EHD ORG CHEM METHOD 16201
PFAS in Water
Matrices: Drinking and Non-potable Waters
Acodes: OC16201, OC16205, OC16206
Method Code (.M)

1. Scope and Application

1.1. Analysis of PFAS in Water by HPLC-MS/MS, WSLH PFAS in Water by Isotope Dilution

1.2. This is a high performance liquid chromatographic triple quadrupole mass spectrometric (HPLC-MS/MS) method applicable to the determination of per- and polyfluorinated compounds in drinking and non-potable waters following a modified version of ISO Method 21675 and Wisconsin PFAS Aqueous (Non-Potable Water) and Non-Aqueous Matrices Method Expectations.

1.3. Compounds and MDLs/MRLs for this method are listed below in ng/L of water:

Analyte	Abbreviation	CAS No.	MDL (ng/L)	MRL (ng/L)
Perfluoro-n-butanoic acid	PFBA	375-22-4	0.346	1.00
Perfluoro-n-pentanoic acid	PFPeA	2706-90-3	0.150	1.00
Perfluoro-1-butanesulfonic acid	PFBS	375-73-5	0.231	1.00
1H,1H,2H,2H-Perfluorohexane sulfonic acid	4:2 FTSA	757124-72-4	0.190	1.00
Perfluoro-n-hexanoic acid	PFHxA	307-24-4	0.204	1.00
Perfluoro-1-pentanesulfonic acid	PFPeS	2706-91-4	0.136	1.00
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3-heptafluoropropoxy)-propanoic acid	HFPO-DA	13252-13-6	0.192	1.00
Perfluoro-n-heptanoic acid	PFHpA	375-85-9	0.150	1.00
Perflioro-1-hexanesulfonic acid	PFHxS	355-46-4	0.142	1.00
Dodecafluoro-3H-4,8-dioxanoanoic acid	DONA	919005-14-4	0.128	1.00
1H,1H,2H,2H-Tridecafluorooctane-1-sulfonic acid	6:2 FTSA	27619-97-2	0.272	1.00
Perfluoro-n-octanoic acid	PFOA	335-67-1	0.108	1.00
Perfluoro-1-heptanesulfonic acid	PFHpS	375-92-8	0.190	1.00
Perfluoro-1-octanesulfonic acid	PFOS	1763-23-1	0.143	1.00
Perfluoro-n-nonanoic acid	PFNA	375-95-1	0.148	1.00
Potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	756426-58-1	0.182	1.00
1H,1H,2H,2H-Perfluorodecanesulfonic acid	8:2 FTSA	39108-34-4	0.262	1.00
Perfluoro-n-decanoic acid	PFDA	335-76-2	0.163	1.00
Perfluoro-1-nonanesulfonic acid	PFNS	68259-12-1	0.182	1.00
N-methyl perfluorooctanesulfonamidoacetic acid	N-MeFOSAA	2355-31-9	0.219	1.00
N-ethyl perfluorooctanesulfonamidoacetic acid	N-EtFOSAA	2991-50-6	0.212	1.00
Perfluorooctanesulphonamide	FOSA	754-91-6	0.155	1.00
Perfluoro-n-undecanoic acid	PFUnA	2058-94-8	0.222	1.00
Perfluoro-1-decanesulfonic acid	PFDS	335-77-3	0.257	1.00
Potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9	0.149	1.00
Perfluoro-n-dodecanoic acid	PFDoA	307-55-1	0.271	1.00
Perfluoro-1-dodecanesulfonic acid	PFDoS	79780-39-5	0.398	1.00
Perfluoro-n-tridecanoic acid	PFTrDA	72629-94-8	0.193	1.00
N-Methyl Perfluorooctanesulfonamide	N-MeFOSA	31506-32-8	1.00	2.00
2-(N-methylperfluoro-1-octanesulfonamido)-ethanol	N-MeFOSE	24448-09-7	0.281	1.00
N-Ethyl Perfluorooctanesulfonamide	N-EtFOSA	4151-50-2	0.694	2.00
2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol	N-EtFOSE	1691-99-2	0.212	1.00
Perfluoro-n-tetradecanoic acid	PFTeDA	376-06-7	0.175	1.00

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2. Summary of Method:

2.1. Labeled internal standards are added to a water sample and per- and polyfluoroalkyl substances (PFAS) present in water samples are extracted by a solid phase extraction, eluted with MeOH and an ammonia/MeOH solution, and evaporated to approximately 1 mL under nitrogen gas. The contents of the tube are brought to 1 mL with MeOH. Prior to analysis by HPLC-MS/MS, the contents are transferred to a polypropylene autosampler vial via polypropylene pipette. Separation of the analytes is achieved using gradient elution chromatography. After elution from the HPLC column, analytes are detected using a turbo ion spray triple quadrupole mass spectrometer in the negative ionization mode.

2.2. Method Considerations:

2.2.1. The reference method (ISO 21675) states that the compounds in the lab blank must be $^{1}/_{10}$ of the lowest concentration in the batch of samples. This method uses the requirements set forth by WDNR which state that any compounds that are detected must be <½ of the MRL or $<^{1}/_{10}$ of the sample concentration, whichever is higher.

2.2.2. If interference is seen in the primary quantification ion transition, it is possible to use the secondary ion transition to quantify sample results. This deviation must be noted alongside results for any affected samples. If a secondary ion transition is used to quantify results, the transition must have a calibration curve that meets the requirements of §9.

2.2.3. Method reporting limits (MRLs) are not determined using the MDL*($^{10}/_{3}$) method outlined in EHD QA 116. MRLs are currently set at 1.00 ng/L or by multiplying the MDL by two and rounding up to the nearest calibration standard, whichever is higher.

3. Safety and Waste Management:

3.1. General safety practices for all laboratory operations are outlined in the Chemical Hygiene Plan for Environmental Sciences (LABWIDE SAFETY 102).

3.2. Waste Disposal

3.2.1. Sample extracts and diluted standards in organic solvents may be discarded with other organic solvent waste. Neat standards at high concentrations in organic solvent may be discarded with other organic solvent waste or lab-packed.

3.2.2. Aqueous waste from extraction and un-extracted aqueous samples may be discarded in a laboratory sink.

3.2.3. Used WAX and ENVI-Carb SPE columns may be discarded with regular waste/garbage.

3.3. Waste Disposal References

3.3.1. EHD DIV-WIDE GENOP 038 | Waste Management

3.3.2. University of Wisconsin Chemical Safety and Disposal Guide located at https://ehs.wisc.edu/disposal-services/.

4. Sampling Handling and Preservation:

4.1. Samples must be collected in a 250 mL polypropylene container filled to the neck of the bottle and must be iced or refrigerated at 0-6°C in transport. The sample must be refrigerated at 0-6°C as soon as possible, and it must be shielded from light from the time of

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collection until analysis. If the sample temperature is >6°C upon receipt at WSLH, the sample will be qualified and analyzed by the usual procedure outlined below.

4.2. If equipment is used in the field to collect samples, the sample collector may wish to collect a field equipment blank by rinsing field equipment with PFAS-free water and collecting the rinse in a separate bottle. This equipment blank must be analyzed if the associated samples contain detections greater than the Method Reporting Limit (MRL). See §8.6.5.

4.3. Site-specific field blanks must be collected at the same time samples are collected. The field blank must be analyzed if the associated samples contain detections greater than the MRL. See §8.6.3.

4.4. Samples must be extracted within 28 days of collection. Extracts must be stored in the dark at 0-6°C until analysis. The hold time for the extracts is 30 days.

5. Interferences: Matrix interference may be caused by contaminants that are present in the sample. The extent of matrix interference is unknown until further sample analysis is completed.

6. Reagents and Standards:

6.1. Reagents

6.1.1. Methanol (must be HPLC grade or higher for mobile phase)

6.1.2. Ammonium Acetate (must be HPLC grade or higher for mobile phase)

6.1.3. 18 MΩ·cm resistivity water

6.1.4. Acetic acid (99.9% mass fraction)

6.1.5. Ammonia solution (28-30% mass fraction)

6.2. Reagent and bottle preparation

6.2.1. Mobile Phase 1 (2mM Ammonium Acetate in Water with 5% MeOH): Quantitatively transfer 0.154 g of ammonium acetate to ~50mL of 18 MΩ·cm water in a 1000mL volumetric flask, add 50mL MeOH, and dilute the flask to volume with 18 MΩ·cm water. Volumes may also be measured with graduated cylinders into non-volumetric glassware.

6.2.2. Acetate buffer (5mM, pH 4): Add 0.396 g of ammonium acetate to ~950mL of 18.2 MΩ·cm water in a 1L volumetric flask. Adjust to pH 4 using glacial acetic acid, and dilute to 1 liter with 18 MΩ·cm water.

6.2.3. Ammonium Hydroxide/MeOH solution (approximately 60mM NH_4OH): Add 1.0mL of concentrated ammonium hydroxide solution (§6.1.5) to a 250mL volumetric flask and dilute with MeOH.

6.2.4. Sample bottle identification: Every box of bottles that is purchased has a unique lot number associated with it (usually provided by the manufacturer). This lot number will be put on every bottle that goes out into the field which will be the same lot number bottle that is used in all QC to ensure no contamination from the bottle is seen. This is done by performing a normal lab blank test on a bottle to make sure it passes all QC (see 8.6.1). Alternatively, containers may be rinsed with an appropriate amount of MeOH + 25 µL internal standard mix and the resulting rinse solution concentrated to 1mL and injected. A rinse with MeOH represents the most likely scenario in which contaminating PFAS from the container would be extracted, and it is therefore reasonable to expect that no additional PFAS would

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contribute to samples undergoing routine extraction procedures. The results of this test will be kept in the folder:

M:\EHD\ESS(4900)\ESS Org(4940)\Method Related Documents\PFAS\QC Bottle Checks

6.3. Standards

6.3.1. Since PFAS are known to adsorb onto glass surfaces, any type of glass should not be used in any step in the extraction or standard-making process. Polypropylene bottles should be used to store and prepare all standards. Polypropylene vials (1.2 mL – used for storing samples) and autosampler vials (~300 uL) should be used for samples and QC. All standards should be prepared on ice and stored in the smallest container available to limit headspace and the risk of evaporation.

6.3.2. Concentrations of compounds that exist as salts in standards must be corrected to the concentration of the corresponding acid. See EHD ORG CHEM GENOP 0074 §6.4.

6.3.3. Calibration and second source standards are prepared in 80:20 MeOH:H_2O to reflect approximate composition of final extracts. All other standards are prepared in 100% MeOH.

