APPLICATIONS

Rapid Extraction and Analysis of PPCPs from Sediments by QuEChERS and LC/MS/MS

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Introduction

Pharmaceutical and personal care products (PPCPs) have been detected in a multitude of aquatic environments. This broad group of compounds contains many biologically active substances that are capable of negatively impacting a water source. Among these compounds are numerous potential endocrine-disruptors. Once released in the environment, PPCP compounds will partition between the water phase and the sediment. Therefore, the sediment can act as a removal route for PPCPs in the water column. To better understand the fate and transport of these compounds, it is necessary to look at both the water and the solid phase.

There are several methods available for the extraction and analysis of PPCPs in aqueous samples. However, very few procedures are available for extracting these compounds in more complex solid matrices such as sediments. Typical methods used are soxhlet extraction, Pressurized Liquid Extraction (PLE), ultrasonic, and microwave assisted extraction. These methods tend to take longer and consume significant amount of solvents. In 2003, a new extraction procedure called QuEChERS (Quick-Easy-Cheap-Effective-Rugged-and Safe) was introduced. It was originally developed to extract pesticides in food matrices but has since found applications in the environmental field.

We developed a modified version of the QuEChERS method to extract PPCPs from marine and river sediment samples followed by Liquid Chromatography-Tandem Mass Spectrometry (LC/MS/MS) analysis. The result is a rapid, simple, and efficient extraction and analysis of 25 PPCP contaminants with reporting limits in the low ng/g range. The use of the modified extraction and clean up method resulted in higher sample throughput, faster extraction times, and greatly reduced solvent consumption compared to conventional solid matrix extraction methods.

Experimental Conditions

Reagents and Chemicals

Anhydrous Magnesium Sulfate
Sodium Acetate

Salts

QuEChERS Sorbents and Kits

- QuEChERS Extraction– In a 50 mL plastic centrifuge tube, combine 2 g of Anhydrous Magnesium Sulfate and 1.5 g Sodium Acetate (modified mix) or use approximately 3.5 g of AOAC 2007.01 roQ™ extraction packet (Part no. AH0-9043)
- QuEChERS dSPE Clean-up – In a 15 mL centrifuge tube, combine 1.5 g Magnesium sulfate, 0.4 g PSA sorbent, and 0.4 g C18 sorbent (omit C18 for +ve mode samples) or use Phenomenex roQ dSPE kit (Part no. KS0-8926) for -ve and (Part no. KS0-8928) for +ve
- Primary/Secondary Amine (PSA) dSPE Sorbent–Phenomenex Sepra™ PSA Sorbent (Part no. 04G-0610)
- C18 dSPE Sorbent – Phenomenex Sepra C18E sorbent (Part no. 04G-4348)

Sample Preparation

QuEChERS Extraction

1. Weigh 2.0 g + 0.02 g of suitably dried sediment in a 50 mL polypropylene vessel and spike with internal standard. Prepare a second tube the same way for each sample (each sample needs two different clean up steps; one for PPCP+ and the other for PPCP- analysis).

   For Method Blanks, weigh 2.0 g + 0.02 g of sand and spike with internal standards.

   For Laboratory Control Sample (LCS) and Matrix Spikes (MS), weigh 2.0 g + 0.02 g of sand and sediment respectively, and spike with the PPCP Spiking solution at desired spike level. Add 1.5 mL of acetonitrile and mix to allow the spiked compounds to interact with the entire sample. Dry the samples under a gentle stream of purified air or nitrogen. Spike the samples with internal standards prior to extraction.

2. Add 10 mL deionized water and vortex. Add 10 mL of acidified acetonitrile (1 % acetic acid in acetonitrile) to the slurry and vortex.
3. Add the extraction salts (1.5 g Sodium Acetate and 2 g $MgSO_4$) to the slurry and vortex for one minute.
4. Centrifuge the samples for 5 minutes at 4000 rpm.
5. Place the samples in a rack and freeze at -20 °C for 1-2 hours. This freezing step allows for easier extraction of the supernatant.
6. Transfer 8-9 mL of the acetonitrile supernatant into a roQ QuEChERS dSPE clean up tube (Part no. KS0-8926) and vortex for one minute. PPCP NEG supernatant goes to PSA/C18 cleanup (Part no. KS0-8926) and PPCP POS goes to PSA only cleanup (Part no. KS0-8928)
7. Centrifuge the tubes for 10 minutes at 3000 rpm.
8. Filter 5 mL of the supernatant through a 0.2 micron syringe filter into a glass test tube.
9. Reduce the extract under gentle stream of purified air of nitrogen. The temperature of the water bath should not exceed 35 ºC and air flow rate should not exceed 4 L/min. Reduce the sample to dryness and remove the samples from the water bath immediately after drying. ***Do not allow the samples to be blown down for an extended period of time***.

