Analyze EPA Method 533 PFAS Reliably with Resprep WAX SPE

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Abstract

Low-level analysis of per-and polyfluoroalkyl substances (PFAS) requires high sensitivity and low background levels throughout the entire workflow from sample collection, transportation, and storage to analytical instrumentation and sample preparation. Resprep polymeric WAX SPE cartridges allow for accurate low-level quantitation of PFAS compounds in drinking water while meeting the requirements of EPA Method 533.

Introduction

Due to the low levels required by many regulatory agencies, the analysis of PFAS in drinking water often employs solid phase extraction (SPE) coupled with LC-MS/MS to reach low detection limits. EPA Method 533 [1] uses weak anion exchange (WAX) SPE to better retain short-chain acid compounds and obtain part-per-trillion (ppt) detection limits. However, due to the ubiquitous nature of PFAS compounds, the selection and screening of all materials and consumables that contact samples, including SPE cartridges, is critical for maintaining a clean background and allowing continued low-level analysis. In this assessment, Resprep WAX cartridges were used with a Resprep QR-12 vacuum manifold to extract samples for EPA Method 533 PFAS analysis using a Shimadzu Nexera LC and 8045 MS/MS.

Related Products

- *PFAS delay column (cat. # 27854)*
- *Force C18 1.8 µm, 50 mm x 2.1 mm (cat.# 9634252)*
- *EPA 533 PFAS calibration standard (cat.# 30736)*
- *Resprep polymeric WAX SPE cartridges (cat. # 28291)*
- *Resprep QR-12 vacuum manifold (cat. # 28298-VM)*
- *Quick replace liners (cat. # 28310-VM)*
- *Resprep sample delivery system (cat. # 26250)*
- *Polypropylene vials (cat.# 23246)*
- *Polyethylene caps (cat.# 23247)*
- *Chemker vacuum pump (cat.# 27427)*

