Extraction of Over-the-Counter Pharmaceuticals from Water and Sludge Cake Using ISOLUTE® QuEChERS Prior to LC/MS Analysis

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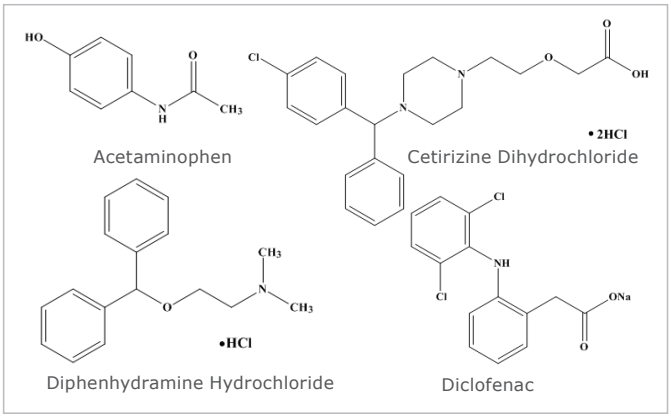

Figure 1. Structures of over-the-counter (OTC) drugs

Introduction

The environmental persistence of pharmaceuticals is of increasing interest, with little known regarding removal during wastewater treatment. This study investigates the potential of commercially available QuEChERS kits for the extraction and clean-up of a suite of common over-the-counter (OTC) pharmaceuticals from water and sludge cake. Extracts are analysed using LC-MS/MS.

ISOLUTE® QuEChERS products provide clean and efficient extraction for a variety of complex samples, reducing sample preparation to approximately 20 minutes to extract concentration (evaporation stage)

Analytes

Acetaminophen, cetirizine dihydrochloride, diphenhydramine hydrochloride and diclofenac.

Sample Preparation Procedure

Format:

QuEChERS 50 mL Centrifuge Tube (Q0000-50V)
ISOLUTE® QuEChERS 15 g EN Extraction Tubes (Q0020-15V)
QuEChERS EN Fruit and Vegetable (Q0035-15V)
QuEChERS EN Waxed Fruit and Vegetables (Q0060-15V)
QuEChERS EN Pigmented Fruit and Vegetables (Q0080-15V)
QuEChERS EN Highly Pigmented Fruit and Vegetables (Q0090-15V)

Sample Preparation:

3.76 mL water, 20 µL of cetirizine, diphenhydramine and diclofenac and 140 µL of acetaminophen were dispensed into a 50 mL centrifuge tube. For the sludge experiments, 2.5 g of lyophilised sludge was weighed into the tube before the addition of water and pharmaceuticals in the quantity described above. 10 mL of neat acetonitrile was added, along with the contents of the EN extraction tube. The tube was shaken manually for 1 minute then centrifuged at 4000 rpm for 5 minutes.

Dispersive-SPE:

The supernatant (approx. 7 mL) was transferred to one of the four d-SPE kits, vortexed for 1 minute at 1400 rpm, then centrifuged at 4000 rpm for 5 minutes. The final extracted supernatant was transferred to a clean 15 mL centrifuge tube, and evaporated to dryness under nitrogen. The sample was re-constituted in 1.875 mL of 50:50 acetonitrile and water.

Calibration Line Preparation:

For each compound, a 1 mg/mL calibration solution was prepared by diluting a 10 mg/mL stock solution to a volume of 1.5 mL. Seven calibration standards were made over a linear range.

HPLC Conditions

Instrument: Thermo Finnigan Surveyor Autosampler with an MS Pump

Column: Thermo Hypersil Gold, 3 µm, 100 x 1 mm column with a Hypersil C18 3µm guard cartridge

Mobile Phase: A: 0.1% formic acid in water
B: acetonitrile

Flow Rate: 50 µL/minute

Injection: 5 µL

Gradient: Initial 95% A: 5% B, hold for 2 minutes
Linear ramp to 100% B in 28 minutes
100% B, hold for 10 minutes
Linear ramp to initial conditions in 1 minute
Hold for 10 minutes
Total run time: 51 minutes

Sample temperature: 4 °C

Table 1. Mean retention times (min) for compounds and internal standards (IS) using the LC-MS/MS method described

Compound	WATER Retention time (min)	SLUDGE CAKE Retention time (min)
Acetaminophen	1.59	1.75
Acetaminophen-(methyl-d3) [IS]	1.59	1.75
Diphenhydramine Hydrochloride	15.64	15.40
Cetirizine Dihydrochloride	18.13	17.90
4((4-Chlorophenyl)Phenylmethyl)-1-Piperazine Ethanol Dihydrochloride [IS]	17.75	17.45
Diclofenac	22.93	22.95

MS Conditions

Instrument: Thermo Finnigan LCQ Classic

Ionisation mode:: ESI+

Desolvation Temperature: 200 °C

Table 2. ESI source settings

ESI Source	
Sheath gas flow rate	85 arbitrary units
Aux gas flow rate	0
Spray voltage	3.5 kV
Capillary temperature	200 °C
Capillary voltage	10 V
Tube lens offset	5 V

Table 3. Compound details and selected reaction monitoring (SRM) transitions

Drug	MW (g/mol)	m/z	SRM Transition
Acetaminophen	151.06	152 $[M+H]^+$	152 → 110
Cetirizine Dihydrochloride	461.81	389 $[M+H-2HCl]^+$	389 → 201
Diphenhydramine Hydrochloride	291.82	256 $[M+H-HCl]^+$	256 → 167
Diclofenac Sodium	318.31	296 $[M+H-Na]^+$	296 → 277

Results

The EN Waxed Fruit and Vegetable d-SPE kit (P/N Q0060-15V) was found to give the best results in terms of overall analyte recovery and extract cleanliness. However, recoveries of certain drugs (diclofenac sodium and cetirizine dihydrochloride) were relatively low, possibly due to their selective retention on the d-SPE sorbent.

