Determination of Table 3 *Plus* Compounds by LC/MS/MS

Chemours Fluoroproducts Analytical Method
Revision date 1/10/2019

Instrument Setup

(Note: Trapping column installed on Line A. Delay column installed between mixer and multisampler.)

Instrument:	Agilent 1290 Infinity II LC with 6470 Triple Quad MS
Analytical Column:	Agilent InfinityLab Poroshell 120 EC-C18 (2.1 x 50 mm, 2.7 µm); P/N: 699775-902
Guard Column:	Agilent InfinityLab Poroshell 120 EC-C18 (2.1 x 5 mm, 2.7 µm); P/N: 821725-911
Trapping Column:	Restek Ultra Aqueous C18 (4.6 x 50 mm, 5 µm); P/N: 9178555
Delay Column:	Agilent Zorbax Eclipse Plus C18 (4.6 x 50 mm, 3.5 µm); P/N: 959943-902

Consumables

(Note: Avoid using glass. All standards, samples, and stock solutions should be prepped and stored in HDPE or PP containers. Part numbers are provided below, but substitutions of similar products can be made.)

LC vials:	Microsolv, Cat. No. 9502S-PP-Clear
LC caps:	VWR, P/N: 82028-424
Scintillation vials:	VWR, P/N: 66021-692 *(used for sample and standard dilutions)*
HDPE bottles:	Thermo Scientific, P/N: 2104-0004 *(used for calibration stocks)*
Luer-Lock syringes:	Microsolv, P/N: 58903-S-K
0.2 um GHP filters:	Pall Acrodisc, P/N: 4554 *(or substitute with same dimensions and filter material)*

Sample Preparation

All standards and stocks are prepared with a 50:50 methanol:water diluent. A 10-point calibration curve is made from a 100 ppb mixed stock standard (containing the twenty-four Table 3 Plus analytes) at the following dilutions: 0.01 ppb, 0.05 ppb, 0.1 ppb, 0.25 ppb, 0.5 ppb, 1 ppb, 5 ppb, 10 ppb, 25 ppb, and 50 ppb. Prepped standards should be filtered in the same way as the samples.

Note: The 100 ppb mixed stock standard is prepared in ultrapure water. The stock standard should be stored in a refrigerator when not in use. After allowing the stock to warm to room temperature, an aliquot is diluted 2x with methanol to produce a 50 ppb mixed standard in a

50:50 methanol:water matrix; this stock is then used for spiking and to prepare subsequent dilutions for the calibration curve.

All samples are tested for pH when received to ensure that they are neutral (pH 6-8). If necessary, dilute solutions of KOH or H_2SO_4 are used to adjust pH accordingly. Samples are prepared in duplicate with duplicate spikes at different dilutions depending on the expected concentration of the analytes. Each sample prep starts with a 2x dilution in methanol to produce a 50:50 methanol:water matrix. Subsequent dilutions are made with a 50:50 methanol:water mix. A 2x dilution will be appropriate for most groundwater analyses (trace levels expected). If expected concentration is unknown, err on side of caution and use large dilution factor initially. If not detected, reprep with smaller dilution factor. Example preparations are listed below.

2x dilution and spike: 2.5 mL of sample is diluted with 2.5 mL of LC/MS grade methanol in a scintillation vial, then vortexed and filtered with a 0.2 um GHP syringe filter. To prepare spikes, 2.5 mL of sample is diluted with 2.4 mL of LC/MS grade methanol and 100 µL of 100 ppb spike solution in a scintillation vial, then vortexed and filtered with a 0.2 um GHP filter. Final expected concentration of spike in vial is 2.0 ppb.

100x dilution and spike: 2.5 mL of sample is diluted with 2.5 mL of LC/MS grade methanol in a scintillation vial, then vortexed to make a 2x prep. 100 µL of the 2x prep is diluted to 5.0 mL using 50:50 methanol:water, then vortexed and filtered with a 0.2 um GHP syringe filter. To prepare spikes, 100 µL of the 2x prep is diluted with 4.8 mL of 50:50 methanol:water and 100 µL of 100 ppb spike solution in a scintillation vial, then vortexed and filtered with a 0.2 um GHP filter. Final expected concentration of spike in vial is 2.0 ppb.

