

Targeted analysis of PFAS (Per- and Polyfluoroalkyl Substances) compounds in water with direct large volume injection

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Direct injection analysis of PFAS

Purpose: Thermo Fisher Scientific has developed an LC-MS/MS method for the quantitation and confirmation of 52 PFAS compounds in a single run, with Limit of Quantitation (LoQ) at 0.2 ng/L by direct injection in both drinking and non-drinking water matrices.

Methods: A direct aqueous injection method was designed for drinking, surface, and ground water samples using Thermo Scientific™ Vanquish™ Core UHPLC system coupled to a Thermo Scientific™ TSQ Altis Plus™ triple quadrupole mass spectrometer.

Results: In general, large-volume injection methods are less common for PFASs compared to offline extraction methods, therefore we report the accuracy, repeatability, and recovery of several months of continuous water sample analysis to demonstrate the robustness and accuracy. The method can meet the regulatory requirements, both in terms of PFAS monitored and LoQs, for all EU regulations.

Introduction

Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS) are a large group of synthetic compounds containing chains of linked carbon and fluorine atoms. Exposure to PFAS has been linked with a variety of health effects, including altered immune and thyroid function, liver disease, lipid and insulin dysregulation, kidney disease, adverse reproductive and developmental outcomes, and cancer¹. European Parliament and Council of the European Union released a new directive that sets the limit of PFAS in drinking water to 0.5 µg/L for all PFAS compounds identified, and 0.1 µg/L for a subset of PFAS compounds that are particularly concerning for humans². Due to rapidly evolving regulatory initiatives across various regions and countries, the number of PFAS compounds that will require monitoring is expected to increase and level to be detected in certain regulation are sub ng/L.

Materials and methods

Sample preparation

The PFAS standards and internal standards were obtained by Wellington Laboratories and were prepared in UHPLC-MS methanol. The mixed stock standard solution had a concentration of 10 ng/L and stored in PFAS free polypropylene (PP) containers at 4° C. Prior to the use or analysis, the solutions were brought to room temperature and shaken thoroughly. The stock standard was further diluted to end with a 50:50 (vol:vol) water/methanol with 0.1% acetic acid to obtain a 10-point curve ranging from 0.25 – 100 ng/L.

Drinking, surface, and ground water samples were obtained from various sources in the United States and The Netherlands and were prepared as per the standard.

LC conditions

PFAS are a large set of components including both long chain and short chain analytes. To run all the 54 components in a single run, the Vanquish HPLC has been configured as describe in figure 1 with a dedicated plumbing for the analysis of PFAS. Moreover, User Define Program of rack shaking has been implemented in the software to improve the solubility of long chain PFAS (Figure 2):

- PFAS Delay Column: 2.1 x 50 mm, 2.6 µm Thermo Scientific™ Accucore™ C18 column
- Analytical Column: 2.1 x 100 mm, 2.2 µm Thermo Scientific™ Acclaim™ RSLC C18 Polar Advantage column
- Column Temp: 40° C
- Mobile Phase A: H₂O with 2% MeOH + 2 mM Am. Acetate + 0.1% HOAc
- Mobile Phase B: MeOH with 2% H₂O + 2 mM Am. Acetate + 0.1% HOAc
- Injection Volume: 500 µL
- Sample Temp: 25° C
- User Defined Program