   Add 50 µL of Acetone to the dry sample and vortex to dissolve any residue. Add 950 µL of 50 % Methanol-Water solution, and transfer to a clean autosampler vial using a clean pasteur pipette. The sample is now ready for analysis.

LC/MS/MS Conditions

Positive Mode

Column: Kinetex® 2.6 µm C18
Dimensions: 50 x 2.1 mm
Part No.: 00B-4462-AN
Mobile Phase: A: 0.1 % Formic acid
B: 0.1 % Formic acid in methanol

Gradient:

Time (min)	% B
0	10
4.0	100
5.0	100
5.01	10
8.0	10

Injection: See Figure
Flow Rate: 0.4 mL/min
Temperature: Ambient
Detector: SCIEX Triple Quad™ 4500
Detection: ESI Positive - MS/MS

Sample:

1. Atenolol
2. Trimethoprim
3. Caffeine
4. Sulfamethoxazole
5. Metoprolol
6. Primidone
7. Meprobamate
8. Propranolol
9. TCEP
10. Phenytoin
11. Carbamazepine
12. Erythromycin
13. DEET
14. Fluoxetine
15. Carisoprodol
16. Diazepam
17. TCPP
18. Oxybenzone
19. TDCPP

Figure 1.
PPCP extract -50 ng/g, positive mode (2 µL inj.)

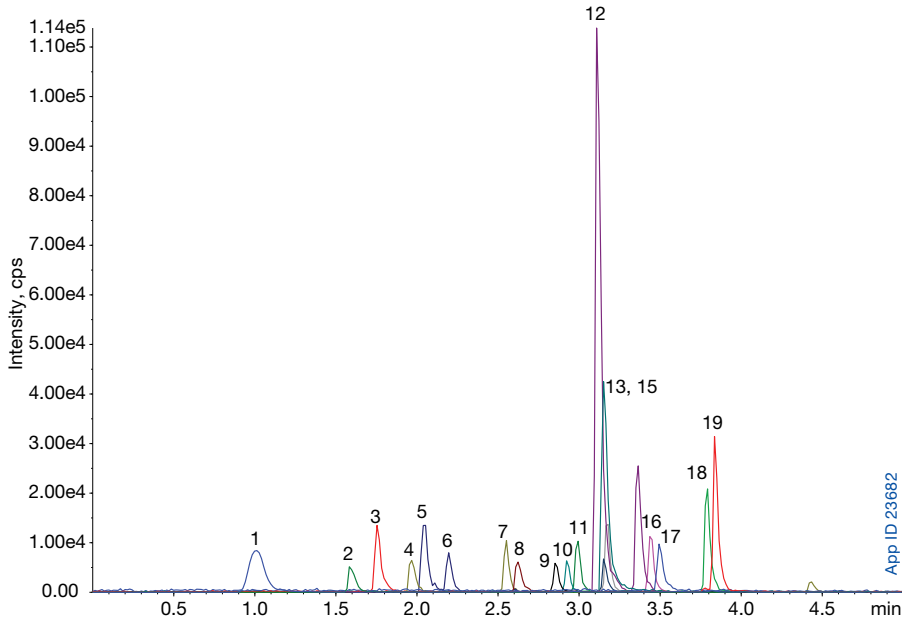
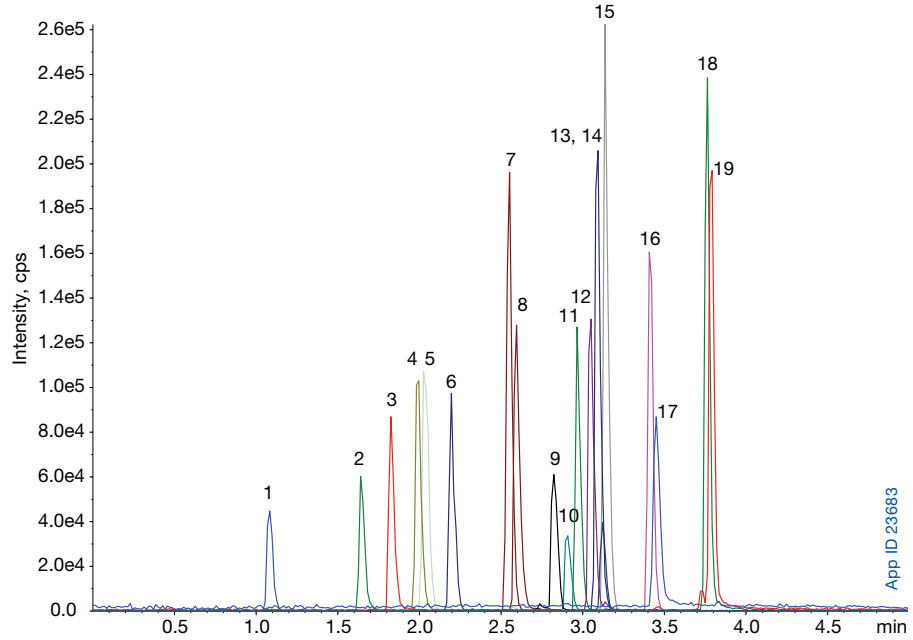


