, these parameters can be adjusted mid-sequence so long as they are applied to all data. Use the LC/MS/MS data system software to generate a linear regression curve for each of the relevant analytes. This curve may be concentration weighted, if necessary. Forcing zero is not allowed for this analysis.

*Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies.
Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete.
Users of the SOP should verify the copy in possession is the current version of the SOP before use.*

COPYRIGHT © 2020 Pace Analytical Services, LLC

9.2.2.5.1 Calibration points at the top or the bottom of the curve may be dropped to achieve recovery requirements across the remaining points provided the minimum number of calibration points are still being used based on the curve fit. Dropping high concentration points lowers the upper QL of the calibration and may require that more dilutions are performed. Dropping low calibration points may elevate the reporting limit for samples associated with this calibration. The RL must be met without exception.

9.2.2.6 Analyte quantification uses the isotope dilution technique for the analytes having commercially available isotopically labeled analogs. The internal standard technique is used when a labeled analog is not commercially available for the target analyte. Details in analytes quantification refer to Section 10.5.

9.2.3 **ICAL Evaluation**

9.2.3.1 Calibration factors have RSD that is ≤ 20% for all analytes

9.2.3.2 Linear regressions have a coefficient of determination that is r^2 ≥0.99 and a minimum of five non-zero concentration standards is used.

9.2.3.3 Do not force linear regression through zero.

9.2.3.4 For each calibration standard, reprocess the target (native) analyte against the chosen calibration function. The reprocessed recoveries are expected to be within ±30% of true value. For some data uses, the lowest concentration standard reprocessed recoveries are expected to be within ±50% of true value.

9.2.3.5 The lowest concentration ICAL standard must be ≤ reporting level (RL).

9.2.3.6 S/N Ratio: ≥ 10: 1 for all quantification ions and S/N Ratio of ≥ 3:1 for confirmation ions

9.2.3.7 Demonstration and documentation of acceptable initial calibration is required before any samples are analyzed. After the initial calibration is successful, a CCV is required at the beginning and end of each period in which analyses are performed, and after every tenth field sample.

9.2.4. **Relative Standard Error (RSE)** – Percent error between the calculated and expected amounts of an analyte should be ≤ 30% for all standards. For some data uses, ≤ 50% may be acceptable for the lowest calibration point.

9.2.5. **Initial Calibration Verification**

9.2.5.1. Initial Calibration Verification (ICV) – analyze an ICV sample from a source different from the source of the CAL standards with each new ICAL before sample analysis. If a second vendor is not available, then a different lot of the standard should be used. The ICV should be prepared and analyzed just like a CCV. Acceptance criteria for the ICV are identical to the CCV; the calculated amount for each analyte must be ± 30% of the expected value. If measured analyte concentrations are not of acceptable accuracy, check the entire analytical procedure to locate and correct the problem.

COPYRIGHT © 2020 Pace Analytical Services, LLC

9.2.6. **Continuing Calibration Verification**

9.2.6.1. CCVs are run at the beginning, end, and bracketing every 10 field samples. Blanks, rinses, and spiked QC (LCS/LCSD/MS/MSD) are not considered field samples, and so can be run in addition to 10 field samples in a CCV window.

9.2.6.2. The opening CCV for any batch must be below or at the RL (CS-1), all further CCVs cycle between mid and high level calibration point.

9.2.6.3. Calculate the concentration of each analyte in the CCV. The calculated amount for each analyte must be within ± 30% of the true value. Determine that the absolute areas of the quantitation ions of the EIS and IIS are within ±50% from the mid-point measured during initial calibration. On days when ICAL is not performed, the peak areas must be within ±50% of the peak area measured in daily initial CCV. If any of the EIS and IIS areas has changed by more than these amounts, adjustments must be made to restore system.

9.2.6.3.1. For Wisconsin and other non-DoD compliance samples, the calculated amount for each analyte must be within ± 30% of the true value except for the lowest ICAL point, for which the calculated amount for each analyte must be within ± 50% of the true value.

9.2.6.4. If the CCV fails high for a particular analyte, and the field sample shows no detection for that analyte, samples may be reported without re-analysis.

9.2.7. **Laboratory Control Sample/Laboratory Control Sample Duplicate (LCS/LCSD)**

9.2.7.1. A LCS is required with each extraction batch. See DoD acceptance criteria for LCS targets in Appendix E for aqueous and solid matrices. If the LCS results do not meet the criteria listed in Appendix E for method analytes, then all data for the problem analyte(s) must be considered invalid for all samples in the extraction batch. For target analytes not included in the DoD Limits for batch control table (Appendix C of QSM 5.3, Table B-15), limits of 70-140% recovery will be used as acceptance criteria. For tissue batches, the recoveries are expected to be within 60-140%.

9.2.7.2. For Wisconsin and other non-DoD compliance samples, the recoveries are expected to be within 60-135%, except for the low range (1 – 2x RL) where the recoveries are expected to be within 50-150% in aqueous and solid batches. For tissue batches, spike the LCS at midrange. For tissue batches, the recoveries are expected to be within 60-135% with the following exceptions: for PFHxDA, PFODA, and NMeFOSA, the recoveries are expected to be within 50-135%; for PFDS, PFDoS, and 4:2 FTS, the recoveries are expected to be within 40-135%.

9.2.8. **EIS Recovery**

9.2.8.1. The EIS is fortified into all samples, CCVs, MBs, LCSs, MSs, MSDs, and FD prior to extraction. It is also added to the CAL standards. The EIS is a means of assessing method performance from extraction to final chromatographic measurement.

9.2.8.2. A minimal signal to noise ratio of 10:1 is expected for each EIS. Do not report results with a qualifier if this minimum is not achieved.

COPYRIGHT © 2020 Pace Analytical Services, LLC

9.2.8.3. EIS recovery must be in ±50% of the mid-point ICAL when the day the ICAL was performed. When EIS recovery from a sample, blank, or CCV failed the criteria, check 1) calculations to locate possible errors, 2) standard solutions for degradation, 3) contamination, and 4) instrument performance. Correct the problem and re-analyze the extract.

9.2.8.4. For Wisconsin and other non-DoD compliance analysis, all EIS compounds must recover within the range 25-150%, except 13C8-PFOSA, d3-MeFOSA, d5-EtFOSA, d7-MeFOSE, and d9-EtFOSE, which must recover within the range 10-150%. Recovery will be based on area counts.

9.2.8.5. If the EIS recoveries in a chromatographic run do not meet these criteria, inject a second aliquot of that extract from a new capped auto-sampler vial.

9.2.8.6. If the reinjected aliquot produces an acceptable EIS recoveries, report results for that aliquot.

9.2.8.7. If recoveries are acceptable for QC samples, but not field samples, the field samples must be re-prepped and reanalyzed (greater dilution may be needed). If recoveries are unacceptable for QC samples, correct problem, and reanalyze all associated failed field samples.

9.2.8.8. Apply Q-flag and discuss in the Case Narrative only if reanalysis confirms failures in exactly the same manner.

9.2.8.9. If the extract re-analysis meets the EIS recovery criterion, report only data for the re-analyzed extract.

9.2.8.10. If the extract re-analysis fails the criterion, the analyst should check the calibration by injecting the last CAL standard that passed. If the CAL standard fails the criteria, re-calibration is in order. If the CAL standard is acceptable, extraction of the sample should be repeated provided the sample is still within the holding time. If the re-extracted sample also fails the recovery criterion, report all data for that sample as suspect recovery to inform the data user that the results are suspect due to EIS recovery. Alternatively, collect a new sample and re-analyze.

9.2.9. **IIS Recovery**

9.2.8.1 The IIS is fortified into all samples, CCVs, MBs, LCSs, MSs, MSDs, and FD prior to extraction. It is also added to the CAL standards. The IIS is a means of assessing instrument performance.

9.2.8.2 A minimal signal to noise ratio of 10:1 is expected for each IIS. Do not report results with a qualifier if this minimum is not achieved.

9.2.8.3 IIS recovery must be in ±50% of the mid-point ICAL when the day the ICAL was performed. When IIS recovery from a sample, blank, or CCV is failed the criteria, check 1) calculations to locate possible errors, 2) standard solutions for degradation, 3) contamination, and 4) instrument performance. Correct the problem and re-analyze the extract.

9.2.8.4 If the EIS recoveries in a chromatographic run do not meet these criteria, inject a second aliquot of that extract from a new capped auto-sampler vial.

