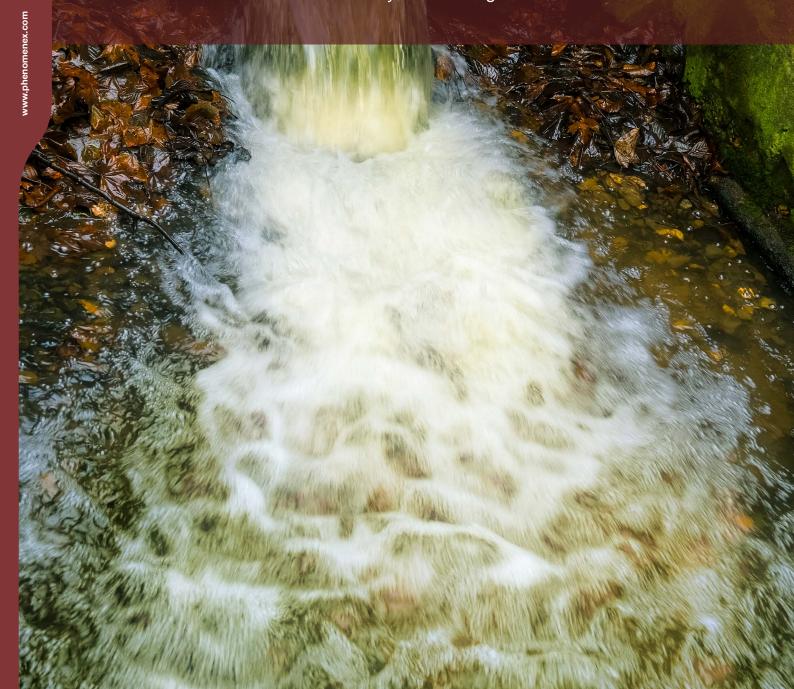
Perfluoroalkyl Substances (PFAS) Testing Guide

Wastewater, Sediment, and Soil

As the PFAS story continues it is becoming more widely recognized that drinking water is not the only environmental media of concern. From its primary sources in fire suppression foams, industrial discharges and consumer products, PFAS is also widely found to occur in soils, sediments, surface water, groundwater and wastewater discharges, illustrating the widespread dispersion and persistence of this unique class of compounds. These discoveries have required the development and application of more advanced sample preparation, chromatography and mass spectrometry techniques to overcome the challenges of matrix and spectral interferences. In this section, two recent applications have been selected to illustrate the analytical challenges of these more difficult matrices.



SPE for DOD QSM 5.3

Per- and Polyfluoroalkyl Substances (PFAS) Extraction by LC-MS/MS Using Strata PFAS for a Stacked Solid Phase Extraction (SPE) Solution

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Overview

PFAS are a class of highly stable synthetic organic compounds used in a wide variety of industrial and commercial applications. They are also highly stable in the environment and strongly bioaccumulate. As a result, they have become ubiquitous throughout the global environment and are often referred to in popular media as "Forever Chemicals". Consequently, PFAS levels need to be tested in drinking water and more recently methods have been developed to measure PFAS in other environmental matrices that require more complex clean-up solutions, such as wastewater, soils and sediments.



The United States Department of Defense (DOD) is dealing with very extensive PFAS contamination owing to the widespread use of PFAS based Aqueous Film Forming Foam (AFFF) used as fire suppression foams at many military installations. As a result, DOD has developed its own PFAS analytical guidelines to deal with the unique environmental monitoring and clean-up challenges found on their installations. These guidelines, contained within the DOD QSM 5.1/5.3 documentation (Department of Defense QSM (osd.mil), feature a unique sample clean-up and concentration approach not found in EPA Methods which are designed only for drinking water application.