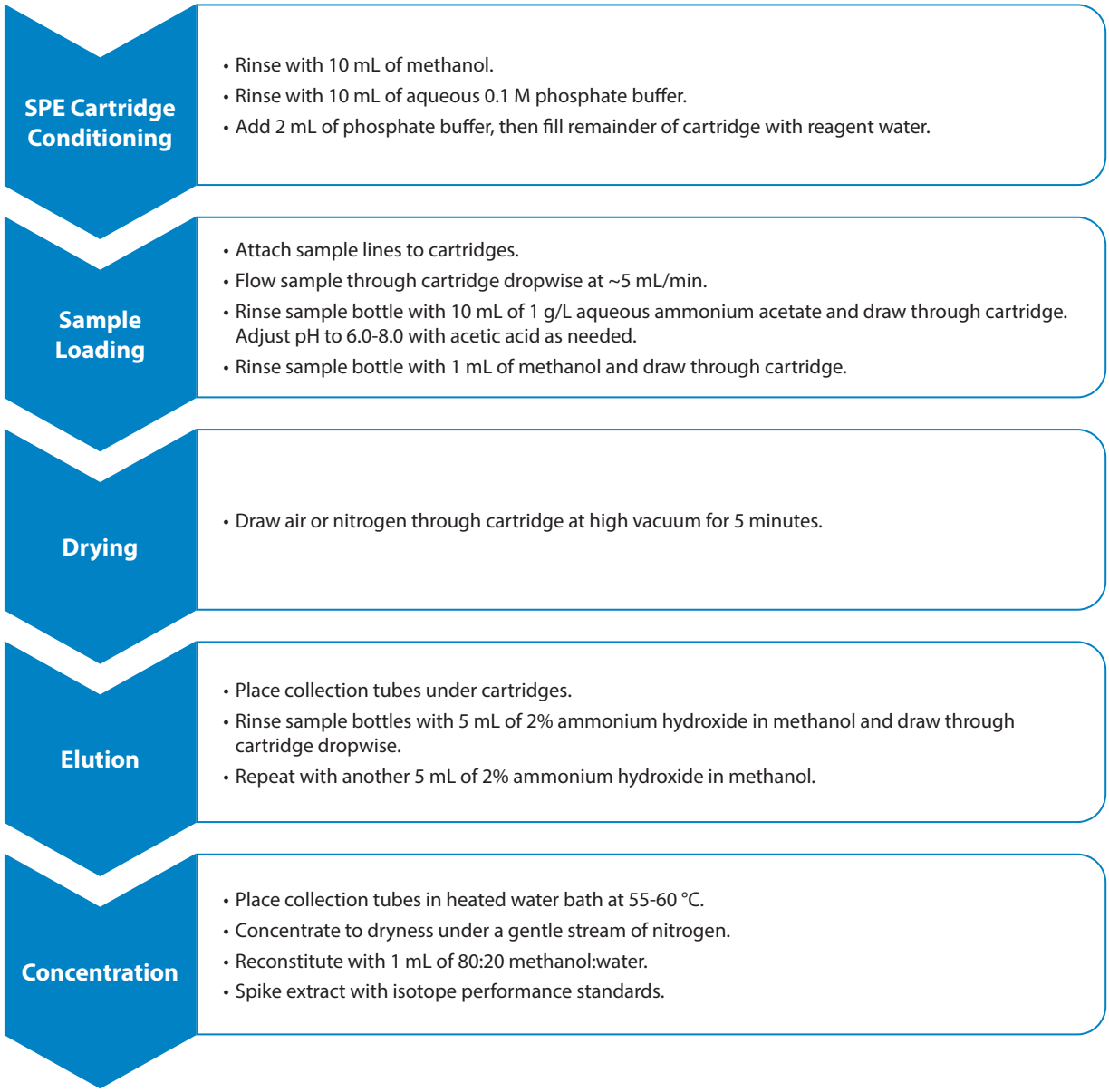
Experimental

Sample Preparation

Spikes and blanks were prepared in polypropylene bottles using 250 mL deionized water spiked with isotope dilution standards as per EPA Method 533, Section 7.16.1. The 6 mL SPE cartridges, which contained 500 mg of 30 µm WAX (cat.# 28291), were placed on a Resprep QR-12 vacuum manifold (cat.# 28298-VM) that was fitted with quick-replace liners (cat.# 28310-VM). Resprep sample delivery system lines (cat.# 26250) were used to transfer the samples to the SPE cartridges. While both the quick-replace liners and sample delivery lines contain PTFE, which can be a potential source of PFAS contamination, investigation of blanks taken using all lines and all ports on the manifold showed no detectable PFAS leaching. Thorough and regular blank checking of all SPE components and solvents, especially when using new lots of materials, is recommended to ensure that background contamination is below acceptable levels.

After preparation of the samples and setup of the SPE system, the samples were extracted following the instructions in EPA Method 533, Section 11.4, which is summarized in Figure 1.

Figure 1: Sample Preparation Procedure for EPA Method 533 PFAS Analysis



Analytical System

After extraction, the samples were analyzed by LC-MS/MS under the EPA Method 533 PFAS analysis conditions shown below. The use of a PFAS delay column is important to prevent any PFAS contamination upstream of the injector from coeluting with the samples. Thorough blank checking of the analytical system was performed and showed no detectable PFAS contamination.

Instrument Conditions for EPA Method 533 PFAS Analysis

System: Shimadzu Nexera X2/Shimadzu LCMS-8045

Columns:

- PFAS delay column (cat.# 27854)
- Analytical column: Force C18, 1.8 µm x 50 mm x 2.1 mm (cat.# 9634252)

Injection volume: 3 µL

Mobile phase A: Water, 5 mM ammonium acetate

Mobile phase B: Methanol

Flow rate: 0.4 mL/min

Temperature: 40 °C

Gradient:

Time (min)	%B
0	20
6	95
6.6	95
6.61	20
7.5	20

Ion source: electrospray

Ion mode: ESI-

Mode: MRM

Method Detection Limits (MDL)

The method detection limit was calculated from the analysis of seven blank replicates and seven low-level spikes, as outlined in EPA's Definition and Procedure for the Determination of the Method Detection Limit, Revision 2 [2]. The spikes were made at 0.5 ng/L using a 2 µg/mL stock solution of native PFAS compounds (EPA 533 PFAS calibration standard, cat.# 30736). The standard deviations of the spike and blank results were multiplied by the Student's *t*-value of 3.143 to determine the MDL, and the higher of the results between the spikes and blanks was selected as the MDL.