Figure 2.
PPCP standard mix - 50 ppb, positive mode (10 µL inj.)



Mass Spectrometer Parameters

Table 1.
Source Parameters (positive mode)

Source Parameters	Settings
Temperature	500 °C
Gas 1 (GS1)	55
Gas 2 (GS2)	55
Curtain Gas	20
Ionization Energy (POS)	5000 V
Collision Gas	Medium

Table 2.
MRM Transitions (positive mode)

Compound	MRM Transition
Atenolol	267.3 → 145.3
Caffeine	195.2 → 138.2
Carbamazine	237.1 → 194.1
Carisoprodol	261.0 → 176.0
DEET	192.2 → 119.0
Diazepam	285.0 → 154.0
Dilantin	253.0 → 182.1
Erythromycin	716.0 → 158.1
Fluoxetine	310.2 → 44.1
Meprobamate	219.0 → 158.0
Metoprolol	268.2 → 116.0
Oxybenzone	229.2 → 151.0
Primidone	219.2 → 162.0
Propranolol	260.0 → 115.9
Sulfamethoxazole	253.9 → 156.0
TCEP	284.9 → 222.8
TCPP	327.2 → 174.7
TDCPP	431.0 → 99.0
Trimethoprim	291.0 → 261.0

Figure 3.
PPCP standard mix- 100 ppb (10 µL injection), negative mode

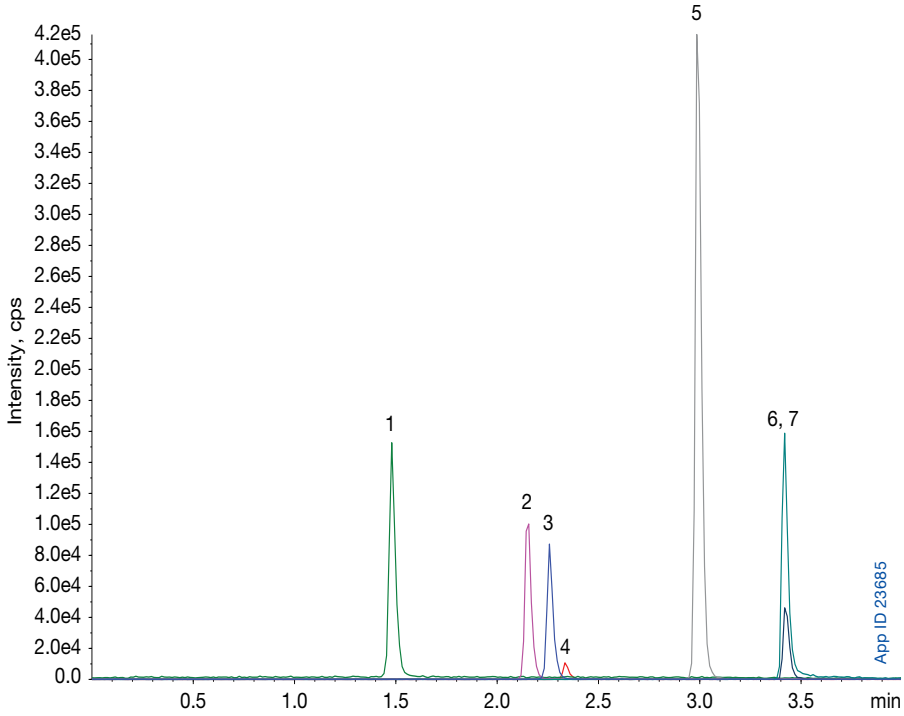
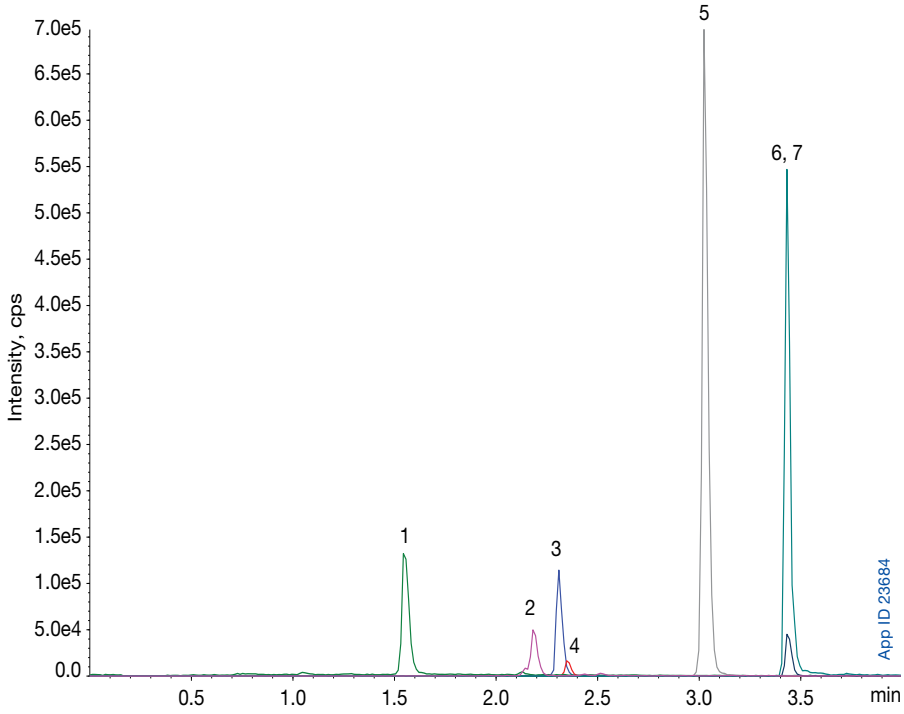