6.3.4. Target compound working stock solution - To make the working stock standard solution, a premade mix (18 compounds) that has both branched and linear forms of the compounds (EPA-537PDS-R1, Wellington Laboratories) at 2 µg/mL is combined with 15 individual stock standards. Individual stock standards are purchased at a concentration of 50 µg/mL or 100 µg/mL; standards purchased at 100 µg/mL are diluted to 2 µg/mL (Table 1) before being combined with the premade mix to create a 100 ng/mL solution with all compounds (Table 2 – this is PFASSpk1 in Horizon). These standards should be re-evaluated and/or replaced after 12 months or if signs of degradation are observed or if any of the individual components of the mixture pass their expiration dates. The 100 ng/mL target compound mix is used to make the calibration standards as well as the QC spikes. A tenfold dilution of the target mix in MeOH is used to make the lowest calibration level.

6.3.5. Internal standard (IS) working stock solution

6.3.5.1. A pre-made mix of nineteen isotopically labeled standards (MPFAC-24ES) at 1 µg/mL each and five individual compounds at 50 µg/mL are purchased from Wellington Laboratories. The individual compounds are diluted to 2 µg/mL (Table 3) and combined with the 1 µg/mL 19-compound mix.

6.3.5.2. To prepare 1 mL of internal standard mix, 80 µL of MPFAC-24ES, 10 µL of 2 µg/mL $^{13}C_3$HFPO-DA, 400 µL of 2 µg/mL d_3-N-MeFOSA, and 40 µL each of 2 µg/mL d_5-N-EtFOSA, d_7-N-MeFOSE, and d_9-N-EtFOSE are combined with 380 µL of MeOH (Table 4). These volumes can be scaled up to produce more IS mix as long as each component is increased proportionally. See Table 4.

6.3.5.3. Twenty-five (25) µL of this standard is added to each sample and QC prior to extraction.

6.3.6. Calibration standards - Combine and dilute the solutions in Sections 6.3.4 and 6.3.5 to produce the calibration solutions in Table 5 and Table 6. These solutions permit

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the relative response (labeled to target) and response factor to be determined as a function of concentration.

6.3.7. Second source/initial calibration verification standard (ICV) - Another source of all compounds must be used to check the original concentrations of the purchased standards. This can be either a different supplier or a different lot number from the same supplier (if available). A premade mixture that contains 28 compounds at 1000 ng/mL (PFAC30PAR) and separate lots of PFDoS (50 µg/mL, LPFDoS1119), MeFOSA (50 µg/mL, NMeFOSA1020M), EtFOSA (50 µg/mL, NEtFOSA1120M), MeFOSE (50 µg/mL, NMeFOSE1020M), & EtFOSE (50 µg/mL, NEtFOSE1020M), all purchased from Wellington Laboratories, are combined for a total of 33 second source compounds (Table 7).

6.3.8. When standards are not being used, store the solutions in the dark at <5°C. Standards may be stored <0°C. Before use, make sure each standard is brought up to room temperature and the vial or bottle is vortexed extensively. All stock standards expire when the earliest manufacturer's expiration date occurs. Standards prepared from stocks expire one year after preparation, when the parent standard expires, or when the preparation solvent expires (whichever is soonest).

7. Apparatus:

7.1. 250-mL HDPE sample bottles - avoid use of Teflon septa/cap liners

7.2. Various beakers

7.3. Analytical Balance capable of accurately weighing to the nearest 0.01 g and capable of weighing up to 500 g.

7.4. 15-mL conical screw-capped polypropylene centrifuge tubes, graduated

7.5. Vacuum extraction manifold

7.6. Vacuum system

7.7. PromoChrom SPE-03 Automated Extraction System (AES)

7.8. SPE cartridge, Waters Corporation Oasis WAX Cartridge 6CC/150 mg (PN: Fisher 50-781-150)

7.9. Valve liners (PN: Fisher 57059)

7.10. Valve stems (PN: Supelco 57146-U)

7.11. Solvent reservoirs, 70 mL, Biotage ISOLUTE (PN: 120-1009-F)

7.12. Adaptors to connect solvent reservoir to SPE column (PN: Supelco 21018-U)

7.13. Nitrogen blow-down apparatus with heated water bath

7.14. Vortex mixer

7.15. Disposable polypropylene pipettes

7.16. Polypropylene Autosampler vials (PN: Waters 186002626)

7.17. Screw caps that have polypropylene septa (PN: Waters 186004169)

7.18. Supelco Supelclean ENVI-Carb Cartridge (Supelco part no. 57092)

7.19. Syringe filters (Glass fiber, 13 mm, Foxx Life Sciences PN: 37C-2116-OEM)

7.20. Syringe filters (Polypropylene, 13mm, Foxx Life Sciences PN: 37B-2116-OEM)

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7.21. Disposable syringes (PN: Fisher 03-377-20)

7.22. PromoChrom nylon in-line filters (PromoChrom PN: F-Ny-30)

7.23. PromoChrom anti-clogging frits (PromoChrom PN: CF-06)

7.24. Waters Acquity UPLC

7.25. Applied Biosystems/SCIEX Q-Trap 5500 Triple quadrupole mass spectrometer

7.26. SCIEX Exion LC

7.27. SCIEX 7500 triple quadrupole mass spectrometer

7.28. HPLC/MS/MS Instrument Conditions: The following conditions/parameters are for analysis performed on a Waters Acquity UPLC followed by an Applied Biosystems/SCIEX Q-Trap 5500 triple quadrupole mass spectrometer

7.28.1. General Method Parameters

7.28.1.1. Synchronization Mode: LC Sync

7.28.1.2. Auto-Equilibration: Off

7.28.1.3. Acquisition Duration: 13 minutes 30 seconds

7.28.1.4. Number of Scans: 810

7.28.1.5. Period In File: 1

7.28.1.6. Acquisition Module: Acquisition Method

7.28.1.7. Software version: Analyst 1.7.1 or SCIEX OS 2.2.0.5738

7.28.2. Source height setting-3, Source L/R setting-5

7.28.3. Waters Acquity Pump Method

7.28.3.1. Pump Model: Waters Acquity UPLC

7.28.3.2. Column: Acquity UPLC BEH C18 1.7 MM 2.1x50 mm Column, (Waters Part# 186002350)

7.28.3.3. Waters Acquity UPLC Pump Method Properties

Minimum Pressure (psi)	0.0
Maximum Pressure (psi)	~12000
Mobile Phase A	2 mM ammonium acetate in H_2O:MeOH (95:5)
Mobile Phase B	Methanol

7.28.3.4. Step Table

Step	Total Time (min)	Flow Rate (ml/min)	A (%)	B (%)
0	0.00	0.200	95	5
1	0.50	0.200	95	5
2	1	0.250	80	20
3	4	0.350	50	50
4	6	0.350	40	60
5	7	0.400	30	70
6	8.5	0.400	10	90
7	9	0.350	0	100
8	10.5	0.300	0	100

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9	11.5	0.200	95	5
10	13.5	0.200	95	5

7.28.4. MS/MS Method Properties:

7.28.4.1. Period 1:

7.28.4.1.1. Scans in Period: 810

7.28.4.1.2. Relative Start Time: 0.00 msec

7.28.4.1.3. Experiments in Period: 1

7.28.4.2. Period 1 Experiment 1:

Scan Type:	MRM (MRM)
Scheduled MRM	Yes
Polarity:	Negative
Scan Mode:	N/A
Ion Source:	Turbo Spray
Resolution Q1:	Unit
Resolution Q3:	Unit
Intensity Thres.:	0.00 cps
Settling Time:	0.0000 msec
MR Pause:	5.0070 msec
MCA:	No
Step Size:	0.00 Da

7.28.4.3. MRM Parameters (DNR33_SchMRM_20%.dam): Primary (quantitation) ion transitions denoted by "-1", secondary (confirmation) ion transitions denoted by "-2", internal standard ion transitions denoted by "M", "M#", or "d#" at the start of compound name. PFBA, PFPeA, N-MeFOSE, & N-EtFOSE do not have confirmation ion transitions.

Compounds	Internal Standard Used to Quantitate	Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)	DP	CE	CXP
PFBA-1	$^{13}C_4$PFBA	216.962	168.906	3-250	-25	-14	-17
$^{13}C_4$PFBA	---	216.900	172.000	3-250	-50	-14	-15
PFPeA-1	$^{13}C_5$PFPeA	262..908	218.904	3-250	-35	-12	-21
$^{13}C_5$PFPeA	---	268.000	223.000	3-250	-15	-12	-9
PFBS-1	$^{13}C_3$PFBS	298.839	80.000	3-250	-25	-70	-9
PFBS-2	$^{13}C_3$PFBS	298.839	99.000	3-250	-25	-36	-9
$^{13}C_3$PFBS	---	301.849	79.978	3-250	-100	-78	-5
4:2 FTSA-1	$^{13}C_2$-4:2 FTSA	326.853	306.926	3-250	-85	-28	-19
4:2 FTSA-2	$^{13}C_2$-4:2 FTSA	326.853	80.974	3-250	-95	-50	-7
$^{13}C_2$-4:2 FTSA	---	329.000	81.000	3-250	-20	-55	-7
PFHxA-1	$^{13}C_5$PFHxA	312.898	269.000	3-250	-45	-14	-13
PFHxA-2	$^{13}C_5$PFHxA	312.898	119.000	3-250	-45	-30	-7
$^{13}C_5$PFHxA	---	318.000	273.000	3-250	-25	-14	-9
PFPeS-1	$^{13}C_3$PFHxS	348.950	79.945	3-250	-25	-86	-9
PFPeS-2	$^{13}C_3$PFHxS	348.950	98.933	3-250	-25	-40	-9