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

9.2.8.5 If the reinjected aliquot produces an acceptable IIS recovery, report results for that aliquot.

9.2.8.6 If recoveries are acceptable for QC samples, but not field samples, the field samples must be re-prepped and reanalyzed (greater dilution may be needed). If recoveries are unacceptable for QC samples, correct problem, and reanalyze all associated failed field samples.

9.2.8.7 If the extract re-analysis meets the IIS recovery criterion, report only data for the re-analyzed extract.

9.2.10. Additional calibration procedures (where applicable) can be found in ENV-POL-CORQ-0005 *Acceptable Calibration Practices for Instrument Testing* (or equivalent replacement).

9.3. Sample Preparation

9.3.1. Some of the PFAS adsorb to surfaces, including polypropylene. Therefore, the aqueous sample bottles must be rinsed with the elution solvent. The bottle rinse is passed through the cartridge to elute the method analytes and is then collected.

NOTE: The SPE cartridges and sample bottles described in this section are designed as single use items and should be discarded after use. They may not be refurbished for reuse in subsequent analyses.

9.3.2. **Solid Sample Preparation**

9.3.2.1. Homogenize the entire solid sample received in the sample container in which it was collected in by stirring the solid with a clean spatula or other implement.

9.3.2.2. 5 g of solid sample is weighed in a tared 50-mL polypropylene bottle

9.3.2.3. Add a 100 µL aliquot of the PFAC_EIS to all field and QC samples at the very beginning of the procedure.

9.3.2.4. QC samples for each batch include a MB, LCS and MS/MSD which are extracted along with each prep batch.

9.3.2.4.1. MB is required for each prep batch. Each batch contains a LCS and a pair of MS/MSD. LCS/MS/MSD spike at concentrations ≥ LOQ and ≤ the mid-level calibration concentration. If insufficient sample is available for a pair of MS/MSD, an MS, Dup, and LCSD at the same level of LCS may be used.

9.3.2.4.2. The LCS/LCSD/MS/MSD is spiked with 20 µL of the PFAC_Native Spike 2.

9.3.2.5. 5 mL of 0.2% ammonia/methanol is added to all samples and QC, bottles are sealed and put on an ultrasonicator for 20 minutes and then shake for one hour.

9.3.2.6. Centrifuge the samples and QC for 5 minutes after shake.

9.3.2.7. Decant the supernatant layer in a 50-mL polypropylene bottle with 50 mg of ENVI-Carb powder.

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

9.3.2.8. Repeat sections 9.3.2.5 with 4 mL of 0.2% ammonia/methanol and centrifuge. All supernatant are collected and combined.

9.3.2.9. The combined supernatant is shaken for one hour and then centrifuge for 5 minutes after shake.

9.3.2.10. Clean the filter with 10 mL of 1% of ammonia/acetonitrile.

9.3.3. Condition the pre-cleaned filter with 10 mL methanol. Pass the combined supernatant through the filter. Rinse the filter with additional 1 mL 0.2% ammonia/methanol. Collect the filtrate and turn on the vacuum for 10 minutes.

9.3.4. **Aqueous Sample Preparation**

9.3.4.1. Sample volume is determined gravimetrically. The full sample bottle is weighed and the empty bottle is weighted after extraction. The sample volume is the difference between the full and empty bottle weights. Sample density is assumed at 1 g/mL. When the sample has significant solids, the laboratory should account for the weight or volume displaced by the solid in the initial sample volume determination and include this information in the report.

9.3.4.2. pH is taken using strips in the lab. This is accomplished via the use of common laboratory grade pH strips (Whatman Indicator Paper pH 0-14 Type CF Cat. No. 2613-991). Adjust the pH to ~5 with acetic acid or 10 mM sodium hydroxide solution when necessary.

9.3.4.3. Add a 100 µL aliquot of the PFAC_EIS to all field and QC samples at the very beginning of the procedure, before extraction, centrifuging, filtering or phase separation takes place. Cap and invert and mix.

9.3.4.4. Ideally, whole samples will pass through the cartridge as received. If particulates in the sample is greater than one percent, centrifuge the sample and take the liquid phase through the SPE after spiking the PFAC_EIS.

9.3.4.5. QC samples for each batch include a MB, LCS and MS/MSD which are extracted along with each prep batch.

9.3.4.5.1. MB is required for each prep batch.

9.3.4.5.2. Each batch contains a LCS and a pair of MS/MSD. LCS/MS/MSD spike at concentrations ≥ LOQ and ≤ the mid-level calibration concentration. If insufficient sample is available for a pair of MS/MSD, an MS, Dup, and LCSD at the same level of LCS may be used.

9.3.4.5.3. The LCS/LCSD/MS/MSD is spiked with 20 µL of the PFAC_Native Spike 2.

9.3.4.6. Proceed with SPE procedure in 9.3.6.

9.3.5. **Tissue Sample Preparation**

9.3.5.1. Homogenization will be performed on the entire tissue sample in accordance with ENV-SOP-GBAY-0129 *Sample Homogenization, Compositing and Sub-Sampling* (or equivalent replacement), at the Pace Green Bay laboratory.

9.3.5.2. 2 g of tissue sample is weighed in a tared 50-mL HDPE bottle.

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

9.3.5.3. Add a 100 µL aliquot of the PFAC_EIS to all field and QC samples (canola oil and SRM) at the very beginning of the procedure.

9.3.5.4. QC samples for each batch include a MB, LCS, MS/MSD, and SRM which are extracted along with each prep batch.

9.3.5.4.1. MB is required for each prep batch. Each batch contains a LCS and a pair of MS/MSD. LCS/MS/MSD spike at concentrations at concentrations ≥ LOQ and ≤ the mid-level calibration concentration. If insufficient sample is available for a pair of MS/MSD, an MS, Dup, and LCSD at the same level of LCS may be used.

9.3.5.4.2. The LCS/LCSD/MS/MSD is spiked with 40 µL of the PFAC_Native Spike 2.

9.3.5.5. 7 mL of 1% ammonia/acetonitrile is added to all samples and QC, bottles are sealed and put on a shaker for 16 hours.

9.3.5.6. Centrifuge the samples and QC for 5 minutes after shake.

9.3.5.7. Decant the supernatant layer in a 50-mL polypropylene bottle with 100 mg of ENVI-Carb powder and shake for 1 hour and then centrifuge for 5 minutes after shake

9.3.5.8. Clean the 250 mg ENVI-Carb cartridge with 10 mL of 1% ammonia/acetonitrile.

9.3.5.9. Condition the pre-cleaned cartridge with 10 mL methanol. Pass the supernatant through the cartridge. Rinse the filter with additional 1 mL 1% ammonia/acetonitrile.

9.3.5.10. Collect the filtrate and dilute the filtrate with 125 mL H_2O and adjust pH to ~5. Proceed with SPE procedure in 9.3.6.

9.3.6. **Cartridge SPE Procedure**

9.3.6.1. Cartridge clean-up – Rinse each cartridge with 20 mL of 1% ammonia/acetonitrile solution.

9.3.6.2. Cartridge conditioning – Do NOT allow cartridge packing material to go dry during any of the conditioning steps. Condition each cartridge with 10 mL of 0.2% ammonia/methanol solution following with 10 mL of methanol. Next, rinse each cartridge with 10 mL of reagent water following with 10 mL of acetate buffer, without allowing the water to drop below the top edge of the packing. If the cartridge goes dry during the conditioning phase, the conditioning must be started over. Turn on the vacuum, and begin adding sample to the cartridge through the attached plastic sample transfer reservoir.

9.3.6.3. Sample extraction – Adjust the vacuum so that the approximate flow rate is 6-10 mL/min. Do not allow the cartridge to go dry before all the sample has passed through.

9.3.6.4. Sample bottle and cartridge rinse – Rinse the sample bottles with two 5-mL aliquots of reagent water, then draw each aliquot through the plastic sample transfer

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies.
Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete.
Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

reservoir and the cartridges. Draw air through the cartridge for 25 min at high vacuum (10-15 in. Hg).

NOTE: If transfer tubes are used in place of the sample transfer tubes to pass the samples through the cartridges, these reservoirs must be treated like the empty plastic sample transfer reservoirs. After the entire sample has passed through the cartridge, the tubes must be rinsed to waste with reagent water.

