

Startup Guide for the Analysis of Perfluorinated Alkyl Substances (PFAS) in Environmental Samples

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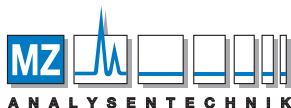
I. INTRODUCTION

Per- and Polyfluorinated Alkyl Substances (PFAS), formerly called Perfluorinated Compounds (PFCs), encompass a group of thousands of highly fluorinated compounds used in many industrial processes and consumer products. PFAS compounds were introduced in the 1940s and are commonly used in surfactants, nonstick and/or water repellent coatings, and even in firefighting foams. Two of the more commonly known PFAS compounds, PFOS (perfluorooctanesulfonic acid) and PFOA (perfluorooctanoic acid), are most well known for their use as precursors in the production of Teflon™ Coatings. Since the C-F bond is very stable, this family of compounds are also very stable, allowing them to be classified as persistent organic pollutants (POPs). They are used globally making them of high environmental impact worldwide. PFAS enter the environment through manufacturing processes, waste discharge, waste/chemical spills, use and disposal of consumer products, fire fighter training exercises, and fire suppression events using aqueous film forming foam (AFFF) sprays.

PFAS are persistent and bioaccumulative and have been detected in humans, wildlife, and the environment and can even be found in isolated regions of the world, such as the Arctic. Over the decade, thousands of PFAS have been created.

Figure 1 highlights a few of the more well-known classes of PFAS as well as examples of each. Each class is made up of many isomers that vary by their CF₂ chain length and as well as the substitution pattern (linear chain vs. branched chain).

Detection requirements for PFAS have been getting more challenging as advisory and regulatory limits, to protect public health and the environment, continue to be created and updated. For example, on June 15, 2022, the US EPA tightened its lifetime health advisory levels (HALs) in drinking water for perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS). The recommendation is 0.004 ng/L (part per trillion, ppt) for PFOA and 0.02 ng/L for PFOS, both of which are categorized as interim levels.



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These levels are dramatically more stringent than the 70 ng/L (ppt) total that the EPA recommended in 2016. In early 2021, the EU began enforcing the revised Drinking Water Directive (EU) 2020/2184 whereby a select group of 20 PFAS have a recommended summed limit of 0.1 µg/L (100 ng/L) with a limit of 0.5 µg/L (500 ng/L) for total PFAS observed. This directive requires individual PFAS to be detected in the single digit ng/L range. Due to toxicological concerns of exposure to the legacy PFAS used for many decades, PFAS like PFOS and PFOA are being discontinued from use. Even so, their persistence in the environment and human tissues will continue to require monitoring for their presence. The legacy PFAS are being replaced by new emerging PFAS, for example GenX, that will need continued monitoring as well.

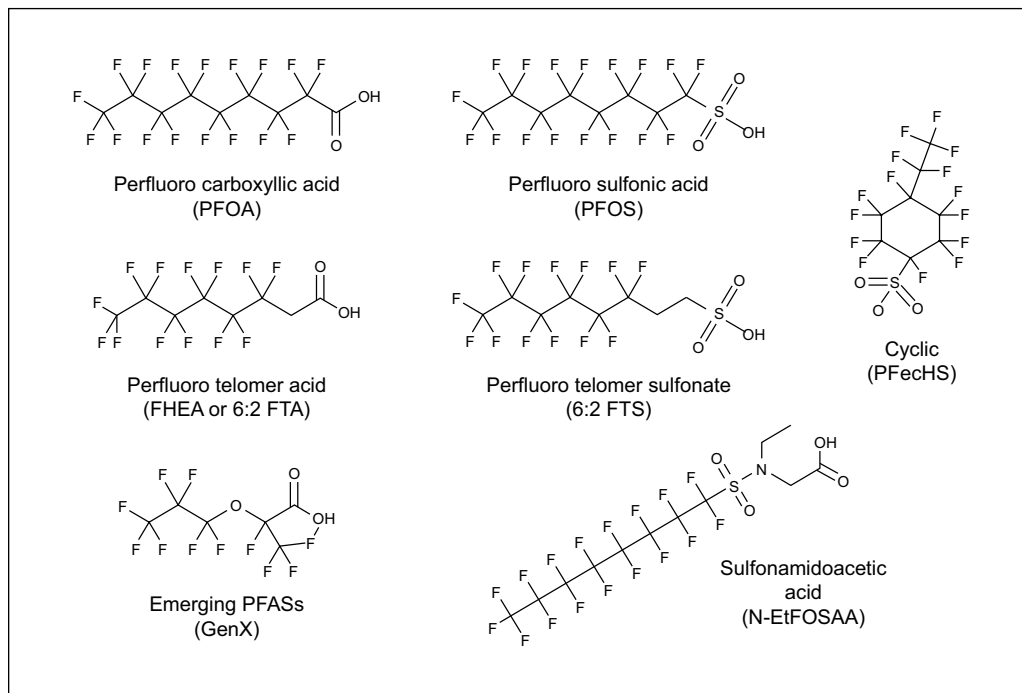


Figure 1. A sample of different PFAS classes and structures.

II. WATER ANALYSIS METHOD OPTIONS

Water analysis is typically broken into two classes:

- Drinking water
- Non-potable (or environmental) water (*i.e.*, surface water, ground water, wastewater)

The approaches for both water classes can overlap, although drinking water can have different requirements which will be covered in the following information.

There are two main approaches used for PFAS analysis in water samples. Either approach is appropriate and depends on the laboratory's requirements and resources.

1. One approach is to perform sample preparation using solid phase extraction (SPE) to enrich or concentrate the sample prior to LC-MS analysis. Using this approach, the laboratory can use a mid-range sensitivity mass spectrometer for analysis (*i.e.* Xevo™ TQ-S micro MS).

There are established methods that use the SPE approach including EPA 537.1, EPA 533, ISO 25101, and ISO 21675. The EPA 537.1 and 533 methods are only appropriate for drinking water analysis and are restrictive in the sample preparation steps used. EPA 537.1 requires extraction using an SDVB phase cartridge (Sep-Pak™ PS2) and covers 18 PFAS. EPA 533 requires a weak anion exchange (WAX) phase cartridge and covers 25 PFAS.