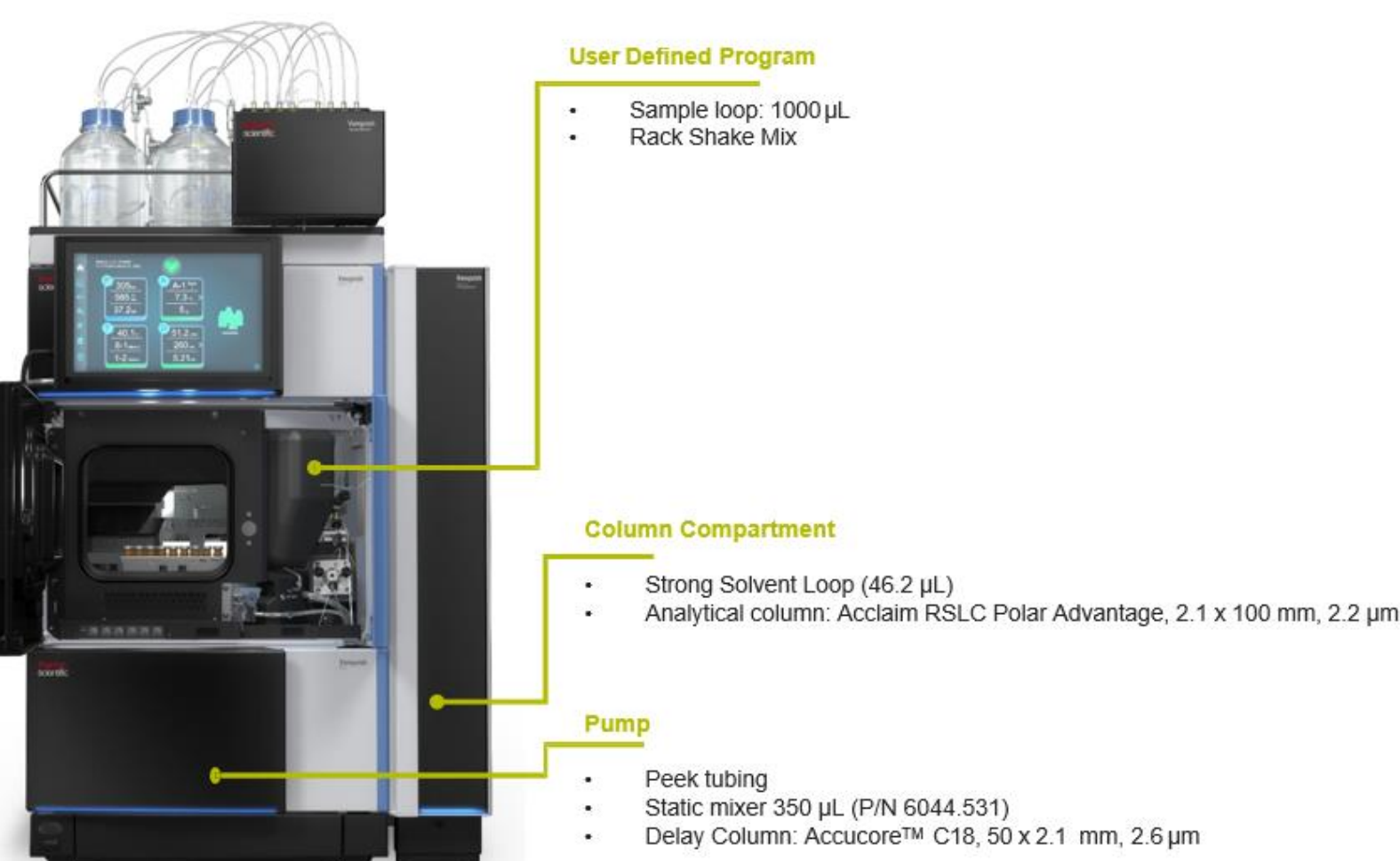


Figure 1: The Vanquish Core UHPLC system shown here modified for large volume injections of PFAS

