

Applikationsbericht

## A Large Volume Injection Technique Using Simplified Sample Preparation for Perfluorinated Alkyl Substance (PFAS) in Soils in Accordance with ASTM 7968

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## Abstract

In this application note we describe the use of the ASTM 7968 method applied to the Waters Xevo TQ-XS Triple Quadrupole Mass Spectrometer for the sensitive and reliable analysis of PFAS in soil. This method utilizes a limited sample preparation technique for increased throughput of soil samples, along with a large volume injection onto a highly sensitive LC-MS/MS system to achieve the sensitivity required for this analysis.

## Benefits

- A total solution for detection and quantitation of legacy and emerging PFAS in soils following the ASTM 7968 method
- This solution can increase sample throughput due to minimal sample preparation while meeting routine requirements for the sensitive analysis of PFAS in the low ng/kg (ppt) range

- Results include time and resource savings

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## Introduction

Perfluoroalkyl substances (PFAS) are ubiquitous environmental pollutants garnering widespread attention around the globe. They are most commonly found in water and soils but are contaminants of concern in many sample types. PFAS enter the environment from a variety of sources, including industrial waste discharge, use and disposal of consumer goods containing PFAS and PFAS precursors, and use of firefighting foams. Their chemical properties make them highly resistant to degradation and extremely bioaccumulative. Risks to human health have been associated with exposure to PFAS and their impact is being closely monitored by organizations globally.

Human exposure to PFAS can occur in many ways, including ingestion through contaminated drinking water and food grown in the presence of contaminated soil and water. Therefore, emphasis has been put on understanding and monitoring the exposure levels contributed by these sources. Remediation and source apportionment efforts also rely on robust solutions for determining environmental PFAS levels in water and soils.

Solutions for analysis of PFAS in various types of water samples have been proposed in previous application notes.<sup>1,2,3</sup> In this application note we describe the use of the ASTM 7968 method applied to the Waters Xevo TQ-XS Triple Quadrupole Mass Spectrometer for the sensitive and reliable analysis of PFAS in soil. This method utilizes a limited sample preparation technique for increased throughput of soil samples, along with a large volume injection onto a highly sensitive LC-MS/MS system to achieve the sensitivity required for this analysis.

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## Experimental

Due to the widespread use of PFAS in manufactured products, special care must be taken when analyzing samples for PFAS. Challenges in sample collection, preparation, and analysis all must be addressed due to required detection limits being in the ng/kg (ppt) range. Caution should be taken to avoid any Teflon or PTFE containing materials, as well as any clothing or items designed to be waterproof. High-density polypropylene (HDPE) containers and vials should be used from collection through analysis. Personal care products (i.e.,

lotions, makeup, etc.) should not be used before contacting samples. As practical, it is recommended that any laboratory supplies be checked for PFAS contamination before use.

Contamination is unavoidable from the chromatographic system as there are vital components of the LC that are manufactured using or in the presence of PFAS containing materials. System PFAS contamination cannot be completely removed, but steps can be taken to reduce and chromatographically delay contamination. The Waters PFAS Analysis Kit (p/n: 176004548) must be installed on the UPLC system prior to use for PFAS analysis. The kit is comprised of PFAS-free components (such as PEEK tubing to replace the conventional Teflon-coated solvent lines) and an isolator column that helps to delay any residual background interferences from co-eluting with the analytical peak. Installation of the PFAS Analysis Kit is straightforward and quick.<sup>4</sup>

Soil samples were provided by U.S. EPA Region 5. Samples included sand, silt, lean clay, and fat clay. All samples were spiked by the EPA with a selection of 24 PFAS compounds at varying concentrations prior to being received in the lab. Samples were extracted and analyzed blind without knowing the pre-spiked concentrations.

Two grams of each sample were received and were prepared in accordance with the ASTM 7968 method.<sup>5</sup> Ten milliliters (10 mL) of 1:1 water:methanol was added to each sample. Sample pH was adjusted to 9–10 using 20  $\mu$ L of ammonium hydroxide. Samples were shaken using a mechanical shaker for one hour, followed by centrifugation at 1900 rpm for 10 min. The entire supernatant was filtered using a disposable polypropylene syringe with a 25-mm dual glass fiber and GHP membrane filter (p/n: WAT200802). Following filtration, 50  $\mu$ L of acetic acid was added to each sample. An aliquot of each sample was transferred to a polypropylene autosampler vial and sealed with a polyethylene cap (p/n: 186005230).

Following extraction, a selection of seven additional emerging PFAS (GenX, ADONA, 9Cl-PF3ONS, 11Cl-PF3OUdS, PFMBBA, PFEESA, and NFDHA) were spiked into the samples at known concentrations to evaluate the incorporation of these emerging PFAS into the method.