EPA Method 1633 requires a two step clean up using both WAX cartridges and graphitized carbon black (GCB).

ISO 25101 and ISO 21675 are methods for drinking water as well as non-potable waters (i.e., surface water, ground water, and wastewater). Both ISO methods are less restrictive on the sample preparation procedure and provide suggestions for the SPE cartridge and extraction procedure, including the Oasis™ WAX Cartridge. ISO 25101 only covers PFOA and PFOS, but ISO 21675 extends a very similar methodology to 30 PFAS.

Generally, the WAX chemistry is better suited for PFAS analysis, especially when looking to analyze a large suite of PFAS in a single method. WAX retains the smaller chain PFAS better lending the best recovery for these compounds when compared to other chemistries. If GCB clean-up is a requirement or additional clean-up is desired, equivalency has been shown with use of dual-phase bilayer cartridges such as Oasis WAX/GCB and GCB/WAX for PFAS analysis. Unless required to follow the EPA 537.1 method using an SDVB chemistry, it is recommended that the WAX chemistry be used for SPE extractions of PFAS.

- The second approach is to perform direct injection prior to LC-MS analysis. Using this approach, the laboratory will need to use a very sensitive mass spectrometer "(i.e. Xevo TQ-XS MS, or Xevo TQ-Absolute MS). This approach is described in the ASTM 8421 method which is suitable for drinking and environmental water samples. The ASTM 8421 method requires only sample dilution and syringe filtration prior to injecting a large volume of sample on the LC-MS. This methodology does not provide the sample enrichment factor that is gained by the SPE methods. This method is appropriate for laboratories that do not need to reach ultra-trace levels of PFAS (<1 ng/L).

	Matrix	Prep	Waters Collateral	Column	Minimum Instrument	Number of Compounds
ASTM 7979/8421	SW, GW, WW	Dilute, filter	App Note 720006329	CSH Phenyl Hexyl 2.1x100mm	TQ Absolute	24/44
ASTM 7968/8535	Soils	Dilute, filter	App Note 720006764	CSH Phenyl Hexyl 2.1x100mm	TQ Absolute	24/44
EPA 533	DW	Oasis WAX	App Note 720006808	BEH C₁₈ 2.1x100mm	TQ-S micro	25
EPA 537.1	DW	Sep-Pak PS2	App Note 720006695	BEH C₁₈ 2.1x100mm	TQ-S micro	18
EPA 1633	Non-potable water (SW, GW, WW), soil, solids, tissue	Oasis WAX/GCB or GCB/WAX	App Note 720008117	BEH C₁₈ 2.1x50mm	TQ-S micro	40
EPA 8327	Non-potable water	Dilute, filter	App Note 720006329	CSH Phenyl Hexyl 2.1x100mm	TQ Absolute	24
EU 2020/2184	Drinking water	Direct inject	App Note 720007413	ACQUITY Premier BEH Shield RP18 Column	TQ Absolute	20
EU 2022/2388	Food	Oasis WAX	App Note 720007482	BEH C₁₈ 2.1x100mm	TQ Absolute	30
ISO 25101	DW, GW, SW	Oasis WAX	App Note 720006471	BEH C₁₈ 2.1x100mm	TQ-S micro	2
ISO 21675	DW, GW, SW, WW < 2 g/L solid particulate matter	Oasis WAX	App Note 720006471	BEH C₁₈ 2.1x100mm	TQ-S micro	30

III. CONTAMINATION SOURCES

Since PFAS are commonly used in the manufacture of a wide range of consumer products and laboratory supplies, one must be very cautious to eliminate any potential sources of contamination during sample collection, transport, preparation, and analysis. Samples should be collected in high density polyethylene (HDPE) containers pre-washed with methanol. Common sources of laboratory contamination that should be avoided include:

- Aluminum foil
- Glass transfer pipettes
- Glass autosampler vials
- Vial caps with Teflon seals
- Pipette tips labelled as “low retention”

Polypropylene vials with polyethylene caps should be used for sample analysis (P/N: [186005230](#)). When possible, all consumables should be tested for PFAS contamination before use.

Care should be taken when choosing solvents for the analysis (for sample preparation and mobile phases). Analysts should be aware that most LC-MS grade solvents are bottled in containers that contain a certain level of fluorinated compounds and usually have a Teflon seal in the cap.

Water purification systems also have the potential to introduce PFAS contaminants. Solvents should be tested before use.

The LC-MS system itself produces sources of contamination due to the presence of Teflon coated parts. This is mitigated by modifying the LC with a PFAS kit (see PFAS Kit section).

See the Waters white paper [720007905EN](#) for full procedures and best practices for testing, controlling, and mitigating PFAS contamination in typical laboratory environments.

IV. PFAS KIT

Liquid chromatographs used for PFAS analysis must be equipped with a PFAS Installation Kit to avoid PFAS contamination from the LC. There are three key components to the PFAS Kit that must be installed on the LC system prior to use. Installation is quick and straightforward.

1. PEEK™ Solvent Tubing: The typical tubing that comes on an LC is Teflon coated. Any mobile phase and wash lines that will be used during sample analysis must be replaced with the PEEK Tubing from the PFAS Kit. Installation of this tubing consists of unscrewing the Teflon tubing from the connection port on the LC pump and connecting the PEEK Tubing in its place (**Figure 2**). The following solvent lines need to be replaced:

On ACQUITY quaternary solvent manager (QSM) systems:

- Solvent line A
- Solvent line B
- Solvent line C
- Solvent line D
- Seal wash line
- Purge/weak needle wash line
- Strong needle wash line*

For more details, reference Waters Technology Brief, [720005701EN](#).

On ACQUITY binary solvent manager (BSM) systems:

- Solvent line A1
- Solvent line A2
- Solvent line B1
- Solvent line B2
- Seal wash line
- Purge/weak needle wash line
- Strong needle wash line*

*To change the strong needle wash line on a flow through needle (FTN) system, the side panel of the pump must be removed to access (Figure 3).