9.3.6.5. Sample bottle and cartridge elution – Turn off and release the vacuum. Lift the extraction manifold top and insert a rack with collection tubes into the extraction tank to collect the extracts as they are eluted from the cartridges. Rinse the sample bottles with 3 mL of 0.2% ammonia/methanol twice and elute the analytes from the cartridges by pulling the additional 3 mL of 0.2% ammonia/methanol through the sample plastic reservoirs and the cartridges. Turn the vacuum on for 20 minutes between each elution. The elution solvent used to rinse the sample bottles must be swirled down the sides of the reservoirs while eluting the cartridge to ensure that any method analytes on the surface of the reservoirs are transferred to the extract.

NOTE: After centrifuging, it is expected that the solid phase remains in the bottom of the container when rinsing the container with elution solvent. If rinsing the container disrupts the solid phase significantly, the container can be centrifuged again before removing the solvent for use during the elution step.

9.3.7. Extract concentration – Concentrate the extract to approximately 0.8 mL for water and solid extract and approximate 0.2 mL for tissue extract under a gentle stream of nitrogen without a heated water bath. Add 100 µL of PFAC_IIS and filled the sample vial to 1 mL mark with 537 Mix. Then vortex for 5-10 seconds. Transfer a ~100 µL to a 300 µL polypropylene autosampler vial with a plastic pipette. The remaining extract is stored at 0-6 °C.

9.4. Analysis

9.4.1. Establish operating conditions equivalent to those summarized in Appendix C. Instrument conditions and columns should be optimized prior to the initiation of the IDOC.

9.4.2. Establish an appropriate retention time window for each analyte. This should be based on measurements of actual retention time variation for each method analyte in Calibration (CAL) standard solutions analyzed on the LC over the course of time. A value of plus or minus three times the standard deviation of the retention time obtained for each method analyte while establishing the initial calibration and completing the IDC can be used to calculate a suggested window size. However, the experience of the analyst should weigh heavily on the determination of the appropriate retention window size.

9.4.3. Retention Time (RT) acceptance – RT of each analyte and EIS analyte must fall within 0.4 minutes (±0.2 minutes) of the predicted retention times from the daily CCV or, on days when ICAL is performed, from the midpoint standard of the ICAL. Analytes must elute within 0.1 minute of the associated EIS. This criterion applies only to analyte and labeled analog pairs.

*Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies.
Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete.
Users of the SOP should verify the copy in possession is the current version of the SOP before use.*

COPYRIGHT © 2020 Pace Analytical Services, LLC

9.4.4. Calibrate the system by either the analysis of a calibration curve or by confirming the initial calibration is still valid by analyzing a CCV. If establishing an initial calibration, complete the IDC.

9.4.5. Begin analyzing field samples, including QC samples, at their appropriate frequency by injecting 3 µL of final sample extractant, under the same conditions used to analyze the ICAL standards.

9.4.6. At the conclusion of data acquisition, use the same software that was used in the calibration procedure to identify peaks of interest in predetermined retention time windows. Use the data system software to examine the ion abundances of the peaks in the chromatogram. Identify an analyte by comparison of its retention time with that of the corresponding method analyte peak in a reference standard. Comparison of the MS/MS mass spectra is not particularly useful given the limited ±0.5 amu mass range around a single product ion for each method analyte.

9.4.7. Dilution – When the concentrations of target analytes exceed the highest concentration of ICAL, dilution analyses are required.

9.4.7.1. An appropriate dilution should be in the upper half of the calibration range, or close to the CCV. The diluted extract must maintain the same methanol/water ratio as the original extract

9.4.7.2. If an analyte concentration exceeds the range of the initial calibration curve, the extract is diluted with 537 Mix. Re-inject the diluted extract. Incorporate the dilution factor into the final concentration calculations. Acceptable injection internal standard (IIS) performance is determined from the undiluted sample extract. The resulting data is documented as a dilution, with an increased LOQ.

9.4.8. In validating this method, concentrations were calculated by measuring the product ions listed in Appendix C. Two transitions and the ion transition ratio per analyte shall be monitored and documented with the exception of PFBA and PFPeA. In order to avoid biasing results high due to known interferences for some transitions, the following transitions must be used for the quantification of the following analytes: PFOA: 413 —› 369, PFOS: 499 —› 80, PFHxS: 399 —› 80, PFBS: 299 —› 80, 4:2FTS: 327 —› 307, 6:2FTS: 427 —› 407 8:2FTS: 527 —› 507, N-EtFOSAA: 584 —› 419, N-MeFOSAA: 570 —› 419. If these transitions are not used, the reason must be technically justified and documented (e.g., alternate transition was used due to observed interferences).

9.4.9. Calculate analyte concentrations using the multipoint calibration established in Section 9.2.1.20. Do not use daily calibration verification data to quantitate analytes in samples. Adjust final analyte concentrations to reflect the actual sample volume.

9.4.10. Prior to reporting the data, the chromatogram is reviewed for any incorrect peak identification or poor integration. Modify if necessary.

9.4.11. Calculations must utilize all available digits of precision, but final reported concentrations are rounded to an appropriate number of significant figures (one digit of uncertainty), typically two, and not more than three significant figures.

9.4.12. For native analytes, the Signal to Noise (S/N) ratio should be ≥3:1 for both quantitation and confirmation ions. If S/N is not achieved, the analyte would be reported as not detected.

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

9.4.13. Ion Ratios – For analytes with two ion transitions (quantitation and confirmation) are analyzed, the area ratio between the confirmation and quantitation transitions shall be monitored and documented. The ion ratio for all analytes in each injection should be within ± 50% of the mid ICAL ion ratio for the same analyte in the ICAL. On days ICAL is not performed, the ion ratio should be within ±50% of the initial CCV standard.

9.4.14. Report results in acid form.

9.4.15. Perform a moisture analysis on solid samples (on a subsample different than that used for extraction) and adjust the final concentration of solid sample for the percent moisture.

9.4.16. DoD acceptance criteria for LCS and MS target analytes are listed in Appendix E. If the LCS results do not meet the criteria listed then all data for the problem analyte(s) must be considered invalid for all samples in the extraction batch. For target analytes not included in the DoD Limits for batch control table in QSM 5.3, Table B-15, limits of 70-140% recovery for water and soil, 60-140% recovery for tissue will be used as acceptance criteria.

9.5. Analytical Sequence

9.5.1. Example analytical sequence

Sequence
Instrument Blank (ICB)
Initial Calibration (ICAL)
Instrument Blank (ICB)
Initial Calibration Verification (ICV)
Continuing Calibration Curve (CCV)
Continuing Calibration Blank (CCB)
Qualitative Standard for PFOA (T-PFOA)
Method Blank (MB)
Laboratory Control Spike/Dup (LCS/LCSD)
Matrix Spike/Dup (MS/MSD)
Field Samples
Continuing Calibration Curve (CCV)
Continuing Calibration Blank (CCB)
Field Samples
Continuing Calibration Curve (CCV)
Continuing Calibration Blank (CCB)

10. Data Analysis And Calculations

10.3. Qualitative Identification

10.3.1. Manual Integration

Manual changes to automated integration is called manual integration. Manual integration is sometimes necessary to correct inaccurate automated integrations but must never be used to meet QC criteria or to substitute for proper instrument maintenance and/or method set-up. To assure that all manual integrations are performed consistently and are ethically justified, all manual integrations must be

*Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies.
Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete.
Users of the SOP should verify the copy in possession is the current version of the SOP before use.*

COPYRIGHT © 2020 Pace Analytical Services, LLC

performed, reviewed, and recorded in accordance with corporate SOP ENV-SOP-CORQ-0006, *Manual Integration.*

10.4. Quantitative Identification

10.4.1. Complete chromatographic resolution is not necessary for accurate and precise measurements of analyte concentrations using MS/MS. In validating this method, concentrations were calculated by measuring the product ions listed in Appendix C. Other ions may be selected at the discretion of the analyst.

10.4.2. Prior to reporting the data, the chromatogram should be reviewed for any incorrect peak identification or poor integration.

10.4.3. For PFHxS, PFOS, N-MeFOSAA and N-EtFOSAA, all the chromatographic peaks observed in the standard must be integrated and the areas summed. Chromatographic peaks in all Field Samples and QC samples must be integrated in the same way as the CAL standard for analytes with quantitative standards containing the branched and linear isomers.

10.4.4. For PFOA, identify the branched isomers by analyzing a qualitative standard (T-PFOA) that includes both linear and branched isomers and compare retention times and tandem mass spectrometry transitions. Quantitate Field Samples and QC samples by integrating the total response (i.e., accounting for peaks that are identified as linear and branched isomers) and relying on the initial calibration with a linear isomer quantitative PFOA standard. This qualitative PFOA standard is not used for quantitation. This branched isomer identification check must be repeated any time changes occur that affect the analyte retention times.