Figure 4.
PPCP extract- 50 ng/g, negative mode



Negative Mode

Column:	Kinetex® 2.6 µm C18
Dimensions:	50 x 2.1 mm
Part No.:	00B-4462-AN
Mobile Phase:	A: 40 ppm Ammonium acetate B: Methanol

Gradient:

Time (min)	% B
0	30
4.0	100
4.01	30
0	30

Injection:	10 µL
Flow Rate:	0.6 mL/min
Temperature:	Ambient
Detector:	SCIEX Triple Quad™ 4500
Detection:	ESI Negative - MS/MS

Sample:
1. Naproxen
2. Diclofenac
3. Ibuprofen
4. Bisphenol A
5. Gemfibrozil
6. Triclocarban
7. Triclosan

Mass Spectrometer Parameters

Table 3.
Source Parameters (negative mode)

Source Parameters	Settings
Temperature	600 °C
Gas 1 (GS1)	50
Gas 2 (GS2)	50
Curtain Gas	20
Ionization Energy (POS)	-4500 V
Collision Gas	Medium

Table 4.
MRM Transitions (negative mode)

Source Parameters	Settings
Bisphenol A	227.1 → 132.8
Ibuprofen	205.1 → 161.3
Triclosan	287.0 → 35.1
Diclofenac	294.0 → 249.8
Gemfibrozil	249.2 → 121.0
Naproxen	229.0 → 170.0
Triclocarban	313.0 → 160.0