2. Isolator Column: The isolator column is a 50 mm column installed after the solvent mixer, but before the analytical column. The purpose of this column is to delay any inherent system contamination (from either solvents or internal instrument components) from interfering with the PFAS in the sample. This column will not remove the PFAS contaminants but delay them from co-eluting with the analytical peak. They will be chromatographically separated from the analytical/sample peak (Figure 4).
3. Stainless Steel Coil: "Mixer-to-Isolator Column" Tubing: This is a piece of coiled stainless-steel tubing that is installed between the solvent mixer and isolator column. This tubing helps to further delay any system contaminants.

For more details, reference Waters Technology Brief, [720005701EN](#).

The isolator column can be purchased separately using Part Number [186004476](#) for XBridge™ BEH™ C₁₈ or Part Number [186009407](#) for the Atlantis™ Premier BEH C₁₈ AX Column (recommended isolator column).

For more details on the PFAS Kit, reference Waters Care and Use Manual, [715006386](#).

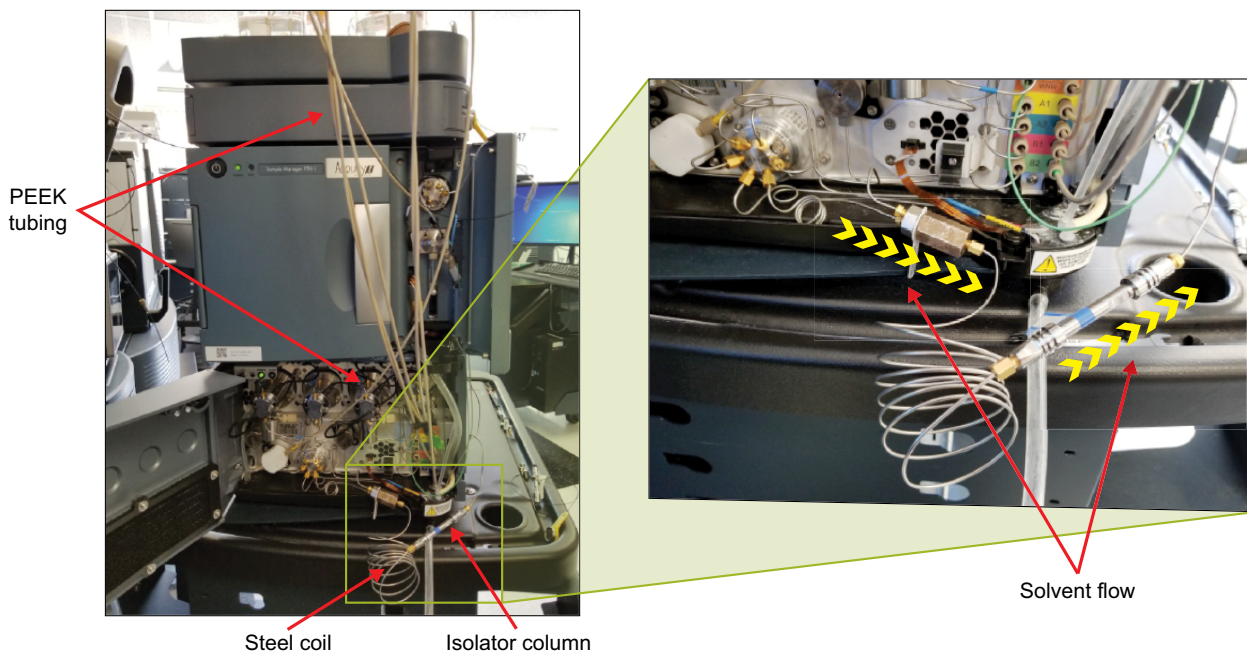


Figure 2. Front view of an ACQUITY™ UPLC™ I-Class System (BSM) showing components of installed PFAS Kit.

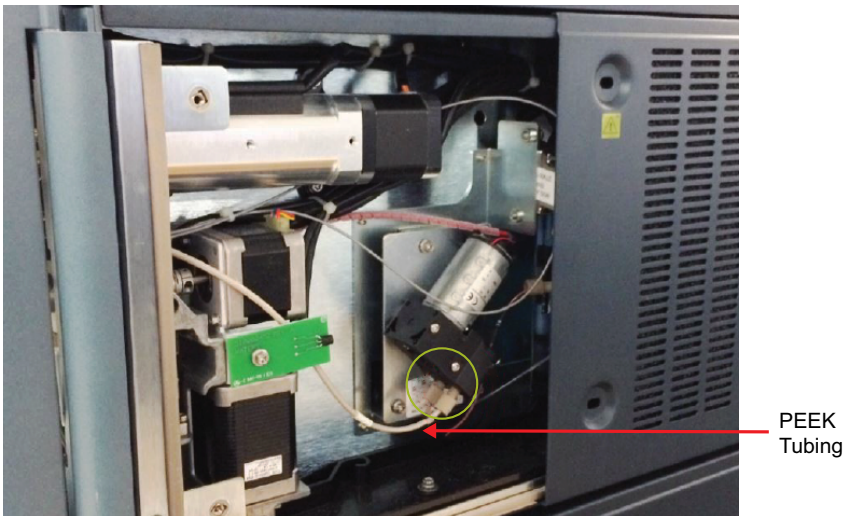


Figure 3. Side view of an ACQUITY UPLC I-Class System (BSM) that shows the placement of the strong needle wash line. The connection circled in red is where the Teflon tubing should be replaced with the PEEK Tubing.