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HFPO-DA-1	$^{13}C_3$HFPO-DA	285.001	168.800	3-250	-5	-8	-5
HFPO-DA-2	$^{13}C_3$HFPO-DA	285.000	185.900	3-250	-40	-23	-17
$^{13}C_3$HFPO-DA	---	287.000	168.800	3-250	-45	-12	-7
PFHpA-1	$^{13}C_4$PFHpA	362.858	318.900	3-250	-40	-14	-13
PFHpA-2	$^{13}C_4$PFHpA	362.858	169.000	3-250	-40	-24	-15
$^{13}C_4$PFHpA	---	367.000	322.000	3-250	-65	-22	-13
PFHxS-1	$^{13}C_3$PFHxS	398.841	79.900	3-250	-5	-86	-7
PFHxS-2	$^{13}C_3$PFHxS	398.841	98.900	3-250	-5	-42	-9
$^{13}C_3$PFHxS	---	402.000	80.000	3-250	-20	-86	-7
DONA-1	$^{13}C_4$PFHpA	376.856	250.900	3-250	-5	-18	-9
DONA-2	$^{13}C_4$PFHpA	376.770	85.000	3-250	-60	-18	-11
6:2 FTSA-1	$^{13}C_2$-6:2 FTSA	426.897	416.968	3-250	-75	-32	-39
6:2 FTSA-2	$^{13}C_2$-6:2 FTSA	426.897	80.936	3-250	-75	-82	-15
$^{13}C_2$-6:2 FTSA	---	429.000	80.999	3-250	-75	-82	-15
PFOA-1	$^{13}C_8$PFOA	412.877	368.900	3-250	-55	-14	-17
PFOA-2	$^{13}C_8$PFOA	412.877	168.900	3-250	-55	-24	-13
$^{13}C_8$PFOA	---	421.000	376.000	3-250	-75	-16	-17
PFHpS-1	$^{13}C_8$PFOS	448.837	79.917	3-250	-145	-94	-5
PFHpS-2	$^{13}C_8$PFOS	448.837	98.909	3-250	-145	-96	-9
PFOS-1	$^{13}C_8$PFOS	489.802	79.900	3-250	-40	-70	-7
PFOS-2	$^{13}C_8$PFOS	480.802	98.900	3-250	-40	-57	-11
$^{13}C_8$PFOS	---	507.000	80.001	3-250	-40	-102	-9
PFNA-1	$^{13}C_9$PFNA	462.817	419.000	3-250	-55	-16	-17
PFNA-2	$^{13}C_9$PFNA	462.817	219.000	3-250	-55	-24	-19
$^{13}C_9$PFNA	---	472.000	427.000	3-250	-55	-16	-13
9Cl-PF3ONS-1	$^{13}C_8$PFOS	530.709	350.800	3-250	-20	-36	-13
9Cl-PF3ONS-2	$^{13}C_8$PFOS	532.746	352.826	3-250	-40	-38	-11
8:2 FTSA-1	$^{13}C_2$-8:2 FTSA	526.910	506.922	3-250	-15	-38	-9
8:2 FTSA-2	$^{13}C_2$-8:2 FTSA	526.910	80.920	3-250	-15	-78	-7
$^{13}C_2$-8:2 FTSA	---	529.000	80.999	3-250	-15	-78	-7
PFDA-1	$^{13}C_6$PFDA	512.830	469.000	3-250	-40	-16	-13
PFDA-2	$^{13}C_6$PFDA	512.830	218.900	3-250	-40	-26	-17
$^{13}C_6$PFDA	---	519.000	474.000	3-250	-55	-16	-15
PFNS-1	$^{13}C_8$PFOS	548.893	79.966	3-250	-90	-112	-9
PFNS-2	$^{13}C_8$PFOS	548.893	98.942	3-250	-90	-106	-7
N-MeFOSAA-1	d_3-N-MeFOSAA	569.863	419.100	3-250	-80	-30	-7
N-MeFOSAA-2	d_3-N-MeFOSAA	569.863	512.000	3-250	-80	-36	-13
d_3-N-MeFOSAA	---	573.000	419.000	3-250	-85	-30	-15
N-EtFOSAA-1	d_5-N-EtFOSAA	583.962	419.000	3-250	-120	-37	-10
N-EtFOSAA-2	d_5-N-EtFOSAA	583.962	525.800	3-250	-120	-37	-10
d_5-N-EtFOSAA	---	589.000	419.000	3-250	-80	-30	-19

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FOSA-1	$^{13}C_8$FOSA	497.854	77.977	3-250	-80	-86	-9
FOSA-2	$^{13}C_8$FOSA	497.854	63.952	3-250	-80	-124	-7
$^{13}C_8$FOSA	---	506.000	78.000	3-250	-90	-78	-7
PFUnA-1	$^{13}C_7$PFUdA	562.846	519.000	3-250	-30	-18	-13
PFUnA-2	$^{13}C_7$PFUdA	562.846	268.900	3-250	-30	-28	-13
$^{13}C_7$PFUdA	---	570.000	525.001	3-250	-90	-18	-15
PFDS-1	$^{13}C_8$PFOS	598.811	79.916	3-250	-25	-120	-9
PFDS-2	$^{13}C_8$PFOS	598.811	98.957	3-250	-25	-108	-15
11Cl-PF3DOUdS-1	$^{13}C_8$PFOS	630.757	451.000	3-250	-95	-38	-19
11Cl-PF3OUdS-2	$^{13}C_8$PFOS	632.715	452.900	3-250	-85	-42	-17
PFDoA-1	$^{13}C_2$PFDoA	612.828	569.000	3-250	-65	-20	-17
PFDoA-2	$^{13}C_2$PFDoA	612.828	168.900	3-250	-65	-36	-19
$^{13}C_2$PFDoA	---	615.000	570.000	3-250	-50	-18	-23
PFDoS-1	$^{13}C_2$PFTeDA	698.730	79.951	3-250	-150	-130	-11
PFDoS-2	$^{13}C_2$PFTeDA	698.730	98.867	3-250	-150	-80	-9
PFTrDA-1	$^{13}C_2$PFDoA	662.782	619.000	3-250	-75	-18	-17
PFTrDA-2	$^{13}C_2$PFDoA	662.782	169.000	3-250	-75	-36	-11
N-MeFOSA-1	d_3-N-MeFOSA	511.973	168.923	3-250	-65	-36	-15
N-MeFOSA-2	d_3-N-MeFOSA	511.973	218.986	3-250	-65	-32	-17
d_3-N-MeFOSA	---	514.931	169.100	3-250	-20	-24	-15
N-MeFOSE	d_7-N-MeFOSE	615.821	59.000	3-250	-50	-80	-7
d_7-N-MeFOSE	---	622.956	59.000	3-250	-60	-78	-7
N-EtFOSA-1	d_5-N-EtFOSA	525.856	168.980	3-250	-55	-40	-11
N-EtFOSA-2	d_5-N-EtFOSA	525.856	218.970	3-250	-55	-38	-5
d_5-N-EtFOSA	---	530.896	168.979	3-250	-70	-38	-11
N-EtFOSE	d_9-N-EtFOSE	629.837	59.000	3-250	-15	-74	-7
d_9-N-EtFOSE	---	638.957	59.000	3-250	-55	-76	-7
PFTeDA-1	$^{13}C_2$PFTeDA	712.763	668.900	3-250	-35	-20	-25
PFTeDA-2	$^{13}C_2$PFTeDA	712.763	168.900	3-250	-35	-36	-11
$^{13}C_2$PFTeDA	---	715.000	670.000	3-250	-65	-20	-27

7.28.5. Parameter Table (Period 1 Experiment 1):

CUR:	30.00
GS1:	30.00
GS2:	30.00
IS:	-4500.00
TEM:	650.00
ihe:	ON
CAD:	Medium
EP	-10.00

7.28.6. Electron Multiplier Settings

Detector Parameters (Negative):

Uncontrolled

CEM	2200.0
DF	200.0

7.29. HPLC/MS/MS Instrument Conditions: The following conditions/parameters are for analysis performed on a SCIEX Exion LC followed by an Applied Biosystems/SCIEX Triple Quad 7500 mass spectrometer

7.29.1. General Method Parameters

7.29.1.1. Acquisition Duration: 15 minutes

7.29.1.2. Ionization start time: 1.5 minutes

7.29.1.3. Ionization stop time: 13 minutes

7.29.1.4. Target Cycle Time: 1000 ms

7.29.1.5. Software version: SCIEX OS 2.0.1

7.29.2. SCIEX Exion LC Pump Method

7.29.2.1. Pump Model: SCIEX Exion LC

7.29.2.2. Column: Acquity UPLC BEH C18 1.7 MM 2.1x50 mm Column, (Waters Part# 186002350)

7.29.2.3. SCIEX Exion LC Pump Method Properties

Minimum Pressure	0 psi
Maximum Pressure	9000 psi
Mobile Phase A	2 mM Ammonium Acetate in H_2O:MeOH (95:5)
Mobile Phase B	Methanol

7.29.2.4. Step Table

Step	Time (min)	Flow (mL/min)	A (%)	B (%)
0	0.0	0.300	95	5
1	0.2	0.300	95	5
2	0.5	0.300	80	20
3	3.0	0.300	65	35
4	3.5	0.300	45	55
5	11.5	0.300	5	95
6	12.5	0.300	5	95
7	12.6	0.300	95	5
8	15.0	0.300	95	5

7.29.3. MS/MS Method Properties

7.29.3.1. Experiment Parameters

Scan Type	MRM
Polarity	Negative

Uncontrolled

Pause Time (ms)	5
Settling time (ms)	0
High mass cooling time (ms)	0
Q1 Resolution	Unit
Q3 Resolution	Unit

7.29.3.2. MRM Parameters (PFAS_DNR33_RT_Sch): Primary (quantitation) ion transitions denoted by "-1", secondary (confirmation) ion transitions denoted by "-2", internal standard ion transitions denoted by M# or $d_{\#}$ prefix. PFBA, PFPeA, N-MeFOSE, & N-EtFOSE do not have confirmation ion transitions.