DOD QSM 5.1/5.3 specifies the use of a polymeric weak anion exchange (WAX) SPE sorbent in combination with graphitized carbon black (GCB) sorbent for the clean-up of solid samples, soils, biota, sediments, or non-drinking water samples. This can be performed by using two individual tubes of WAX and GCB sorbent that are applied sequentially or the use of dispersive SPE (dSPE) utilizing GCB following the WAX SPE tube extraction. Both methods add time to the clean-up procedure and present the opportunity for loss of analytes and introduction of imprecision. In this communication we describe a significant improvement to the guidelines, Strata PFAS SPE, wherein the two sorbents are contained within a single tube, offering the opportunity for decreased sample processing time and increased accuracy and precision. When comparing recoveries for a small subset of analytes for a WAX SPE and dSPE GCB method vs Strata® PFAS, the recovery is greatly improved for Strata PFAS (Table 1).

Strata PFAS is a stacked single cartridge solution with polymeric WAX and GCB sorbents that functions as a traditional Solid Phase Extraction (SPE) cartridge with a built in polishing step to meet the aforementioned DOD guidelines. This SPE product increases lab productivity and reduces the need for multiple extraction tubes when compared to a traditional two tube method.

Table 2 presents typical analyte recovery data from a routine Laboratory Control Sample (LCS) analyzed by a commercial testing laboratory highly experienced with the performance of DOD QSM 5.1/5.3. The LCS had been spiked with all 32 target analytes at 25 µg/L and was analyzed with a batch of field samples to demonstrate method performance and data acceptability. The recovery data show that all 32 analytes were well within method recovery limits with an average recovery of 98.8 % and a mean recovery of 99.0 %, thereby demonstrating acceptability of the use of Strata PFAS in the performance DOD QSM5.1/5.3.

The LCS sample was extracted with Strata PFAS under the conditions shown below and analyzed on a LC-MS/MS system using a Gemini® 3 µm C18 HPLC column under the conditions described below.

SPE Conditions

Cartridge: Strata PFAS (200 mg WAX/50 mg GCB/ 6 mL)
Part No.: CS0-9207
Condition 1: 4 mL 0.3 % Ammonium hydroxide
Condition 2: 4 mL Methanol
Equilibrate: 5 mL Water
Load: Add sample at 4 mL/min
Wash: 2x 4 mL Water
Elute: 2x 4 mL 0.3 % Ammonium hydroxide in Methanol
Evaporate: To dryness and reconstitute to 1 mL with
Methanol/Water (96:4)

LC-MS/MS Parameters

Column: Gemini® 3 µm C18
Dimensions: 50 x 2.0 mm
Part No.: 00B-4439-B0

Mobile Phase: A: 20 mM Ammonium acetate in Water B: Methanol

 Gradient:
 Time (min)
 %E

 0
 5

 0.1
 55

 4.5
 99

 8.0
 99

 8.5
 5

Flow Rate: 0.6 mL/min
Delay column: Luna® 5 µm C18(2) 30 x 3.0 mm

(00A-4252-Y0) installed between the autosampler and mobile phase pump mixer

450

Injection Volume: 10 µL

Mass Spec Parameters

Mass Spec Detector: SCIEX® Triple Quad™ 4500
Ion Source Parameters: Samples were ionized using electrospray in negative ion-mode
Parameter Value
CAD 9
CUR 30
GS1 40
GS2 60
IS Voltage -4500

MRM Transitions for HFPO-DA

Compound	Q1	Q3	RT	DP	CE
HFPO-DA (Quant)	329	185	3.7	-30	-32
HFPO-DA (Qual)	329	169	3.7	-30	-18
13C3 _HEDO_DA	333	195	37	-30	-33

Strata ® PFAS SPE for DOD QSM 5.3 (continued)

Table 1.