Table 5.
Method Performance data for sediments spiked at 10 ng/g

Compound	Average Recovery	%RSD
Trimethoprim	103	1
Primidone	110	5
Erythromycin [-H20]	120	7
Sulfamethoxazole	96	1
Fluoxetine	98	8
Carbamazepine	115	5
Naproxen	99	1
Ibuprofen	106	5
Bisphenol A	86	4
Gemfibrozil	96	3
Triclosan	112	5
Atenolol	107	6
Metoprolol	108	0
Propranolol	103	5
Caffeine	102	9
Phenytoin	99	9
DEET	108	4
TCEP	106	6
TCPP	98	3
TDCPP	119	7
Oxybenzone	92	12
Carisoprodol	100	4
Meprobamate	103	7
Diazepam	101	2
Triclocarban	180	5

Results and Discussion

The modified QuEChERS method proved to be a very simple and efficient method for the determination of PPCPs in sediments. The method shows high recovery and precision with reporting limits in the low ng/g concentration range (1 ng/g based on 2 g initial sample weight). Sample throughput is very high and solvent consumption is significantly lower than conventional extraction methods. 20 samples can easily be extracted within an hour by a single analyst (plus an extra hour if the optional freezing out step is used) and each sample consumes only 10 mL of acetonitrile. (~2-3 hours total from start to finish).

Ionization suppression or enhancement of mass spectral signal due to the co-extracted sample matrix is common in electrospray ionization methods. This problem is reduced by performing an appropriate dispersive-Solid-Phase-Extraction (dSPE) clean-up step on sample extracts. To clean-up the extracts we used PSA for the negative mode assay and a combination of PSA and C18 dSPE sorbents for the positive mode assay. Suspended solid material that can potentially clog or damage the HPLC column or the ESI capillary electrode are eliminated by filtering the acetonitrile extracts through a 0.2 micron syringe filter prior to the reduction step.

Most QuEChERS methods allow for the direct injection of the extract into the analytical instrument. For this method, we employed a sample blow-down and solvent exchange step which slightly increases the total extraction time but gave us a 5X concentration factor.

Upon sample reduction, a brown residue may sometimes be observed with certain samples. This residue can harbor some of the analytes and internal standards, and failure to re-suspend this residue could result in lower recoveries. The 50 % Methanol reconstitution solvent may not be sufficient to dissolve this residue. Adding a small amount of acetone (50 µL) prior to sample reconstitution helps dissolve the residue without adversely affecting HPLC chromatography.

Conclusion

PPCPs are detected in many different aquatic environments. Aside from analyzing the water source directly, sediments must also be analyzed to understand the fate of these compounds. The outlined QuEChERS extraction protocol is able to remove most but not all sediment matrix interferences, resulting in clean – LC/MS/MS friendly - extracts. The protocol also gives high extraction efficiency with recovery values of 86 % or greater for all of the PPCP compounds analyzed.

By applying the outlined QuEChERS extraction protocol with LC/MS/MS to marine sediment, and freshwater sediments PPCPs are rapidly and effectively analyzed.

Acknowledgements

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SANITATION DISTRICTS OF LOS ANGELES COUNTY

References

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5. Method 6810-Pharmaceuticals and Personal Care Products. Eaton, Andrew D, Lenore S. Clesceri, Arnold E. Greenberg, and Mary A. H. Franson. Standard Methods for the Examination of Water and Wastewater. Washington, DC: American Public Health Association, 2013.

Ordering Information
Kinetex® C18 Core-Shell LC Columns

Kinetex 5 µm Columns (mm)		SecurityGuard™ ULTRA Cartridges (mm)		SecurityGuard ULTRA Cartridges (mm)					SecurityGuard ULTRA Cartridges (mm)
Phases	**50 x 2.1**	**3/pk**	**50 x 3.0**	**3/pk**	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**3/pk**
C18	00B-4601-AN	AJ0-8782	00B-4601-Y0	AJ0-8775	00B-4601-E0	00D-4601-E0	00F-4601-E0	00G-4601-E0	AJ0-8768
		for 2.1 mm ID		for 3.0 mm ID					for 4.6 mm ID

Kinetex 2.6 µm Columns (mm)			SecurityGuard ULTRA Cartridges (mm)		SecurityGuard ULTRA Cartridges (mm)				SecurityGuard ULTRA Cartridges (mm)
Phases	**50 x 2.1**	**150 x 2.1**	**3/pk**	**50 x 3.0**	**3/pk**	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**3/pk**
C18	00B-4462-AN	00F-4462-AN	AJ0-8782	00B-4462-Y0	AJ0-8775	00B-4462-E0	00D-4462-E0	00F-4462-E0	AJ0-8768
			for 2.1 mm ID		for 3.0 mm ID				for 4.6 mm ID

