APPLICATIONS

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

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Introduction

Pyrethroids are compounds similar to naturally produced Pyrethrins, and are heavily used as household insecticides. Although these compounds are intended to rapidly breakdown in the environment, it is important to monitor their presence in aquatic environments. Once released in an aquatic environment, pyrethroids will partition between the water phase and the sediment. Therefore, the sediment can act as a removal route for pyrethroids 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 pyrethroids 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 pyrethroids 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 7 pyrethroids 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 1.2 g Magnesium sulfate, 0.4 g C18 sorbent, and 0.4 g PSA sorbent or use roQ part no. KS0-8926
- 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 Protocol

1. Weigh 2.0 g + 0.02 g of suitably dried sediment in a 50 mL polypropylene vessel and spike with internal standard.

   For Method Blanks, weigh 2.0 g + 0.02 g of sand and spike with 20 µL of 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 Pyrethroid 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.

   Note: Concurrent extraction for Alkylphenol polyethoxylate, PPCPs, and Hormone Compounds may be done by adding the appropriate internal standard and spiking solutions.
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.
7. Centrifuge the tubes for 10 minutes at 3000 rpm.
8. Filter 5 mL of the supernatant through a 0.2 micron syringe filter (Part no. AF0-2202-12) into a glass test tube.
9. Reduce the extract under gentle stream of purified air or nitrogen. The temperature of the water bath should not exceed 35 °C 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***.
10. 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

Column: Kinetex® 2.6 µm EVO C18
Dimensions: 50 x 2.1 mm
Part No.: 00B-4725-AN
Mobile Phase: A: 5 mM Ammonium acetate
B: Methanol

Gradient:

Time (min)	% B
0	50
1.0	95
3.0	95
3.01	50
6.0	50

Injection: 5 µL
Flow Rate: 0.7 mL/min
Temperature: Ambient
Detector: Sciex 4500 Triple Quad™
Detection: ESI Positive - MS/MS
Sample:
1. Cyfluthrin
2. Cyhalothrin
3. Cypermethrin
4. Deltamethrin
5. Esfenvalerate
6. cis-Permethrin
7. trans-Permethrin
8. Bifenthrin

Mass Spectrometer Parameters

Table 1.
Source Parameters

Source Parameters	Settings
Temperature	300 °C
Gas 1 (GS1)	60
Gas 2 (GS2)	40
Curtain Gas	20
Ionization Energy (POS)	5500 V
Collision Gas	Medium

Table 2.
MRM Transitions

Compound	MRM Transition
Permethrin (cis + trans)	408.2 → 183.1
Bifenthrin	442.0 → 181.0
Cypermethrin	433.2 → 191.0
Cyfluthrin	451.2 → 191.0
Deltamethrin	523.2 →280.9
Esfenvalerate	437.3 → 166.9
Cyhalothrin	467.2 → 225.0

Table 3.
Method Performance data for sediments spiked at 2.5 ng/g

Compound	Average Recovery	%RSD
Permethrin	105	2
Bifenthrin	108	3
Cypermethrin	99	6
Cyfluthrin	87	6
Deltamethrin	104	4
Esfenvalerate	93	8

Figure 1.
Pyrethroids standards 100 ng/mL using Kinetex 2.6 µm EVO C18

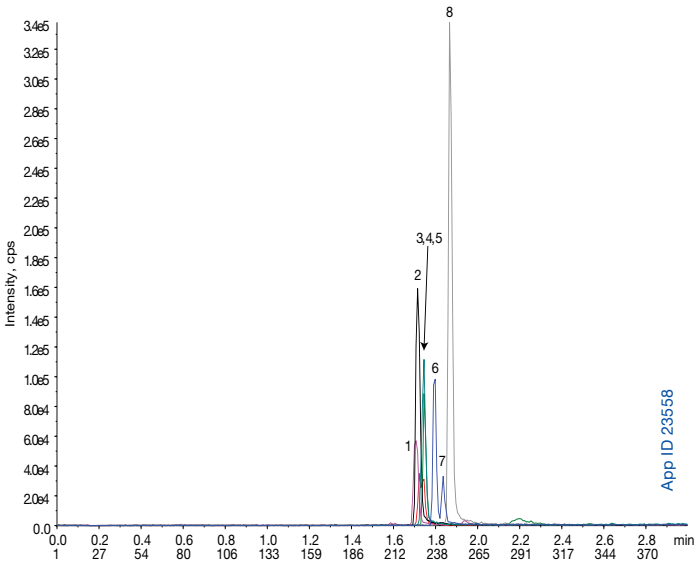
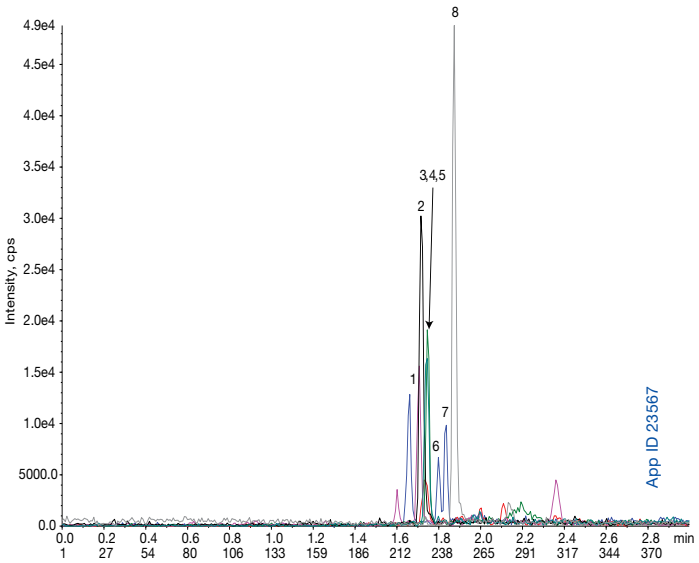