Recovery Comparisons of WAX SPE and dSPE using GCB vs Strata PFAS Single Cartridge Method

Analyte	WAX SPE + dSPE GCB % Recovery	Strata PFAS Stacked Cartridge % Recovery
13C2-PFDoDA	77.0	84.5
13C2-PFTeDA	62.0	84.0
PFODA	38.0	78.3
PFHxDA	63.0	89.3



Table 2.
Recovery of QSM 5.3 Target Analytes from a Laboratory Control Sample Using Strata PFAS SPE (WAX/GCB)

Analyte	Actual Concentration	Sample Result	% Recovery	Method Limits	Pass/Fail
PFBA	25.600	22.640	88	84-135	Pass
PFPeA	25.600	22.157	87	75-138	Pass
PFBS	22.640	22.300	99	81-133	Pass
4:2-FTS	23.920	22.078	92	64-134	Pass
PFHxA	25.600	24.644	96	80-137	Pass
PFPeS	24.000	21.699	90	82-132	Pass
HFPODA	25.600	26.336	103	0-130	Pass
PFHpA	25.600	27.018	106	80-140	Pass
PFHpA	25.600	27.018	106	80-140	Pass
PFHxS	24.200	24.713	102	71-131	Pass
DONA	24.120	26.083	108	70-130	Pass
6:2-FTS	24.280	24.217	100	51-155	Pass
PFHpS	24.360	23.015	94	80-129	Pass
PFOA	25.600	25.043	98	83-138	Pass
PF0S	24.480	22.492	92	54-139	Pass
PFNA	25.600	25.872	101	73-140	Pass
9CI-PF30NS	23.840	21.863	92	70-130	Pass
PFNS	24.560	21.993	90	71-121	Pass
PFNS	24.560	21.993	90	71-121	Pass
PFDA	25.600	25.047	98	78-137	Pass
8:2-FTS	24.520	22.231	91	62-133	Pass
PFOSA	25.600	25.714	100	73-121	Pass
NMEFOSAA	25.600	30.906	121	53-136	Pass
PFDS	24.640	22.873	93	69-124	Pass
PFUnDA	25.600	26.353	103	70-134	Pass
NEtFOSAA	25.600	28.765	112	59-145	Pass
11CI-PF30UdS	24.120	22.625	94	70-130	Pass
PFDoDA	25.600	27.710	108	75-139	Pass
10:2-FTS	24.680	26.626	108	50-124	Pass
PFDoS	24.800	21.509	87	39-121	Pass
PFTrDA	25.600	25.814	101	67-144	Pass
PFTeDA	25.600	25.446	99	79-134	Pass
PFODA	25.600	27.373	107	10-124	Pass
Recovery Range:	87% - 116%				

 Recovery Range:
 87 % - 116 %

 Average Recovery:
 98.8 %

 Mean Recovery:
 99.0 %

Determination of PFAS in Sediments

Determination of Perfluoroalkyl Substances (PFAS) in Sediments by QuEChERS Extraction and HPLC-MS/MS

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Introduction

Perfluoroalkyl substances (PFAS) are a class of highly stable synthetic organic compounds used in a wide variety of industrial and commercial applications including surface treatment for textiles, packaging materials, and non-stick cookware. PFAS are characterized by a hydrophobic fully fluorinated alkyl chain and a hydrophilic functional group. They are persistent in the environment due to the exceptional stability of the C-F bond. Once released in the aquatic environment, these chemicals will partition between the water phase and the sediment.



Currently, there are no federal regulatory limits controlling the discharge of PFAS compounds into the environment. Looking forward, it is possible that at some point EPA may establish requlatory limits for the various PFAS compounds in drinking water, wastewater and solid waste. In anticipation of such future developments, it is prudent to develop robust analytical methods and begin to better understand the fate and transport of these compounds in both the solid and liquid environmental fractions.

There are several methods available for the extraction and analysis of PFAS in aqueous samples, including the EPA Methods 537.1 and 533 previously described in this Guide (5). However, very few procedures are available for extracting these compounds in solid matrices such as sediments (1). Typical methods used are mechanical shaker and ultrasonic-assisted Solid-Liquid Extractions (SLE) 3, 4, 5). The extracts are then subjected to additional cleanup steps, usually by solid phase extraction, such as in the DoD QSM 5.3 approach previously described in this Guide. These are generally solvent-intensive and time-consuming processes. However, in 2003, an extraction procedure called **QuEChERS** (Quick-Easy-Cheap-Effective-Rugged-and-Safe) developed by researchers at the US Department of Agriculture was introduced (6). It was originally developed to extract pesticide residues in food matrices but has since found many other applications in the field of environmental analytical chemistry.