Accuracy and Precision

Accuracy and precision were determined by analyzing five replicate 10 ng/L spikes. The accuracy of the spikes was calculated and compared to the recovery limits of 70-130% from EPA Method 533. The relative standard deviations of the spike results were also determined.

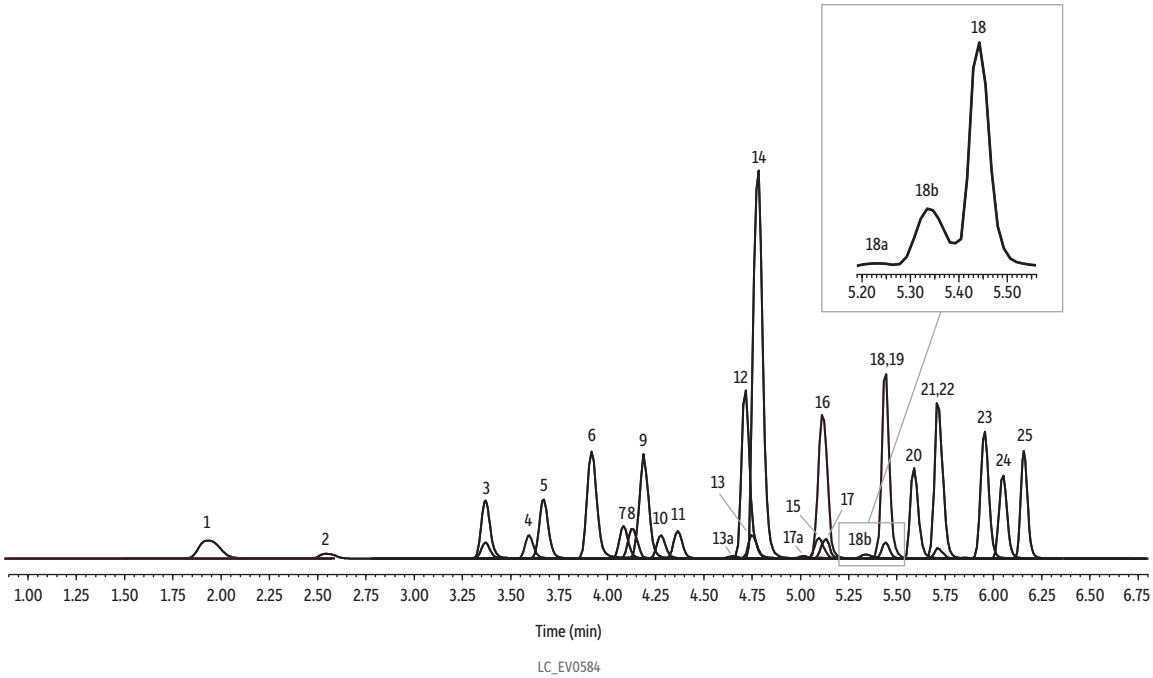
The recovery of the isotope dilution standards was calculated from the spike replicates and compared to the recovery limits of 50-200% from EPA Method 533 for PFAS analysis.

Results and Discussion

Good chromatographic results were obtained for all compounds, as shown in Figure 2. The MDL, accuracy, and precision results for native PFAS analytes are shown in Table I. The calculated MDLs were below the reporting limits shown in Table 7 in EPA Method 533, and the accuracy of the 10 ng/mL spikes ranged from 84 to 119% of the spiked value, well within the 70-130% recovery required by the method. The spikes showed good precision as well, with the results being ≤20% RSD.

Similarly, the recoveries for the isotope dilution standards were also within 20% of the true value and had precision ≤20% RSD. The results are shown in Table II.

