



Meet Requirements of EPA Method 537.1 PFAS **Analysis with Contaminant-Free Workflow**

Equipment

By Landon Wiest

Abstract

Theubiquitous nature of PFAS in the environment makes ensuring a contaminant-free workflow essential. In this application note, we demonstrate that Resprep S-DVB SPE cartridges and related sample preparation products are consistently free of background interferences. In addition, a PFAS delay column effectively removes any contamination that may be present in the instrument. Using the materials and procedure presented here, EPA Method 537.1 requirements for cleanliness, accuracy, and precision were reliably met.

Introduction

Per- andpolyfluoroalkyl substances (PFAS) are being analyzed more now than ever before due to growing concern about human exposure and potential adverse health effects. Their persistent nature and widespread use across many industries and in diverse products, ranging from non-stick kitchenware, weatherproof clothes, and aqueous film-forming foam (AFFF) to food packaging coatings, has made them essentially ubiquitous contaminants worldwide. Accordingly, testing of soils, wastewater, drinking water, and other environmental matrices, along with PFAS testing in foods, continues to increase.

In 2020, the U.S. EPA released Method 537.1 revision 2 [1] for PFAS analysis in drinking water. The method specifies that sample prepara-

tion must be performed using styrene-divinylbenzene (S-DVB) SPE cartridges and that deviation in the extraction procedure is not allowed. Because background PFAS contaminants can leach out of materials anywhere in the sample pathway and interfere with target compound analysis, labs must demonstrate acceptable background, accuracy, and precision results each time a new lot of supplies is used. In addition to this initial qualification, routine blank and QC sample analysis is required to ensure continued performance.

In a typical workflow, the sample comes in contact with multiple surfaces and substances, each of which can be possible sources of PFAS contamination or retention. These include collection vessels, chemicals (Trizma base, solvents, mobile phases, etc.), pipettes, SPE products, manifolds or automated systems, tubing, filters, vials, vial caps, and components within the LC. Even when care is taken to avoid materials known to leach PFAS, such as PTFE, or to which target analytes may adhere, such as glass, the use of clean, high-quality consumables will help prevent downtime due to system suitability failures.

Here, we followed U.S. EPA Method 537.1 and demonstrated a contaminant-free workflow that meets the stringent method requirements. While this data set is based on Method 537.1, similar testing to determine if background PFAS are present is strongly recommended for other PFAS methods [2] due to the pervasiveness of PFAS contamination.

Experimental

Calibration Standards and Quality Control Samples

Fox

PFAS analytical, internal, and surrogate standardswere used to create calibration standards and laboratory fortified blanks as directed by EPA 537.1. Eight calibration standards were created from 0.2–50 ppb corresponding to 0.8–200 ppt in drinking water prior to 250-fold concentration, which occurs during sample preparation. Laboratory reagent blanks (LRB) and laboratory fortified blanks (LFB) were used as per EPA 537.1, sections 9.2.2–9.2.4. LFBs spiked at 40 ppt were used to determine the accuracy and precision of the method.



(800) 369-5524 sales@foxscientific.com Pure Chromatography

www.restek.com

Sample Preparation

Analyticaland surrogate standards were added to 250 mL 18.3 MΩ•cm (megohm) ultrapure reagent water for the LFB samples. The LFB samples were kept in polypropylene containers prior to extraction. The materials used for this workflow are detailed in Table I.

PFAS were extracted by using Resprep S-DVB SPE cartridges (6 mL, 500 mg) attached to a Resprep vacuum manifold. Cartridges were

first conditioned using 15 mL methanol followed by 18 mL reagent water, never allowing the bed to dry. Reservoirs were affixed to the SPE cartridges with adaptors to avoid the use of PTFE transfer lines. Use of reservoirs, as set up in Figure 1, made the addition of the samples more convenient. The full sample preparation procedure is described below and in Figure 2.

For extraction, a flow rate of approximately 10–15 mL/min was established, and care was taken to never allow the particle bed to dry

throughout the extraction process. After the samples had passed through the cartridges, we rinsed each sample bottle with two 7.5 mL aliquots of reagent water. After rinsing the sample bottles, the aliquot was used to rinse each sample reservoir as well to ensure that no PFAS of interest in the sample were left behind.