## Method Conditions

LC system:	ACQUITY UPLC I-Class PLUS fitted with PFAS kit (p/n: 176004548)
Column:	ACQUITY UPLC CSH Phenyl Hexyl 2.1 $\times$ 100 mm, 1.7 $\mu$ m (p/n: 186005407)

Column temp.:	35 °C
Sample temp.:	10 °C
Injection volume:	30 µL
Mobile phase A:	95:5 Water:Methanol + 2 mM ammonium acetate
Mobile phase B:	Methanol + 2 mM ammonium acetate

## Gradient

Time (min)	Flow rate (mL/min)	%A	%B
0	0.3	100	0
1	0.3	80	20
6	0.3	55	45
13	0.3	20	80
14	0.4	5	95
17	0.4	5	95
18	0.3	100	0
22	0.3	100	0

## MS Conditions

MS system:	Xevo TQ-XS
Ionization mode:	ESI-
Capillary voltage:	0.5 kV

Desolvation temp.:	350 °C
Desolvation gas flow:	1100 L/hr
Cone gas flow:	150 L/hr
Source temp.:	100 °C

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## Results and Discussion

The ASTM 7968 method requires that a series of controls be extracted and analyzed with each batch of samples, including reagent blanks, method blanks, lower limit of quantitation (LLOQ) checks, and lab control samples (LCS). A description of each control as well as the criteria to pass is outlined in Figure 1. The soil samples provided were prepped in three batches and controls for each batch passed all criteria.

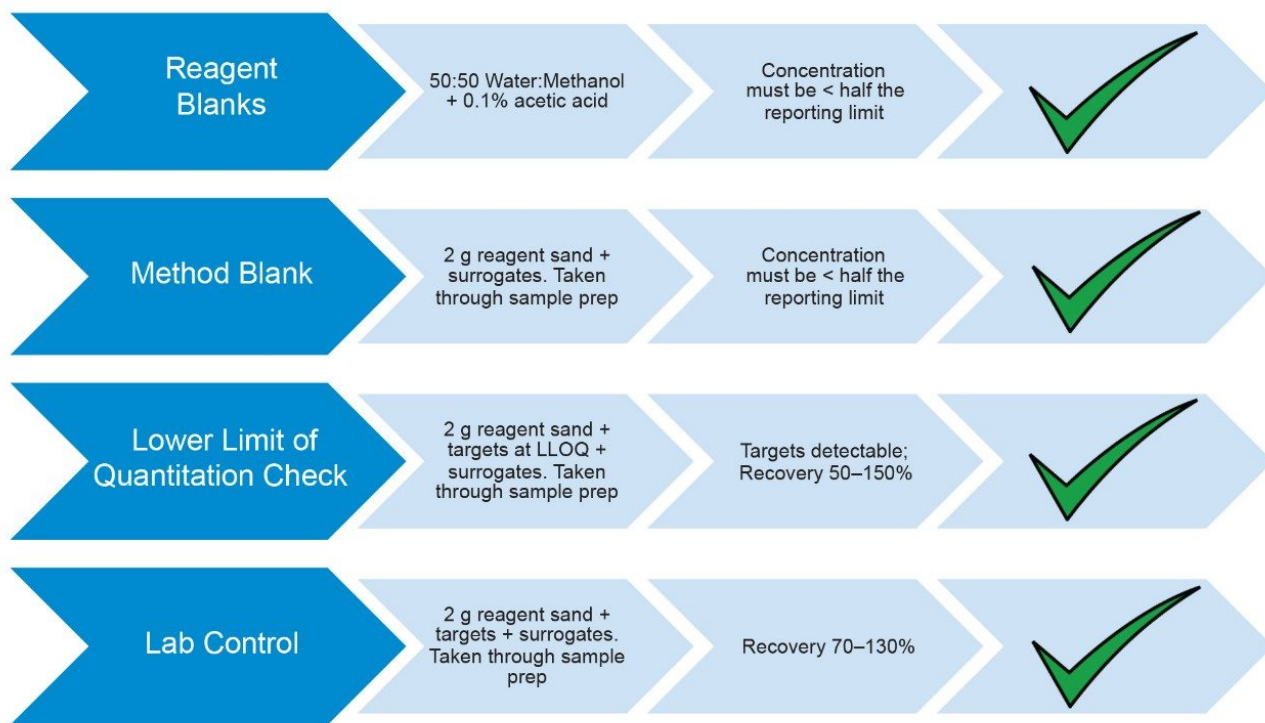


Figure 1. Required control samples included with the preparation and analysis of every batch of samples.