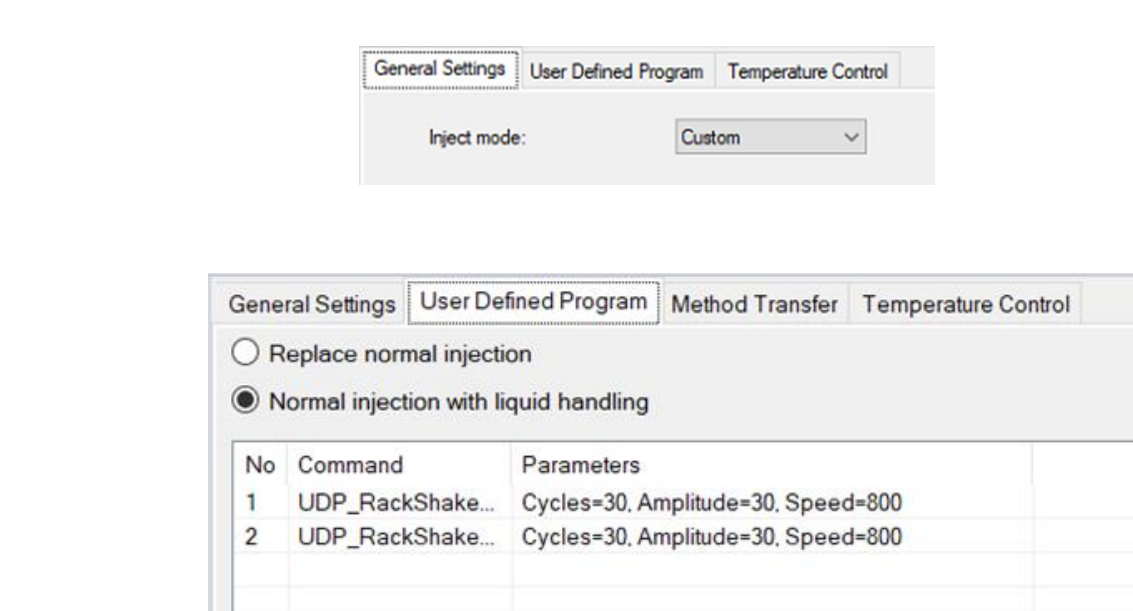


Figure 2: User Define Program automated implemented in the software for rack shaking.

MS conditions

TSQ Altis Plus triple quadrupole mass spectrometer equipped with a HESI ionization probe. Table 1. describe the optimized MS parameters used to cover all 54 component in a single run.

Table 1. Describe MS settings

HESI source:	Negative ionization mode	Cycle time for the negative SRM transitions:	0.5 s
Spray voltage:	500 V	Q1 resolution:	0.7 Da
Sheath gas:	40 arb	Q3 resolution:	1.2 Da
Auxiliary gas:	10 arb	CID gas:	2.5 mTorr
Son Transfer tube Temp:	200° C		
Vaporizer temperature:	300° C		

Data analysis

Thermo Scientific™ Chromeleon™ Chromatography Data System software, version 7.3.2 was used for data acquisition and processing.

Results

Calibration curves from 0.025 ng/L up to 100 ng/L were prepared in drinking water as describe in the previous section to obtain 9 calibration levels.

The Validation of the method was performed by injecting each calibration standard 3 times to assess reproducibility and robustness of the method.

As described in the material and method, rack shaking was implemented. The use of this automated shaking is crucial to maintain in solution long chain PFAS.

Long chain PFAS are not soluble in water and tend to stick to the interface between water and methanol. For this reason, if the solution of 50/50 water methanol is not shaken, we observe peak area decrease over the time. With rack shaking and room temperature the area of the long chain PFAS is nearly back to the level of the fresh solution. In Figure 3 experiments with and without rack shaking and the influence of rack temperature are described. Peak area decrease over the time without rack shaking as describe in figure 3.

