

APPLICATIONS

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

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Introduction

A wide range of estrogenic contaminants have been detected in aquatic environments. Among these are natural and synthetic estrogens and androgens which could potentially cause endocrine disruption in aquatic organisms. Once released in the environment, these steroid hormones will partition between the water phase and sediment. The sediment can therefore act as a removal route for steroid hormones 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 steroid hormones 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 natural and synthetic hormones 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 several steroid hormone 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

Salts

Anhydrous Magnesium Sulfate
Sodium Acetate

QuEChERS Sorbents and Kits

QuEChERS Salt Extraction – In a 50 mL plastic centrifuge tube, combine 2 g of Anhydrous Magnesium Sulfate, and 1.5 g Sodium Acetate (modified mix) or approximately weigh out 3.5 g of AOAC 2007.01 roQ™ extraction packet (AHO-9043)

QuEChERS dSPE Clean-up – In a 15 mL centrifuge tube, combine 1.5 g Magnesium sulfate, 0.4 g C18 sorbent, and 0.4 g PSA sorbent or use Phenomenex roQ part number KSO-8926

Primary/Secondary Amine (PSA) dSPE Sorbent–Phenomenex Septra™ PSA Sorbent (Part # 04G-4610)

C18 dSPE Sorbent – Phenomenex Septra C18E sorbent (Part # 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 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 Steroid 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, polyethoxylates, Pyrethroids, and PPCP 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 of Sodium Acetate and 2.0 g of MgSO₄) 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 (KSO-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 into a glass test tube.
9. Place the test tubes in the N-Evap apparatus. 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. 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, vortex, 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 XB-C18
Dimensions: 50 x 2.1 mm
Part No.: 00B-4696-AN
Mobile Phase: A: Water
 B: Acetonitrile

Gradient	Time (min)	% B
	0	15
	1.0	20
	7.25	60
	8.00	80
	9.50	80
	9.55	15
	14	15

Injection: 12 µL
Flow Rate: 0.5 mL/min
Temperature: 30 °C
Detector: SCIEX 5500 QTRAP®
Detection: MS/MS (ESI + and ESI -)

Sample:

1. Estriol (E3)	5. 17- α -Ethinylestradiol (EE2)
2. 17- β Estradiol (E2)	6. Estrone (E1)
3. Testosterone (TEST)	7. Androstenedione (AND)
4. Equilin (EQ)	

Note: Post column infusion with 0.1 % NH₄OH

Mass Spectrometer Parameters

Table 1.
Source Parameters

Source Parameters	Settings
Temperature	500 °C
Gas 1 (GS1)	35
Gas 2 (GS2)	35
Curtain Gas	30
Ionization Energy (POS)	5000 V
Ionization Energy (NEG)	-4500 V
Collision Gas	High

Table 2.
MRM Transitions

Compound	MRM Transition	Polarity
Estrone (E1)	268.6→144.8	-ve
17 β Estradiol (E2)	271.2→144.7	-ve
17 α -Ethinylestradiol (EE2)	294.9→145.1	-ve
Estriol (E3)	286.8→144.7	-ve
Equilin (EQ)	266.9→142.9	-ve
Testosterone (TEST)	289.0→97.0	+ve
Androstenedione (AND)	287.2→97.2	+ve

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

Compound	Average Recovery	% RSD
Estrone (E1)	95.7	8
17 β Estradiol (E2)	101.7	3.8
17 α -Ethinylestradiol (EE2)	104.7	7.9
Estriol (E3)	93.1	7.6
Equilin (EQ)	95.5	6.8
Testosterone (TEST)	101.7	6.5
Androstenedione (AND)	88.5	6.8