Our laboratory (LACSD) previously developed and validated a QuEChERS sediment extraction procedure for emerging contaminants including: pharmaceutical and personal care products, steroids, alkylphenol ethoxylates, and pyrethroid pesticides 7,8,9). We have successfully applied the same extraction method to determine perfluoroalkyl substances in marine and freshwater sediments.

Materials and Methods

Reagents/Chemicals

- QuEChERS Extraction In a 50 mL plastic centrifuge tube combine 2.0 g of Anhydrous Magnesium Sulfate, and 1.5 g Sodium Acetate or use approximately 3.5 g of AOAC 2007.01 roQ™ extraction packet (part no. AH0-9043)
- QuEChERS dSPE Clean-Up roQ 15 mL dSPE Kit (part no. KS0-8926)

Sample Preparation

QuEChERS Extraction Protocol

- 1. Weigh 2.0g of dried sediment into a polypropylene $container \, and \, spike \, with \, isotopically-labeled \, internal \, standards.$ PPCPs, Steroids, and Pyrethroids can be extracted concurrently with this method by adding the appropriate internal standard and spiking solutions to the samples and QCs 7,8,9.
- 2. Add 10mL deionized water and vortex. Add 10mL acidified acetonitrile (1 % acetic acid) to the slurry and vortex.
- 3. Add the extraction salts (1.5 g Sodium Acetate and 2 g MgSO₄) to the sample and vortex for 1 minute.
- 4. Centrifuge the samples for 5 minutes at 4000 rpm.
- 5. Place the samples in a rack and freeze at -20° for 30-60 minutes. This freezing step allows for easier extraction of the supernatant.
- 6. Transfer 8-9 mL of the acetonitrile supernatant into a roQ QuEChERS PSA/C18 dSPE clean-up tube (Part no. KS0-8926) and vortex for one minute.
- 7. Centrifuge the dSPE tubes for 10 minutes at 3000 rpm.
- 8. Place an aliquot of the extract in a HPLC vial and dilute 1:1 with deionized water. The sample is now ready for analysis.

HPLC-MS/MS Conditions

Gemini® 3µm C18 Column: **Dimensions:** 100 x 3 mm 00D-4439-Y0 Part No.: Phenomenex Krudkatcher™ Ultra Inline Filter: Luna® 5 µm C18 (2) 30 x 2.0 mm **Delay Column:** Part No.: 00A-4252-B0 Mobile Phase: A: 20 mM Ammonium acetate in water B: Methanol Time (min) % B **Gradient:** 10 0.0 1.5 65 8.0 95 8.1 99 12.0 99 Injection: 90 uL Flow Rate: 0.6 mL/min Temperature: 40°C SCIEX® 5500 QTRAP® Detector: MS/MS ESI Negative (sMRM) **Detection:** Analytes: 1. PFBA 2 PFPeA 3. PFBS 4. PFHxA 5. PFPS 6. PFHxS

> 7. PFHpA 8 PFH_{pS} 9. PFOA

Determination of PFAS in Sediments (continued)