Following extraction, we dried the SPE cartridges by drawing air through them while they were still attached to the vacuum manifold.

After drying, collection tubes were placed in the manifold and two 4 mL aliquots of methanol were passed through each SPE cartridge and collected. The collection tubes were removed from the manifold, and the extract was concentrated to dryness under nitrogen flow while heating the samples at 65 °C.

Once the samples were dry, we added 1 mL 96:4 methanol:water solution and internal standard and then vortexed to ensure proper mixing.

After vortexing, aliquots of the concentrated solution were transferred to polypropylene sample vials and capped with polyethylene caps. Samples were then analyzed via an LC-MS/MS equipped with a PFAS delay column (cat.# 27854) and a Raptor C18 LC column (50 mm x 2.1 mm, 2.7 µm; cat.# 9304A52). Method conditions can be found in Figures 3 and 4.

Figure 1: Sample preparation setup using sample reservoirs attached to Resprep S-DVB SPE cartridges mounted on a vacuum manifold.





2 www.restek.com



(800) 369-5524 sales@foxscientific.com

www.foxscientific.com



Table I: Sample preparation materials used for Method 537.1 PFAS analysis.

Description	Restek Cat.#
Resprep S-DVB SPE cartridges (6 mL, 500 mg)	28937
Resprep vacuum manifold (12 or 24 port)	28298-VM, 28299-VM*
Reservoirs (polypropylene)	26015
Connectors (polypropylene)	26007
Vials (polypropylene)	23245
Vial caps (polyethylene)	23247

*A 12-port manifold was used in this study, but either manifold can be employed because the SPE cartridges do not contact the manifold directly; they only contact the quick-replace disposable liners that are used for both manifold styles.







Results and Discussion

Method 537.1 PFAS analysis in drinking water requirements include initial and ongoing demonstration of low system background and suitable accuracy and precision to ensure that the workflow, from sample collection through analysis, is free of contamination and qualified for use. To verify cleanliness, laboratory reagent blanks were prepared for three different lots of Resprep S-DVB SPE cartridges according to the method. As shown in Figure 3, all lots were free of contamination and no target analytes were detected, satisfying the low system background requirement of section 9.2.2. LOD values were 0.2–5 ppt, defined as a signal-to-noise ratio >3 for each compound. LOQ values were estab- lished as signal-to-noise ratios >10 and were found to be 0.5–10 ppt across the range of target analytes.

In addition to demonstrating the consistent cleanliness of Resprep S-DVB cartridges across multiple lots, this experiment proved that no

interfering contaminants leached from any other component in the entire sample prep workflow listed in Table I (vacuum manifold system, vials, and caps, etc.). LC instruments can also contribute background contamination, but none was present in these analyses because the LC was plumbed with PEEK or stainless-steel tubing, and a PFAS delay column was installed. A PFAS delay column prevents interference from any PFAS leaching out of components in the LC system by trapping them and delaying their elution until after the sample analytes have eluted. Retention on a PFAS delay column is strong enough to prevent breakthrough even with extended equilibration times. [3,4]





4 www.restek.com



www.foxscientific.com

Recovery performance for Method 537.1 PFAS analysis was assessed using four laboratory fortified blanks prepared at 40 ppt. A representative LFB chromatogram is presented in Figure 4, which shows that good peak efficiency, selectivity, and asymmetry were obtained. To meet the method requirements for recovery, precision values across the LFB replicates must have %RSD <20%, and accuracy results for the same LFB samples must be within ±30% of the true value. The data presented in Table II demonstrate that method precision and accuracy (Section 9.2.3. and Section 9.2.4. of Method 537.1, respectively) requirements were easily met. Good recoveries indicate that the target analytes were not lost due to adhesion to surfaces encountered in the sample pathway.