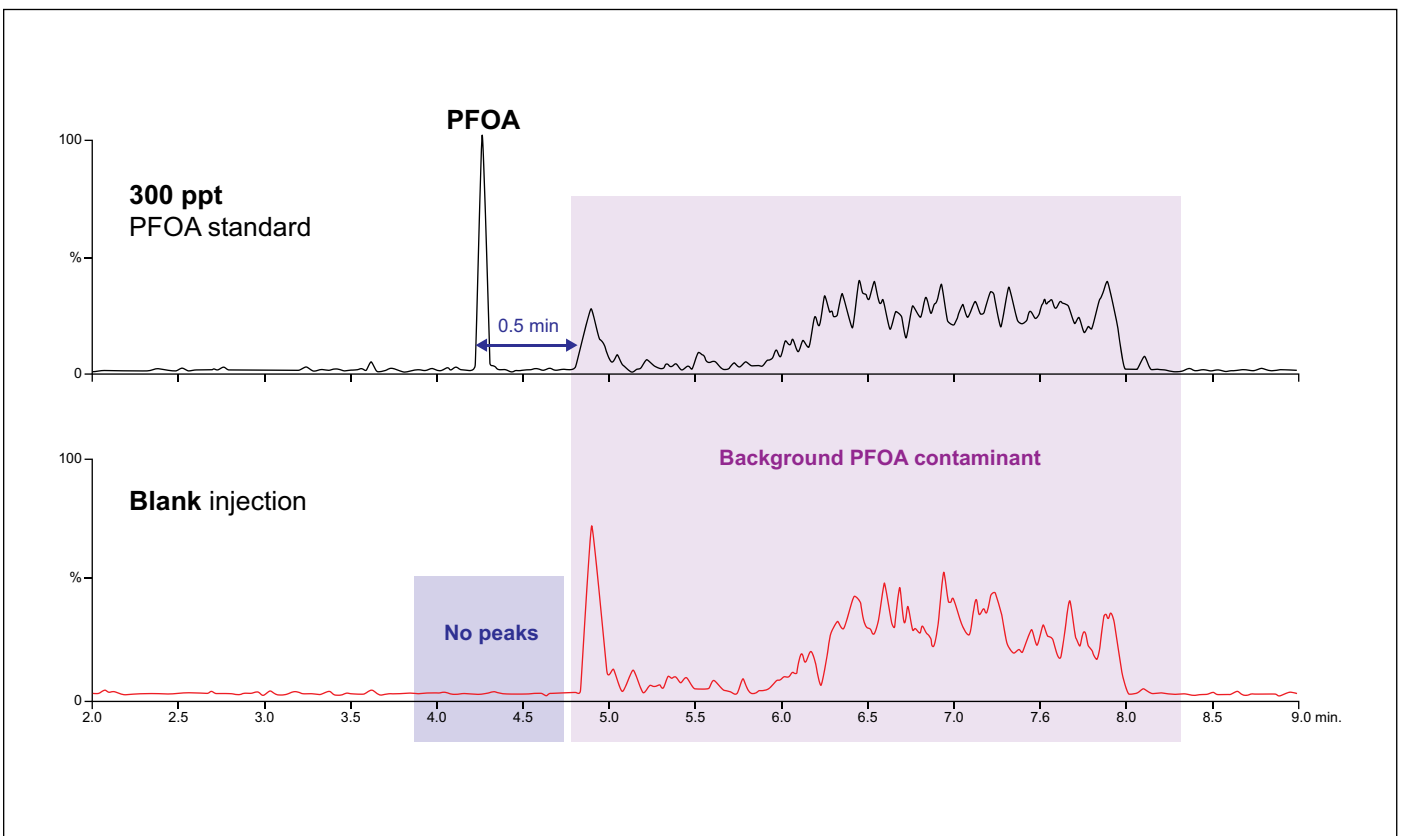


Figure 4. Example of PFOA system contaminant delay with isolator column installed.

V. SPE APPROACHES FOR WATER SAMPLE ANALYSIS

a. EPA 537.1 (for drinking water only)

The protocol for extraction of PFAS from drinking water following EPA 537.1 is listed in **Figure 5**.

The supplies needed for this method are listed below and an example of the SPE extraction manifold system is shown in **Figure 6**.

For more details, reference **Waters Technology Brief, 720006695EN**.

Sample preparation

The following supplies are required for sample preparation:

- SPE manifold (P/N: [WAT200609](#))
- Vacuum pump (P/N: [725000417](#))
- Sep-Pak PS2 Cartridges, 6 cc, 500 mg (P/N: [WAT200610](#))
- Sample reservoirs, 60 mL (P/N: [186005587](#))
- Sample reservoir adaptors (P/N: [WAT054260](#))
- Nitrogen sample evaporation system
- 15 mL polypropylene centrifuge tubes

The following reagents and solutions are required for sample preparation:

- Trizma crystals, pH 7, reagent grade (equivalent to Sigma cat # T-7193)
- PFAS native standards (Wellington Part Number EPA-537PDS-R1 or EPA-537APDS, or similar)
- Isotope labelled PFAS internal standards (Wellington Part Number EPA-537SS-R1 and EPA-537IS, or similar)
- Methanol, LC-MS/MS grade, evaluated for low PFAS background
- Water, LC-MS/MS grade, evaluated for low PFAS background
- 96:4 methanol:water solution

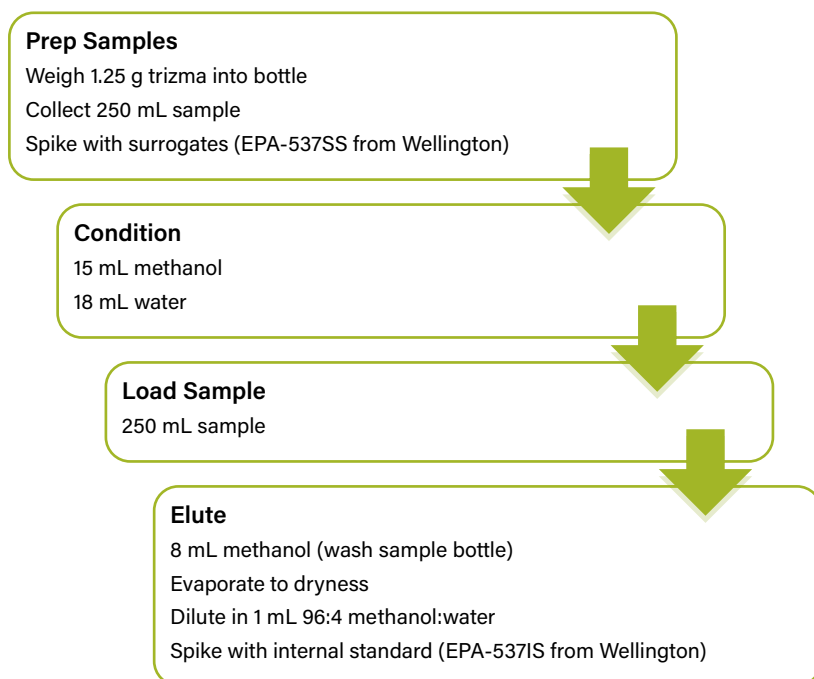


Figure 5. Method for extraction of PFAS from drinking water using Sep-Pak PS2 Cartridges, in accordance with EPA 537.1.