The lower limit of quantitation (LLOQ) for this study was determined using the calibration range analyzed with the samples, which was derived from the suggested calibration range in the ASTM 7968 method. ASTM 7968 requires the LLOQ to be the lowest point in the calibration curve. Therefore, the lowest point of the curve (5 ng/L or 25 ng/kg) was the LLOQ determined for most PFAS in this particular evaluation, with a few having slightly higher LLOQs. However, this does not reflect the sensitivity or detection limit for this method using the Xevo TQ-XS. The calculated detection limits (Table 1) are generally 5–10 times lower than the prescribed calibration range for this method. This could allow for modifications to the method (i.e., further dilution of samples, lower injection volume, and smaller sample mass) for further improvements in method ruggedness. As part of the controls, the LLOQ levels were evaluated in extracted reagent sand with each sample batch. The recoveries for the LLOQ checks (Table 1) were all within a range of 77–135%, easily meeting the allowable range for LLOQ samples of 50–150%. Table 1 also highlights the reporting range as described in ASTM 7968, demonstrating all compounds analyzed using the Xevo TQ-XS were within the requirements. Finally, Table 1 also highlights the linearity of all compounds analyzed with all having  $R^2$  values  $>0.995$ , except 6:2 FTS with an  $R^2$  value of 0.994. A selection of

calibration curves is shown in Figure 2 along with the quantitative and qualitative MRM chromatograms at the LLOQ for the compounds shown.



Compound	LLOQ in vial (ng/L)	LLOQ in soil (ng/kg)	Limit of detection (ng/L)	Reporting range* (ng/kg)	Recovery of LLOQ (ng/L)	R <sup>2</sup>
PFBA	25	125	10.0	125-1000	100.0	0.999
PFPeA	5	25	0.8	125-1000	98.9	0.999
PFHxA	5	25	0.8	25-1000	85.6	0.999
PFHpA	5	25	0.8	25-1000	93.7	0.999
PFOA	5	25	0.6	25-1000	99.2	0.999
PFNA	5	25	0.2	25-1000	95.1	0.998
PFDA	5	25	1.3	25-1000	99.3	0.998
PFUnDA	5	25	0.2	25-1000	91.2	0.998
PFDODA	5	25	1.1	25-1000	77.2	0.999
PFTriDA	5	25	1.9	25-1000	99.0	0.999
PFTreDA	5	25	2.1	25-1000	109.8	0.996
PFBS	5	25	0.2	25-1000	94.3	0.999
PFPeS	5	25	0.6	25-1000	83.9	0.999
PFHxS	5	25	0.6	25-1000	80.4	0.999
PFHpS	5	25	1.1	25-1000	111.9	0.998
PFOS	5	25	1.1	25-1000	135.8	0.998
PFNS	5	25	2.3	25-1000	128.0	0.995
PFDS	5	25	1.7	25-1000	133.0	0.997
FOSA	5	25	1.1	25-1000	94.3	0.999
N-Et-FOSAA	5	25	1.9	25-1000	102.8	0.997
N-Me-FOSAA	5	25	0.4	25-1000	124.2	0.999
4:2 FTS	5	25	1.3	25-1000	87.8	0.999
6:2 FTS	5	25	1.9	25-1000	NA	0.994
8:2 FTS	5	25	0.6	25-1000	95.2	0.997
ADONA	5	25	0.7	-	-	0.999
9Cl-PF3ONS	5	25	1.3	-	-	0.997
11Cl-PF3OUdS	5	25	0.9	-	-	0.998
GenX	5	25	5.0	-	-	0.999
PFMBA	5	25	0.7	-	-	0.995
PFEESA	5	25	1.1	-	-	0.998
NFDHA	10	50	10.0	-	-	0.995

Table 1. Method specifications for analysis using ASTM 7968 on the Xevo TQ-XS. In vial and in sample LLOQ values used in this study, detection limit capabilities, recoveries of LLOQ samples,

and calibration linearity. (\*The reporting range designated in the ASTM 7968 method.)