Analyte	%RSD*	Mean Recovery**
Perfluorobutanesulfonic acid (PFBS)	11.9%	91.1%
Perfluorohexanoic acid (PFHxA)	7.96%	99.4%
Hexafluoropropylene oxide dimer acid (HFPO-DA)	6.34%	94.4%
Perfluoroheptanoic acid (PFHpA)	4.19%	92.7%
Perfluorohexanesulfonic acid (PFHxS)	11.9%	89.4%
4,8-Dioxa-3H-perfluorononanoic acid (ADONA)	5.18%	96.6%
Perfluorooctanoic acid (PFOA)	5.21%	91.6%
Perfluorononanoic acid (PFNA)	6.79%	97.2%
Perfluorooctanesulfonic acid (PFOS)	6.78%	87.8%
9-Chlorohexadecafluoro-3-oxanone-1-sulfonic acid (9CI-PF3ONS)	8.59%	85.1%
Perfluorodecanoic acid (PFDA)	6.96%	93.6%
N-methyl perfluorooctanesulfonamidoacetic acid (N-MeFOSAA)	10.1%	82.8%
N-ethyl perfluorooctanesulfonamidoacetic acid (N-EtFOSAA)	16.5%	106%
Perfluoroundecanoic acid (PFUnA)	2.30%	97.5%
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF30UdS)	5.47%	87.6%
Perfluorododecanoic acid (PFDoA)	5.7 3%	99.0%
Perfluorotridecanoic acid (PFTrDA)	12.7%	89.1%
Perfluorotetradecanoic acid (PFTA)	8.90%	89.7%

**Recovery must within ±30% of the true value.

Conclusion

Thedata presented here clearly demonstrate that Resprep S-DVB SPE cartridges and the other sample preparation products used in this workflow for EPA Method 537.1 PFAS analysis were consistently free of background contaminants. In addition, use of a PFAS delay column effectively removed any PFAS background contamination that was potentially present in the LC instrument. Based on the results shown here, use of these workflow consumables will reduce interfering background contamination, leading to reliable system qualification and more accurate analysis and reporting.

References

[1] J. Shoemakerand D. Tettenhorst, U.S. EPA Method 537.1 Rev 2., Method 537.1 Determination of selected per- and polyflourinated alkyl substances in drinking water by solid phase extraction and liquid chromatography/tandem mass spectrometry (LC/MS/MS), 2020. https://cfpub.epa.gov/si/si_public_file_download.cfm?p_download_id=539984&Lab=CESER [2] Restek Corporation, Product guide for PFAS analysis: a methods-based reference to lab supplies for PFAS testing (EVAR3498-UNV), (2021). https://www.restek.com/globalassets/pdfs/literature/ evar3498-unv.pdf

[3] Restek Corporation, Eliminate the impact of instrument-related PFAS interferences by using a delay column, (2019). https://www.restek.com/Technical-Resources/Technical-Library/Environmental/enviro_EVAR3001-UNV.

[4] Restek, PFAS Analysis – Why a Delay Column is Important, Video. https://www.restek.com/Technical-Resources/Technical-Library/Video-Library/PFAS-Analysis-Why-a-Delay-Column-is-Important



6 www.restek.com



Raptor C18 LC Columns (USP L1)

- A traditional end-capped C18 ideal for general-purpose use in reversed-phase chromatography.
- Wide pH range (2–8) provides excellent data quality for many applications, matrices, and compounds.
- Offers the highest hydrophobic retention of any Raptor phase.
- Part of Restek's Raptor LC column line featuring 1.8, 2.7, and 5 µm SPP core-shell silica.

ID	Length	qty.	cat.#
1.8 µm Particles			
	30 mm	еа	9304232
2.4	50 mm		9304252
2.1 mm	100 mm	еа	9304212
	150 mm		9304262
2.0	50 mm	еа	930425E
3.0 mm	100 mm	•	930421E
2.7 µm Particles		еа	
	30 mm	еа	9304A32
24	50 mm	ea	9304A52
2.1 mm	100 mm	еа	9304A12
	150 mm	ea	9304A62
	30 mm	еа	9304A3E
	50 mm		9304A5E
3.0 mm	100 mm	еа	9304A1E
	150 mm		9304A6E
	30 mm	еа	9304A35
	50 mm	•	9304A55
4.6 mm	100 mm	еа	9304A15
	150 mm	•	9304A65
5 µm Particles		ea	
_ •	50 mm	еа	9304552
2.1 mm	100 mm	ea	9304512
	150 mm	еа	9304562
	30 mm	ea	930453E
	50 mm	еа	930455E
3.0 mm	100 mm	ea	930451E
	150 mm	еа	930456E
	50 mm	ea	9304555
	100 mm	ea	9304515
4.6 mm	150 mm	ea	9304565
	250 mm	еа	9304575