Mass Spectrometer Parameters

Table 1. **MRM Transitions and Compound Dependent Parameters**

Compound Name	Q1	Q3	DP	CE
Perfluorobutanoic acid (PFBA)	213	169	-71	-14
Perfluoropentanoic acid (PFPeA)	263	219	-71	-12
Perfluorohexanoic acid (PFHxA)	313	269	-60	-14
Perfluoroheptanoic acid (PFHpA)	363	319	-62	-15
Perfluoroctanoic acid (PFOA)	413	369	-91	-12
Perfluorononanoic acid (PFNA)	463	419	-79	-15
Perfluorodecanoic acid (PFDA)	513	469	-83	-17
Perfluoroundecanoic acid (PFUdA)	563	519	-60	-17
Perfluorododecanoic acid (PFDoA)	613	569	-50	-21
Perfluorotridecanoic acid (PFTrDA)	663	619	-49	-18
Perfluorotetradecanoic acid (PFTeDA)	713	669	-63	-20
Perfluorobutanesulfonate (PFBS)	299	80	-94	-70
Perfluoropentantesulfonate (PFPeS)	349	80	-96	-66
Perfluorohexanesulfonate (PFHxS)	399	80	-92	-75
Perfluoroheptanesulfonate (PFHpS)	449	80	-75	-84
Perfluorooctanesulfonate (PFOS)	499	80	-78	-96
Perflluorooctanesulfonamide (PFOSA)	498	78	-60	-84
Perfluorononanesulfonate (PFNS)	549	80	-87	-100
Perfluorodecanesulfonate (PFDS)	599	80	-55	-100
Perfluoro-n-13C4-butanoic acid (M4PFBA)	217	171.9	-71	-13
Perfluoro-n-13C5-pentanoic acid (M5PFPeA)	268	222.7	-71	-12
Perfluoro-n-13C5-hexanoic acid (M5PFHxA)	318	272.9	-60	-13
Perfluoro-n-13C4-heptanoic acid (M4PFHpA)	367	321.8	-62	-14
Perfluoro-n-13C8-octanoic acid (M8PFOA)	421	376	-91	-12
Perfluoro-n-13C9-nonanoic acid (M9PFNA)	472	427	-79	-17
Perfluoro-n-13C6-decanoic acid (M6PFDA)	519	474	-83	-21
Perfluoro-n-13C7-undecanoic acid (M7PFUdA)	570	525	-60	-17
Perfluoro-n-13C2-dodecanoic acid (M2PFDoA)	615	570	-50	-24
Perfluor-n-13C2-tetradecanoic acid (M2PFTeDA)	715	670	-63	-25
Perfluro- ¹³ C3-butanesulfonate (M3PFBS)	302	80	-94	-55
Perfluoro- ¹³ C3-hexanesulfonate (M3PFHxS)	402	80	-92	-85
Perfluoro-¹3C8-octanesulfonate (M8PFOS)	507	80	-78	-100

Note: DP = Declustering Potential

Table 2. **MS Source Parameters**

Source Parameters	Settings
Temperature	400 °C
Gas 1	50
Gas 2	50
Curtain Gas	35
Ionization Energies	-4500 V
Collision Gas	High

Table 3. Method Performance Data for Sediments Spiked at 1 ng/g of the Target Analytes (n=4)

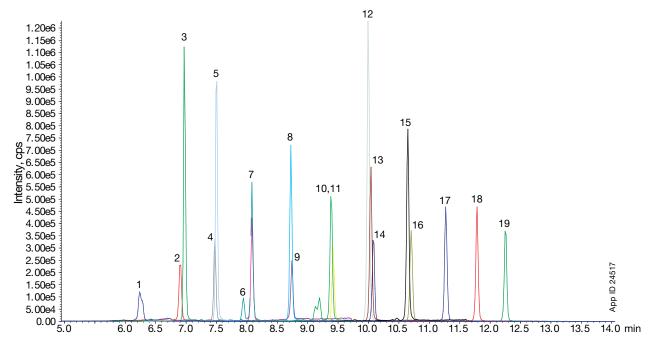
Compound	Average % Recovery	% RSD
PFBA	91.7	0.76
PFPeA	86.3	6
PFHxA	89.4	1.2
PFHpA	93.1	2.9
PFOA	98.3	1.5
PFNA	93	1.6
PFDA	87.7	4.5
PFUdA	92.3	2.1
PFDoA	92.5	4.1
PFTrDA	88.2	2.1
PFTeDA	87.6	2.1
PFBS	86.3	2.1
PFPeS	96.2	3.2
PFHxS	81.3	5
PFHpS	92.3	2.6
PFOS	92.1	2.6
PFOSA	104.5	6.3
PFNS	89.8	6.8
PFDS	87.3	6.7