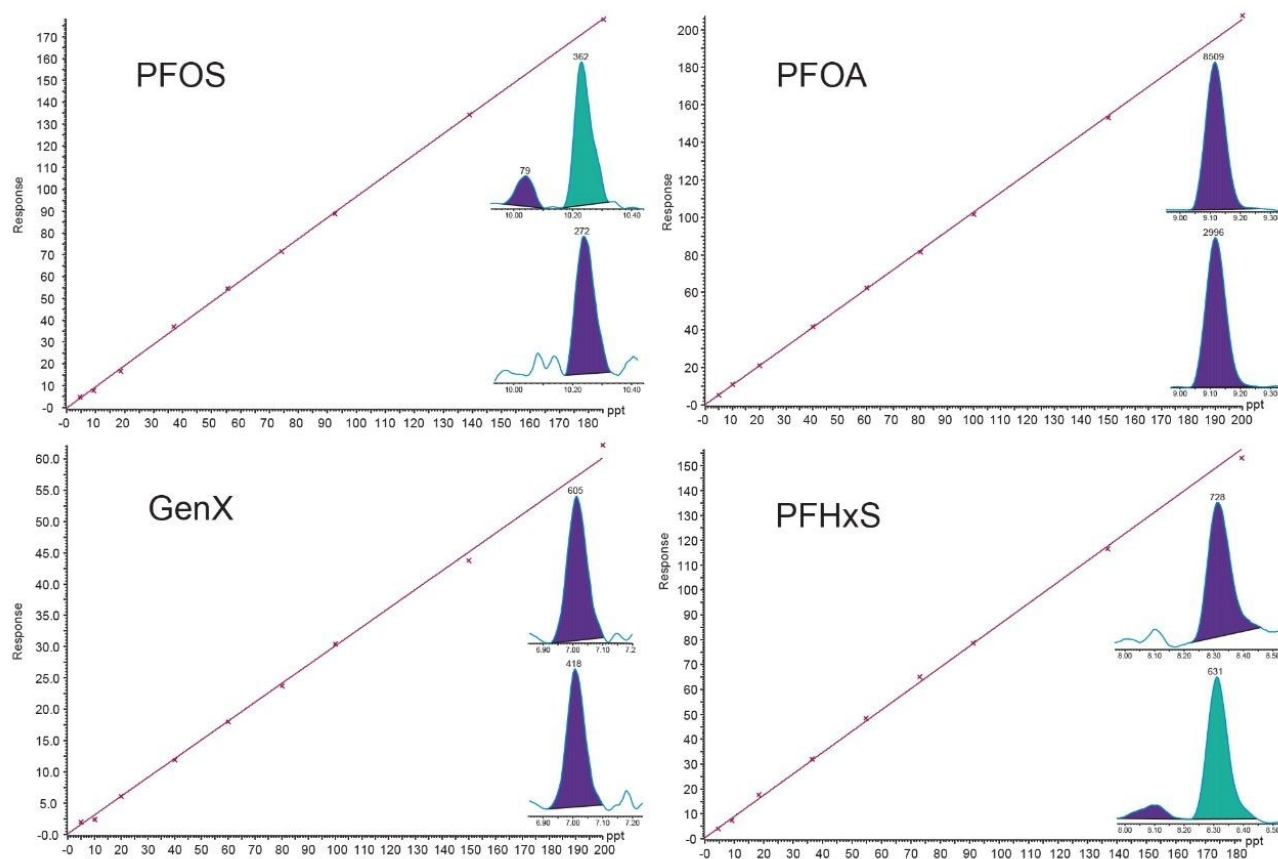


Figure 2. Calibration curves for a selection of PFAS included in the method and chromatograms of the quantitative and qualitative transitions at the LLOQ.

In the ASTM 7968 method, percent recoveries are calculated based on the isotope labelled surrogate standards spiked into the soil samples prior to sample preparation. The method requires recoveries within the range of 70–130%. Method recoveries for this evaluation are demonstrated in Figure 3 showing the range of isotope labelled surrogates used in this study. Recoveries are highlighted in all four soil matrices analyzed. The error bars indicate the standard deviation of the recoveries from 15 samples of each soil type. All PFAS were recovered within the 70–130% range, with only 6:2 FTS and 8:2 FTS having standard deviations of the recovery values that

fall slightly above 130%. 6:2 FTS is a known contaminant in many laboratories and 8:2 FTS is one of the more difficult PFAS compounds to ionize using electrospray, making the variability slightly higher for each of these.