Figure 2: Analysis of 25 ng/mL Standard for EPA Method 533



Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion
1. Perfluoro-*n*-butanoic acid (PFBA)	1.933	25	213	169
2. Perfluoro-3-methoxypropanoic acid (PFMPA)	2.544	25	229	85
3. Perfluoro-*n*-pentanoic acid (PFPeA)	3.369	25	263	219
4. Perfluorobutanesulfonic acid (PFBS)	3.594	25	299	80
5. Perfluoro-4-methoxybutanoic acid (PFMBA)	3.669	25	279	85
6. Perfluoro(2-ethoxyethane)sulfonic acid (PFEESA)	3.919	25	315	135
7. Perfluoro-3,6-dioxaheptanoic acid (NFDHA)	4.084	25	295	201
8. 1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	4.129	25	327	307
9. Perfluorohexanoic acid (PFHxA)	4.189	25	313	269
10. Perfluoro-1-pentanesulfonic acid (PFPeS)	4.278	25	349	80
11. Perfluoro(2-methyl-3-oxahexanoic) acid (HFPO-DA)	4.365	25	285	169
12. Perfluoroheptanoic acid (PFHpA)	4.715	25	363	319
13. Perfluoro-1-hexanesulfonic acid (PFHxS)	4.750	25	399	80
14. 4,8-dioxa-3H-perfluorononanoic acid (ADONA)	4.78	25	277	251
15. 1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	5.096	25	427	407
16. Perfluoro-1-heptanesulfonic acid (PFHpS)	5.132	25	449	80
17. Perfluorooctanoic acid (PFOA)	5.115	25	413	369
18. Perfluorooctanesulfonic acid (PFOS)	5.441	25	499	80
19. Perfluorononanoic acid (PFNA)	5.439	25	463	419
20. 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	5.588	25	531	351
21. 1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	5.712	25	527	507
22. Perfluorodecanoic acid (PFDA)	5.712	25	513	469
23. Perfluoroundecanoic acid (PFUnA)	5.954	25	563	519
24. 11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	6.049	25	631	451
25. Perfluorododecanoic acid (PFDoA)	6.158	25	613	569

Column	Force C18 (cat.# 9634252)
Dimensions:	50 mm x 2.1 mm ID
Particle Size:	1.8 µm
Pore Size:	100 Å
Temp.:	40 °C
Standard/Sample	EPA 533 PFAS calibration standard (cat.# 30736)
Diluent:	80:20 Methanol:water
Conc.:	25 ng/mL
Inj. Vol.:	3 µL
Mobile Phase	
A:	Water, 5 mM ammonium acetate
B:	Methanol

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	80	20
6.00	0.4	5	95
6.60	0.4	5	95
6.61	0.4	80	20
7.50	0.4	80	20

Detector	Shimadzu 8045
Ion Source:	Electrospray
Ion Mode:	ESI-
Mode:	MRM
Instrument	Shimadzu Nexera X2
Notes	Branched isomers for PFOA, PFOS, and PFHxS labeled as peak number "a" and "b." PFAS delay column used (cat.# 27854).

Table I: Results from MDL, Precision, and Accuracy Experiments for Native PFAS

Compound	Abbreviation	MDL (ng/L)	Accuracy (%)	%RSD
Perfluorobutanoic acid	PFBA	8.5	95	20
Perfluoro-3-methoxypropanoic acid	PFMPA	0.2	119	20
Perfluoropentanoic acid	PFPeA	0.2	114	10
Perfluorobutane sulfonate	PFBS	0.3	94	14
Perfluoro-4-methoxybutanoic acid	PFMBA	1.1	88	8
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	0.2	84	12
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	0.2	103	15
1H, 1H, 2H,2H-perfluorohexane sulfonate	4:2 FTS	0.3	97	19
Perfluorohexanoic acid	PFHxA	0.1	98	11
Perfluoropentane sulfonate	PFPeS	0.2	96	13
Hexafluoropropylene oxide dimer acid	HFPO-DA	1.0	89	13
Perfluoroheptanoic acid	PFHpA	0.4	101	17
Perfluorohexane sulfonate	PFHxS	0.3	110	15
4,8-Dioxa-3H-perfluorononanoic acid	ADONA	1.1	100	7
1H, 1H, 2H,2H-perfluorooctane sulfonate	6:2 FTS	0.7	105	8
Perfluoroheptane sulfonate	PFHpS	0.5	101	12
Perfluorooctanoic acid	PFOA	0.2	112	9
Perfluorooctane sulfonate	PFOS	0.3	109	5
Perfluorononanoic acid	PFNA	0.5	101	10
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	0.2	93	7
Perfluorodecanoic acid	PFDA	0.4	111	6
1H, 1H, 2H,2H-perfluorodecane sulfonate	8:2 FTS	0.6	109	5
Perfluoroundecanoic acid	PFUnA	0.8	110	6
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	0.3	95	11
Perfluorododecanoic acid	PFDoA	1.1	94	7

Table II: Results from Precision and Accuracy Experiments for Isotope Dilution Standards.