CE = Collision Energy

Determination of PFAS in Sediments

(continued)

Figure 1.
Extracted ion chromatogram of sediments spiked with 1.0 ng/g of the target analytes



Results and Discussion

QuEChERS is a vortex-assisted solid-liquid extraction procedure that uses acetonitrile, salts, and buffering agents for extraction, phase-separation, and pH adjustment respectively. Extracts are subsequently transferred to a dispersive solid phase extraction (dSPE) tube containing a drying agent (MgSO₄) and SPE sorbents such as C18 or PSA for sample cleanup.

The modified QuEChERS method presented here is a simple, efficient, and cost-effective method for determining PFAS levels in sediments. Accuracy and precision were assessed using four replicates of sediments spiked with the target analytes. Average % recoveries are all within the 80-120 % range and % RSDs for all analytes are below 10 % (**Table 3**). Reporting limits were set at 0.05 ng/g dry weight based on a 2.0 g initial sample weight.





References

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- Estil, S., et. al., A. Rapid Extraction of Steroids and Pyrethroids from Sediments by QuEChERS and LC/MS/MS (2016) Phenomenex Application Note: TN-0098
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Acknowledgements

Special thanks to Syljohn Estil and to the Sanitation Districts of Los Angeles County – San Jose Creek Water Quality Laboratory for contributing this method.

Product Guide

Table 1.
Phenomenex PFAS Products Referenced or Applicable in Official Methods

Regulatory Method	Product	Part Number
	Strata® SDB-L 500 mg/6 mL	<u>8B-S014-HCH</u>
USEPA 537.1: Determination of Selected Per-and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/ Tandem Mass Spectrometry (LC/MS/MS) (5)	Gemini® 3µm C18, 50 x 3 mm or	00B-4439-B0
	Luna® Omega 1.6 µm PS C18 100 x 2.1 mm	00D-4752-AN
	Strata-X-AW 500 mg/6 mL	<u>8B-S038-HCH</u>
USEPA Method 533: Determination of Per- and Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution, Anion Exchange Solid Phase Extraction and LC-MS/MS. (1)	Gemini 3 µm C18 50 x 2 mm or	<u>00B-4758-Y0</u>
	Luna Omega 1.6 µm PS C18 100 x 2.1 mm	00D-4752-AN
US Food and Drug Administration: Determination of 16 Perfluoroalkyl and Polyfluoroalkyl Substances(PFAS) in Food using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). (2)	Strata-XL-AW 200 mg/3 mL	8B-S051-FBJ
US Department of Agriculture: Screening, Determiation and Confirmation of PFAS by UPLC-MS-MS (3)	Luna C8(2) 3 µm 50 x 2 mm	00B-4248-B0
US Department of Defense: Quality Systems Manual (QSM) for Environmental Laboratories (4)	Strata PFAS (WAX/GCB) 200 mg/50 mg/6 mL, 30/box 500 mg/50 mg/6 mL, 30/box	CSO-9207 CSO-9208
	Gemini 3 µm C18 50 x 2 mm	00B-4439-B0

References

- Method 537.1: Determination of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) | Science Inventory LUS EPA
- 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 | Methods Approved to Analyze Drinking Water Samples to Ensure Compliance with Regulations | US EPA
- 3. Determination of 16 Perfluoroalkyl and Polyfluoroalkyl Substances in Food using Liquid Chromatography-Tandem Mass Spectrometry (fda.gov)
- Screening, Determination and Confirmation of PFAS by UPLC-MS-MS
 (usda gov)
- 5. https://denix.osd.mil/edqw/documents/manuals/qsm-version-5-3-final/