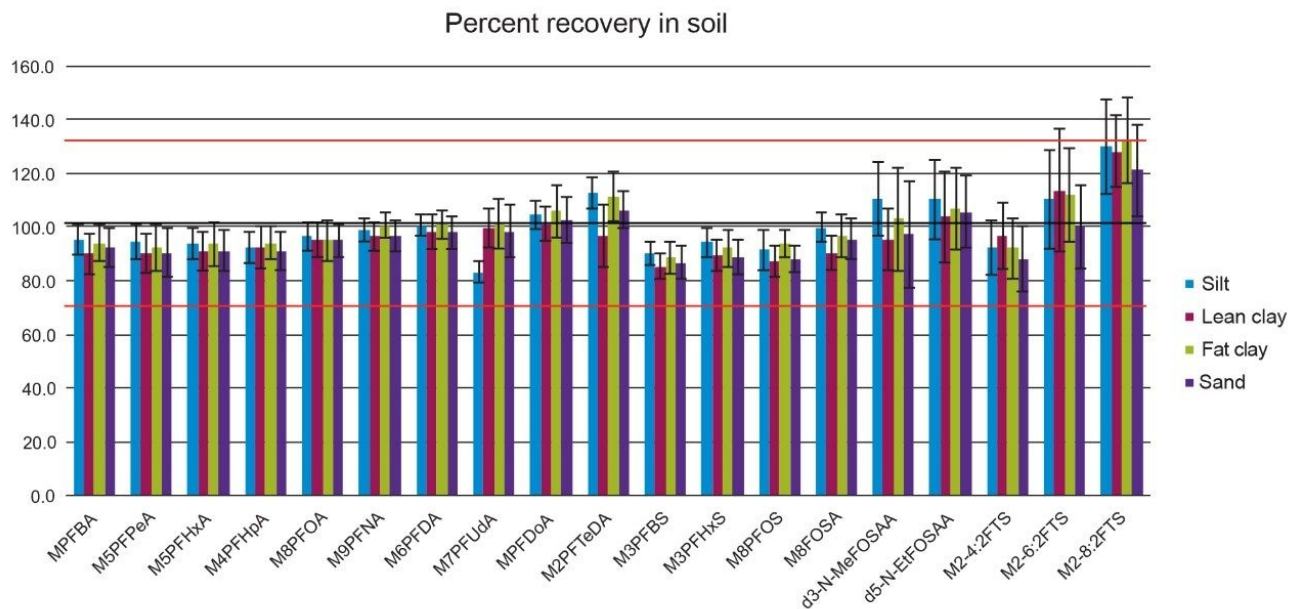


Figure 3. Percent recovery and standard deviations (n=15) for each surrogate standard spiked prior to sample pre-treatment. The red lines indicate the acceptable recovery range of 70–130% designated in ASTM 7968.

The range of 24 PFAS pre-spiked into the samples were all easily detectable in all four soil types as well as the seven emerging PFAS added to the samples. An example of all PFAS detected in a lean clay sample is shown in Figure 4. Quantitation of samples was performed in TargetLynx using external calibration, as designated by ASTM 7968. An example of the quantitation of selected control samples and soil samples is shown in Figure 5. Percent recovery and ion ratios values are automatically calculated in TargetLynx. Although not required by this particular method, ion ratio information can be used as an added level of verification when compounds are identified in samples.