Stationary Phase Category: C18, octadecylsilane (L1) Ligand Type: End-capped C18 Particle: 1.8 µm, 2.7 µm, or 5 µm superficially porous silica (SPP or "core-shell") Pore Size: 90 Å Carbon Load: 9% (1.8 µm), 7% (2.7 µm), 5% (5 µm) End-Cap: yes Surface Area: 125 m2/g (1.8 µm), 130 m2/g (2.7 µm), or 100 m2/g (5 µm) Recommended Usage: pH Range: 2.0–8.0 Maximum Temperature: 80 °C Maximum Temperature: 80 °C Maximum Temperature: 400 bar/5,000 psi* (1.8 µm), 600 bar/8,700 psi (2.7 µm); 400 bar/5,800 psi (5 µm) * For maximum lifetime, recommended maximum pressure for 1.8 µm particles is 830 bar/12,000 psi. **Proper ties:**

- Compatible with moderately acidic to neutral mobile phases (pH 2–8).
- Excellent data quality in food, environmental, bioanalytical, and other applications.

Switch to a C18 when:

- You need a general-purpose column for reversed-phase chromatography.
- You need to increase retention of hydrophobic compounds.

PFAS Delay Column

• Traps system-related PFAS, preventing interference and ensuring accurate trace-level analysis of PFAS in samples.

ea . ea

ea

ea

- Universal compatibility; works with
 - Any HPLC or UHPLC up to 15,000 psi (1034 bar),
 - Both FPP and SPP analytical columns,
 - All stationary phases.
- Highly retentive of system-related PFAS; no breakthrough even with extended equilibration times.
- Easy installation with standard fittings.

ID	Length	qty.	cat.#
5 µm Particles			
2.1 mm	50 mm	ea.	27854





27854

Particle: 5 µm, spherical, fully porous pH Range: 2.5 to 8 Maximum Temperature: 80 °C Maximum Pressure: 1034 bar/15,000 psi





26007

Resprep SPE Tube Parts & Accessories

Resprep tubes, frits, caps, and connectors for your method development needs.

Description	Material	Porosity	Volume	qty.	cat.#
	polypropylene		1 mL	50-pk.	26010
	polypropylene		3 mL	50-pk.	26011
с , т.	polypropylene		6 mL	50-pk.	26012
Empty Tubes	polypropylene		15 mL	50-pk.	26013
	polypropylene		sample reservoir, 25 mL	12-pk.	26014
	polypropylene		sample reservoir, 75 mL	12-pk.	26015
	polyethylene	20	1 mL, 6 mm	100-pk.	26016
	polyethylene	μm	3 mL, 9 mm	100-pk.	26017
F 11	polyethylene	20	6 mL, 1.2 cm	100-pk.	26018
Frits	polyethylene	μm	15 mL, 1.6 cm	100-pk.	26019
	polyethylene	20	25 mL, 2.0 cm (For 20 mL packed	100-pk.	26020
	nolvethylene	μm	tubes.)	12-nk	26001
	nolvethylene	20	1 mL	12-pk.	20001
	polyetilylelle	μm	3 mL	12-pk.	20002
Tube Caps	polyethylene	20	6 mL	т2-рк.	20003
I	polyethylene	20	15 mL	12-pk.	26004
	polyethylene		25 mL (For 20 mL packed tubes.)	12-pk.	26005
Fomale Luer End Cana	polypropylene	μm	universal	12-pk.	26000
Female Luer End Caps	polypropylene		1, 3, 6, 10, or 15 mL		26007
Commentant			12, 25 mL		26008
Connectors	polypropylene		60 mL	 	26009



28298-VM

Resprep Quick-Replace SPE Vacuum Manifolds (12- or 24-Port)

- Disposable, quick-replace valve liners ensure a clean flow path and eliminate crosscontamination of samples extracted on the same port.
- Individual screw-type valves in each SPE port provide precise flow control.
- Easily modified sample collection rack supports a wide variety of collection vessels.
- Screw-type, solvent-resistant vacuum gauge and bleed valve offer better sealing and vacuum control.
- Compatible with any standard male luer end SPE cartridge.