10.4.5. Peaks that are consistent with branched isomers have been observed with other target analytes, in particular PFOA. Quantitate of PFOA by integrating the total response (i.e. accounting for peaks that are identified as linear and branched isomers) and relying on the initial calibration with the linear-isomer quantitative standard is acceptable.

10.4.6. All analytes are quantified using the isotope dilution or internal standard technique.

10.4.7. The native analytes are quantified by comparison of their responses to the mass-labelled internal standards. Relative response factors are calculated from analyses of standard mixtures containing native analytes at six concentration levels, and the concentration remains at a constant level for each internal standard. The target analytes response factors are calculated by comparing the response from the native ion mass monitored to the response from the ion mass of the corresponding isotopically labelled internal standard (See Appendix D for reference).

10.5. Calculations

See the laboratory SOP ENV-SOP-MIN4-0171 *Laboratory Calculations* (or equivalent replacement) for equations for common calculations.

Linear Calibration Using Average Response Factors

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

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

Equation 1

$$RF_i = (A_a Q_s)/A_s Q_a$$

Where, RF = Response factor
A_a = Sum of integrated areas for analyte
Q_s = Quantity of labeled standard
A_s = Sum of integrated areas for labeled standard
Q_a = Quantity of analyte

The levels of native analytes in the samples are quantified using the following equations:

Equation 2

$$C = (A_n Q_{is})/A_{is} \times W \times RF$$

Where, RF = Response factor
A_n = Sum of integrated areas for target isomer
Q_{is} = Quantity of labeled internal standard added to the sample
A_{is} = Sum of integrated areas for labeled internal standard
W = Sample amount
C = Concentration of target isomer

Equation 3

$$\text{Average Response Factor} = \overline{RF} = \left(\frac{\sum_{i=1}^{n} RF_i}{n}\right)$$

Where, n = Number of calibration levels
RF_i = Response factor for the ith level

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

Equation 4

$$RSD\ (\%) = \frac{SD}{\overline{RF}} \times 100\%$$

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

$$SD = \sqrt{\frac{\sum_{i=1}^{N}(RF_i - \overline{RF})^2}{N-1}}$$

Linear regression fit: *y* = m*x* + b

Equation 5

$$A_x/A_{is} = m(C_x/C_{is}) + b$$

Where, A_x = Response area for analyte

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

A_{is} = Response area for the internal standard
C_x = Analyte concentration of calibration standard
C_{is} = Internal standard concentration
m = Slope
b = *y*-intercept

The levels of native analytes in the samples are quantified using the following equation:

Equation 6

$$C_{sx} = (A_x/A_{is} - b)*C_{is}/m$$

Where, C_{sx} = Unknown sample analyte concentration
A_x = Response area for analyte
A_{is} = Response area for the internal standard
C_{is} = Internal standard concentration
m = Slope
b = *y*-intercept

NOTE: For Wisconsin samples, report sample results and all quality control blank results to MDL and included the RL with each results. Quality results reported between the MDL and RL as estimated concentration.

11. Quality Control and Method Performance

11.1. Quality Control

The following QC samples are prepared and analyzed with each batch of samples. Refer to Appendix B for acceptance criteria and required corrective action.

QC Item	Frequency
Method Blank (MB)	1 per batch of 20 or fewer samples. If batch exceeds, 20 samples, every 20.
Laboratory Control Sample (LCS)	1 per batch of 20 or fewer samples. If batch exceeds, 20 samples, every 20.
Laboratory Control Sample Duplicate (LCSD)	As needed
Matrix Spike (MS)	1 per batch of 20 or fewer samples. If batch exceeds, 20 samples, every 20.
Matrix Spike Duplicate (MSD)	1 per batch of 20 or fewer samples. If batch exceeds, 20 samples, every 20.
Field Duplicate	1 per batch of 20 or fewer samples. If batch exceeds, 20 samples, every 20.
Extraction Internal Standard	All samples and QC
Injection Internal Standard	All samples and QC
Standard Reference Material	1 per batch of 20 or fewer tissue samples. If batch exceeds, 20 samples, every 20.

11.2. Instrument QC

COPYRIGHT © 2020 Pace Analytical Services, LLC

The following Instrument QC checks are performed. Refer to Appendix B for acceptance criteria and required corrective action.

QC Item	Frequency
Tune	Every 6 months or when ICAL won't pass, the peak shape is significantly off, major maintenance is performed, or instrument is moved.
Initial Calibration	At instrument set up, after CCV failure
Initial Calibration Verification	Once per calibration at mid-level of ICAL
Initial Calibration Blank	One following the highest standard analyzed and prior to ICV
Continuing Calibration Verification	At the beginning, end, and bracketing every 10 field samples
Continuing Calibration Blank	1 after each CCV
RT Window	RT of each analyte and EIS analyte must fall within 0.4 minutes (±0.2 minutes) of the predicted retention times from the daily CCV or, on days when ICAL is performed, from the midpoint standard of the ICAL.
Relative Retention Time	Analytes must elute within 0.1 minute of the associated EIS. This criterion applies only to analyte and labeled analog pairs

11.3. Method Performance

11.3.1. Method Validation

11.3.1.1. Detection Limits

Detection limits (DL) and limits of quantitation (LOQ) are established at initial method setup and verified on an on-going basis thereafter. Refer to Pace ENV corporate SOP ENV-SOP-CORQ-0011 *Method Validation and Instrument Verification*.

11.4. Analyst Qualifications and Training

11.4.1. Employees that perform any step of this procedure must have a completed Read and Acknowledgment Statement for this version of the SOP in their training record. In addition, prior to unsupervised (independent) work on any client sample, analysts that prepare or analyze samples must have successful initial demonstration of capability (IDOC) and must successfully demonstrate on-going proficiency on an annual basis. Successful means the initial and on-going DOC met criteria, documentation of the DOC is complete, and the DOC record is in the employee's training file. Refer to laboratory SOP ENV-SOP-MIN4-0165 *Orientation and Training Procedures* (or equivalent replacement) for more information.

11.4.2. For each analyte, the mean accuracy is true value ±30%. The RSD must be less than 20%. If any target analyte fails to meet this criterion, the source of the problem must be corrected and the test repeated

11.4.2.1. For Wisconsin and other non-DoD compliance samples, the mean accuracy is true value ±35%. The RSD must be less than or equal to 30%. If any target analyte fails to meet this criterion, the source of the problem must be corrected and the test repeated

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

12. Data Review and Corrective Action

12.1. Data Review

Pace's data review process includes a series of checks performed at different stages of the analytical process by different people to ensure that SOPs were followed, the analytical record is complete and properly documented, proper corrective actions were taken for QC failure and other nonconformance(s), and that test results are reported with proper qualification.

The review steps and checks that occur as employee's complete tasks and review their own work is called primary review.

All data and results are also reviewed by an experienced peer or supervisor. Secondary review is performed to verify SOPs were followed, that calibration, instrument performance, and QC criteria were met and/or proper corrective actions were taken, qualitative ID and quantitative measurement is accurate, all manual integrations are justified and documented in accordance with the Pace ENV's SOP for manual integration, calculations are correct, the analytical record is complete and traceable, and that results are properly qualified.

A third-level review, called a completeness check, is performed by reporting or project management staff to verify the data report is not missing information and project specifications were met.

Refer to laboratory SOP ENV-SOP-MIN4-0092 *Data Review Process* (or equivalent replacement) for specific instructions and requirements for each step of the data review process.

12.2. Corrective Action

Corrective action is expected any time QC or sample results are not within acceptance criteria. If corrective action is not taken or was not successful, the decision/outcome must be documented in the analytical record. The primary analyst has primary responsibility for taking corrective action when QA/QC criteria are not met. Secondary data reviewers must verify that appropriate action was taken and/or that results reported with QC failure are properly qualified.

Corrective action is also required when carryover is suspected and when results are over range.

Samples analyzed after a high concentration sample must be checked for carryover and reanalyzed if carryover is suspected. Carryover is usually indicated by low concentration detects of the analyte in successive samples analyzed after the high concentration sample.

Sample results at concentrations above the upper limit of quantitation must be diluted and reanalyzed. The result in the diluted samples should be within the upper half of the calibration range. Results less than the mid-range of the calibration indicate the sample was over diluted and analysis should be repeated with a lower level of dilution. If dilution is not performed, any result reported above the upper range is considered a qualitative measurement and must be qualified as an estimated value.