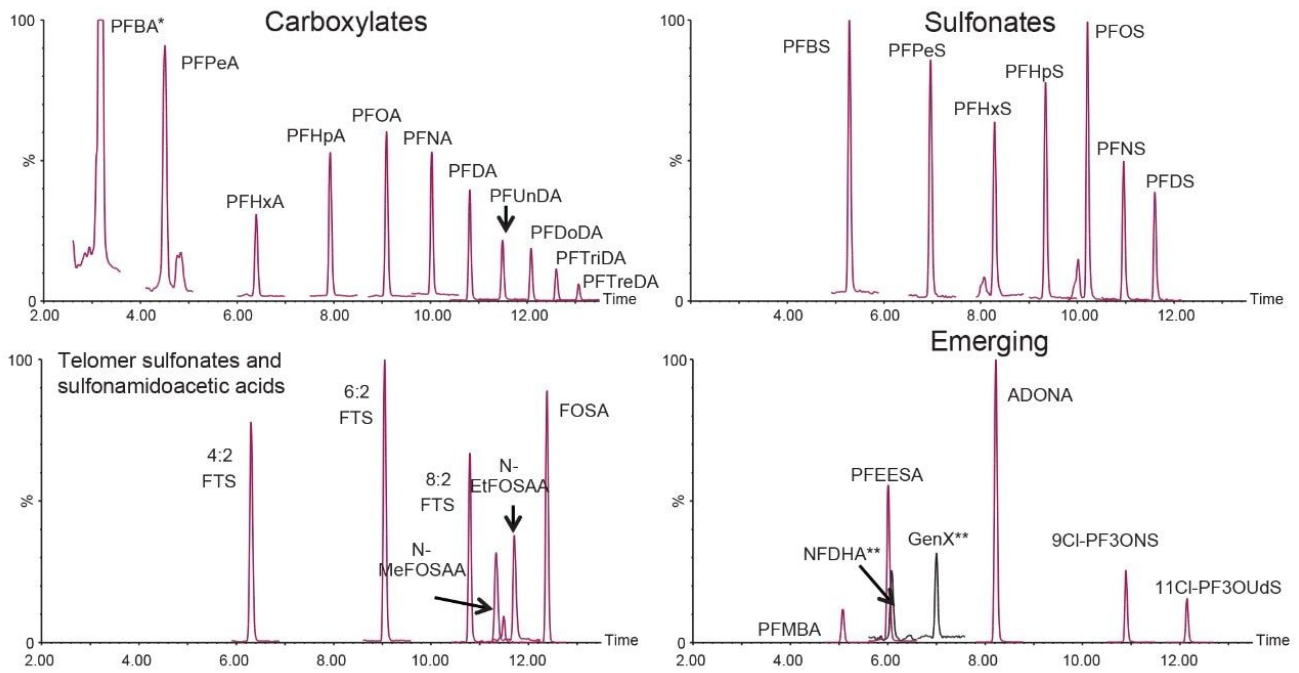


Figure 4. Chromatograms demonstrating the PFAS detected in a lean clay sample including emerging PFAS not currently included in the ASTM 7968 method.

\*This compound is shown off scale.

\*\*Compounds are shown magnified.

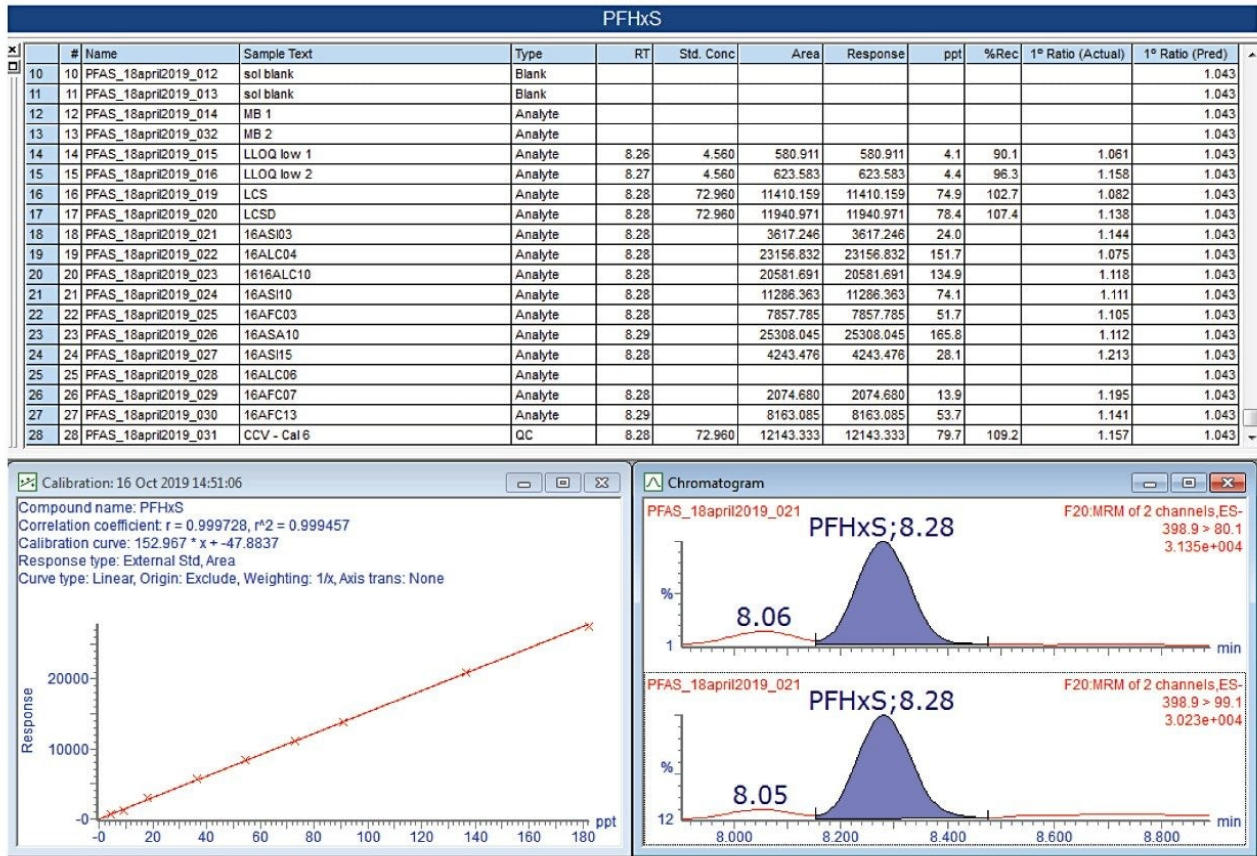


Figure 5. TargetLynx example for linear PFHxS showing the quantitation of select control samples and soil samples. Branched isomer shown as peak labelled with RT of 8.05.