Compound	Internal Standard Used for Quantitation	Q1 Mass (Da)	Q3 Mass (Da)	Dwell Time (ms)	CE	CXP
PFBA	$^{13}C_4$PFBA	213	169	3-200	-14	-10
$^{13}C_4$PFBA	---	217	172	3-200	-12	-9
PFPeA	$^{13}C_5$PFPeA	263	219	3-200	-12	-14
$^{13}C_5$PFPeA	---	268	223	3-200	-12	-12
PFBS-1	$^{13}C_3$PFBS	299	80	3-200	-67	-8
PFBS-2	$^{13}C_3$PFBS	299	99	3-200	-37	-11
$^{13}C_3$PFBS	---	302	80	3-200	-59	-9
4:2 FTSA-1	$^{13}C_2$-4:2 FTSA	327	307	3-200	-27	-19
4:2 FTSA-2	$^{13}C_2$-4:2 FTSA	327	81	3-200	-68	-9
$^{13}C_2$-4:2 FTSA	---	329	81	3-200	-71	-9
PFHxA-1	$^{13}C_5$PFHxA	313	269	3-200	-14	-13
PFHxA-2	$^{13}C_5$PFHxA	313	119	3-200	-26	-11
$^{13}C_5$PFHxA	---	318	273	3-200	-14	-13
PFPeS-1	$^{13}C_3$PFHxS	349	80	3-200	-80	-9
PFPeS-2	$^{13}C_3$PFHxS	349	99	3-200	-40	-11
HFPO-DA-1	$^{13}C_3$HFPO-DA	285	169	3-200	-12	-8
HFPO-DA-1	$^{13}C_3$HFPO-DA	285	185	3-200	-21	-9
$^{13}C_3$HFPO-DA	---	287	169	3-200	-11	-9
PFHpA-1	$^{13}C_4$PFHpA	363	319	3-200	-14	-17
PFHpA-2	$^{13}C_4$PFHpA	363	169	3-200	-25	-11
$^{13}C_4$PFHpA	---	367	322	3-200	-14	-19
PFHxS-1	$^{13}C_3$PFHxS	399	80	3-200	-95	-9
PFHxS-2	$^{13}C_3$PFHxS	399	99	3-200	-75	-11
$^{13}C_3$PFHxS	---	402	80	3-200	-95	-9
DONA-1	$^{13}C_4$PFHpA	377	251	3-200	-16	-14
DONA-2	$^{13}C_4$PFHpA	377	85	3-200	-40	-10
6:2 FTSA-1	$^{13}C_2$-6:2 FTSA	427	407	3-200	-32	-21
6:2 FTSA-2	$^{13}C_2$-6:2 FTSA	427	81	3-200	-91	-9

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$^{13}C_2$-6:2 FTSA	---	429	81	3-200	-88	-9
PFOA-1	$^{13}C_8$PFOA	413	369	3-200	-14	-20
PFOA-2	$^{13}C_8$PFOA	413	169	3-200	-26	-12
$^{13}C_8$PFOA	---	421	376	3-200	-15	-24
PFHpS-1	$^{13}C_8$PFOS	449	80	3-200	-100	-11
PFHpS-2	$^{13}C_8$PFOS	449	99	3-200	-90	-11
PFNA-1	$^{13}C_9$PFNA	463	419	3-200	-15	-29
PFNA-2	$^{13}C_9$PFNA	463	169	3-200	-26	-9
$^{13}C_9$PFNA	---	472	427	3-200	-14	-8
PFOS-1	$^{13}C_8$PFOS	499	80	3-200	-105	-11
PFOS-2	$^{13}C_8$PFOS	499	99	3-200	-100	-13
$^{13}C_8$PFOS	---	507	80	3-200	-107	-9
9Cl-PF3ONS-1	$^{13}C_8$PFOS	531	351	3-200	-39	-21
9Cl-PF3ONS-2	$^{13}C_8$PFOS	533	352.9	3-200	-38	-10
8:2 FTSA-1	$^{13}C_2$-8:2 FTSA	527	507	3-200	-39	-31
8:2 FTSA-2	$^{13}C_2$-8:2 FTSA	527	81	3-200	-94	-9
$^{13}C_2$-8:2 FTSA	---	529	81	3-200	-109	-9
PFDA-1	$^{13}C_6$PFDA	513	469	3-200	-17	-31
PFDA-2	$^{13}C_6$PFDA	513	169	3-200	-30	-9
$^{13}C_6$PFDA	---	519	474	3-200	-16	-37
PFNS-1	$^{13}C_8$PFOS	549	80	3-200	-120	-11
PFNS-2	$^{13}C_8$PFOS	549	99	3-200	-95	-13
N-MeFOSAA-1	d_3-N-MeFOSAA	570	419	3-200	-30	-25
N-MeFOSAA-2	d_3-N-MeFOSAA	570	512	3-200	-28	-32
d_3-N-MeFOSAA	---	573	419	3-200	-28	-22
FOSA-1	$^{13}C_8$FOSA	498	78	3-200	-85	-11
FOSA-2	$^{13}C_8$FOSA	498	64	3-200	-174	-6
$^{13}C_8$FOSA	---	506	78	3-200	-84	-9
N-EtFOSAA-1	d_5-N-EtFOSAA	584	419	3-200	-27	-12
N-EtFOSAA-2	d_5-N-EtFOSAA	584	526	3-200	-26	-32
d_5-N-EtFOSAA	---	589	419	3-200	-28	-28
PFDS-1	$^{13}C_8$PFOS	599	80	3-200	-125	-14
PFDS-2	$^{13}C_8$PFOS	599	99	3-200	-120	-14
PFUnA-1	$^{13}C_7$PFUdA	563	519	3-200	-16	-10
PFUnA-2	$^{13}C_7$PFUdA	563	169	3-200	-32	-9
$^{13}C_7$PFUdA	---	570	525	3-200	-17	-36
11Cl-PF3OUdS-1	$^{13}C_8$PFOS	631	451	3-200	-42	-25
11Cl-PF3OUdS-2	$^{13}C_8$PFOS	633	452.9	3-200	-42	-13
PFDoA-1	$^{13}C_2$PFDoA	613	569	3-200	-20	-20
PFDoA-2	$^{13}C_2$PFDoA	613	169	3-200	-34	-14
$^{13}C_2$PFDoA	---	615	570	3-200	-18	-11

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N-MeFOSA-1	d_3-N-MeFOSA	512	169	3-200	-36	-9
N-MeFOSA-2	d_3-N-MeFOSA	512	219	3-200	-34	-12
d_3-N-MeFOSA	---	515	169	3-200	-37	-9
N-MeFOSE	d_7-N-MeFOSE	616	59	3-200	-65	-6
d_7-N-MeFOSE	---	623	59	3-200	-68	-6
PFDoS-1	$^{13}C_2$PFTeDA	699	80	3-200	-155	-15
PFDoS-2	$^{13}C_2$PFTeDA	699	99	3-200	-135	-15
PFTrDA-1	$^{13}C_2$PFDoA	663	619	3-200	-18	-25
PFTrDA-2	$^{13}C_2$PFDoA	663	169	3-200	-34	-11
N-EtFOSA-1	d_5-N-EtFOSA	526	169	3-200	-38	-8
N-EtFOSA-2	d_5-N-EtFOSA	526	219	3-200	-34	-11
d5-N-EtFOSA	d_9-N-EtFOSE	531	169	3-200	-38	-9
N-EtFOSE	d_9-N-EtFOSE	630	59	3-200	-75	-6
d_9-N-EtFOSE	---	639	59	3-200	-63	-29
PFTeDA-1	$^{13}C_2$PFTeDA	713	669	3-200	-20	-22
PFTeDA-2	$^{13}C_2$PFTeDA	713	169	3-200	-38	-14
$^{13}C_2$PFTeDA	---	715	670	3-200	-21	-13

7.29.3.3. Parameter table

CUR (psi)	40
GS1 (psi)	40
GS2 (psi)	70
IonSpray Voltage (V)	2000
Temperature (°C)	400
CAD gas	14
EP	-10
CEM (V)	1900

8. Quality Control

8.1. For general quality control procedures, see the Quality Assurance Manual. For specific quality control acceptance limits that apply to laboratory control samples, surrogates, calibration check standards, matrix spikes, and duplicates for this analytical procedure please consult the LIMS. For details, see the standard operating procedure EHD ORG CHEM QA 0001 | Horizon and QA

8.2. Before any analysis is performed, the MS/MS detector must pass a polypropylene glycol (PPG) tune check. A standard containing 300 µM of SCIEX Mixed PPG solution is analyzed. The spectra should meet the recommended SCIEX operating criteria before samples are analyzed. A tune check should be performed at least once annually and after PM or other service. See example SCIEX tuning criteria in §8.3 below.

8.3. SCIEX tuning criteria for PPGs in negative Turbo Ion Spray mode (NOTE: cps = counts per second)

8.3.1. Negative Mode PPGs (per SCIEX PM criteria):

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Target Mass (Da)	Intensity Required (cps)	Peak Width	Mass Shift (Da)
933.6360 (Q1)	**≥ $1.0x10^7$**	**0.6-0.8**	**< 0.1**
933.6360 (Q3)	**≥ $8.0x10^6$**	**0.6-0.8**	**< 0.1**

8.3.2. Instrument tuning is performed by a qualified service technician, and the final report can be found on the instrument computer at SERVICE (V:)\PM Data or in a binder located above the instrument PC.

8.4. Initial demonstration of capability (IDC)

8.4.1. An IDC must be completed before any field samples may be analyzed. An initial calibration curve may be linear (at least five points) or quadratic (at least six points). Both types of curves must have a coefficient of determination (R^2) that is greater than or equal to 0.99. The curve is not forced through zero and may be weighted by concentration. Using the generated curves, each calibration point for each analyte must calculate between 70-130% of the true value (except for the lowest point, which must calculate between 50-150%). If any of these criteria fail, sample analysis cannot be performed.

8.4.2. Initial calibration blank (ICB) –an ICB must be injected directly after the highest calibration standard to ensure that no contamination is seen that could bias results. The results of the ICB must be less than ½ of the MRL.

8.4.3. Analyze a second source/initial calibration verification standard. The second source must have the same compounds as the primary calibration standards, but a different vendor or a different lot number from the compounds in the calibration standards must be used. Recovery must be 70-130% for all compounds; otherwise, the calibration curve is invalid and sample analysis cannot proceed. The second source must be injected directly after the ICB.

8.4.4. Initial demonstration of low system background – If a new lot of SPE cartridges, solvents, tubes, pipettes or autosampler vials are used, a MB must be analyzed to show that each item is free of contamination. The results for all compounds must be less than ½ of the MRL.

8.4.5. Retention times (RT) for all target compounds and internal standards must be determined. See EHD ORG CHEM GENOP 0074 §5.1.5-5.2 for the procedure for establishing RT windows.

8.4.6. Initial demonstration of precision (IDP) – Four to seven laboratory control spikes (LCSs) will be prepared near the midpoint of the initial calibration curve and extracted like a normal sample. The relative standard deviation of the results of the replicates must be less than 30%.

8.4.7. Initial demonstration of accuracy (IDA) – Calculate the average recovery for the same four to seven samples extracted for the IDP. The recovery must be within 65-135% of the true value.

8.4.8. Extracted internal standard recoveries must be 50-150% of the average of the calibration standards or the IDC must be repeated. The exceptions to this are FOSA, NMeFOSA, NEtFOSA, NMeFOSE, and NEtFOSE where internal standard recoveries are expected to be 20-150% of the average of the calibration standards.

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8.5. Method Detection Limit (MDL) study- Seven LCSs are spiked at a level one to five times the estimated LOD. The estimated LOD is roughly determined using the recoveries of the initial calibration standards. The LCSs (as well as seven lab blanks) are extracted using the established method. The MDL is calculated by following the standard operating procedure EHD DIV-WIDE QA 116 | LOD Procedures.