Refer to Appendix B for a complete summary of QC, acceptance criteria, and recommended corrective actions for QC associated with this test method.

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies.
Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete.
Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

13. Pollution Prevention And Waste Management

Pace proactively seeks ways to minimize waste generated during our work processes. Some examples of pollution prevention include but are not limited to: reduced solvent extraction, solvent capture, use of reusable cycletainers for solvent management, and real-time purchasing.

The EPA requires that laboratory waste management practice to be conducted consistent with all applicable federal and state laws and regulations. Excess reagents, samples and method process wastes must be characterized and disposed of in an acceptable manner in accordance with Pace's Chemical Hygiene Plan / Safety Manual.

14. Modifications

A modification is a change to a reference test method made by the laboratory. For example, changes in stoichiometry, technology, quantitation ions, reagent or solvent volumes, reducing digestion or extraction times, instrument runtimes, etc. are all examples of modifications. Refer to Pace ENV corporate SOP ENV-SOP-CORQ-0011 *Method Validation and Instrument Verification* for the conditions under which the procedures in test method SOPs may be modified and for the procedure and document requirements.

15. Responsibilities

Pace ENV employees that perform any part this procedure in their work activities must have a signed Read and Acknowledgement Statement in their training file for this version of the SOP. The employee is responsible for following the procedures in this SOP and handling temporary departures from this SOP in accordance with Pace's policy for temporary departure.

Pace supervisors/managers are responsible for training employees on the procedures in this SOP and monitoring the implementation of this SOP in their work area.

16. Attachments

Appendix A – Target Analyte List and Routine LOQ

Appendix B – QC Summary

Appendix C – Typical MS/MS Method Conditions

Appendix D – PFAS Analyte and Recommended Extracted Internal Standard Used for Quantification

Appendix E – Method PFAS by LCMSMS Compliant with DoD QSM Batch Control Limits

Appendix F – DoD QSM Requirements

17. References

Department of Defense Department of Energy Consolidated Quality Systems Manual (QSM) for Environmental Laboratories, Version 5.3, Table B-15, 2019.

COPYRIGHT © 2020 Pace Analytical Services, LLC

Wisconsin Department of Natural Resources, Wisconsin PFAS Aqueous (Non-Potable Water) and Non-Aqueous Matrices Method Expectations, EA-19-0001, December, 2019.

DoD Guidance for PFAS Analysis in Biota. April, 2020.

USEPA, Method 537.1, Version 1.0 "Determination of selected per- and polyfluorinated alkyl substances in drinking water by solid phase extraction and liquid chromatography/tandem mass spectrometry (LC/MS/MS)"; November 2018.

USEPA, Technical Advisory, "Technical Advisory - Laboratory Analysis of Drinking Water Samples for Perfluorooctanoic Acid (PFOA) Using EPA Method 537 Rev. 1.1"; September 2016.

JT Baker, Application Technical Support Group, Endothall extraction using BAKERBOND Speedisk SAX, PN-8058-06, 2006.

Pace Quality Assurance Manual- most current version.

TNI Standard, *Management and Technical Requirements for Laboratories Performing Environmental Analyses*, EL-V1-2009.

TNI Standard, *Management and Technical Requirements for Laboratories Performing Environmental Analyses*, EL-VI-2016-Rev.2.1.

USEPA, "Manual for the Certification of Laboratories Analyzing Drinking Water"; Fifth Edition, January 2005.

USEPA, "Supplement 1to the Fifth Edition of the Manual for the Certification of Laboratories Analyzing Drinking Water"; June 2008.

40 CFR Appendix B to Part 136, *Definition and Procedure for the Determination of the Method Detection Limit - Rev 2*, August 28, 2017.

18. REVISION HISTORY

This Version:

Section	Description of Change
NA	Original version of document.

This document supersedes the following document(s):

Document Number	Title	Version
NA	Original version of document.	

COPYRIGHT © 2020 Pace Analytical Services, LLC

Appendix A: Target Analyte List and Routine LOQ[1]

LOQs for all analytes are listed below. Current MDLs are listed in LIMS and are available by request from the Quality Manager.

Analyte	Acronym(s)[3]	CAS#	LOQ		
			Water (ng/L)	Solid (ng/kg)	Tissue (ng/kg)
Perfluorobutanoic acid	PFBA	375-22-4	2	100	250
Perfluoropentanoic acid	PFPeA	2706-90-3	2	100	250
Hexafluoropropylene oxide dimer acid	HFPO-DA[2] PFPrOPrA	13252-13-6	2	100	250
Perfluorohexanoic acid	PFHxA	307-24-4	2	100	250
Perfluoroheptanoic acid	PFHpA	375-85-9	2	100	250
Perfluorooctanoic acid	PFOA	335-67-1	2	100	250
Perfluorononanoic acid	PFNA	375-95-1	2	100	250
Perfluorooctanesulfonamide	PFOSAm PFOSA FOSA	754-91-6	2	100	250
N-methylperfluorooctane sulfonamide	MeFOSA[2] N-MeFOSA NMeFOSA	31506-32-8	2	100	250
Perfluorodecanoic acid	PFDA	335-76-2	2	100	250
N-ethylperfluorooctane sulfonamide	EtFOSAm[2] N-EtFOSA NEtFOSA	4151-50-2	2	100	250
Perfluoroundecanoic acid	PFUnDA PFUnA PFUdA	2058-94-8	2	100	250
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA N-MeFOSAA	2355-31-9	2	100	250
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA N-EtFOSAA	2991-50-6	2	100	250
Perfluorododecanoic acid	PFDOA PFDoA PFDoDA	307-55-1	2	100	250
N-methylperfluorooctane sulfonamidoethanol	MeFOSE[2] N-MeFOSE NMeFOSE	24448-09-7	2	100	250
N-ethylperfluorooctane sulfonamidoethanol	EtFOSE[2] N-EtFOSE NEtFOSE	1691-99-2	2	100	250
Perfluorotridecanoic acid	PFTrDA PFTriA PFTrA	72629-94-8	2	100	250
Perfluorotetradecanoic acid	PFTDA PFTeDA PFTA PFTeA	376-06-7	2	100	250
Perfluorohexadecanoic acid	PFHxDA[2]	67905-19-5	2	100	250
Perfluorooctandecanoic acid	PFODA[2]	16517-11-6	2	100	250
Perfluorobutanesulfonic acid	PFBS	375-73-5	1.77	88.5	221.3
Perfluoropentanesulfonic acid	PFPeS	2706-91-4	1.88	94	235
Perfluorohexanesulfonic acid	PFHxS	355-46-4	1.82	91	227.5
Perfluoroheptanesulfonic acid	PFHpS	375-92-8	1.90	95	237.5
Perfluorooctanesulfonic acid	PFOS	1763-23-1	1.85	92.5	231.3
Perfluorononanesulfonic acid	PFNS	68259-12-1	1.92	96	240
Perfluorodecanesulfonic acid	PFDS	335-77-3	1.93	96.5	241.3
Perfluorododecanesulfonic acid	PFDoS[2] PFDoDS	79780-39-5	1.94	97	242.5

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

Analyte	Acronym(s)[3]	CAS#	LOQ		
			Water (ng/L)	Solid (ng/kg)	Tissue (ng/kg)
4:2 Fluorotelomer sulfonic acid	4:2 FTS 4:2 FTSA 4:2FTS	757124-72-4	1.87	93.5	233.8
6:2 Fluorotelomer sulfonic acid	6:2 FTS 6:2 FTSA 6:2FTS	27619-97-2	1.90	95	237.5
8:2 Fluorotelomer sulfonic acid	8:2 FTS 8:2 FTSA 8:2FTS	39108-34-4	1.93	96.5	241.3
10:2 Fluorotelomer sulfonic acid	10:2 FTS[2] 10:2 FTSA	120226-60-0	1.93	96.5	241.3
4,8-Dioxa-3H-perfluorononanoic acid	DONA[2] ADONA	919005-14-4	1.89	94.5	236.3
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS[2] F-53B Major	756426-58-1	1.86	93	232.5
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS[2] F-53B Minor	763051-92-9	1.88	94	235

[1] Values in place as of effective date of this SOP. LOQs are subject to change. For the most up to date LOQ, refer to the LIMS or contact the laboratory.

[2] DoD currently does not have guidance for the compound, as of 2019 QSM 5.3, Table B-15.

[3] All possible acronym variations are listed as the acronym used and/or referenced may vary depending on the State data is being reported to.