Description	qty.	cat.#
Resprep QR-12 Quick-Replace vacuum manifold Includes: cover with 12 flow control valves & gasket; glass basin with vacuum gauge & valve assembly: collection rack (base, 3 support rods, center plate, 10 mm test tube plate, 12 clips); plate for 16 mm test tubes; 12 test tubes (10 x 75 mm); 12 liner guides (stainless steel); 100 quick-replace disposable liners (PTFE)	kit	28298-VM
Resprep QR-24 Quick-Replace vacuum manifold Includes: cover with 24 flow control valves & gasket; glass basin with vacuum gauge & valve assembly; collection rack (base, 2 support rods, center plate, 10 mm test tube plate, 8 clips); plate for 16 mm test tubes; 24 test tubes (10 x 75 mm); 24 liner guides (stainless steel); 100 quick-replace disposable liners (PTFE)	kit	28299-VM





Resprep S-DVB SPE Cartridge (Reversed Phase)

- High-purity material with highest reproducibility and lowest blank values due to an optimized manufacturing process.
- Excellent recovery rates, especially for the enrichment of pharmaceuticals and active ingredients, due to the spherical particle shape, homogeneous surface, and optimized pore structure.
- Hydrophobic styrene-divinylbenzene (SDVB) copolymer, pH stability 1–14.
- Recommended analytes: PFAS in drinking water; pharmaceuticals/active ingredients from tablets, creams, and water/wastewater; drugs from blood, plasma, serum, and urine; trace analysis of herbicides, pesticides, PAHs, PCBs, and phenols from water.
- Ideal for EPA Method 537.1 PFAS in drinking water; meets method performance requirements.

Description	Packing	Volume	qty.	cat.#
Resprep S-DVB	500 mg spherical styrene-divinylbenzene (SDVB) copolymer	6 mL	30-pk.	28937

Limited-Volume 2.0 mL, 9 mm Screw-Thread Polypropylene Vials

• Available in 1.5 mL or 700 µL volumes. • Limited-volume design fits all 2.0 mL, 12 x 32

mm, vial-based autosamplers. • Compatible with all 9 mm screw-thread caps. • PTFE-free

-ideal for PFAS analysis (e.g., EPA 537) and other PFAS-sensitive methods. cat.# 23242 23245 23243 23246

Description	Туре	Volume	Color	Size	qty.
	9 mm Screw-Thread	1.5 mL	Clear	12 x 32 mm	100-pk.
Limited-Volume 2.0 mL, 9 mm Screw-	9 mm Screw-Thread	1.5 mL	Clear	12 x 32 mm	1000-pk.
Thread Polypropylene Vials	9 mm Screw-Thread	700 µL	Clear	12 x 32 mm	100-pk.
	9 mm Screw-Thread	700 µL	Clear	12 x 32 mm	1000-pk.

2.0 mL, 9 mm Solid-Top Polyethylene Caps

- Compatible with all 9 mm screw-thread vials.
- Molded, 10 mil, solid, pierceable cap.
- PTFE-free—ideal for PFAS analysis (e.g., EPA 537) and other PFAS-sensitive methods.