Kinetex 1.7 µm Columns (mm)				SecurityGuard ULTRA Cartridges (mm)
Phases	**50 x 2.1**	**100 x 2.1**	**150 x 2.1**	**3/pk**
C18	00B-4515-AN	00D-4475-AN	00F-4475-AN	AJ0-8782

Ordering Information

roQ™ Extraction Kits

Extraction Kits contain fifty easy-pour salt packets and fifty 50 mL stand-alone centrifuge tubes

Description	Unit	Part No.
EN 15662 Method Extraction Kits		
4.0 g $MgSO_4$, 1.0 g NaCl, 1.0 g SCTD, 0.5 g SCDS	50/pk	KS0-8909*
AOAC 2007.01 Method Extraction Kits		
6.0 g $MgSO_4$, 1.5 g NaOAc	50/pk	KS0-8911*
Original Non-Buffered Method Extraction Kits		
4.0 g $MgSO_4$, 1.0 g NaCl	50/pk	KS0-8910
6.0 g $MgSO_4$, 1.5 g NaCl	50/pk	KS0-8912

*AOAC and EN Extraction Kits also available in traditional non-collared 50 mL centrifuge tubes, Part No.: KS0-8911-NC and KS0-8909-NC

roQ Extraction Salt Packets

Salt packets only. Centrifuge tubes not included.

Description	Unit	Part No.
AOAC 2007.01 Method Extraction Packets		
6.0 g $MgSO_4$, 1.5 g NaOAc	50/pk	AH0-9043
EN 15662 Method Extraction Packets		
4.0 g $MgSO_4$, 1.0 g NaCl, 1.0 g SCTD, 0.5 g SCDS	50/pk	AH0-9041
Original Non-Buffered Method Extraction Packets		
4.0 g $MgSO_4$, 1.0 g NaCl	50/pk	AH0-9042
6.0 g $MgSO_4$, 1.5 g NaCl	50/pk	AH0-9044

roQ dSPE Kits

dSPE Kits contain pre-weighed sorbents/salts inside 2 mL or 15 mL centrifuge tubes

Description	Unit	Part No.
2 mL dSPE Kits		
150 mg $MgSO_4$, 25 mg PSA, 25 mg C18-E	100/pk	KS0-8913
150 mg $MgSO_4$, 25 mg PSA, 2.5 mg GCB	100/pk	KS0-8914
150 mg, $MgSO_4$, 25 mg PSA, 7.5 mg GCB	100/pk	KS0-8915
150 mg $MgSO_4$, 25 mg PSA	100/pk	KS0-8916
150 mg $MgSO_4$, 50 mg PSA, 50 mg C18-E, 50 mg GCB	100/pk	KS0-8917
150 mg $MgSO_4$, 50 mg PSA, 50 mg C18-E	100/pk	KS0-8918
150 mg $MgSO_4$, 50 mg PSA, 50 mg GCB	100/pk	KS0-8919
150 mg $MgSO_4$, 50 mg PSA	100/pk	KS0-8920
15 mL dSPE Kits		
900 mg $MgSO_4$, 150 mg PSA, 150 mg C18-E	50/pk	KS0-8921
900 mg $MgSO_4$, 150 mg PSA, 15 mg GCB	50/pk	KS0-8922
900 mg $MgSO_4$, 150 mg PSA, 45 mg GCB	50/pk	KS0-8923
900 mg $MgSO_4$, 150 mg PSA	50/pk	KS0-8924
1200 mg $MgSO_4$, 400 mg PSA, 400 mg C18-E, 400 mg GCB	50/pk	KS0-8925
1200 mg $MgSO_4$, 400 mg PSA, 400 mg C18-E	50/pk	KS0-8926
1200 mg $MgSO_4$, 400 mg PSA, 400 mg GCB	50/pk	KS0-8927
1200 mg $MgSO_4$, 400 mg PSA	50/pk	KS0-8928

Bulk roQ QuEChERS Sorbents

Phases	10 g	100 g
C18-E	–	04G-4348
GCB (Graphitized Carbon Black)	04D-4615	04G-4615
PSA	–	04G-4610

APPLICATIONS

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