Continuing calibration verification (CCV) injections were performed after every 10 soil samples to ensure instrument stability. A mid-level concentration point on the calibration curve was utilized as the CCV level. Each CCV injection was prepared in individual vials to ensure that bias wasn't introduced from sample evaporation. The entire sample queue was composed of 90 total injections over a 36-hour run period. The stability of the CCV injections was excellent, as seen in Figure 6 for the example of PFOA. This indicates the system is robust over a long series of injections of samples with high matrix load.

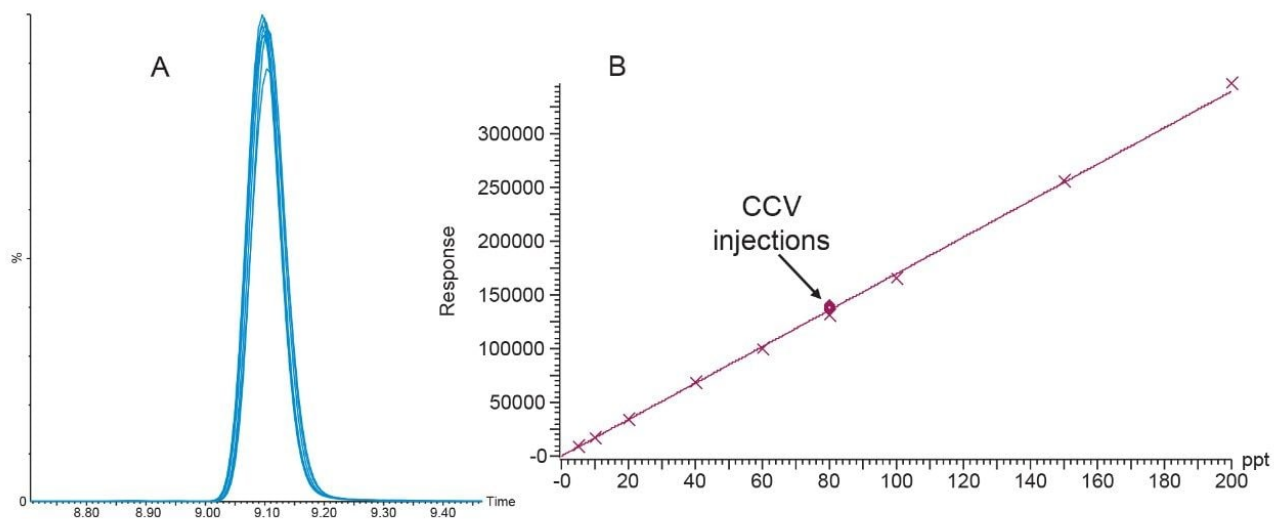


Figure 6. (A) Chromatographic overlay of PFOA peak of six CCV injections and associated calibration point run over a course of approximately 36 hours and (B) the six CCV injections graphed on the calibration curve.

## Conclusion

- The solution, including the Xevo TQ-XS and PFAS Kit, has been shown to be fit for purpose to achieve the requirements of the ASTM 7968 method, simplifying adoption and incorporation of the system and methodology into a routine laboratory setting.
- The ASTM 7968 method allows for high throughput analysis of PFAS in various soil and sediment types.
- Although ASTM 7968 does not include any emerging PFAS, they can easily be incorporated into the methodology for labs that need to expand the testing scope.
- The total solution provided sensitive, accurate, and robust analysis of PFAS in various soils and sediments utilizing very minimal sample preparation, helping to save cost and resources.

## References



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2. Organtini, K.; Rosnack, K.; Stevens, D.; Ross, D. Analysis of Legacy and Emerging Perfluorinated Alkyl Substances (PFAS) in Environmental Water Samples Using Solid Phase Extraction (SPE) and LC-MS/MS. Waters Corporation Application Note 720006471EN. 2019.
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4. Mullin, L.; Burgess, J. Ultra Low-Level Detection of Perfluoroalkyl Substances (PFASs) Using the PFAS Analysis Kit. Waters Corporation Technology Brief 720005701EN. 2016.
5. ASTM D7968, Standard Test Method for Determination of Polyfluorinated Compounds in Soil by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS), ASTM International, West Conshohocken, PA, 2017, [www.astm.org](http://www.astm.org).