Compound	Abbreviation	Accuracy (%)	%RSD
Perfluoro-*n*-[1,2,3,4- $^{13}C_4$]butanoic acid	$^{13}C_4$-PFBA	111	13
Perfluoro-*n*-[1,2,3,4,5- $^{13}C_5$]pentanoic acid	$^{13}C_5$-PFPeA	118	14
Sodium perfluoro-1-[2,3,4- $^{13}C_3$]butanesulfonate	$^{13}C_3$-PFBS	108	16
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2- $^{13}C_2$]hexane sulfonate	$^{13}C_2$-4:2FTS	97	12
Perfluoro-*n*-[1,2,3,4,6- $^{13}C_5$]hexanoic acid	$^{13}C_5$-PFHxA	115	14
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy$^{13}C_3$-propanoic acid	$^{13}C_3$-HFPO-DA	89	14
Perfluoro-*n*-[1,2,3,4- $^{13}C_4$]heptanoic acid	$^{13}C_4$-PFHpA	110	14
Sodium perfluoro-1-[1,2,3- $^{13}C_3$]hexanesulfonate	$^{13}C_3$-PFHxS	114	14
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2-$^{13}C_2$]-octane sulfonate	$^{13}C_2$-6:2FTS	92	7
Perfluoro-*n*-[$^{13}C_8$]octanoic acid	$^{13}C_8$-PFOA	113	14
Sodium perfluoro-[$^{13}C_8$]octanesulfonate	$^{13}C_8$-PFOS	102	7
Perfluoro-*n*-[$^{13}C_9$]nonanoic acid	$^{13}C_9$-PFNA	101	9
Perfluoro-*n*-[1,2,3,4,5,6- $^{13}C_6$]decanoic acid	$^{13}C_6$-PFDA	111	12
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2- $^{13}C_2$]-decane sulfonate	$^{13}C_2$-8:2FTS	118	14
Perfluoro-*n*-[1,2,3,4,5,6,7- $^{13}C_7$]undecanoic acid	$^{13}C_7$-PFUnA	114	13
Perfluoro-*n*-[1,2- $^{13}C_2$]dodecanoic acid	$^{13}C_2$-PFDoA	112	13

Conclusions

EPA Method 533 PFAS analysis in drinking water can be challenging with low-level analysis complicated by background contamination. Resprep WAX cartridges have been shown to provide performance that meets or exceeds the requirements of EPA Method 533, allowing for analysis of PFAS at ng/L levels and lower. Visit www.restek.com/PFAS for additional products, methods, and technical resources.

References

1. U.S. Environmental Protection Agency, Method 533, Determination of per-and polyfluoroalkyl substances in drinking water by isotope dilution anion exchange solid phase extraction and liquid chromatography/tandem mass spectrometry, November 2019. https://www.epa.gov/sites/default/files/2019-12/documents/method-533-815b19020.pdf
2. U.S. Environmental Protection Agency, Definition and procedure for the determination of the method detection limit, revision 2, December 2016. https://www.epa.gov/sites/default/files/2016-12/documents/mdl-procedure_rev2_12-13-2016.pdf

PFAS Delay Column

- Traps system-related PFAS, preventing interference and ensuring accurate trace-level analysis of PFAS in samples.
- Universal compatibility: works with
  - any HPLC or UHPLC up to 15,000 psi (1034 bar);
  - both FPP and SPP analytical columns; and
  - all stationary phases.
- Highly retentive of system-related PFAS; no breakthrough even with extended equilibration times.
- Easy installation with standard fittings.

Catalog No.	Product Name	Units
27854	PFAS Delay Column, 5 µm, 50 x 2.1 mm HPLC Column	ea.

Force C18

- 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.
- High carbon load (20%) offers high hydrophobic retention.

Catalog No.	Product Name	Units
9634252	Force C18, 1.8 µm, 50 x 2.1 mm LC Column	ea.