COPYRIGHT © 2020 Pace Analytical Services, LLC

Appendix B: QC Summary

QC Item	Frequency	Wisconsin Guidance Acceptance Criteria	QSM 5.3, Table B-15 Acceptance Criteria	Corrective Action	Qualification
ICAL	At instrument set up, after CCV failure	Lowest ICAL ± 50% Other points ± 30% For any curve fit other than Average RF (RSD), curve must also pass RSE test at the low and midpoint calibration standard.	All points ±30% of true value. For any curve fit other than Average RF (RSD), curve must also pass RSE test at the low and midpoint calibration standard.	Identify and correct source of problem, repeat	None. Do not proceed with analysis
Curve Refitting	Whenever ICAL is performed	Must meet one of curve fit options presented in Section 10.0.	Must meet one of curve fit options presented in Section 10.0.	Identify and correct source of problem, repeat	None. Do not proceed with analysis
ICV	1 after each ICAL	True value ± 30%	True value ± 30%	Identify source of problem, re-analyze. If repeat failure, repeat ICAL. Analysis may proceed if it can be demonstrated that the ICV exceedance has no impact on analytical measurements. For example, the ICV %R is high, CCV is within criteria, and the analyte is not detected in sample(s).	Qualify analytes with ICV out of criteria.
RT Window Position (Daily)	Once per ICAL and at the beginning of the analytical window.	Position is set using the mid-point of the ICAL on the day ICAL is performed; otherwise mid-point of CCV is used	Position is set using the mid-point of the ICAL on the day ICAL is performed; otherwise mid-point of CCV is used	NA	NA
RT Window Study	At method set-up and after major instrument maintenance	Window is ±0.2 minutes the daily CCV or, on days when ICAL is performed, from the midpoint standard of the ICAL. Analytes must elute within 0.1 minute of the associated EIS	Window is ±0.2 minutes the daily CCV or, on days when ICAL is performed, from the midpoint standard of the ICAL. Analytes must elute within 0.1 minute of the associated EIS	Correct problem and reanalyze samples	NA
Continuing Calibration Curve (CCV)	Daily, before sample analysis, after every 10 field samples, and at end of analytical window.	CCV at low level: True value ±50% Other CCV: True value ±30%	True value ±30%	Perform necessary maintenance and demonstrate stability by analyzing an initial calibration before resuming sample analysis. Samples between passing CCV and failing CCV should be re-analyzed.	Qualify analytes with CCV out of criteria.
Extracted	Every field	Must meet criteria	Must meet criteria	If the CCV fails high for a	Qualify

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

Internal Standards (EIS)	sample, standard and QC sample	specified in Section 9.2.8	specified in Section 9.2.8	particular analyte, and the field sample is non-detect for that analyte, samples may be reported without re-analysis.	outages and explain in case narrative.
Injection Internal Standards (IIS)	Every field sample, standard and QC sample	Must meet criteria specified in Section 9.2.9	Must meet criteria specified in Section 9.2.9	Troubleshoot instrument performance. Reanalyze samples.	Qualify outages and explain in case narrative.
Method Blank (MB)	1 per batch	Analytes ≤1/2 the RL or 1/10th the amount measured in any sample	Analytes ≤1/2 the RL or 1/10th the amount measured in any sample or 1/10th the regulatory limit, whichever is greater	1) If sample ND, report sample without qualification. 2) If sample result >10x MB detects and sample cannot be reanalyzed, report sample with appropriate qualifier indicating blank contamination. 3) If sample result <10x MB detects, report sample with appropriate qualifier to indicate an estimated value. Client must be alerted to give authorization to report this data. 4) Analyte detection or failure of internal standard fails entire batch.	Qualify outages and explain in case narrative
LCS/LCSD	1 spiked at a concentration ≥ LOQ and ≤ the mid-level calibration concentration	True Value ≤ 1-2x RL 50-150% True Value > 2x RL 60-135% RPD ≤ 30%	See Appendix E RPD ≤ 30%	Reanalyze and/or reprepare batch of samples with new LCS. If LCS rec > QC limits and these compounds are non-detect in the associated samples, the sample data may be reported with appropriate data qualifiers. If these criteria are not met, where extra samples are available, a re-extract is analyzed or else data is narrated.	Qualify outages and explain in case narrative
MS/MSD	1 pair/batch spiked at a concentration ≥ LOQ and ≤ the mid-level calibration concentration	True Value ≤ 1-2x RL 50-150% True Value > 2x RL 60-135% RPD ≤ 30%	See Appendix E RPD ≤ 30%	Failures are flagged but do not prevent reporting data if MB and LCS meet criteria.	Qualify outages and explain in case narrative
Field Duplicate (FD)	1 per batch. A MSD may be substituted for a sample	NA	NA	If these criteria are not met, results are labeled suspect due to matrix effects	Qualify outages and explain in case

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

	duplicate if sample is insufficient				narrative
Tune Standard	Every six month, when ICAL won't pass, when the peak shape is significantly off (indicating an MS problem), when major maintenance is performed, or instrument is moved	See section 9.2.1 for reference	See section 9.2.1 for reference	Refer to manufacture criteria	NA
Instrument Blank (ICB)	1 following the highest standard analyzed	< ½ RL	≤ ½ RL	If acceptance criteria are not met after the highest calibration standard, calibration must be performed using a lower concentration for the highest standard until acceptance criteria is met.	Flagging is only appropriate in cases when the sample cannot be reanalyzed and when there is no more sample left.
CCB	1 following the CCV and prior to sample analysis	< ½ RL	NA	If acceptance criteria are not met after the CCV. Clean the system and prepare new CCV if needed.	Flagging is only appropriate in cases when the sample cannot be reanalyzed and when there is no more sample left.

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies.
Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete.
Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

Appendix C: Typical MS/MS Method Conditions

Analyte	Precursor Ion (m/z)	Product Ion[a] (m/z)	RT (min)	Declustering Potential (v)	Collision Energy (v)	Collision Cell Exit Potential (v)
PFBA	212.9	169	2.43	-45	-12	-11
PFPeA	262.9	219	3.33	-25	-12	-11
HFPO-DA	285	169	4.3	-70	-12	-11
HFPO-DA_2	285	185	4.29	-70	-24	-13
PFBS	298.9	80	4.27	-65	-58	-9
PFBS_2	298.9	99	4.27	-65	-40	-9
PFHxA	313	269	4.03	-25	-12	-19
PFHxA_2	313	119	4.02	-25	-28	-19
4:2FTS	327	307	3.75	-65	-28	-13
4:2FTS_2	327	81	3.75	-65	-56	-11
PFPeS	349	80	4.94	-45	-68	-9
PFPeS_2	349	99	4.94	-65	-40	-9
PFHpA	363	319	4.63	-50	-14	-15
PFHpA_2	363	169	4.63	-50	-24	-11
DONA	377	251	4.84	-50	-16	-11
DONA_2	377	85	4.84	-50	-36	-11
PFHxS	399	80	5.52	-55	-84	-9
PFHxS_2	399	99	5.52	-55	-68	-11
PFOA	413	369	5.17	-55	-14	-17
PFOA_2	413	169	5.17	-55	-24	-9
6:2FTS	427	407	4.89	-65	-32	-17
6:2FTS_2	427	81	4.89	-65	-68	-7
PFHpS	449	80	6.07	-105	-92	-9
PFHpS_2	449	99	6.07	-80	-80	-13
PFNA	463	419	5.7	-70	-16	-15
PFNA_2	463	169	5.7	-70	-26	-11
PFOSA	498	78	7.47	-130	-90	-11
PFOS	499	80	6.58	-65	-112	-9
PFOS_2	499	99	6.58	-65	-90	-11
N-MeFOSA	512	169	8.76	-55	-36	-11
N-MeFOSA_2	512	218.9	8.75	-60	-34	-19
PFDA	513	469	6.21	-80	-16	-19
PFDA_2	513	169	6.21	-80	-28	-13
N-EtFOSA	526	169	9.23	-40	-36	-13
N-EtFOSA_2	526	219.15	9.23	-15	-34	-9
8:2FTS	527	507	5.92	-70	-38	-21
8:2FTS_2	527	81	5.92	-70	-92	-9
9Cl-PF3ONS	530.9	351	6.97	-75	-36	-15
9Cl-PF3ONS_2	530.9	83	6.97	-75	-70	-11
PFNS	549	80	7.08	-65	-118	-13
PFNS_2	549	99	7.08	-85	-96	-11
PFUdA	563	519	6.72	-30	-18	-21
PFUdA_2	563	169	6.71	-30	-32	-11
N-MeFOSAA	570	419	6.11	-125	-28	-33
N-MeFOSAA_2	570	483	6.11	-125	-22	-33
N-EtFOSAA	584	419	6.34	-125	-28	-33
N-EtFOSAA_2	584	526	6.33	-125	-28	-33
PFDS	599	80	7.57	-85	-122	-11
PFDS_2	599	99	7.57	-85	-100	-11
PFDoA	613	569	7.21	-25	-18	-23
PFDoA_2	613	169	7.21	-25	-34	-11