## Appendix

Compound	CAS number	Parent	Fragment	CV	CE	RT
PFBA	375-22-4	213.0	169	8	5	3.08
PFPeA	2706-90-3	262.9	218.9	5	5	4.48
PFHxA	307-24-4	312.9	268.9	16	6	6.35
			118.9	16	21	
PFHpA	375-85-9	362.9	318.9	14	8	7.9
			168.9	14	14	
PFOA	335-67-1	412.9	368.9	22	7	9.06
			168.9	22	15	
PFNA	378-99-1	462.9	418.9	18	9	10.01
			218.9	18	15	
PFDA	335-76-2	512.9	468.9	6	9	10.80
			218.9	6	15	
PFUnDA	2058-94-8	562.9	518.9	8	8	11.47
			268.9	8	14	
PFDoDA	307-55-1	612.9	568.9	12	12	12.06
			168.9	12	21	
PFTeDA	72629-94-8	662.9	168.9	14	22	12.59
			218.9	14	20	
PFTroDA	376-06-7	712.9	218.9	14	22	13.05
			168.9	14	20	
PFBS	29420-49-3	298.9	80.1	7	27	5.25
			99.1	7	27	
PFPeS	2706-91-4	348.9	79.9	32	31	6.92
			98.9	32	25	
PFHxS	3871-99-6	398.9	80.1	38	35	8.25
			99.1	38	29	
PFHpS	375-92-6	448.9	79.9	16	34	9.31
			98.9	16	34	
PFOS	1763-23-1	498.9	79.9	30	42	10.18
			98.9	30	40	
PFNS	N/A	548.9	80.1	24	40	10.94
			99.1	24	36	
PFDS	335-77-3	598.9	80.1	46	46	11.58
			99.1	46	46	
FOSA	754-91-6	498.0	77.9	40	29	12.38
N-EFOSAA	2991-50-6	584.0	418.9	34	15	11.68
N-MeFOSAA	2355-31-9	569.9	418.9	36	15	11.33
			168.9	36	27	
4:2 FTS	757124-72-4	326.9	306.9	42	18	6.25
			80.9	42	27	
6:2 FTS	29420-49-3	427.0	406.9	12	22	9.01
			80.1	12	32	
8:2 FTS	39108-34-4	528.9	506.9	28	26	10.77
			80.9	28	37	
ADONA	958445-44-8	376.9	251	12	10	8.18
9Cl-PF3ONS	73606-19-6	531.0	351	14	22	10.87
			82.9	14	20	
11Cl-PF3OUs	73606-19-6	631.0	450.9	16	26	12.12
			82.9	16	26	
GenX	13252-13-6	285.0	119	5	39	6.95
			185	5	7	
PFMBA	863090-89-5	278.9	95	10	10	5.02
NFDHA	15172-58-8	294.9	236	10	5	6.05
			201	5	10	
PFEEA	113507-82-7	314.9	85	5	20	5.99
			135	15	20	
<sup>13</sup> C-PFBA	-	217	172	7	8	3.08
<sup>13</sup> C-PFPeA	-	268	223	11	7	4.48
<sup>13</sup> C <sub>1</sub> -PFHxA	-	318	273	10	6	6.35
			120	10	18	
<sup>13</sup> C <sub>1</sub> -PFHpA	-	367	322	16	7	7.9
			172	16	15	
<sup>13</sup> C <sub>1</sub> -PFOA	-	421	376	6	8	9.06
			172	6	16	
<sup>13</sup> C <sub>1</sub> -PFNA	-	472	427	7	18	10.01
			223	7	18	
<sup>13</sup> C <sub>1</sub> -PFDA	-	519	473.9	25	7	10.80
			219	25	13	
<sup>13</sup> C <sub>1</sub> -PFUnDA	-	569.9	524.9	9	8	11.47
			273.9	9	14	
<sup>13</sup> C <sub>1</sub> -PFDoDA	-	615	569.9	23	10	12.06
			168.9	23	22	
<sup>13</sup> C <sub>1</sub> -PFTeDA	-	715	168.9	18	25	13.05
			219	18	25	
<sup>13</sup> C <sub>1</sub> -PFBS	-	301.9	80.1	34	28	5.25
			99.1	34	24	
<sup>13</sup> C <sub>1</sub> -PFHxS	-	402	80.1	13	38	8.25
			99.1	13	30	
<sup>13</sup> C <sub>1</sub> -PFOS	-	507	80.1	38	34	10.18
			99.1	36	34	
<sup>13</sup> C <sub>1</sub> -FOSA	-	506	77.9	13	28	12.38
D <sub>9</sub> -N-EFOSAA	-	589	418.9	24	17	11.68
			482.9	24	13	
D <sub>9</sub> -N-MeFOSAA	-	573	418.9	17	18	11.33
			515	17	18	
<sup>13</sup> C <sub>2</sub> -4:2 FTS	-	329	309	14	18	6.25
			80.9	14	21	
<sup>13</sup> C <sub>2</sub> -6:2 FTS	-	429	409	48	21	9.01
			80.9	48	27	
<sup>13</sup> C <sub>2</sub> -8:2 FTS	-	529	509	20	27	10.77
			80.9	20	37	
<sup>13</sup> C <sub>1</sub> -GenX	-	287	169	5	12	6.95
			119	5	12	



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