QC sample: A 5 ppb QC sample is prepared from a stock standard solution separate from the one used for calibration following the same dilution and filtering protocol as for samples.

Analytical Method

LC Operating Conditions:

Mobile phase A: 2 mM Ammonium Acetate in 5:95 Acetonitrile:Water

Mobile phase B: Acetonitrile

Needle/seat wash: 50:50 Acetonitrile:Water, Multi-wash option using 2 cycles of seat back flush and needle wash (10 seconds each)

Seal wash: 10:90 Isopropanol:Water

Injection volume: 5.00 µL

Flow rate: 0.500 mL/min

Maximum pressure: 600.00 bar

Solvent gradient:

	Time	A	B
Initial	0.00 min	85%	15%
1	1.00 min	85%	15%
2	5.00 min	10%	90%
3	6.40 min	10%	90%
4	6.50 min	85%	15%

End time: 9.50 mins

Column temp: 50.0°C

MS Operating Conditions:

Ion mode: ESI negative

Scan type: MRM

Gas temp: 150°C

Gas flow: 8 L/min

Nebulizer: 45 psi

Sheath gas heater: 200°C

Sheath gas flow: 8 L/min

Capillary: 3500 V

Nozzle voltage: 0 V

QQQ Acquisition Parameters:

Compound Name	MRM Transition	RT (mins)	Dwell (msec)	Frag (V)	CE (V)	Cell Accelerator (V)
DFSA	175.0 -> 131.0	0.268	50	72	12	2
MMF	139.0 -> 95.0	0.271	50	93	8	1
MTP	175.0 -> 97.0	0.370	50	78	12	1
PPF acid	163.0 -> 118.9	0.394	50	58	8	5
PFMOAA	179.0 -> 84.9	0.437	50	54	12	1
R-EVE	405.0 -> 217.0	0.420	50	100	16	4
Byproduct 4	440.9 -> 241.0	0.509	50	114	28	1
Byproduct 5	439.0 -> 343.0	0.510	50	126	30	1
PMPA	229.0 -> 184.9	0.662	50	55	4	5
PFO2HxA	245.0 -> 85.0	1.046	50	60	8	1
NVHOS	297.0 -> 135.0	1.655	50	123	28	1
PEPA	279.0 -> 234.9	1.702	50	58	4	4
PFECA B	295.0 -> 201.0	3.027	50	55	8	3
PFO3OA	310.9 -> 85.0	3.298	50	54	12	3
PES	314.9 -> 135.0	3.388	50	109	20	5
HFPO-DA	329.0 -> 284.9	3.386	50	75	0	4
HFPO-DA_qualifier	329.0 -> 169.0	3.386	50	75	12	1
PFECA_G	378.9 -> 184.9	3.702	50	87	16	4
PFO4DA	376.9 -> 85.0	3.794	50	66	20	3
Hydro-EVE Acid	427.0 -> 282.9	3.825	50	90	12	3
EVE Acid	407.0 -> 262.9	3.825	50	78	8	2
Byproduct 6	397.0 -> 217.0	3.951	50	122	27	1
Byproduct 2	463.0 -> 262.9	4.072	50	160	32	1
Byproduct 1	443.0 -> 146.9	4.084	50	143	30	1
PFO5DoA	442.9 -> 85.0	4.085	50	66	8	1

Data Processing

Calibration: The full set of 10 calibration standards should be run at the beginning and end of a sequence of samples. All 20 standard injections are used in constructing the calibration curve for quantitation of unknowns. Continuing calibration checks should be performed after every 10 sample injections in the sequence using a mid-level standard; these are not included as points in the calibration curve. The curve should be linear, not forced through the origin, and weighted 1/x. The acceptable accuracy range is 70%-130%.

Blanks: Three blanks should be run at the beginning of each sequence—a methanol blank taken from the vessel used to perform 2x dilutions of aqueous stock standard and samples; a 50:50 methanol:water blank from the vessel used to perform subsequent dilutions; and an environmental blank consisting of ultrapure water taken from the same type of container used for sampling. Target analyte concentrations in all blanks must be less than one-half of the method LOQ.