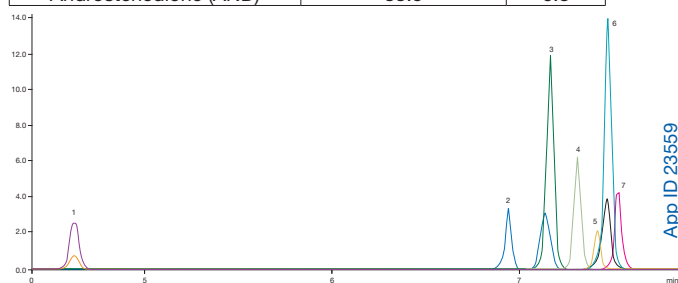


Figure 1.
Extracted Hormones using Kinetex 2.6µm XB-C18 50 x 2.1 mm HPLC column

Results and Discussion

The modified QuEChERS method proved to be a very simple and efficient method for the determination of Estrone (E1), 17- β Estradiol (E2), 17 α -Ethinylestradiol (EE2), Estriol (E3), Equilin (EQ), Testosterone (Test), and Androstenedione (And) in sediments. The method shows high recovery and precision with reporting limits in the low ng/g concentration range (0.125 ng/g based on a 2 g initial sample weight). Recoveries of the analytes ranged between 89 and 105 % with relative standard deviations below 10 %. 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.

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. For this method, we used a combination of PSA and C18 dSPE sorbents to clean-up the extracts. 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 blow-down step.

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. In addition, directly injecting the acetonitrile extracts resulted in significant broadening of chromatographic peaks. This issue can be avoided by doing a solvent exchange with 50 % Methanol-Water solution.

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

Steroid hormones are detected in a multitude of aquatic environments. Aside from analyzing the water source directly, sediments must also be analyzed to understand the fate of these compounds. The outlined modified QuEChERS extraction protocol is able to remove sediment matrix interferences, resulting in clean – LC/MS/MS friendly - extracts. The protocol also gives high extraction efficiency with recovery values of 89% or greater for all of the steroid hormones analyzed.

By applying the outlined modified QuEChERS extraction protocol with LC/MS/MS to marine sediment, steroid hormones are rapidly and effectively analyzed.

Acknowledgements

We would like to provide special thanks to the Sanitation Districts of Los Angeles County – San Jose Creek Water Quality Laboratory for their contributions.



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	AHO-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	AHO-9041
Original Non-Buffered Method Extraction Packets		
4.0 g MgSO ₄ , 1.0 g NaCl	50/pk	AHO-9042
6.0 g MgSO ₄ , 1.5 g NaCl	50/pk	AHO-9044

Kinetex® XB-C18 Core-Shell LC Columns

5 μm Minibore Columns (mm)				SecurityGuard™ ULTRA Cartridges†
Phases	30 x 2.1	50 x 2.1	100 x 2.1	3/pk
XB-C18	00A-4605-AN	00B-4605-AN	00D-4605-AN	AJO-8782

for 2.1 mm ID

5 μm Analytical Columns (mm)					SecurityGuard™ ULTRA Cartridges†
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
XB-C18	00B-4605-E0	00D-4605-E0	00F-4605-E0	00G-4605-E0	AJO-8768

for 4.6 mm ID

2.6 μm Minibore Columns (mm)						SecurityGuard™ ULTRA Cartridges†
Phases	30 x 2.1	50 x 2.1	75 x 2.1	100 x 2.1	150 x 2.1	3/pk
XB-C18	00A-4496-AN	00B-4496-AN	00C-4496-AN	00D-4496-AN	00F-4496-AN	AJO-8782

for 2.1 mm ID

2.6 μm MidBore Columns (mm)						SecurityGuard™ ULTRA Cartridges†
Phases	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	3/pk
XB-C18	00A-4496-Y0	00B-4496-Y0	00C-4496-Y0	00D-4496-Y0	00F-4496-Y0	AJO-8775

for 3.0 mm ID

2.6 μm Analytical Columns (mm)					SecurityGuard™ ULTRA Cartridges†
Phases	50 x 4.6	100 x 4.6	150 x 4.6	3/pk	
XB-C18	00B-4496-E0	00D-4496-E0	00F-4496-E0	AJO-8768	

for 4.6 mm ID

1.7 μm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges†
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
XB-C18	00A-4498-AN	00B-4498-AN	00D-4498-AN	00F-4498-AN	AJO-8782

for 2.1 mm ID

1.7 μm MidBore Columns (mm)					SecurityGuard™ ULTRA Cartridges†
Phases	30 x 3.0	50 x 3.0	100 x 3.0	3/pk	
XB-C18	00A-4498-Y0	00B-4498-Y0	00D-4498-Y0	AJO-8775	

for 3.0 mm ID

†SecurityGuard Ultra Cartridges require holder, Part No.: AJO-9000

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



APPLICATIONS

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