8.6. Ongoing QC requirements

8.6.1. Laboratory Method Blanks (MB) - The analyst must demonstrate that all glassware and reagent interferences are under control. Before a new set of samples is extracted, a laboratory method blank must be analyzed. If the MB produces a peak within an analyte's retention time window that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples. Background from method analytes or other contaminants that interfere with the measurement of method analytes must be less than ½ of the MRL or $^1/_{10}$ of the sample concentration, whichever is greater. If the target compounds are detected above or equal to this level, the data for the problem analytes will be appropriately flagged. At least one MB must be analyzed with each extraction batch. MBs must be rotated through every extraction port on the SPE manifold/automated extraction system.

8.6.2. Field Blanks - A field blank (FB) only needs to be analyzed if there is a result for any compound that is above the MRL. The FB is expected to be less than ½ of the MRL or $^1/_{10}$ of the sample concentration, whichever is greater. It is not necessary to flag FB detections that are between the MDL and ½ of the MRL.

8.6.3. Equipment Blanks – These may be collected once per sampling event when equipment is used in the field to collect samples. An equipment blank (EB) only needs to be analyzed if there is a result for any compound that is above the MRL. The EB is expected to be less than ½ of the MRL or $^1/_{10}$ of the sample concentration, whichever is greater. It is not necessary to flag EB detections that are between the MDL and ½ of the MRL.

8.6.4. Assessing laboratory performance with Laboratory Control Samples (LCS) - An LCS is also analyzed with each extraction batch and can be at three different concentrations (see below). These spikes should rotate between the three per batch or depend on the sample matrix (if high concentrations of PFAS are suspected, use the high spike). Results for the LCS must be within 60-135% of the true value (50-150% for the low LCS). If any of the recoveries for the target compounds fall outside of this range, the data will be appropriately flagged. If recoveries for five more compounds fall outside of acceptable ranges, re-extraction of samples is necessary.

LCS – 1.0 ng/L (2.5 µL of PFASSpk1)

LCS1 – 8.0 ng/L (20 µL of PFASSpk1)

LCS2 – 40 ng/L (100 µL of PFASSpk1)

8.6.5. *Optional* - Assessing analyte recovery with matrix spike (MS) - If sufficient sample is available, the laboratory may add a known concentration to a minimum of 5% of the routine samples or one sample per set, whichever is greater. The spiking concentration should not be less than 2-5 times the background concentration of the sample selected for fortification. A typical matrix spike rotates between 2.5, 20, and 100 µL of the 100 ng/mL mix (1.0, 8.0, and 40 ng/L respectively). Calculate the percent recovery, P, of the concentration for each analyte, after correcting the

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analytical result, X, from the fortified sample for the background concentration, b, measured in the unfortified sample, i.e.:

$$P = 100 (X - b) / \text{fortifying concentration}$$

8.6.6. Assessing precision with Duplicates - If enough sample is collected in a separate bottle, the laboratory will analyze one sample in duplicate per extraction batch. Based upon the analyst's experience, a sample that is expected to contain the analytes above the limit of quantitation should be chosen to analyze in duplicate. The relative percent difference (RPD) of sample and duplicate response is expected to be ± 30% for analytes with concentration greater than 2x MRL and ± 50% for analytes with concentration between 1-2x MRL. If the RPD fails these criteria, data will be appropriately flagged.

8.6.7. Continuing Calibration Verification (CCV) - A calibration standard is analyzed at the beginning of each analysis batch, after every ten field samples, and at the end of the batch. The beginning (low) CCV must be at or below the MRL for all compounds. The subsequent CCVs during the run will rotate between the medium and high calibration standards. If any of these CCVs fail, immediately analyze two additional CCVs. If both pass, then analysis may continue. If either fails (outside of 50-150% for the low CCV and 70-130% for the medium and high CCV), a new curve must be generated. If target or internal standard recoveries in the CCV are above acceptable recovery but there are no detections in the sample, data may be reported without any qualifiers.

Recommended CCV standards:

CCV = Calibration level 2 (1.0 ng/L), 50-150% recovery limits

CCV1 = Calibration level 5 (10 ng/L), 70-130% recovery limits

CCV2 = Calibration level 7 (40 ng/L), 70-130% recovery limits

8.6.8. Continuing Calibration Blank (CCB) – A CCB must be analyzed after each CCV to demonstrate that that instrument is free from contamination or carryover. If method blanks or reagent blanks must be analyzed, they may take the place of a CCB. The results of the CCB must be less than ½ of the MRL.

8.6.9. Internal Standards recovery - The peak area of the internal standards must be monitored in all injections during a run. The response for extracted samples must be within 25-150% of the average area of the calibration standards (if injecting on the same day as calibration) or the most recent CCV (if injecting on a different day than calibration). The only exception to this is the response of five neutral internal standards (FOSA, NMeFOSA, NMeFOSE, NEtFOSA, and NEtFOSE) which must be within 10-150%. If this fails, reinject the sample. If recovery of the repeat injection fails, appropriately flag the data. Internal standard is added to instrument quality control samples (ICB, ICV, CCV, and CCB) prior to analysis. IS recovery for all compounds must be 50-150% in these samples.

The SCIEX OS processing method is able to calculate IS recovery compared to the appropriate reference standard(s) and flag any failures. See EHD ORG CHEM GENOP 0073 §3.9.2 for details of the calculation. Data processed in SCIEX OS will have IS recovery data saved in the results table.

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In order to report quality data using internal standards, a minimum signal to noise ratio of 10:1 is expected for each internal standard. Do not report results if this is not achieved.

8.6.10. Transition ratio calculations – Any analytes over the MDL that have at least a 3:1 signal to noise ratio must also pass transition ratio requirements. The primary to secondary ion transition ratio must be within 50-150% of the value calculated from the midpoint standard during an injection sequence where a calibration curve is generated or the initial CCV on non-calibration sequence. The transition ratio is calculated with the equation below:

$$Transition\ ratio = \frac{quantitation\ ion\ abundance}{confirmation\ ion\ abundance}$$

If the ratio fails, qualified data may still be reported.

The SCIEX OS processing method is able to calculate ion ratios, compare them to the appropriate reference standard, and flag any failures. See EHD ORG CHEM GENOP 0073 §3.9.1 for details of the calculation. Data processed in SCIEX OS will have transition ion ratio data saved in the results table.

8.7. Carryover - Injection of target mix at 100 ng/mL (equivalent to 400 ng/L in water) showed no evidence of carryover > ½ MRL. If a compound result is >400 ng/L and the injection immediately following shows detection of that compound >½ MRL, the sample injected after the high detection sample must be reinjected to ensure the detection was not a result of carryover.

8.8. PT/Blind Samples - The lab treats samples that are known to contain high concentrations of any target analyte(s) by diluting them either prior to extraction or prior to analysis. The same procedure will be followed for PT and Blind samples if any target analyte has historically been known to be outside the typical calibration range or the PTRL (Proficiency Testing Reporting Limit) lists the expected concentrations to be outside the current calibration range. Multiple dilutions of a PT sample are prepared, analyzed, and results compared. If the results of all target analytes are found to be within the normal calibration range, the PT or Blind will be re-analyzed in the undiluted state, if possible. Additionally, PT samples may be extracted alongside a high-level LCS to ensure that PFAS are being extracted efficiently at high concentrations. The PT reporting limits (PTRLs) for a PT or Blind sample may be obtained from QA personnel prior to running the samples

8.9. Batches will typically include samples with the accompanying MB, LCS, FRB (if needed), EB (if needed), and duplicate. An extraction batch is defined as between 1 and 20 field samples with a maximum time of 24 hours between the start of extracting the first and last samples in the batch. Batch QC samples (MB, FRB, EB, and LCS) do not count against the 20-sample limit.

8.10. Data qualification – see EHD ORG CHEM GENOP 0073 | PFAS Data Qualifying Rules for WI DNR QC criteria, acceptable data qualifications, and situations when data will not be reported.

8.11. Identification of Analytes - See EHD ORG CHEM GENOP 0074 | PFAS Compound Identification for details of compound identification, retention time window establishment, and branched isomer peak integration.

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9. Method Calibration

9.1. Working Standard Preparation Procedure

9.1.1. Before using any stock or working standards, they must brought to room temperature and be vortexed for at least 10 seconds to ensure that the compounds are in solution and not adsorbed to the sides of the container.

9.1.2. Calibration standards - the suggested levels are 0.10, 0.25, 0.50, 1.0, 2.5, 5.0, 10, and 25.0 ng/mL (equivalent to 0.40, 1.0, 2.0, 4.0, 10, 20, 40, and 100 ng/L in 250 mL of water). The Applied Biosystems Analyst or SCIEX OS data system is used to prepare an internal standard calibration curve that is not forced through zero for each analyte. At least five standards must be used to generate a linear curve, and at least six standards must be used to generate a quadratic curve. The regression of the curve should default to quadratic and only be switched to linear if fewer than six curve points are available. Calibration points below MDL for a given compound may be included in the calibration curve as long as signal-to-noise, peak shape, RT and all other considerations of peak quality are acceptable and consistent with other calibration standards. An $R^2 \geq 0.99$ ($R \geq 0.995$) will be used as guidance to verify the acceptability of the curve.

9.2. Second Source/Initial Calibration Verification (ICV) - The second source must have the same compounds as the primary calibration standards but either a different vendor or a different lot number from the compounds in the calibration standards. Recovery must be 70-130% for all compounds, otherwise the calibration curve is invalid and sample analysis cannot proceed. The ICV must be analyzed directly after the ICB. If the initial ICV injection fails, immediately analyze two additional ICV injections. If both injections pass the 70-130% recovery limits, then analysis may continue. If either of the two fail, a new calibration curve must be generated.

9.3. Initial Calibration Blank (ICB) – Inject the ICB immediately following the highest calibration standard. Results must be < ½ MRL.

9.4. The working calibration curve must be verified on each working day by the injection of one or more calibration standards at the beginning and end of each analytical run, and after the analysis of 10 samples if 10 or more samples are analyzed in an analysis day. See §8.6.8 for recovery criteria and procedure for failed CCV.

9.5. If any sample results are higher than the highest calibration standard, a dilution that brings the result within the boundaries of the curve must be analyzed.

9.6. Branched/Linear isomer standards – See EHD ORG CHEM GENOP 0074 | PFAS Compound Identification §5.1.5.1 for details on branched isomer peak retention time window establishment and §5.3 for details on branched isomer peak integration.