Method and/or solvent blanks are also used to monitor for carryover between samples, particularly those with analyte levels detected at high concentrations. A typical sequence consists of duplicate preps followed by their respective spikes, then a 50:50 methanol:water blank, and repeating with subsequent sample prep/spike segments. Blanks in between sample segments should be analyzed to quickly identify carryover contamination and are subject to the same concentration criteria as described in the paragraph above.

Duplicates: Duplicate preps of the same sample must have a %RPD of ≤25% at mid- and high-range concentrations and ≤50% at the minimum reporting limit (see equation below).

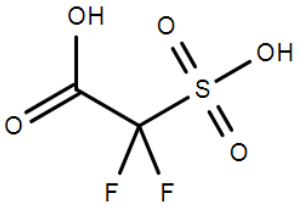
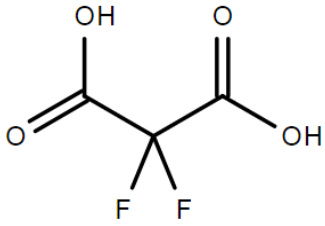
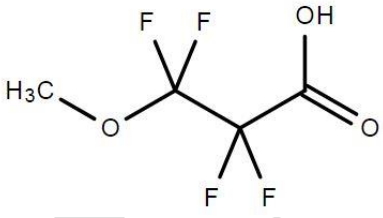
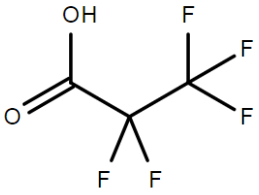
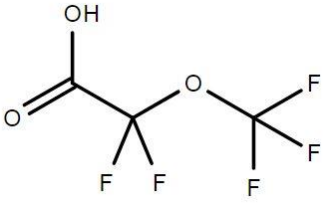
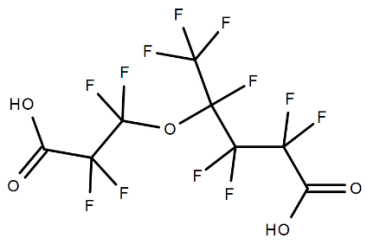
$$\%RPD = \frac{(\text{sample result} - \text{duplicate result}) * 100}{(\text{sample result} + \text{duplicate result})/2}$$

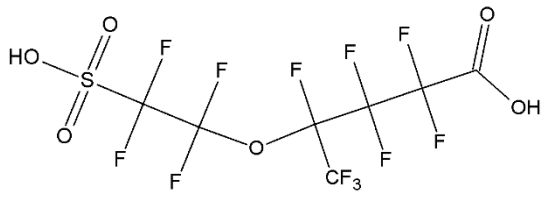
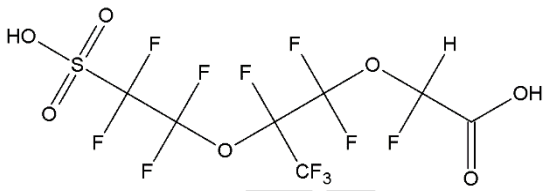
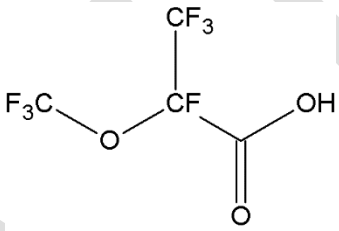
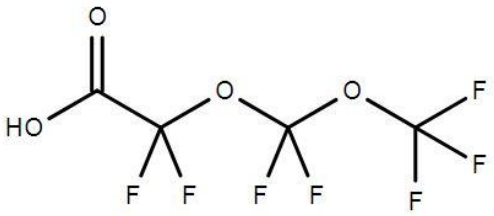
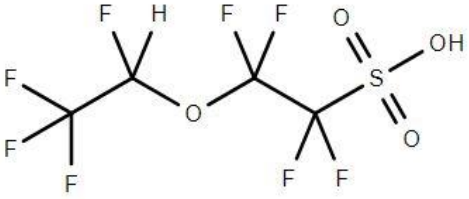
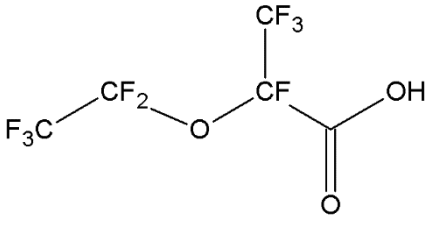
Results outside this range require a duplicate reprep. Both results should be reported, along with the average.