Description	Туре	Cap Size	Color	qty.	cat.#
2.0 ml .0 mm Solid Top Polyothylong Conc	Screw-Thread	9 mm	Clear	100-pk.	23244
	Screw-Thread	9 mm	Clear	1000-pk.	23247





Note: Polypropylene vials and caps prevent sample contamination from PTFE-coated septa. However, since polypropylene caps do not reseal, evaporation occurs after injection. Multiple injections from the same vial are therefore not possible.





www.restek.com

RESTEK



9



25097

Low-Pressure Slip-On Inlet Filter for Mobile Phase Reservoir

A 316 stainless-steel tip with a Tefzel collar seals to a corrosion-resistant 316 stainless-steel filter element. The slip-on filter easily attaches to the pump inlet line, without the use of wrenches. The universal 1/8" OD tip accommodates standard PTFE tubing inner diameters. The cylindrical filter is standard 10 μ m porosity. Fits Altex, ISCO, LDC, Varian, Waters, PerkinElmer, and other pumps.

Description	qty.	cat.#
Slip-On Inlet Filter	ea.	25008

Survival Kit for HPLC, Stainless Steel

For start-up and maintenance in all HPLC systems.

The stainless-steel survival kit contains a wide range of tubing, fittings, and tools necessary to set up and maintain your HPLC system: a selection of lengths and IDs of 1/16" tubing, nuts, ferrules, a ValvTool wrench, and a zero-dead-volume union.

Kit includes:

- HPLC Capillary Tubing, SS, ¹/₁₆" x 0.005" x 5 cm, 3-pk.
- HPLC Capillary Tubing, SS, ¹/₁₆" x 0.005" x 10 cm, 3-pk.
- HPLC Capillary Tubing, SS, ¹/₁₆" x 0.005" x 20 cm, 3-pk.
- HPLC Capillary Tubing, SS, ¹/₁₆" x 0.005" x 30 cm, 3-pk.
- HPLC Capillary Tubing, SS, ¹/₁₆" x 0.007" x 5 cm, 3-pk.
- HPLC Capillary Tubing, SS, ¹/₁₆" x 0.007" x 10 cm, 3-pk.
- HPLC Capillary Tubing, SS, ¹/₁₆" x 0.007" x 20 cm, 3-pk.
- HPLC Capillary Tubing, SS, ¹/₁₆" x 0.007" x 30 cm, 3-pk.
- HPLC Capillary Tubing, SS, ¹/₁₆" x 0.010" x 5 cm, 3-pk.
- HPLC Capillary Tubing, SS, ¹/₁₆" x 0.010" x 10 cm, 3-pk.
- HPLC Capillary Tubing, SS, ¹/₁₆" x 0.010" x 20 cm, 3-pk.

• HPLC Capillary Tubing, SS, $^{1}/_{16}$ " x 0.010" x 30 cm, 3-pk. • HPLC Capillary Tubing, SS, $^{1}/_{16}$ " x 0.020" x 5 cm, 3-pk. • HPLC Capillary Tubing, SS, $^{1}/_{16}$ " x 0.020" x 10 cm, 3-pk. • HPLC Capillary Tubing, SS, $^{1}/_{16}$ " x 0.020" x 20 cm, 3-pk. • HPLC Capillary Tubing, SS, $^{1}/_{16}$ " x 0.020" x 30 cm, 3-pk. • $^{1}/_{16}$ " Rheodyne Style Nut, 10-pk. • $^{1}/_{16}$ " Rheodyne Style Ferrule, 10-pk. • ValvTool Wrench, ea. • Ferrules, $^{1}/_{16}$ " Stainless Steel, 10-pk. • Nuts, $^{1}/_{16}$ " Stainless Steel, 10pk. • Zero-Dead-Volume Internal Union, ea.

Description	qty.	cat.#
Survival Kit for HPLC	kit	25097





Questions? Contact us or your local Restek representative (www.restek.com/contact-us).

Restek patents andtrademarks are theproperty ofRestekCorporation. (Seewww.restek.com/Patents-Trademarksforfull list.) Othertrademarksin Restek literatureoronits website are the property of their respective owners. Restek registered trademarks are registered in the U.S. and may also be registered in other countries. To unsubscribe from future Restek communications or to update your preferences, visit www.restek.com/subscribe To update your status with an authorized Restek distributor or instrument channel partner, please contact them directly.

 $\ensuremath{\mathbb{C}}$ 2021 Restek Corporation. All rights reserved. Printed in the U.S.A.

www.restek.com