10. Sample Preparation Procedure

10.1. Samples should only be collected in polypropylene bottles that have polypropylene caps. 250-mL is the typical size bottle; bottles for sampling are provided by WSLH.

10.2. Samples with low turbidity may be extracted with an automated extraction system (AES). If samples contain light particulates, they may still be extracted on the AES if a PromoChrom in-line filter is used to prevent contamination and damage to AES lines/pumps. If in-line filters are used to extract any samples in a batch, the associated MB and LCS must be extracted using in-line filters. See §10.3 for the manual extraction procedure. See §10.4 for the automated extraction procedure.

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10.3. Weighing the samples – Each sample (with cap included) must be weighed prior to and after extraction to obtain the total volume of the sample (assuming the water sample is 1 g/mL). The balance must be calibrated with the appropriate weights before weighing any samples.

10.4. **Manual Extraction**—manual extraction is typically reserved for samples that have more solid particulate than can be added to and filtered by the SPE cartridge. The liquid portion of the sample is decanted and extracted; the solid portion is rinsed with methanol, and the rinse is passed through the SPE cartridge during the final elution step

10.4.1. Add 25 µL of the internal standard mix to each sample and QC. Add a proper amount of target-compound stock mix (PFASSpk1) to the LCS and MS (if included). A minimum fifteen-minute equilibrium time must be allowed from the time the target compounds and IS are added to the samples to the time the samples are extracted. The amount in the LCS will rotate between a low, medium, and high spike (see §8.6.2 for spike volumes/concentrations). Recap the bottles and mix well.

10.4.2. SPE cartridges are attached to a valve liner and then inserted through SPE valve stems in the vacuum manifold. A solvent reservoir to which sample is added is attached to the top of the SPE cartridge via an adaptor. Prior to use, valve stems and reservoir adaptors must be rinsed. Place stems and adaptors in a beaker, pour enough MeOH to cover completely, swirl beaker contents, then pour off MeOH. Repeat this twice more, then lay stems and adaptors out to dry before using. Once dry, place valve stems in vacuum manifold ports. Attach the valve liner to the bottom of the cartridge and carefully insert until the valve liner locks into place in the valve stem.

10.4.3. Before sample is drawn through the SPE, the cartridge must be conditioned. At no point during the conditioning and sample transfer can the SPE packing material be allowed to go dry. If the cartridge goes dry during the conditioning phase, conditioning must be restarted with a new cartridge. Using a vacuum manifold with no vacuum on, 4 mL of ammonia/methanol is passed through dropwise, followed by 4 mL of methanol, and lastly 4 mL of 18-mΩ H_2O water. Close the valve stems to stop any flow. Add a small amount of water to each column to ensure the packing material does not go dry. Attach solvent reservoirs to SPE columns with adaptor plugs.

10.4.4. If more solids than can be filtered through the SPE cartridge exist in a sample, centrifuge the sample to form a plug of solid particulate on the bottom of the sample container. If necessary, homogenize the sample and transfer to a new container for centrifugation. Retain the original sample container to rinse with methanol in §10.4.5. PromoChrom anti-clogging frits may also be inserted into the SPE cartridge to alleviate clogging from particulates. Pour approximately 50 mL of the sample into the solvent reservoir. Open up the valve stems and adjust the vacuum so that the approximate flow rate is 3-6 mL/min (Roughly 1-2 drops/second out the bottom of the valve liner). Extract the entire sample. If clogging of the SPE cartridge occurs and the entire sample is not able to be completely extracted, weigh the bottle with remaining sample to determine the amount extracted. Dispose of the remaining sample and continue the extraction in §10.4.6.

10.4.5. After the sample bottle is empty (solid particulates may remain), close the valve stems and remove the solvent reservoir (with the adaptor attached) from the SPE column and place on top of the sample bottle so that the solvent from the rinse steps

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drains into the sample bottle. Using two disposable polypropylene pipettes, rinse the solvent reservoir with two 2.5 mL aliquots of methanol. Coat all surfaces of the solvent reservoir. With the 5 mL total that is now in the sample bottle, rinse the entire inner surface to make sure any PFAS that have adhered to the bottle or particulates in the sample get onto the SPE column. If necessary, vortex the container with methanol to thoroughly rinse the solid plug and centrifuge the container to re-settle the particulate. Set the sample bottle aside for §10.4.7.

10.4.6. Add 4 mL of water to each column followed by 4 mL of the 25 mM acetate buffer that was prepared in §6.2.2. Discard both rinses. Open up valve stems all the way, increase the vacuum to 15 psi, and leave for at least two minutes to make sure all residual water has been removed from the SPE columns.

10.4.7. Turn off the vacuum and close valve stems. Insert a rack that contains labeled polypropylene centrifuge tubes under the cartridges. Make sure the valve liners are going into the opening of the centrifuge tubes in order to collect the eluent. Take each 5 mL rinse of methanol in the sample bottle and add to the corresponding SPE column. Turn the vacuum back on very low and open up valve stems so that the solvent flows very slowly through the tube, into the cartridge, and into the polypropylene tubes in a dropwise fashion (approximately 1 mL/min or 1 drop every 3 seconds). This is followed by elution at the same rate of 4 mL of the ammonia/methanol mixture that was made in §6.2.3. After elution is complete, proceed to §10.6.

10.5. **Automated Extraction**

10.5.1. Add 25 µL of the internal standard mix to each sample and QC. Add a proper amount of target-compound stock mix (PFASSpk1) to the LCS and MS (if included). A minimum fifteen-minute equilibration time must be allowed from the time the target compounds and IS are added to the samples to the time the samples are extracted. The amount in the LCS will rotate between a low, medium, and high spike (see §8.6.2 for spike volumes/concentrations). Recap the bottles and mix well.

10.5.2. Each AES can extract up to eight samples at a time, and may operate lines 1-4, 5-8, or 1-8 depending on what is selected. Multiple AES may be used simultaneously for the same batch. Install empty 250-mL bottles on the AES, and rinse the lines 1-2 times before each extraction by running the "clean sys" method. Refill methanol and PFAS-free water reservoirs as necessary. It is acceptable to use old sample bottles and leave old SPE cartridges in place while rinsing the AES lines.

10.5.3. After AES rinsing, install all sample bottles. Using a lancet or thumbtack, make a small hole at the bottom of each sample bottle (now facing upward as installed in the bottle rack) to ensure that the AES pump does not create a vacuum in the bottles as it pulls sample through the lines. Make note of the sample bottle positions, and place labeled 15-mL centrifuge tubes in the corresponding positions of the front vial rack. Ensure that this rack can move freely by adjusting the slack in the solvent waste lines. Install new Oasis WAX SPE cartridges on each of the sample lines in use. Ensure that the N_2 gas supply in the fume hood directly to the left of the AES is turned on to ~25 psi.

10.5.4. Select method "OC16201-Surface Water", and refill solvent reservoirs as necessary.

Solvent 1 — Methanol, HPLC grade

Solvent 2 — Water, filtered to 18-MΩ·cm resistivity

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Solvent 3 — 60mM Ammonium hydroxide in methanol

Solvent 4 — 25mM ammonium acetate buffer

10.5.5. When the extraction begins, the AES automatically pre-conditions each SPE cartridge in three separate steps with 4mL ammonium hydroxide in methanol, methanol, and water, rinsing at a flow rate of 5mL/min for each solvent. The samples are then added to the SPE cartridges in ~4 mL increments at a flow rate of 3 mL/min. The AES is programmed to pull 270-290 mL of sample to ensure that the entire sample is extracted. After entire samples pass through the cartridges, 4 mL each of water and 25 mM ammonium acetate buffer are automatically added at 3 mL/min in separate steps to prepare the cartridges for elution. The AES pushes 20 mL of air at 10 mL/min through each cartridge, and then nitrogen is blown through each cartridge for 10 minutes. The AES adds 5 mL of methanol to each sample bottle and waits until the system is manually restarted. Before manually restarting, lift each bottle from the rack and rinse the bottle with the MeOH added by the AES, rotating the bottle to coat the entire inner surface. After restarting, the AES adds the rinse methanol to the SPE cartridges at a rate of 0.5 mL/min. The AES automatically collects the eluent in the 15 mL centrifuge tubes. Finally, the AES adds 4 mL 60mM ammonium hydroxide in methanol to each cartridge and collects the eluent at 0.5 mL/min. After elution is complete, proceed to §10.6.

10.6. Each sample must pass through a carbon-phase SPE cartridge after the initial SPE cleanup. Install one clean ENVI-Carb cartridge for each sample on the vacuum extraction manifold. Each carbon SPE cartridge must be rinsed with at least 6 mL 60mM ammonium hydroxide in methanol before samples are added. Turn the vacuum on low, and allow the ammonium hydroxide in methanol to flow through each cartridge at about 3-5 mL/min. Close the valve of each cartridge to stop the flow as the rinse solvent reaches the level of the frit in the cartridge. Place labelled 15-mL polypropylene centrifuge tubes below each cartridge to collect the filtered sample extracts. Add each sample extract to a rinsed cartridge, and open the valves to collect the filtered extracts in the 15-mL tubes at about 3-5 mL/min. Carbon-phase filtration should be completed immediately after the initial SPE step.

10.7. Take the extract and blow it down to between 0.5-1 mL under a gentle stream of nitrogen (~0.7 L/min) in a heated water bath at 60-65°C. Do not allow samples to dry completely. Add MeOH to get to exactly 1 mL.

10.8. To remove particulates that may damage the LCMS system, samples must be filtered prior to analysis. To filter a sample, attach a glass fiber filter or polylpropylene syringe filter to the end of a disposable 1mL syringe. Remove the plunger from the syringe, and add at least 2.5 mL of methanol to the top of the syringe by using a disposable pipette. The solvent will start dripping out of the bottom of the filter. Gripping only the top of the plunger, put the plunger back into the syringe and push the remaining solvent through the syringe filter. Remove the plunger. Thoroughly vortex the sample to be filtered. Holding the syringe and filter over an open 1.2mL or 2mL polypropylene cryovial, transfer the samples using a disposable polypropylene pipette into the top of the syringe. Gently push the sample through the syringe filter with the plunger. Filter sample immediately after rinsing syringe/filter; do not allow filter to go dry. Take a portion of this mixture after vortexing well and transfer to a 300-uL polypropylene autosampler vial. Cap with polypropylene caps.

10.8.1. Glass fiber filters (37C-2116-OEM) and polypropylene filters (37B-2116-OEM) were tested for adsorption of analytes by filtering low-level calibration standards, and they demonstrated no significant adsorption of target or mass-labelled

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compounds. Filters are also used to filter MBs and demonstrate no contamination > ½ MRL as long as they are properly rinsed with 2.5mL of MeOH.