Figure 2.
Pyrethroid Extracts 50 ng/g using Kinetex 2.6 µm EVO C18



Results and Discussion

The modified QuEChERS method proved to be a very simple and efficient method for the determination of Pyrethroids in sediments. The method shows high recovery and precision with reporting limits in the low ng/g concentration range (0.1-0.5 ng/g based on 2g 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 a combination of PSA and C18 dSPE sorbents was used. 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. Concentrating the dSPE extract allowed for a smaller initial sample size, which is important for reducing ion-suppression.

Most QuEChERS methods allow for the direct injection of the extract into the analytical instrument. For this method, we employed a sample reduction 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 will contain 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.

Acknowledgements

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SANITATION DISTRICTS OF LOS ANGELES COUNTY

Conclusion

Pyrethroids are widely used insecticides that can potentially migrate into a multitude of 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 87 % or greater for all of the pyrethroids analyzed.

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

References

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2. Lehotay S., et. al., Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruit and vegetables. Journal of Chromatography A (2010). 1217: 2548-2560
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4. Cerqueira, M., et. al., Evaluation of QuEChERS method for the extraction of pharmaceuticals and personal care products in treatment sludge with determination by UPLC-ESI-MS/MS. Chemosphere (2014). 107: 74-82.Iker, J.M.; Cox, M. *The Language of Biotechnology: A Dictionary of Terms*, 2nd ed.;
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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	KSO-8909*
AOAC 2007.01 Method Extraction Kits		
6.0 g $MgSO_4$, 1.5 g NaOAc	50/pk	KSO-8911*
Original Non-Buffered Method Extraction Kits		
4.0 g $MgSO_4$, 1.0 g NaCl	50/pk	KSO-8910
6.0 g $MgSO_4$, 1.5 g NaCl	50/pk	KSO-8912

*AOAC and EN Extraction Kits also available in traditional non-collared 50 mL centrifuge tubes, Part No.: KSO-8911-NC and KSO-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	KSO-8913
150 mg $MgSO_4$, 25 mg PSA, 2.5 mg GCB	100/pk	KSO-8914
150 mg, $MgSO_4$, 25 mg PSA, 7.5 mg GCB	100/pk	KSO-8915
150 mg $MgSO_4$, 25 mg PSA	100/pk	KSO-8916
150 mg $MgSO_4$, 50 mg PSA, 50 mg C18-E, 50 mg GCB	100/pk	KSO-8917
150 mg $MgSO_4$, 50 mg PSA, 50 mg C18-E	100/pk	KSO-8918
150 mg $MgSO_4$, 50 mg PSA, 50 mg GCB	100/pk	KSO-8919
150 mg $MgSO_4$, 50 mg PSA	100/pk	KSO-8920
15 mL dSPE Kits		
900 mg $MgSO_4$, 150 mg PSA, 150 mg C18-E	50/pk	KSO-8921
900 mg $MgSO_4$, 150 mg PSA, 15 mg GCB	50/pk	KSO-8922
900 mg $MgSO_4$, 150 mg PSA, 45 mg GCB	50/pk	KSO-8923
900 mg $MgSO_4$, 150 mg PSA	50/pk	KSO-8924
1200 mg $MgSO_4$, 400 mg PSA, 400 mg C18-E, 400 mg GCB	50/pk	KSO-8925
1200 mg $MgSO_4$, 400 mg PSA, 400 mg C18-E	50/pk	KSO-8926
1200 mg $MgSO_4$, 400 mg PSA, 400 mg GCB	50/pk	KSO-8927
1200 mg $MgSO_4$, 400 mg PSA	50/pk	KSO-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

(Continued in back)

APPLICATIONS

Ordering Information
Kinetex® EVO 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
EVO C18	00B-4633-AN	AJ0-9298	00B-4633-Y0	AJ0-9297	00B-4633-E0	00D-4633-E0	00F-4633-E0	00G-4633-E0	AJ0-9296
		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
EVO C18	00B-4725-AN	00F-4725-AN	AJ0-9298	00B-4725-Y0	AJ0-9297	00B-4725-E0	00D-4725-E0	00F-4725-E0	AJ0-9296
			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
EVO C18	00B-4726-AN	00D-4726-AN	00F-4726-AN	AJ0-9298

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