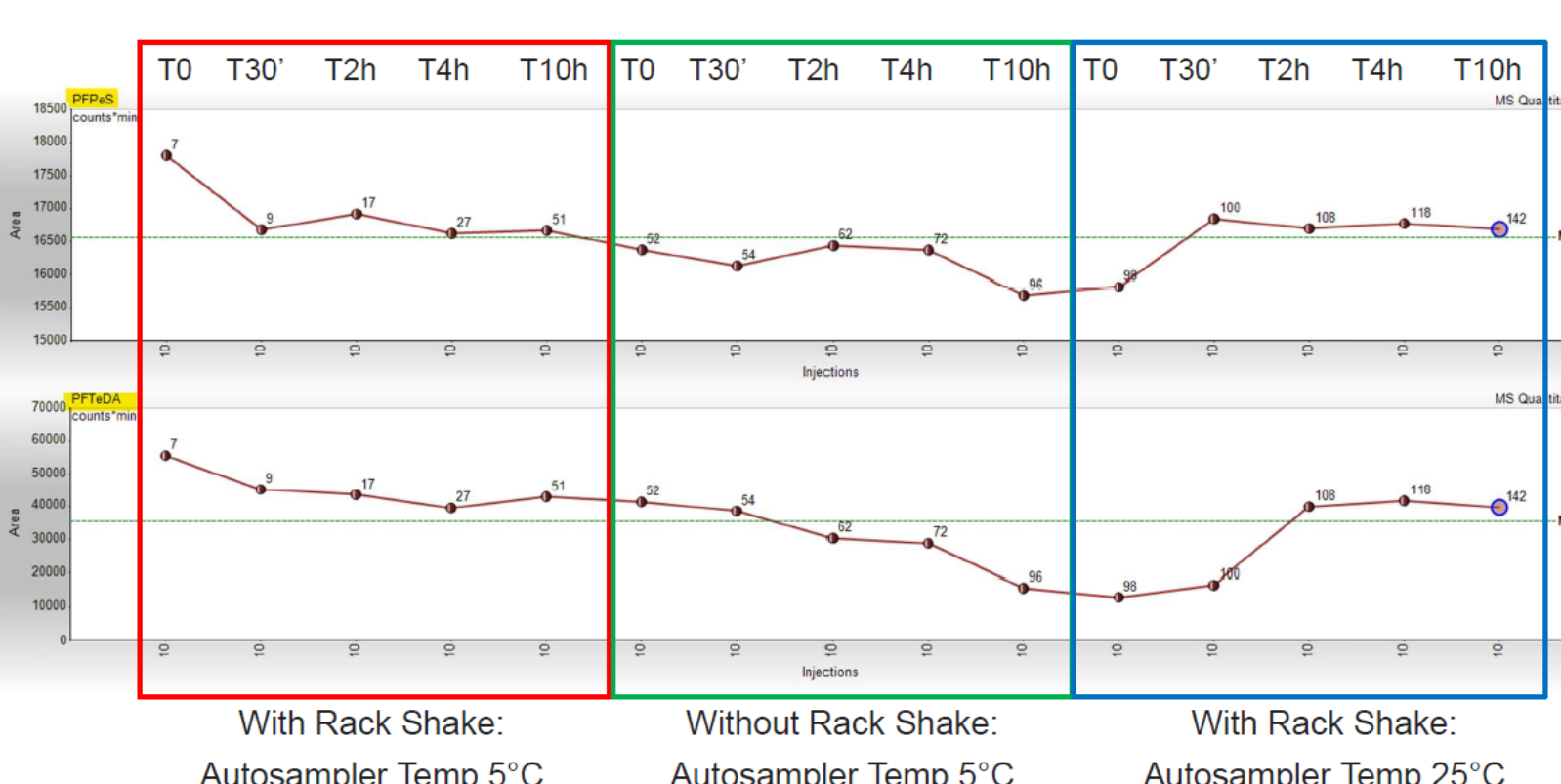


Figure 3 Influence of rack shaking and temperature on long chain PFAS

To have ultimate sensitivity and to comply with all regulation in EU an injection volume of 500 µL was used. Taking advantage of the easy plumbing and the adjustment of gradient delay volume of the Vanquish platform we were able to achieve good peak shape for all components (Figure 4).