10.9. Vortex autosampler vials prior to loading into the autosampler. Vials are single use unless immediately recapped.

11. Calculations: Calculations are performed using a multilevel calibration with the Applied Biosystems Analyst or SCIEX OS software.

11.1. Calibration points below MDL for a given compound may be included in the calibration curve as long as signal-to-noise, peak shape, RT and all other considerations of peak quality are acceptable and consistent with other calibration standards.

11.2. Curves will default to quadratic regression and will only be changed to linear if not enough valid curve points exist for quadratic regression (six curve points are required for quadratic, five curve points are required for linear).

11.3. For target compounds, the signal to noise ratio (S/N) is expected to be greater than or equal to 3:1 for quantitation and confirmation ions. If this is not achieved, the peak is not used and the analyte is reported as < MRL.

11.4. See GENOP 0074 | PFAS Compound Identification for other calculations including compound concentration adjustments for results of labelled internal standards

12. Corrective Action Procedure:

12.1. For a detailed explanation of the corrective action procedure, please refer to the NELAC QA Manual Organic Chemistry Section: O:\SOP\EHD\Division Wide\Final\

12.2. QC Failures, instrument problems, or analytical problems:

12.2.1. Corrective action shall be documented either in the instrument logbook, the report generated from the instrument, or in an occurrence management form. Most incidences can be isolated to the instrument. Instruments could require repairing, adjusting, servicing instrument hardware/software, or a combination of actions.

12.2.1.1. The analyst should document on the lab report a concise explanation for resolving the immediate issue(s).

12.2.1.2. The instrument logbook can be found at: **M:\EHD\ESS(4900)\ESS Org(4940)\Logbooks\PFAS\Maintenance Log for Waters HPLC & QTRAP.doc**

12.2.2. Each batch of samples is assessed against the required QC for data reporting integrity. All instrument problems, analytical problems, or QC failures should be addressed by answering the following questions.

12.2.2.1. **What was the source of the problem?**

12.2.2.1.1. The analyst should determine and document what the failure was. Example: blank contamination, lab matrix spike failure, matrix spike failure, precision failure, surrogate spike failure, etc.

12.2.2.1.2. The source of the issue or failure may not always be known, but a comment should be documented stating that the issue was investigated; include a comment of why corrective action cannot be performed. (e.g., checks OK – unknown

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cause; failure investigated – unknown cause – reanalysis not possible due to… etc.)

12.2.2.2. **What corrective action was performed?** The analyst should formulate and document an immediate assessment as how best to proceed. Some solutions could be recalibration, check standard assessment, interference assessment, qualify data, reanalysis, instrument maintenance, stock standard response assessment, etc.

12.2.2.3. **What was done to verify the corrective action?** All assumptions of correction need to be verified and documented. If possible, reanalysis after corrective action will provide conclusive evidence that corrective action worked. ***Note: Not all QC failures can be readily explained. Analyst experience weighs heavily in determining the proper corrective action.***

13. Data Management: Data is collected, and calculations are made on a PC-based system running SCIEX Analyst Software or SCIEX OS software by the analyst. PFAS analyte data is transferred to the LIMS by the analyst (or designee). It is then reviewed by peers or the section supervisor before being released.

13.1. Updating a Quantitation Method (Analyst)

13.1.1. Create a Results Table containing at least the calibration standards or the initial CCV to be used for Quantitation Method (QM) reference; select a previously-used QM for initial quantitation parameters.

13.1.2. In the newly created Results Table, begin peak review for the QM reference standard. Select a peak by dragging the cursor across its width (without Manual Integration enabled) and pressing Select Peak. Select Apply and then Accept. In the quantitation parameters, the Expected RT should update to the RT of the selected peak. After selecting each peak (including IS) in the reference standard (note: the peak selection tool will often not include branched isomers; this integration must be adjusted later), right click in the peak review pane and select Update Method. All samples in the Results Table will be re-integrated with the updated method, and any additions to the same Results Table will be integrated with that method.

13.1.3. If desired, select Tools > Results Table > Export Method… and save the new QM (it is acceptable to save over an old QM to save space). This step is necessary to save the quantitation parameters; otherwise, the QM referencing will apply only to the created Results Table. Because a new QM reference will generally be used for each new Results Table, saving is not necessary.

13.2. Updating a Processing Method (SCIEX OS) – see EHD ORG CHEM GENOP 0074 | PFAS Compound Identification

13.3. Horizon:

13.3.1. When Creating a Batch, include all QC that will be analyzed. This includes MB, FRB, FD, LFB (low, medium and high), LFSM (low, medium, and high), and LFSMD (low, medium, and high).

13.3.2. If a QC sample fails, add an additional one to the batch (giving it a second number) and upload both into Horizon.

13.3.3. OK the failed QC so it will count when new limits are run.

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13.3.4. These new limits will be entered by QC personnel or a LIMS Admin, not by the Chemist.

13.4. Upload into Horizon:

13.4.1. In Analyst, right click anywhere on the table to bring up a drop-down menu. Select "full" to get all compounds and all injections into one table. Highlight the entire table, copy the data into an Excel file, and save it as a .CSV file.

13.4.2. In SCIEX OS, right click and copy the entire table. Copy the data into an Excel file and save it as a .CSV file.

13.4.3. Add the batch number (number only) to cell A1. Make sure that the Sample Name is in column B, Calculated Concentration is in column D, the Acquisition Date is in column H, and the Analyte Peak Name is in column J.

13.4.4. The Sample Name (row B) must contain the Horizon number of any sample or QC or the upload will not work correctly.

13.4.5. Move a copy of the .CSV file to the appropriate instrument interface folder: WLSH Network > slhhorizprdapp> InstrumentResults> ORG_LCMS > Individual Analyst Folder

13.4.6. Horizon will automatically move the data from the instrument interface folder to the LIMS Results Upload queue. If the .CSV file does not disappear from the interface folder within 60 seconds, check that the file is formatted correctly. Contact a LIMS Administrator with further issues.

13.4.7. Prior to selecting samples to upload from the Horizon Data Pipeline, ensure the correct instrument is selected in the analyst's Instrument Interface. "LC-MS/MS QTRAP" is the SCIEX 5500 LCMS system and "LC-MS/MS 7500" is the SCIEX 7500 LCMS system.

13.5. LIMS Results Upload

13.5.1. In Horizon, select Data > Results Upload

13.5.2. Enter the batch number in the search bar, and hit Enter

13.5.3. Highlight all data for upload and select Upload Results. The data will automatically transfer to the corresponding samples in the batch, and each sample's Active Status should now be "In Review".

13.6. Calculations in Horizon

13.6.1. Horizon cannot perform an appropriate calculation for the expected values of the QCS using the amounts of standard that are actually spiked into a water sample. Due to the calculations in Horizon, the volumes that are used in the QCSs are 250 times less than what they actually are. The Horizon calculations were tested to make sure the numbers are correct when reporting the final result.

13.7. Standards in Horizon for PFASs in water:

13.7.1. PFASSpk1 - Perfluorinated Compounds Stock 1 100 ng/mL (Table 2)

13.8. Data is reported in ng/L of water. All compounds are reported in their acid form.

13.9. Standard logbook information is listed in Tables 1-7 below.

14. Definitions: General definitions of terms that may be used in this method can be found in the following documents.

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14.1. Check reference methods (§16).

14.2. TNI Standard, EL-V1M2-2016-Rev. 2.1, Section 3.0, Terms and Definitions, The NELAC Institute, 2016 O:\Teams\EHD QC Team\Accreditation\NELAC\2016 TNI Standard + guidance doc\STD-ELV1-2016-Rev2,1_LabRegs

14.3. Chapter NR 149, LABORATORY ACCREDITATION, Wisconsin State Legislative Reference Bureau, Register February, 2021 No. 782, effective June 29, 2021. See Section NR149.03 Definitions.

15. Method Performance:

15.1. Where applicable, the laboratory's initial accuracy and precision data (MDLs and IDCs) were generated in compliance with the reference method and the Departments standard operating procedure

15.1.1. MDL procedure (EHD DIV-WIDE QA 116 | LOD Procedures)

15.1.2. IDC procedure (EHD DIV-WIDE QA 115 | Initial and Ongoing DOC Procedures)

15.2. Data generated within the last two years will be located in a filing cabinet across from cubicle SC213 or stored in the basement. Any data older than three years is archived, stored at Wisconsin State Record facilities and then destroyed after meeting its required retention and disposition schedule according to "Record Retention and Disposition Schedule" (O:\RDA's\Final RDA\EHD_2017_WSLH.pdf).

16. References:

16.1. Wisconsin PFAS Aqueous (Non-Potable Water) and Non-Aqueous Matrices Method Expectations, Version 12.16.2019, Per and Polyflorinated Alkyl (PFAS) Analysis Using Isotope Dilution by LC/MS/MS, Document ID EA-19-0001.

16.2. Determination of selected perfluorinated alkyl acids in drinking water by solid phase extraction and liquid chromatography/tandem mass spectrometry (LC/MS/MS). Method 537.1 EPA/600/R-08/092.

16.3. "Methods for the Determination of Organic Compounds in Drinking Water," US EPA/600/4-88/039, 1995.