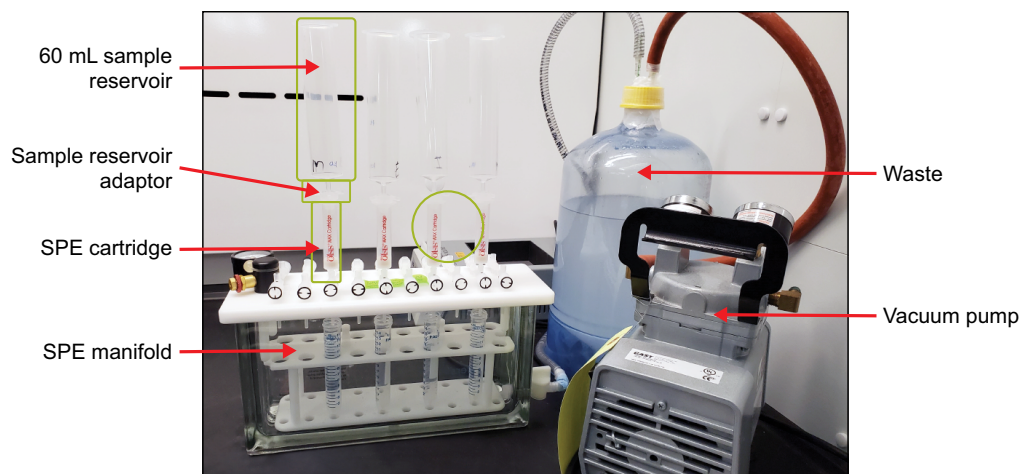


Figure 6. SPE setup suitable for large volume extractions of water samples.

Instrument setup

For this analysis method, a tandem quadrupole mass spectrometer is required. At a minimum, the Xevo TQ-S micro MS is required.

An LC modified with the PFAS Kit is required for analysis. See Section IV for details on the PFAS Kit.

b. EPA 533 (for drinking water only)

More information on the analysis method can be found in Technology Brief [720006808EN](#).

The protocol for extraction of PFAS from drinking water following EPA 533 is listed in **Figure 7**.

The supplies needed for this method are listed below and an example of the SPE extraction manifold system is shown in **Figure 6**.

Sample preparation

The following supplies are required for sample preparation:

- SPE manifold (P/N: [WAT200609](#))
- Vacuum pump (P/N: [725000417](#))
- Oasis WAX Cartridges, 6 cc, 500 mg (P/N: [186009346](#) or [186009347](#))
- Sample reservoirs, 60 mL (P/N: [186005587](#))
- Sample reservoir adaptors (P/N: [WAT054260](#))
- Nitrogen sample evaporation system
- 15 mL polypropylene centrifuge tubes

The following reagents and solutions are required for sample preparation:

- Ammonium acetate, HPLC grade (equivalent to Sigma cat # 372331)
- Ammonium Hydroxide, approximately 25% ammonia (equivalent to Millipore Sigma cat # AX1308-7)
- PFAS standards (Waters, Wellington P/N: PFAC30PAR, or similar plus additional compounds)
- Isotope labelled PFAS internal standards (Wellington Part Number EPA-E33ES and EPA-533IS, or similar)
- Methanol, LC-MS/MS grade, evaluated for low PFAS background
- Water, LC-MS/MS grade, evaluated for low PFAS background

- 1 g/L ammonium acetate solution
- 2% (v/v) ammonium hydroxide in methanol solution
- 0.1 M phosphate buffer, pH 7.0 (prepared in house or Sigma cat # P5244)