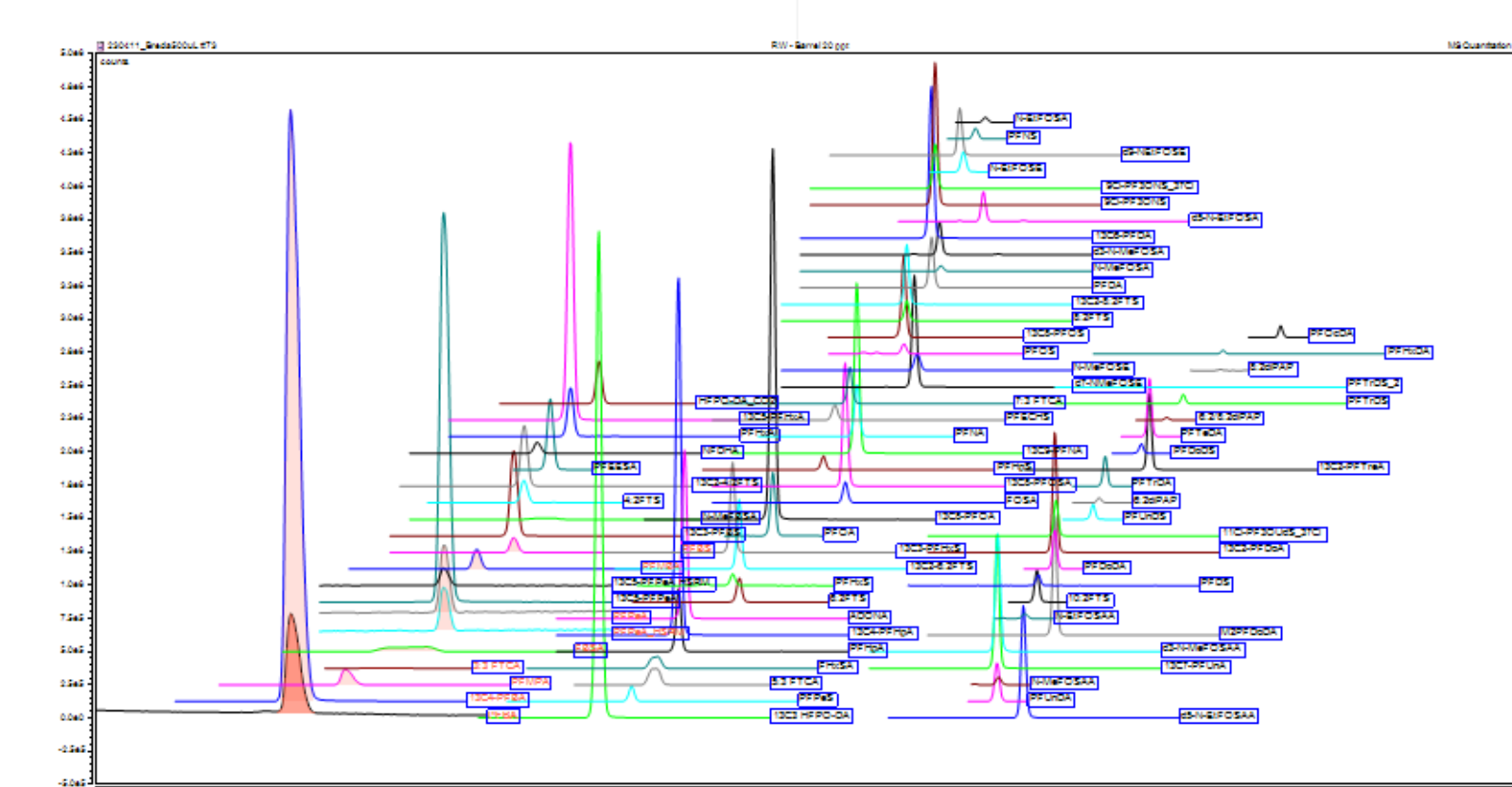


Figure 4 Chromatogram of 500µL injection of 20 ppt spiked rainwater showing excellent peak shape for early eluting PFAS

Recently European country such as Netherland and Belgium have set their limit of detection in various water types < 0.2ng/L³. To rich those levels SPE is suggested in the regulation.

As SPE, online and offline is expensive and time consuming, users are requesting direct injection approach with high throughput to be more effective in delivering result and overcome contamination.

With the configuration and injection volume describe we were able to rich the requested LOD for the majority of the components as describe in Table 2.

For the purposes of this poster, LoQ is defined as both %RSD (precision) and average %bias (accuracy) based on at least 5 replicates, < 20%.

Table 2 report Calibration range, linearity LoD (defined as peak area grater then 3xblank response), LoQ and MDL.

To assess the applicability of the method to various water matrix such as rainwater, barrel, tap and puddle were collected and recovery of PFAS was calculated. Recoveries are nearly all in the 70-130 % range. The exceptions, where recovery is higher than 130%, are due to high levels of PFAS in the blank matrix Table 3. To show the precision of the method replicate injection of the 5 ng/L standard in various bland of drinking water were injected along the validation sequence. 5 replicate of 5ng/L were taken in account.

In most of the cases, as shown in Table 4 RSD calculated on the measured amount is <10%. When grater than 10% we have to mention the native component did not have appropriate isotope analogue to be added to the method.

Table 2. Calibration Range, Linearity, LoD, LoQ and MDL for all components using 500µL injection. 6:2 FTS had significant contamination 10:2 FTS used incorrect retention time windows and contamination