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies.
Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete.
Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

N-MeFOSE	616	59	8.58	-20	-76	-5
10:2FTS	627	607	6.92	-50	-44	-25
10:2FTS_2	627	81	6.91	-50	-108	-9
N-EtFOSE	630	59	9.03	-20	-58	-27
11Cl-PF3OUdS	630.9	451	7.94	-90	-40	-19
11Cl-PF3OUdS_2	630.9	99	7.94	-90	-92	-5
PFTrDA	663	619	7.69	-75	-20	-25
PFTrDA_2	663	169	7.69	-75	-34	-9
PFDoS	699	80	8.46	-30	-134	-9
PFDoS_2	699	99	8.46	-20	-132	-11
PFTeDA	713	669	8.17	-85	-20	-27
PFTeDA_2	713	169	8.17	-85	-36	-13
PFHxDA	813	769	9.23	-30	-22	-33
PFHxDA_2	813	169	9.23	-30	-38	-9
PFODA	913	869	9.72	-5	-22	-35
PFODA_2	913	169	9.72	-5	-42	-11

Analyte_2 Ions used for confirmation purposes.

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies.
Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete.
Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

Appendix D: PFAS Analyte and Recommended Extracted Internal Standard Used for Quantification

Analyte	EIS Name
PFBA	13C4_PFBA
PFPeA	13C5_PFPeA
HFPO-DA	13C3_HFPO-DA
PFBS	13C3_PFBS
PFHxA	13C5_PFHxA
4:2FTS	13C2_4:2FTS
PFPeS	13C3_PFHxS
PFHpA	13C4_PFHpA
DONA	13C8_PFOA
PFHxS	13C3_PFHxS
PFOA	13C8_PFOA
6:2FTS	13C2_6:2FTS
PFHpS	13C3_PFOS
PFNA	13C9_PFNA
PFOSA	13C8_PFOSA
PFOS	13C8_PFOS
N-MeFOSA	d3-N-MeFOSA
PFDA	13C6_PFDA
N-EtFOSA	d5-N-EtFOSA
8:2FTS	13C2_8:2FTS
9Cl-PF3ONS	13C8_PFOS
PFNS	13C8_PFOS
PFUdA	13C7_PFUdA
N-MeFOSAA	d3-MeFOSAA
N-EtFOSAA	d5-EtFOSAA
PFDS	13C8_PFOS
PFDoA	13C2_PFDoA
N-MeFOSE	d7-N-MeFOSE
10:2FTS	13C2_8:2FTS
N-EtFOSE	d9-N-EtFOSE
11Cl-PF3OUdS	13C8_PFOS
PFTrDA	13C2_PFDoA
PFDoS	13C8_PFOS
PFTeDA	13C2_PFTeDA
PFHxDA	13C2_PFHxDA
PFODA	13C2_PFHxDA

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies.
Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete.
Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

Appendix E: Method PFAS by LCMSMS Compliant with DoD QSM Batch Control Limits

CAS	Analyte Acronym	Aqueous Matrix		Solid Matrix		Tissue Matrix[3]	
		Lower Control Limit (%REC)	Upper Control Limit (%REC)	Lower Control Limit (%REC)	Upper Control Limit (%REC)	Lower Control Limit (%REC)	Upper Control Limit (%REC)
2991-50-6	N-EtFOSAA	61	135	61	139	60	140
2355-31-9	N-MeFOSAA	65	136	63	144	60	140
757124-72-4	4:2 FTS	63	143	62	145	60	140
27619-97-2	6:2 FTS	64	140	64	140	60	140
39108-34-4	8:2 FTS	67	138	65	137	60	140
375-73-5	PFBS	72	130	72	128	60	140
375-22-4	PFBA	73	129	71	135	60	140
335-77-3	PFDS	53	142	59	134	60	140
335-76-2	PFDA	71	129	69	133	60	140
307-55-1	PFDoA	72	134	69	135	60	140
375-92-8	PFHpS	69	134	70	132	60	140
375-85-9	PFHpA	72	130	71	131	60	140
355-46-4	PFHxS	68	131	67	130	60	140
307-24-4	PFHxA	72	129	70	132	60	140
68259-12-1	PFNS	69	127	69	125	60	140
375-95-1	PFNA	69	130	72	129	60	140
754-91-6	PFOSA	67	137	67	137	60	140
1763-23-1	PFOS	65	140	68	136	60	140
335-67-1	PFOA	71	133	69	133	60	140
2706-91-4	PFPeS	71	127	73	123	60	140
2706-90-3	PFPeA	72	129	69	132	60	140
376-06-7	PFTeDA	71	132	69	133	60	140
72629-94-8	PFTrDA	65	144	66	139	60	140
2058-94-8	PFUdA	69	133	64	136	60	140
31506-32-8	N-MeFOSA[1]	68	141	70	140	60	140
4151-50-2	N-EtFOSA[2]	70	140	70	140	60	140
120226-60-0	10:2FTS[2]	70	140	70	140	60	140
13252-13-6	HFPO-DA[2]	70	140	70	140	NA	NA
919005-14-4	DONA[1]	70	140	70	140	60	140
756426-58-1	9Cl-PF3ONS[2]	70	140	70	140	60	140
763051-92-9	11Cl-PF3OUDS[2]	70	140	70	140	60	140
24448-09-7	N-MeFOSE[2]	70	140	70	140	60	140
1691-99-2	N-EtFOSE[2]	70	140	70	140	60	140
67905-19-5	PFHxDA[2]	70	140	70	140	60	140
16517-11-6	PFODA[2]	70	140	70	140	60	140
79780-39-5	PFDoS[2]	70	140	70	140	60	140

[1] DoD currently does not have guidance for the analyte in solid matrix, as of 2019 QSM 5.3, Table B-15.

[2] DoD currently does not have guidance for the analyte in both aqueous and solid matrix, as of 2019 QSM 5.3, Table B-15

[3] DoD currently does not have guidance for the analyte in tissue matrix, as of 2019 QSM 5.3, Table B-15

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

Appendix F: DoD QSM Requirements

Table B-15. Per- and Polyfluoroalkyl Substances (PFAS) Using Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) With Isotope Dilution or Internal Standard Quantification in Matrices Other Than Drinking Water

QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria	Comments
Initial Calibration (ICAL) *(Continued)*		ICAL must meet one of the two options below: Option 1: The RSD of the RFs for all analytes must be ≤ 20%. Option 2: Linear or non-linear calibrations must have r^2 ≥ 0.99 for each analyte.			
Retention Time window position establishment	Once per ICAL and at the beginning of the analytical sequence.	Position shall be set using the midpoint standard of the ICAL curve when ICAL is performed. On days when ICAL is not performed, the initial CCV is used.	NA.	NA.	Calculated for each analyte and EIS.
Retention Time (RT) window width	Every field sample, standard, blank, and QC sample.	RT of each analyte and EIS analyte must fall within 0.4 minutes of the predicted retention times from the daily calibration verification or, on days when ICAL is performed, from the midpoint standard of the ICAL. Analytes must elute within 0.1 minutes of the associated EIS. This criterion applies only to analyte and labeled analog pairs.	Correct problem and reanalyze samples.	NA.	Calculated for each analyte and EIS.