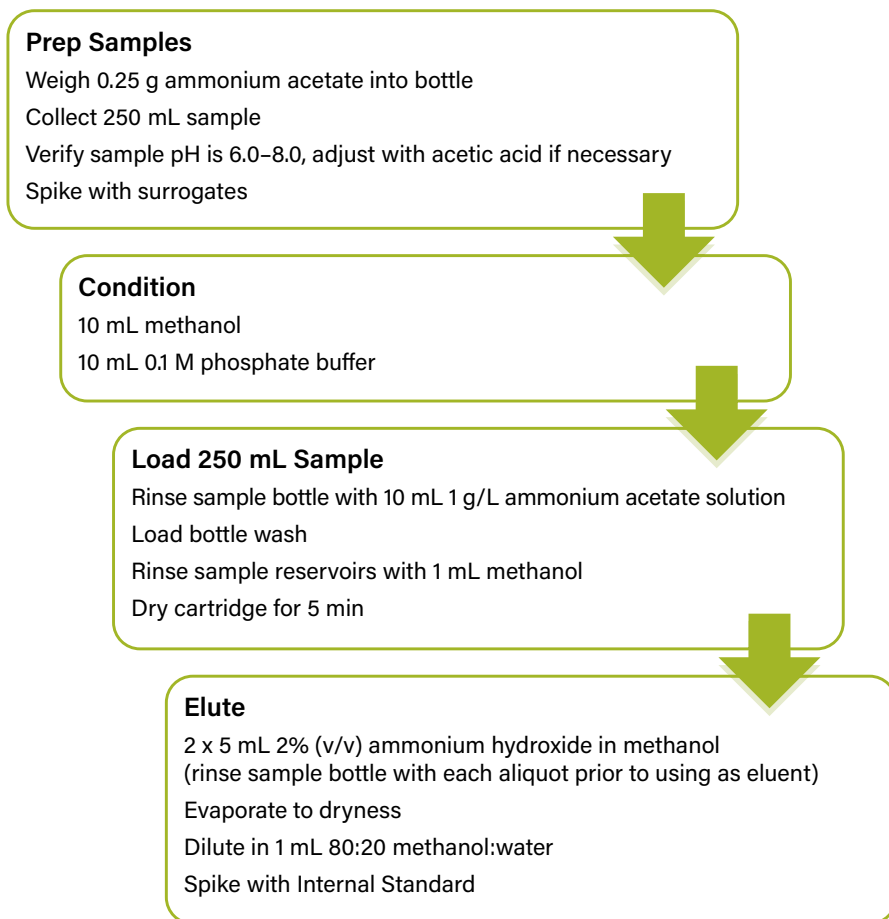


Figure 7. Method for the extraction of PFAS from drinking water using Oasis WAX Cartridges, in accordance with EPA 533.

Instrument setup

For this analysis method, a tandem quadrupole mass spectrometer is required. At a minimum, the Xevo TQ-S micro MS is required.

An LC modified with the PFAS Kit is required for analysis. See Section IV for details on the PFAS Kit.

c. ISO 25101 and ISO 21675

The protocol for extraction of PFAS from drinking and environmental water adapted from the ISO methods is listed in **Figure 8**.

The supplies needed for this method are listed below and an example of the SPE extraction manifold system is shown in **Figure 6**.

Sample preparation

The following supplies are required for sample preparation:

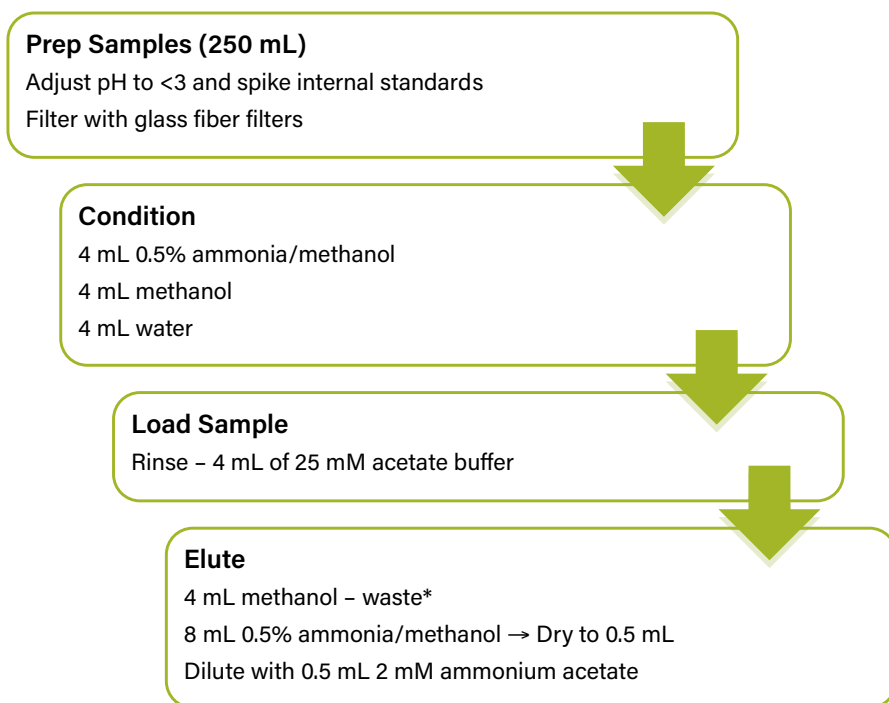
- SPE manifold (P/N: [WAT200609](#))
- Vacuum pump (P/N: [725000417](#))

For more details, reference **Waters Application Note, 720006471EN**.

- For ≤250 mL samples: Oasis WAX Cartridges, 6 cc, 150 mg (P/N: [186009344](#) or [186009345](#))
- For >250 mL samples: Oasis WAX Cartridges, 6 cc, 500 mg (P/N: [186009346](#) or [186009347](#))
- Sample reservoirs, 60 mL (P/N: [186005587](#))
- Sample reservoir adaptors (P/N: [WAT054260](#))
- Nitrogen sample evaporation system
- 15 mL polypropylene centrifuge tubes

The following reagents and solutions are required for sample preparation:

- Acetic acid, 99.9% (equivalent to Sigma cat # A6283)
- Ammonium acetate, HPLC grade (equivalent to Sigma cat # 372331)
- Ammonium Hydroxide, approximately 25% ammonia (equivalent to Millipore Sigma cat # AX1308-7)
- PFAS native standards (Wellington Part Number PFAC-30PAR or similar)
- Isotope labelled PFAS internal standards (Wellington Part Number MPFAC-24ES or similar)
- Methanol, LC-MS/MS grade, evaluated for low PFAS background
- Water, LC-MS/MS grade, evaluated for low PFAS background
- 0.5% (m/v) ammonium hydroxide in methanol solution (equivalent to 2% [v/v] solution)
- 25 mM acetate buffer, pH 4.0



**Collect, as neutrals may be eluted during this step.*

Figure 8. Method for the extraction of PFAS from drinking and environmental water using Oasis WAX Cartridges adapted from ISO 25101 and ISO 21675.

Instrument setup

For this analysis method, a tandem quadrupole mass spectrometer is required. At a minimum, the Xevo TQ-S micro MS is required.

An LC modified with the PFAS Kit is required for analysis. See Section IV for details.

VI. DIRECT-INJECT APPROACH FOR WATER SAMPLE ANALYSIS

The protocol for preparation of drinking and environmental water following ASTM 8421 and 7979 is listed in **Figure 9**.

The supplies needed for this method are listed below.

Sample preparation

The following supplies are required for sample preparation:

- Nylon GF Syringe Filter (P/N: WAT200800, or equivalent)
- Polypropylene tubes, 15 mL

The following reagents and solutions are required for sample preparation:

- PFAS standards (Wellington P/N: PFAC-30PAR or similar)
- Isotope labelled PFAS surrogates (Wellington p/n: MPFAC-24ES or similar)
- Acetic acid, 99.9% (equivalent to Sigma cat # A6283)
- Methanol, LC-MS/MS grade, evaluated for low PFAS background
- Water, LC-MS/MS grade, evaluated for low PFAS background

For more details, reference **Waters Application Note, 720006329EN**.