Compound Name	Calibration range ppt	Linear correlation (R2)	LOD ppt	LOQ ppt	MDL ppt
PFBA	0.2 - 400	0.995	0.2	1	0.26
PFPeA	0.1 - 200	0.997	0.1	0.2	0.08
PFHxA	0.05 - 100	0.997	0.05	0.1	0.04
PFHpA	0.05 - 100	0.998	0.05	0.1	0.04
PFOA	0.05 - 100	0.997	0.05	0.05	0.03
PFNA	0.025 - 100	0.997	0.05	0.1	0.04
PFDA	0.025 - 100	0.997	0.05	0.05	0.05
PFUnDA	0.025 - 100	0.996	0.025	0.05	0.05
PFDoDA	0.025 - 100	0.997	0.025	0.05	0.08
PFTtDA	0.025 - 100	0.996	0.025	0.05	0.07
PFTeDA	0.025 - 100	0.992	0.05	0.1	0.04
PFHxDA	0.025 - 100	0.99	0.05	0.1	0.01
PFOcDA	0.025 - 100	0.989	0.1	0.25	0.25
PFBS	0.022 - 88.7	0.996	0.022	0.044	0.05
PFPeS	0.024 - 94.1	0.997	0.047	0.094	0.06
PFHxS	0.025 - 100	0.996	0.025	0.1	0.08
PFHpS	0.024 - 95.3	0.996	0.048	0.095	0.06
PFOS	0.025 - 100	0.997	0.025	0.05	0.07
PFNS	0.024 - 96.2	0.997	0.024	0.048	0.11
PFDS	0.025 - 100	0.998	0.05	0.1	0.04
PFUnDS	0.024 - 96.8	0.997	0.097	0.097	0.06
PFDoDS	0.025 - 100	0.997	0.05	0.1	0.09
PFTtDS	0.05 - 100	0.997	0.025	0.05	0.1
PFECHS	0.025 - 100	0.995	0.05	0.1	0.09
11Cl-PF3OUdS	NF - NF_Max	0.996	0.025	0.1	0.04
9Cl-PF3ONS	0.025 - 100	0.997	0.025	0.05	0.06
FBSA	0.025 - 100	0.996	0.025	0.1	0.04
FhKSA	0.025 - 100	0.996	0.025	0.05	0.05
FOSA	0.025 - 100	0.995	0.025	0.05	0.03
N-MeFBSA	0.1 - 100	0.997	0.1	0.5	0.36
N-EtFOSA	0.025 - 100	0.995	0.05	0.25	0.21
N-MeFOSA	0.025 - 100	0.997	0.025	0.1	0.08
N-MeFOSE	1 - 1000	0.996	1	1	0.78
N-EtFOSE	0.25 - 1000	0.995	1	2.5	1.28
N-MeFOSAA	0.025 - 100	0.997	0.025	0.05	0.07
N-EtFOSAA	0.025 - 100	0.996	0.05	0.05	0.08
3:3 FTCA	2.5 - 100	0.997	2.5	2.5	1.54
5:3 FTCA	0.025 - 100	0.997	0.025	0.1	0.08
7:3 FTCA	0.025 - 100	0.995	0.025	0.05	0.08
NFDHA	0.025 - 100	0.997	0.025	0.05	0.07
ADONA	0.025 - 100	0.997	0.025	0.1	0.03
PFMPA	0.05 - 100	0.996	0.05	0.05	0.06
PFMBA	0.025 - 100	0.996	0.025	0.05	0.03
PFEESA	0.025 - 100	0.999	0.025	0.025	0.06
HFPO-DA	0.025 - 100	0.997	0.025	0.05	0.05
4:2 FTS	0.094 - 375	0.998	0.094	0.094	0.15
6:2 FTS		Contamination			
8:2 FTS	0.096 - 192	0.999	0.096	0.096	0.15
10:2 FTS		Outside window			
6:2diPAP	0.025 - 100	0.992	0.025	0.05	0.03
6:2/8:2diPAP	0.025 - 100	0.991	0.1	0.5	0.17
8:2diPAP	0.05 - 100	0.998	0.1	1	17.5

Table 3. % recovery of PFAS drinking and surface waters using 500µL injection volume