Table 2.
Recommended HPLC Products for Routine PFAS Analysis

Description and Function	Product	Part Number
	Kinetex® 5 µm EVO C18 100 x 2.1 mm	00D-4633-AN
Analytical Column (UHPLC)	Luna Omega C18 1.6 µm 50 x 2.1	00B-4752-AN
	Gemini 3 µm C18 50 x 3 mm	<u>00B-4439-Y0</u>
Analytical Column	Gemini 3 µm C18 50 x 3 mm	00B-4439-Y0
Analytical Column (> 100 μL injection)	Gemini 3 µm C18 100 x 3 mm	00D-4439-Y0
Analytical Column (improved. Imwt acids)	Luna Omega 3 µm PS C18 50 x 3 mm	00B-4758-Y0
Delay Column	Kinetex 5 μm EVO C18, 50 x 2.1 mm	00B-4633-AN 00A-4252-YO
SecurityGuard	Luna Omega PS C18 4 x 3.0/10 pack for ID: 3.2-8.0 mm 4 x 2.0/10 pack for ID: 2.0-3.0 mm	AJ0-7606 AJ0-7605

Product Guide (continued)

Table 3. Recommended SPE Products

Description and Function	Product	Part Number
SPE Cartridge for EPA 537.1	Strata® SDB-L 500 mg/6 mL, 30/box	<u>8B-S014-HCH</u>
SPE Cartridge for EPA 533	Strata-X-AW 33um Polymeric Weak Anion, 500 mg/6 mL, 30/box	8B-S038-HCH
SPE Cartridge (Rev. Phase, High Perf.)	Strata-XL 500 mg/6 mL, 30/box	<u>8B-S043-HCH</u>
SPE Stacked Cartridge (D0D QSM 5.3)	Strata PFAS (WAX/GCB) 200 mg/50 mg/6 mL, 30/box	<u>CS0-9207</u>
SPE Stacked Cartridge (DOD QSM 5.3)	Strata PFAS (WAX/GCB) 500 mg/50 mg/6 mL, 30/box	CS0-9208
SPE Cartridge (WAX for DOD QSM 5.3)	Strata-XL-AW 500 mg/6 mL, 30/box	8B-S051-HCH
GCB** Cartridge (GCB for DOD QSM 5.3)	Strata GCB 250 mg/6 mL, 30/box	8B-S528-FCH
SPE Cartridge (WAX* for FDA Method)	Strata-XL-AW 100 µm 200 mg/3 mL, 50/box	8B-S051-FBJ
(*WAX = Weak Anion Exchange) (**GCB = Graphitized Carbon Black)		

Table 4. Recommended QuEChERs Products

Description and Function	Product	Part Number
QuEChERs Extraction (Soil/Sediment)	roQ QuEChERs Extraction Kit	KS0-8911
QuEChERs dSPE (Soil/Sediment)	roQ QuEChERs dSPE Kit, 15 mL	KS0-9516
QuEChERs Extraction (Dairy/Eggs/Fish)	roQ QuEChERs Extraction Kit	KS0-8910
QuEChERs dSPE (Dairy/Eggs/Fish)	roQ QuEChERs dSPE Kit	KS0-9511

Table 5. Recommended Accessories

Description and Function	Product	Part Number
SPE Sample Reservoir	75 mL Sample Reservoir	<u>H0-7005</u>
Large Volume SPE	Adaptor Cap for 12,20, 60 mL SPE Tubes	AH0-7379
Autosampler Vials	Polypropylene, 300 µm + PE Starburst Cap	AR0-9995-12-C
Polypropylene Vials	Vial 9mm Screw Thd PP 2mL, 1000 Pk	AR0-89C7-13
Vial Caps	Cap 9 mm Solid Top Black Unlined	8B-S528-FCH
PEEK Capillary Tubing	Capillary Tubing Kit, Various Sizes	AT0-1964
PEEK Tubing Cutter	Cutter for PEEK Capillary Tubing	AT0-1110



Product Guide (continued)

Currently offered by Phenomenex in USA and Canada only. Other regions coming soon.

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