EPA 533 PFAS Calibration Standard

Contains:
11-chloroeicosafluoro-3-oxaundecane-1sulfonic acid (11Cl-PF3OUdS) (763051-92-9)
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS) (39108-34-4)
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS) (757124-72-4)
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS) (27619-97-2)
4,8-dioxa-3H-perfluorononanoic acid (ADONA) (919005-14-4)
9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS) (756426-58-1)
2-(Heptafluoropropoxy)2,3,3,3tetrafluoropropionic acid (HFPO-DA) (13252-13-6)
Perfluoro-3,6-dioxaheptanoic acid (NFDHA) (151772-58-6)
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA) (113507-82-7)
Perfluoro-5-oxahexanoic acid (PFMPA) (377-73-1)
Perfluoro-4-methoxybutanoic acid (PFMBA) (863090-89-5)
Perfluorobutanesulfonic acid (PFBS) (375-73-5)
Perfluorobutanoic acid (PFBA) (375-22-4)
Perfluorodecanoic acid (PFDA) (335-76-2)
Perfluorododecanoic acid (PFDOA) (307-55-1)
Perfluoroheptanesulfonic acid (PFHpS) (375-92-8)
Perfluoroheptanoic acid (PFHpA) (375-85-9)
Perfluorohexanesulfonic acid (PFHxS)* (355-46-4)
Perfluorohexanoic acid (PFHxA) (307-24-4)
Perfluorononanoic acid (PFNA) (375-95-1)
Heptadecafluorooctanesulfonic acid (PFOS)* (1763-23-1)
Perfluorooctanoic acid (PFOA)* (335-67-1)
Perfluoropentanesulfonic acid (2706-91-4)
Perfluoropentanoic acid (PFPeA) (2706-90-3)
Perfluoroundecanoic acid (PFUnA) (2058-94-8)

*Technical grade compound containing both branched and linear isomers; see certificate for details.

Catalog No.	Concentration	Solvent	Volume	Units
30736	2 µg/mL	Methanol (1 mM KOH)	1 mL/ampul	ea.

Resprep Polymeric SPE Cartridge, WAX

- Silica-free, bonded polymeric material—no unwanted secondary silica interactions, even with basic compounds.
- High surface area—higher loading capacity compared to silica-based sorbents.
- Stable over a wide pH range (0–14)—won't hydrolyze under extreme conditions.
- Water-wettable—streamlined conditioning and equilibration steps drastically reduce solvent usage and sample prep time.
- No flow-rate dependence—maintains retention and capacity after conditioning, even if dried out from vacuum or positive pressure flows.
- Choose cartridges for high loading capacity; 96-well plates for high throughput and automation.

Catalog No.	Product Name	Units
28291	Resprep Polymeric SPE Cartridge, WAX, 6 mL/500 mg, 30 µm	30-pk.

Resprep QR-12 Vacuum Manifold

Catalog No.	Product Name	Units
28298-VM	Resprep QR-12 Vacuum Manifold, includes cover with flow control valves & gasket (cat.# 28316-VM); collection rack (cat.# 28318-VM); plate for 16 mm test tubes (cat.# 28319-VM); 100-pk. Quick Replace liners, PTFE (cat.# 28310-VM); 12-pk. liner guide (cat.# 28312-VM); 12-pk. test tubes (cat.# 28315-VM).	kit

Quick Replace Liners

Catalog No.	Product Name	Units
28310-VM	Quick Replace Liners, PTFE, for Resprep QR Vacuum Manifolds	100-pk.

Resprep Sample Delivery System

- Compatible with Resprep 1, 3, 6, and 15 mL SPE cartridges.
- Six PTFE transfer lines (⅛" OD x 1⁄16" ID x 36" long); each is banded with a different color for easy sample identification.
- Specified in EPA drinking water methods.
- Tested to pH of 1 to ensure no contaminants leach from system.

Catalog No.	Product Name	Units
26250	Resprep Sample Delivery System	6-pk.

Polypropylene Vials

Catalog No.	Product Name	Units
23246	Limited-Volume Screw-Thread Polypropylene Vials, 9 mm, 700 µL, 12 x 32 mm	1000-pk.

Polyethylene Caps

Catalog No.	Product Name	Units
23247	2.0 mL, 9 mm Solid-Top Polyethylene Caps, Screw-Thread, 10 mil thick membrane, Clear	1000-pk.

Chemker Vacuum Pump

Catalog No.	Product Name	Units
27427	Chemker 300 PTFE Vacuum Pump, 18 L/min, AC220 V, 50 Hz	ea.

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