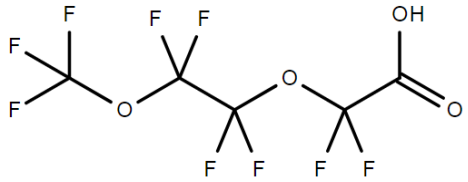
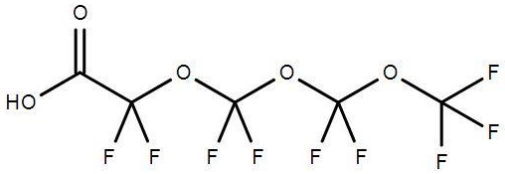
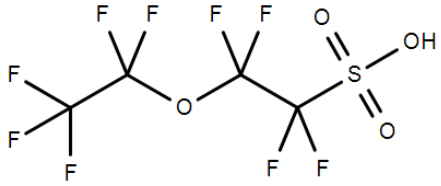
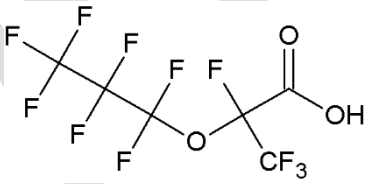
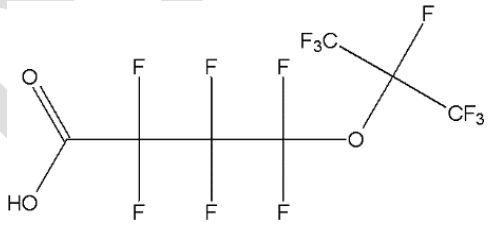
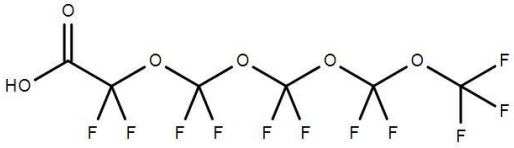
Spikes: Acceptable spike recovery is in the range of 70%-130%. Spike recovery outside this range does not necessitate reprep, but results should be flagged for further investigation due to possible matrix effects.

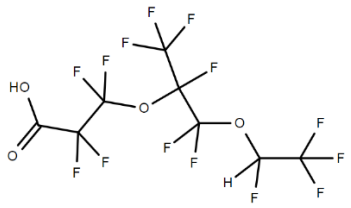
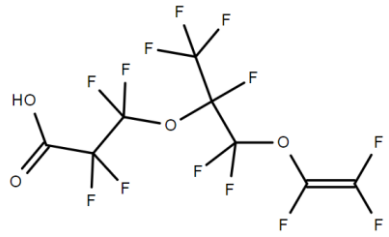
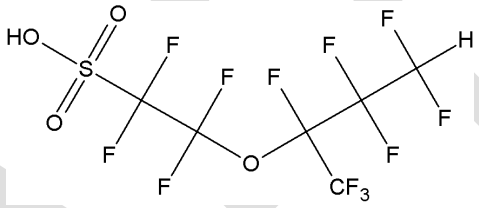
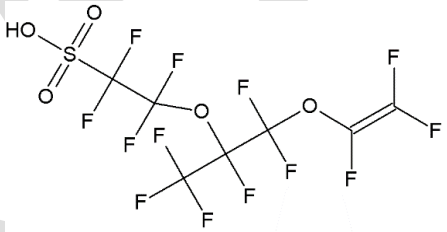
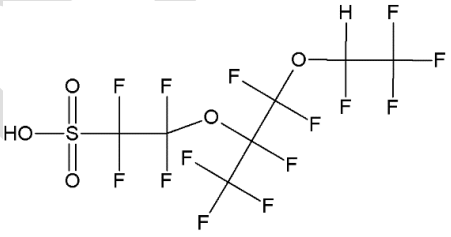
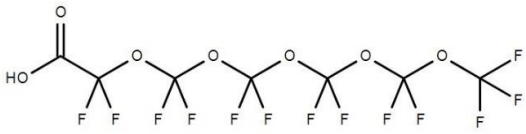
QC Sample: A QC sample of known concentration should be prepped from a standard stock solution separate from the one used for the calibration. Results must be within 70%-130% of the true value.