Compound Name	Recovery (%)				
	Rain Water	Barrel	Puddle	Tap	Guadalupe
PFBA	93.35	90.7	88.85	81.31	102.88
PFPeA	88.09	95.13	91.62	94.43	88.49
PFHxA	89.32	94.39	90.4	91.67	82.52
PFHpA	88.37	93.61	92.23	93.78	102.66
PFOA	89.54	94.47	95.61	94.99	104.4
PFNA	86.54	92.78	94.58	91.54	104.16
PFDA	85.62	91.14	93.28	90.98	104.17
PFUnDA	87.51	94.35	145.04	91.59	104.1
PFDoDA	86.45	90.25	191.82	90.41	103.52
PFTtDA	92.32	92.96	114.1	95.1	104.96
PFTeDA	83.9	95.19	122.62	95.78	109.31
PFHxDA	128.2	156.91	151.5	145.88	118.69
PFOcDA	131.05	146.99	108.09	93.47	79.55
PFBS	86.11	91.31	85.17	92.25	87.36
PFPeS	90.75	96.49	91.56	93.52	84.44
PFHxS	85.44	90.76	86.6	90.24	104.56
PFHpS	90.28	97.41	93.86	91.15	91.76
PFOS	88.17	91.93	89.09	92.12	102.97
PFNS	84.57	88.81	91.55	91.81	93.96
PFDS	84.24	92.01	92.86	94.24	98.32
PFUnDS	89.63	97.4	95.53	97.55	97.96
PFDoDS	75.1	84.38	83.9	86.15	92.2
PFTtDS	78.05	85.76	90.49	89.73	89.69
PFECHS	92.51	99.13	97.03	92.94	94.01
11Cl-PF3OUdS	88.96	93.69	95.14	95.6	104.38
9Cl-PF3ONS	90.03	93.46	87.54	94.59	94.79
FBSA	85.77	94.42	88.38	89.93	190.03
FhKSA	80.26	94.63	94.53	86.24	85.55
FOSA	84.34	89.64	83.2	91.39	101.83
N-MeFBSA	82.28	89.33	83.06	97.34	117.49
N-EtFOSA	83.58	86.64	82.12	87.65	102.6
N-MeFOSA	83.67	88.28	79.92	88.52	96.52
N-MeFOSE	83.6	88.49	83.69	87.08	100.87
N-EtFOSE	88.07	92.36	85.11	89.78	104.97
N-MeFOSAA	85.08	89.78	86.14	88.46	100.99
N-EtFOSAA	84.14	93.03	87.1	89.67	99.85
3:3 FTCA	81.76	94.7	89.38	90.6	227.81
5:3 FTCA	85.11	93.52	86.44	92	97.02
7:3 FTCA	87.74	90.47	83.18	92.37	106.06
NFDHA	75.32	82.12	76.17	87.04	125.44
ADONA	91.15	96.52	91.91	96.44	101.07
PFMPA	84.86	91.9	87.41	92.04	86.57
PFMBA	79.8	84.62	79.98	86.94	112.52
PFEESA	94.69	100.2	94.89	95.47	93.63
HFPO-DA	86.39	91.58	87.18	91.79	99.52
4:2 FTS	91.03	92.84	93.81	95.25	104.6
6:2 FTS	464.01	83.39	515.83	131.71	167.35
8:2 FTS	86.4	91.85	89.57	91.76	104.19
10:2 FTS	86.62	92.31	94.01	89.62	99.23
6:2diPAP	89.97	105.49	116.09	101.69	108.36
6:2/8:2diPAP	76.61	101.59	111.14	88.29	81.64
8:2diPAP	82.4	115.03	174.75	95.94	63.67

Table 4. %RSD for 5 ppt spike for all PFAS in a variety of drinking and mineral waters. 6:2 FTS has significant contamination

Compound Name	Evian %	Solan %	Barle Duc %	Spa %
PFBA	16.36	9.16	8.54	12.8
PFPeA	1.01	1.35	3.77	2.38
PFHxA	1.85	1.32	5.87	2.91
PFHpA	2.06	3.56	4.18	2.98
PFOA	1.8	1.8	2.97	3.05
PFNA	1.83	1.37	3.11	3.77
PFDA	1.37	0.94	4.05	2.28
PFUnDA	2	1.16	3.47	3.17
PFDoDA	3.34	2.21	4.26	3.21
PFTtDA	4.46	1.95	4.45	2.33
PFTeDA	6.3	1.84	5.87	5.77
PFHxDA	6.58	5.25	11.83	24.13
PFOcDA	15.6	19.2	8.93	38.41
PFBS	5.75	3.12	5.28	2.16
PFPeS	4.19	4.28	5.77	1.8
PFHxS	4.23	5.6	4.86	2.95
PFHpS	5.29	5.06	5.13	3.03
PFOS	5.82	2.29	5.52	6.55
PFNS	6.41	1.48		