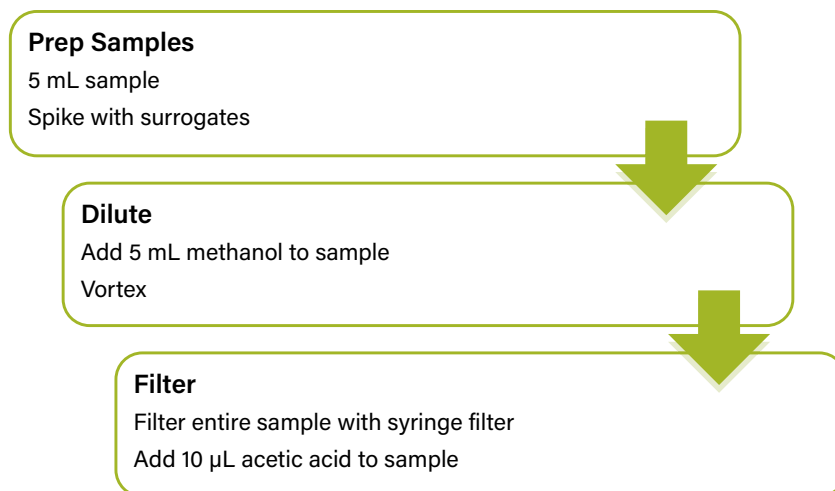


Figure 9. Method for sample preparation of drinking and environmental water in accordance with ASTM 8421.

Instrument setup

A tandem quadrupole mass spectrometer is required for this analysis method. The Xevo TQ-XS or Xevo Absolute MS is required for maximum sensitivity.

An LC modified with the PFAS Kit is required for analysis. See Section IV for details.

To be able to perform the required 30 μL injections, the sample manager must be equipped with a 50 μL extension loop for FTN (P/N: [430002012](#)).

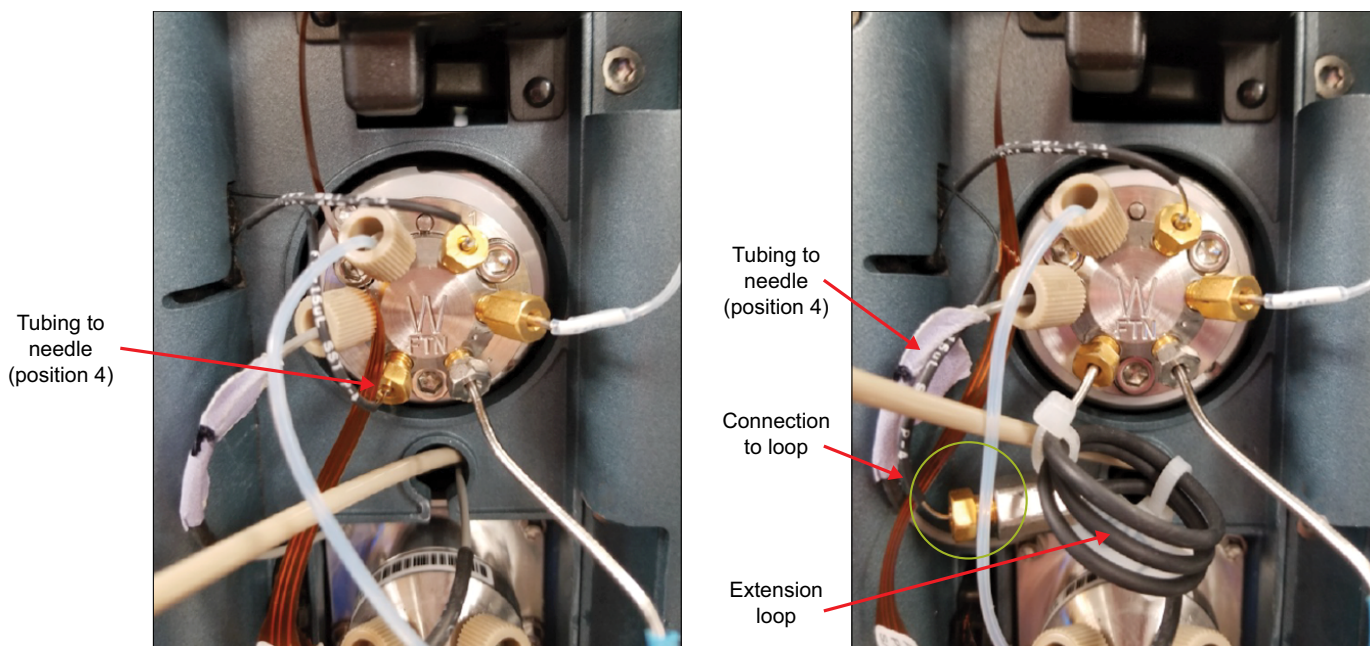


Figure 10. Installation of the 50 μL extension loop for large volume injections. Left shows valve without the loop and right shows the loop installed.

VII. SPE APPROACHES FOR COMPLEX SAMPLES IN AQUEOUS AND SOLID MATRICES

A. EPA 1633 FOR NON-POTABLE WATER SAMPLES

More information on the analysis method can be found in Application Notes [720008117EN](#) and [720008143EN](#).

The protocol for extraction of PFAS from non-potable water (ground, surface, and wastewater) following EPA 1633 is listed in Figure 11.