Appendix A: Compound Details

Compound Name	Molecular Formula	Structure	CASRN
DFSA	$C_2H_2F_2O_5S$		422-67-3
MMF	$C_3H_2F_2O_4$		1514-85-8
MTP	$C_4H_4F_4O_3$		93449-21-9
PPF Acid	$C_3HF_5O_2$		422-64-0
PFMOAA	$C_3HF_5O_3$		674-13-5
R-EVE	$C_8H_2F_{12}O_5$		N/A

Byproduct 4	$C_7H_2F_{12}O_6S$		N/A
Byproduct 5	$C_7H_3F_{11}O_7S$		N/A
PMPA	$C_4HF_7O_3$		13140-29-9
PFO2HxA	$C_4HF_7O_4$		39492-88-1
NVHOS	$C_4H_2F_8O_4S$		1132933-86-8
PEPA	$C_5HF_9O_3$		267239-61-2

PFECA B	$C_5HF_9O_4$		151772-58-6
PFO3OA	$C_5HF_9O_5$		39492-89-2
PES	$C_4HF_9O_4S$		113507-82-7
HFPO-DA	$C_6HF_{11}O_3$		13252-13-6
PFECA G	$C_7HF_{13}O_3$		801212-59-9
PFO4DA	$C_6HF_{11}O_6$		39492-90-5

Hydro-EVE Acid	$C_8H_2F_{14}O_4$		773804-62-9
EVE Acid	$C_8HF_{13}O_4$		69087-46-3
Byproduct 6	$C_6H_2F_{12}O_4S$		N/A
Byproduct 1	$C_7HF_{13}O_5S$		29311-67-9
Byproduct 2	$C_7H_2F_{14}O_5S$		749836-20-2
PFO5DoA	$C_7HF_{13}O_7$		39492-91-6

Appendix B: Recommendations for Instrument Maintenance

Before starting a sequence, run an isopropanol blank and 3 method blanks (50:50 methanol:water) to flush the system and establish a "clean" baseline. At the end of a sequence, run a "steam clean" line and end with an "idle" line (see screenshot below).

		Sample Name	Sample Position	Dilution	Method	Data File	Sample Type
100	✔	50 ppb Table 3 std_2	P6-B8	1	Table3Compounds\Table3Cmpds.m	50 ppb Table 3 std_2.d	Calibration
101	✔	Blank (50-50)_23	Vial 5	1	Table3Compounds\Table3Cmpds.m	Blank (50-50)_23.d	DoubleBlank
102	✔	Steam	No Injection	1	Steam Clean.m	Steam.d	Blank
103	✔	Idle	No Injection	1	Idle Method.m	Idle.d	Blank

Both of these methods should be set up to utilize lines A2 and B2, which are 95:5 water:methanol and 100% methanol, respectively. This will help to flush out any salts or other buildup that has accumulated in the system. It is recommended to run the idle method while the system is not actively being used.

After the idle method has completed and while the source temperatures are still low, open the spray chamber and wipe down all surfaces – especially the spray shield and surrounding area – with a 50:50 mixture of isopropanol and ultrapure water on a Kimwipe. This should be done daily to prevent buildup.

A 50:50 acetonitrile:water mixture works well as a needle/seat rinse to minimize carryover. A solution of 90:10 water:isopropanol should be used as the seal wash solvent, which should be primed if bubbles are observed in the clear tubing between pump heads.

Solvent reservoirs should be periodically rinsed with isopropanol before rinsing and refilling with the intended solvent. If possible, store solvents in amber bottles to inhibit microbial growth, and prepare fresh solvents if not used within 1-2 weeks.