16.4. "Quality Assurance Manual", NELAC QA Manual. O:\SOP\EHD\Division Wide\Final\

16.5. TNI Standard, EL-V1-2016-Rev. 2.1, The NELAC Institute, 2016, O:\Teams\EHD QC Team\Accreditation\NELAC\2016 TNI Standard + guidance doc\STD-ELV1-2016-Rev2,1_LabRegs

16.6. Chapter NR 149, LABORATORY ACCREDITATION, Wisconsin State Legislative Reference Bureau, Register February, 2021 No. 782, effective June 29, 2021

16.7. PFAS in Water forms and instructions, **M:\EHD\ESS(4900)\ESS Org(4940)\Forms and Sampling Instructions\Final\PFAS in Water**

16.8. QC Bottle Checks Template, **M:\EHD\ESS(4900)\ESS Org(4940)\Method Related Documents\PFAS\QC Bottle Checks**

16.9. Instrument manufacture manuals can be found at **M:\EHD\ESS(4900)\ESS Org(4940)\Method Related Documents\Instrument Support Documents**

16.10. PFAS Logbooks, **M:\EHD\ESS(4900)\ESS Org(4940)\Logbooks\PFAS\Maintenance Log for Waters HPLC & QTRAP.doc**

16.11. EHD ORG CHEM GENOP 0073 | PFAS Data Qualifying Rules

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16.12. EHD ORG CHEM GENOP 0074 | PFAS Compound Identification

16.13. EHD ORG CHEM GENOP 0036 | Standard Log System

16.14. EHD ORG CHEM IOP 0503 | PromoChrom SPE-03 Systems

16.15. EHD ORG CHEM IOP 0505 | Biotage TurboVap LV

16.16. EHD ORG CHEM IOP 0506 | SCIEX 5500 Mass Spec with Waters UPLC

16.17. EHD ORG CHEM IOP 0507 | SCIEX 7500 Mass Spec & Exion LC

16.18. EHD ORG CHEM QA 0008 | QA Audit of Analytical Data

16.19. EHD ORG CHEM QA 0001 | Horizon LIMS and QA

16.20. EHD DIV-WIDE GENOP 0034 | Manual Integration

16.21. EHD DIV-WIDE GENOP 038 | Waste Management

16.22. EHD DIV-WIDE QA 115 | Initial and Ongoing DOC Procedures

16.23. EHD DIV-WIDE QA 116 | LOD Procedures

16.24. Record Retention and Disposition Schedule (O:\RDA's\Final RDA\EHD_2017_WSLH.pdf)

16.25. LABWIDE SAFETY 102 | Chemical Hygiene Plan

16.26. University of Wisconsin Chemical Safety and Disposal Guide, http://ehs.wisc.edu/disposal-services/.

17. Tables, figures, diagrams, charts, checklists, appendices (tables include concentrations from example stock standards; concentrations may change slightly as different stocks are used):

Table 1 – Preparation of ~2 µg/mL Intermediate Solutions from 100 µg/mL Stocks

Compound	Concentration of Stock (µg/mL)	Amount added (µL)	Final Volume (mL)	Concentration (µg/mL)
N-MeFOSA	99	20	1	1.98
N-EtFOSA	94	20	1	1.88

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Table 2 – Preparation of 100 ng/mL Target Compound Solution

Compound	Concentration of stock (µg/mL)	Amount Added (µL)	Final volume (mL)	Final Concentration (ng/mL)
PFHxA	2	250	5	100
PFHpA	2			100
PFOA	2			100
PFNA	2			100
PFDA	2			100
PFUnA	2			100
PFDoA	2			100
PFTrDA	2			100
PFTeDA	2			100
PFBS	1.77			88.5
PFHxS	1.82			91.2
PFOS	1.85			92.6
N-MeFOSAA	2			100
N-EtFOSAA	2			100
HFPO-DA	2			100
DONA	1.834			91.7
9Cl-PF3ONS	1.86			93
11Cl-PF3OUdS	1.88			94
PFBA	50	20		200
PFPeA	50	10		100
PFPeS	46.9	10		93.8
PFHpS	47.6	10		95.2
PFNS	48	10		96
PFDS	48.2	10		96.4
PFDoS	48.4	10		96.8
4:2 FTSA	47.5	10		95
6:2 FTSA	50	10		94
8:2 FTSA	50	10		95.8
FOSA	50	10		100
N-MeFOSA	1.98	250		99
N-EtFOSA	1.88	250		94
N-MeFOSE	50	10		100
N-EtFOSE	50	10		100

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Table 3 – Preparation of 2 µg/mL Intermediate IS Solutions From 50 µg/mL Stocks

Compound	Stock Concentration (µg/mL)	Volume to add (µL)	Final Volume (mL)	Final Conc. (µg/mL)
$^{13}C_3$-HFPO-DA	50	200	5	2
d_3-N-MeFOSA	50	200	5	2
d_5-N-EtFOSA	50	200	5	2
d_7-N-MeFOSE	50	200	5	2
d_9-N-EtFOSE	50	200	5	2

Table 4 – Preparation of Working ~80 ng/mL IS Mix

Compound	Stock Concentration (µg/mL)	Volume to add (µL)	Final Volume (mL)	Final Conc. (ng/mL)
$^{13}C_4$-PFBA	1	80	1	80
$^{13}C_5$-PFPeA	1			80
$^{13}C_5$-PFHxA	1			80
$^{13}C_4$-PFHpA	1			80
$^{13}C_8$-PFOA	1			80
$^{13}C_9$-PFNA	1			80
$^{13}C_6$-PFDA	1			80
$^{13}C_7$-PFUdA	1			80
$^{13}C_2$-PFDoA	1			80
$^{13}C_2$-PFTeDA	1			80
$^{13}C_8$-FOSA	1			80
d_3-N-MeFOSAA	1			80
d_5-N-EtFOSAA	1			80
$^{13}C_3$-PFBS	0.932			74.56
$^{13}C_3$-PFHxS	0.948			75.84
$^{13}C_8$-PFOS	0.958			76.72
$^{13}C_2$-4:2FTSA	0.938			75.04
$^{13}C_2$-6:2FTSA	0.951			76.08
$^{13}C_2$-8:2FTSA	0.960			76.8
$^{13}C_3$-HFPO-DA	2	20		40
d_3-N-MeFOSA	2	400		800
d_5-N-EtFOSA	2	40		80
d_7-N-MeFOSE	2	40		80

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d_9-N-EtFOSE	2	40		80

Table 5 – Preparation of Calibration Standards Using 100 ng/mL Target Mix

Level (ng/mL of target compounds)	Concentration of standard in water (ng/L)	Amount of 100 ppb target compound mix (µL)	Amount of internal standard mix (µL)	Amount of MeOH (µL)	Amount of H_2O (µL)
Level 8 (25 ng/mL)	100	250	25	525	200
Level 7 (10 ng/mL)	40	100	25	675	200
Level 6 (5 ng/mL)	20	50	25	725	200
Level 5 (2.5 ng/mL)	10	25	25	750	200
Level 4 (1 ng/mL)	4	10	25	765	200
Level 3 (0.5 ng/mL)	2	5	25	770	200
Level 2 (0.25 ng/mL)	1	2.5	25	772.5	200

Table 6 – Preparation of Calibration Standards Using 10 ng/mL Target Mix

Level (ng/mL of target compounds)	Concentration standard in water (ng/L)	Amount 10 ppb mix (µL)	Amount IS mix (µL)	Amount MeOH (µL)	Amount H_2O (µL)
Level 1 (0.1 ng/mL)	0.4	10	25	765	200

Table 7 – Second Source Individual Compounds

Compound	Vendor and Lot	Concentration (ng/mL)
PFBA	Wellington PFAC300719	10
PFPeA	Wellington PFAC300719	10
PFBS	Wellington PFAC300719	8.87
4:2 FTSA	Wellington PFAC300719	9.37
PFHxA	Wellington PFAC300719	10
PFPeS	Wellington PFAC300719	9.41
HFPO-DA	Wellington PFAC300719	10
PFHpA	Wellington PFAC300719	10
PFHxS	Wellington PFAC300719	9.14
DONA	Wellington PFAC300719	9.45
6:2 FTSA	Wellington PFAC300719	9.51
PFOA	Wellington PFAC300719	10
PFHpS	Wellington PFAC300719	10

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PFOS	Wellington PFAC300719	9.28
PFNA	Wellington PFAC300719	10
9Cl-PF3ONS	Wellington PFAC300719	9.33
8:2 FTSA	Wellington PFAC300719	9.60
PFDA	Wellington PFAC300719	10
PFNS	Wellington PFAC300719	9.62
N-MeFOSAA	Wellington PFAC300719	10
N-EtFOSAA	Wellington PFAC300719	10
FOSA	Wellington PFAC300719	10
PFUnA	Wellington PFAC300719	10
PFDS	Wellington PFAC300719	9.65
11Cl-PF3OUdS	Wellington PFAC300719	9.43
PFDoA	Wellington PFAC300719	10
PFDoS	Wellington LPFDoS1119	10
PFTrDA	Wellington PFAC300719	10
PFTeDA	Wellington PFAC300719	10
MeFOSA	Wellington NMeFOSA1020M	10
EtFOSA	Wellington NEtFOSA1120M	10
MeFOSE	Wellington NMeFOSE1020M	10
EtFOSE	Wellington NEtFOSE1020M	10

Uncontrolled

Appendix 1

Method OC16201 PFAS IN WATER EXTRACTION LOG

Batch:		Method:	**OC16201**		
Start Date/Time:		End Date/Time:		Analyst:	

Extraction:

Internal Standard ID:		Lab Control Spike ID:	
Extractor ID(s):		SPE Column Lot:	
Syringe Filter Lot:		ENVI-Carb Column Lot:	

Other Sample Information:

QC Number on sample/field blank/lab blank bottles:			
15mL collection tube lots:			
Temp. of water bath:		SN of thermometer:	
TurboVap ID:			

Extraction Solvent/Reagent Information:

MeOH (Extraction):	VWR	Lot, Exp Date:	
Acetate Buffer:			
60mM NH_4OH in MeOH:			
Water Source:			**RO218A**
2mM Ammonium Acetate in H_2O:MeOH (95:5) (MP):			
MeOH (MP):	VWR	Lot, Exp Date:	

Weighing full/empty bottles:

Balance ID:		Date:		Analyst:	

Pipettes (circle used):

W13060E (1-10µL)	SD05386 (1-10µL)	S12416G (5-25µL)	SD06065 (5-25µL)	U12178B (10-100µL)
SG05360 (10-100µL)	S12062M (100-250µL)	SE05358 (50-250µL)	QJ05350 (100-1000µL)	QJ05352 (100-1000µL)
SA05211 (100-1000µL)	H24427J (100-1000µL)	H24527J (100-1000µL)	Q27823K (Repeater)	

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ID	Horizon ID	Extraction Position	MS	DUP	FRB	IS Amt. (µL)	Spike Amt. (µL)	Initial Weight (g)	Final Weight (g)	Samp. Vol. (mL)
MB			---	---	---					250
LCS			---	---	---					250
1.										
2.										
3.										
4.										
5.										
6.										
7.										
8.										
9.										
10.										
11.										
12.										
13.										
14.										

Additional Extraction Notes:

Uncontrolled

Appendix 2

PFAS GHS Labelling

Solution	GHS Pictograms
Methanol	
NH_4OH in Methanol	
Ammonium Acetate in H_2O with 5% Methanol	
5mM Ammonium Acetate Buffer	

Uncontrolled

Appendix 3

Analyst Certification Statement for Current Technical Methods

Analyst Name: _____

I, the undersigned, certify that I have read, understood, and agreed to perform the most recent version of the above test method.

_____ _____

Analyst Date

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