COPYRIGHT © 2020 Pace Analytical Services, LLC

Table B-15. Per- and Polyfluoroalkyl Substances (PFAS) Using Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) With Isotope Dilution or Internal Standard Quantification in Matrices Other Than Drinking Water

QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria	Comments
Instrument Sensitivity Check (ISC)	Prior to analysis and at least once every 12 hours.	Analyte concentrations must be at LOQ; concentrations must be within ±30% of their true values.	Correct problem, rerun ISC. If problem persists, repeat ICAL.	Flagging is not appropriate.	No samples shall be analyzed until ISC has met acceptance criteria. ISC can serve as the initial daily CCV.
Initial Calibration Verification (ICV)	Once after each ICAL, analysis of a second source standard prior to sample analysis.	Analyte concentrations must be within ±30% of their true value.	Correct problem, rerun ICV. If problem persists, repeat ICAL.	Flagging is not appropriate.	No samples shall be analyzed until calibration has been verified.
Continuing Calibration Verification (CCV)	Prior to sample analysis, after every 10 field samples, and at the end of the analytical sequence.	Concentration of analytes must range from the LOQ to the mid-level calibration concentration. Analyte concentrations must be within ±30% of their true value.	Immediately analyze two additional consecutive CCVs. If both pass, samples may be reported without reanalysis. If either fails, or if two consecutive CCVs cannot be run, perform corrective action(s) and repeat CCV and all associated samples since last successful CCV. Alternately, recalibrate if necessary; then reanalyze all associated samples since the last acceptable CCV.	If reanalysis cannot be performed, data must be qualified and explained in the Case Narrative. Apply Q-flag to all results for the specific analyte(s) in all samples since the last acceptable calibration verification.	Results may not be reported without valid CCVs. Instrument Sensitivity Check (ISC) can serve as a bracketing CCV.

COPYRIGHT © 2020 Pace Analytical Services, LLC

Table B-15. Per- and Polyfluoroalkyl Substances (PFAS) Using Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) With Isotope Dilution or Internal Standard Quantification in Matrices Other Than Drinking Water

QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria	Comments
Instrument Blanks	Immediately following the highest standard analyzed and daily prior to sample analysis.	Concentration of each analyte must be ≤ ½ the LOQ. Instrument Blank must contain EIS to enable quantitation of contamination.	If acceptance criteria are not met after the highest calibration standard, calibration must be performed using a lower concentration for the highest standard until acceptance criteria is met. If sample concentrations exceed the highest allowed standard and the sample(s) following exceed this acceptance criteria (>1/2 LOQ), they must be reanalyzed.	Flagging is only appropriate in cases when the sample cannot be reanalyzed and when there is no more sample left.	No samples shall be analyzed until instrument blank has met acceptance criteria. Note: Successful analysis following the highest standard analyzed determines the highest concentration that carryover does not occur. When the highest standard analyzed is not part of the calibration curve, it cannot be used to extend out the calibration range, it is used only to document a higher concentration at which carryover still does not occur.

COPYRIGHT © 2020 Pace Analytical Services, LLC

Table B-15. Per- and Polyfluoroalkyl Substances (PFAS) Using Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) With Isotope Dilution or Internal Standard Quantification in Matrices Other Than Drinking Water

QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria	Comments
Extracted Internal Standard (EIS) Analytes	Every field sample, standard, blank, and QC sample.	Added to solid sample prior to extraction. Added to aqueous samples, into the original container, prior to extraction. For aqueous samples prepared by serial dilution instead of SPE, added to final dilution of samples prior to analysis. Extracted Internal Standard Analyte recoveries must be within 50% to 150% of ICAL midpoint standard area or area measured in the initial CCV on days when an ICAL is not performed.	Correct problem. If required, re-extract and reanalyze associated field and QC samples. If recoveries are acceptable for QC samples, but not field samples, the field samples must be re-extracted and analyzed (greater dilution may be needed). Samples may be re-extracted and analyzed outside of hold times, as necessary for corrective action associated with QC failure.	Apply Q-flag and discuss in the Case Narrative only if reanalysis confirms failures in exactly the same manner.	Failing analytes shall be thoroughly documented in the Case Narrative. EIS should be 96% (or greater) purity. When the impurity consists of the unlabeled analyte, the EIS can result in a background artifact in every sample, standard and blank, if the EIS is fortified at excessive concentrations.
Method Blank (MB)	One per preparatory batch.	No analytes detected >½ LOQ or > 1/10th the amount measured in any sample or 1/10th the regulatory limit, whichever is greater.	Correct problem. If required, re-extract and reanalyze MB and all QC samples and field samples processed with the contaminated blank. Samples may be re-extracted and analyzed outside of hold times, as necessary for corrective action associated with QC failure. Examine the project-specific requirements. Contact the client as to additional measures to be taken.	If reanalysis cannot be performed, data must be qualified and explained in the Case Narrative. Apply B-flag to all results for the specific analyte(s) in all samples in the associated preparatory batch.	Results may not be reported without a valid MB. Flagging is only appropriate in cases where the samples cannot be reanalyzed.

COPYRIGHT © 2020 Pace Analytical Services, LLC

Table B-15. Per- and Polyfluoroalkyl Substances (PFAS) Using Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) With Isotope Dilution or Internal Standard Quantification in Matrices Other Than Drinking Water

QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria	Comments
Laboratory Control Sample (LCS)	One per preparatory batch.	Blank spiked with all analytes at a concentration ≥ LOQ and ≤ the mid-level calibration concentration. A laboratory must use the DoD/DOE QSM Appendix C Limits for batch control if project limits are not specified. If the analyte(s) are not listed, 70-140% will be used for NPW and SL and 60-140%. for tissue	Correct problem, then re- extract and reanalyze the LCS and all samples in the associated preparatory batch for failed analytes if sufficient sample material is available. Samples may be re-extracted and analyzed outside of hold times, as necessary for corrective action associated with QC failure. Examine the project-specific requirements. Contact the client as to additional measures to be taken.	If reanalysis cannot be performed, data must be qualified and explained in the Case Narrative. Apply Q-flag to specific analyte(s) in all samples in the associated preparatory batch.	Results may not be reported without a valid LCS. Flagging is only appropriate in cases where the samples cannot be reanalyzed.
Matrix Spike (MS)	One per preparatory batch. Not required for aqueous samples prepared by serial dilution instead of SPE.	Sample spiked with all analytes at a concentration ≥ LOQ and ≤ the mid-level calibration concentration. A laboratory must use the DoD/DOE QSM Appendix C Limits for batch control if project limits are not specified. If the analyte(s) are not listed, 70-140% will be used for NPW and SL and 60-140%. for tissue	Examine the project-specific requirements. Contact the client as to additional measures to be taken.	For the specific analyte(s) in the parent sample, apply J-flag if acceptance criteria are not met and explain in the Case Narrative.	For matrix evaluation only. If MS results are outside the limits, the data shall be evaluated to determine the source(s) of difference (i.e., matrix effect or analytical error).

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies. Uncontrolled copies are not tracked or replaced when new versions are released or the SOP is made obsolete. Users of the SOP should verify the copy in possession is the current version of the SOP before use.

COPYRIGHT © 2020 Pace Analytical Services, LLC

Table B-15. Per- and Polyfluoroalkyl Substances (PFAS) Using Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) With Isotope Dilution or Internal Standard Quantification in Matrices Other Than Drinking Water

QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria	Comments
Matrix Spike Duplicate (MSD) or Matrix Duplicate (MD)	For MSD: One per preparatory batch. For MD: Each aqueous sample prepared by serial dilution instead of SPE.	For MSD: Sample spiked with all analytes at a concentration ≥ LOQ and ≤ the mid-level calibration concentration. A laboratory must use the DoD/DOE QSM Appendix C Limits for batch control if project limits are not specified. If the analyte(s) are not listed, 70-140% will be used for NPW and SL and 60-140%. for tissue RPD ≤ 30% (between MS and MSD or sample and MD).	Examine the project-specific requirements. Contact the client as to additional measures to be taken.	For the specific analyte(s) in the parent sample, apply J-flag if acceptance criteria are not met and explain in the Case Narrative.	The data shall be evaluated to determine the source of difference. For Sample/MD: RPD criteria only apply to analytes whose concentration in the sample is ≥ LOQ. The MD is a second aliquot of the field sample that has been prepared by serial dilution.
Post Spike Sample	Only applies to aqueous samples prepared by serial dilution instead of SPE that have reported value of < LOQ for analyte(s).	Spike all analytes reported as < LOQ into the dilution that the result for that analyte is reported from. The spike must be at the LOQ concentration to be reported for this sample as < LOQ. When analyte concentrations are calculated as < LOQ, the post spike for that analyte must recover within 70- 130% of its true value.	When analyte concentrations are calculated as < LOQ, and the spike recovery does not meet the acceptance criteria, the sample, sample duplicate, and post spike sample must be reanalyzed at consecutively higher dilutions until the criteria is met.	Flagging is not appropriate.	When analyte concentrations are calculated as < LOQ, results may not be reported without acceptable post spike recoveries.