The supplies needed for this method are listed below and an example of the SPE extraction manifold system is shown in Figure 6:

Sample preparation

The following supplies are required for sample preparation:

- SPE manifold (P/N: [WAT200609](#))
- Vacuum pump (P/N: [725000417](#))
- 6 cc 200 mg Oasis WAX, 50 mg GCB (Oasis WAX/GCB for PFAS Analysis) Cartridges (P/N: 186011110 and 186011111)

- 60 mL Sample Reservoirs ([P/N: 186005587](#))
- Sample reservoir adaptors ([P/N: WAT054260](#))
- Glass wool
- 15 mL polypropylene centrifuge tubes
- pH test strips

The following reagents will be required for sample preparation:

- PFAS native standards (Wellington Part Numbers PFAC-MXF, PFAC-MXG, PFAC-MXH, PFAC-MXI, and PFAC-MXJ)
- Isotope labelled PFAS internal standards (Wellington Part Numbers MPFAC-HIF-ES and MPFAC-HIF-IS)
- Methanol, LC-MS/MS grade, evaluated for low PFAS background
- Water, LC-MS/MS grade, evaluated for low PFAS background
- Ammonium Hydroxide, approximately 25 - 30% ammonia (equivalent to Millipore Sigma cat # AX1308-7)
- Formic acid
- Acetic acid

The following solutions will be required for sample preparation:

- 50% formic acid
- 0.3 M formic acid
- 0.1 M formic acid
- 1:1 0.1 M formic acid:methanol
- 1% (v/v) ammonium hydroxide in methanol

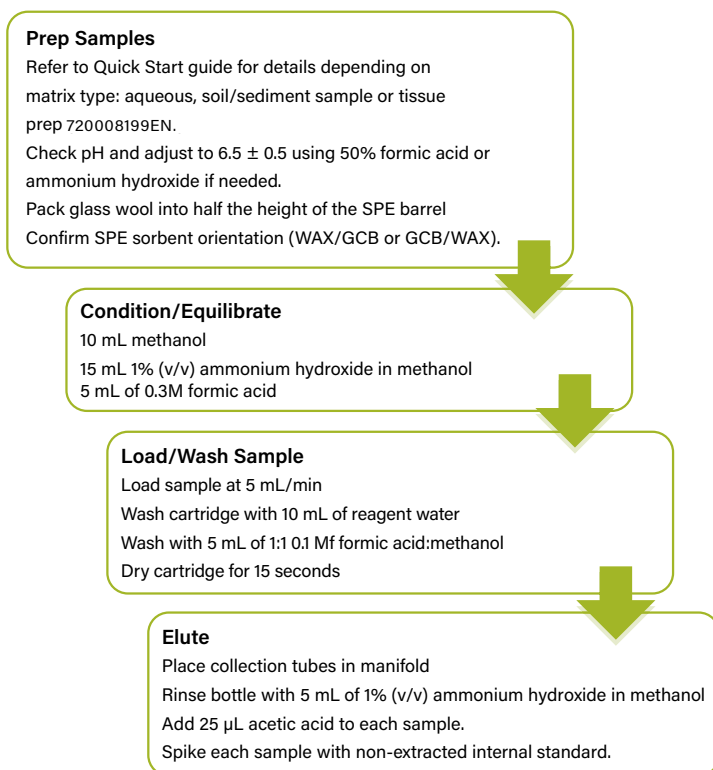


Figure 11. Method for extraction of PFAS from non-potable water, solids, biosolids, and tissue using Oasis WAX/GCB or GCB/WAX Cartridges in accordance with EPA 1633.

Instrument setup

For this analysis method, a tandem quadrupole mass spectrometer is required. At a minimum, Xevo TQ-S micro is required, although the TQ-Absolute is recommended. Analysis information in the application note is with the Xevo TQ-Absolute.

An LC modified with the PFAS Kit is required for analysis. See Section IV for details on the PFAS Kit.

B. EPA 1633 FOR SOLIDS, BIOSOLIDS, AND TISSUE SAMPLES

More information on the analysis method can be found in Application Notes [720008117EN](#) and [720008320EN](#).

The protocol for extraction of PFAS from solids, biosolids, and tissue samples following EPA 1633 is listed in Figure 11.

Sample preparation

The following supplies will be required for sample preparation:

- SPE manifold (Part Number [WAT200609](#))
- Vacuum pump (Part Number [725000417](#))
- 6 cc 50 mg GCB, 200 mg Oasis WAX (Oasis GCB/WAX for PFAS Analysis) Cartridges (Part Number 186011112 OR 186011113)
- 60 mL Sample Reservoirs (Part Number [186005587](#))
- Sample reservoir adaptors (Part Number [WAT054260](#))
- Nitrogen sample evaporation system
- Glass wool
- 50 mL polypropylene centrifuge tubes
- 15 mL polypropylene centrifuge tubes
- pH test strips

The following reagents will be required for sample preparation:

- PFAS native standards (Wellington Part Numbers PFAC-MXF, PFAC-MXG, PFAC-MXH, PFAC-MXI, and PFAC-MXJ)
- Isotope labelled PFAS internal standards (Wellington Part Numbers MPFAC-HIF-ES and MPFAC-HIF-IS)
- Methanol, LC-MS/MS grade, evaluated for low PFAS background
- Water, LC-MS/MS grade, evaluated for low PFAS background
- Acetonitrile, LC-MS/MS grade, evaluated for low PFAS background
- Ammonium Hydroxide, approximately 25–30% ammonia (equivalent to Millipore Sigma cat # AX1308-7)
- Potassium Hydroxide (KOH)
- Formic acid
- Acetic acid

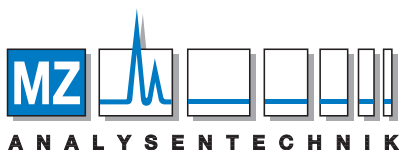
The following solutions will be required for sample preparation:

- 50% formic acid
- 0.3 M formic acid
- 0.1 M formic acid
- 1:1 0.1 M formic acid:methanol
- 1% (v/v) ammonium hydroxide in methanol
- 0.3 % (v/v) ammonium hydroxide in methanol
- 0.05 M KOH in methanol

Instrument setup

For this analysis method, a tandem quadrupole mass spectrometer is required. At a minimum, Xevo TQ-S micro is required, although the TQ-Absolute is recommended. Analysis information in the application note is with the Xevo TQ-Absolute.

An LC modified with the PFAS Kit is required for analysis. See Section IV for details on the PFAS Kit.



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