Significant matrix effects were observed for extracts from sewage sludge compared with water (data not shown).

Example chromatograms for extracts of a) water and b) sludge cake are shown in **Figures 2 and 3** respectively.

An example of the calibration line constructed for acetaminophen in water is shown in **Figure 4**.

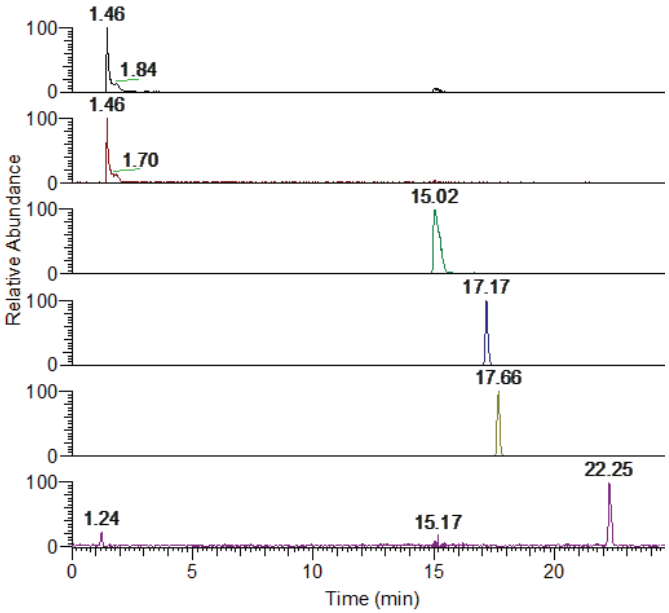

Figure 2. Extracted precursor ion chromatograms for over-the-counter (OTC) drugs in water in positive ion mode after preparation with EN Waxed Fruit and Vegetable d-SPE kit

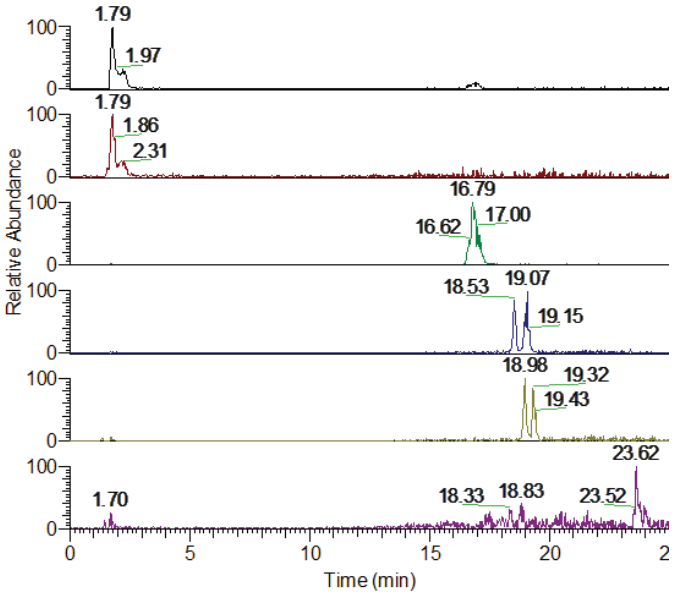

Figure 3. Extracted precursor ion chromatograms for over-the-counter (OTC) drugs in sludge in positive ion mode after preparation with EN Waxed Fruit and Vegetable d-SPE kit

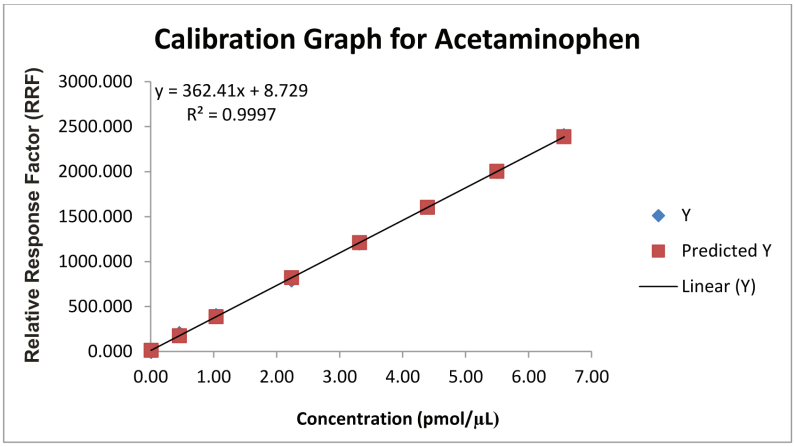

Figure 4. Typical calibration graph for Acetaminophen in water expressed on a linear scale

Recovery calculations were based upon the ratio of peak area for the spike before extraction and the spike after extraction experiments, with the latter adjusted to account for the evaporation step in the sample preparation method.

Table 4. Recovery and analyte performance in water and sludge using EN Waxed Fruit and Vegetable d-SPE kit

	WATER		SLUDGE CAKE
Compound	**Recovery%**	**R^2**	**Recovery%**
Acetaminophen	61.04	0.9997	52.69
Cetirizine Dihydrochloride	16.08	0.9906	3.35
Diphenhydramine Hydrochloride	57.01	0.9932	46.19
Diclofenac	18.79	0.9929	15.55

Coefficient of determination (r^2) calculations were based on calibration lines including a “zero” standard, spiked standards between 200–2400 pmol/µL for acetaminophen, 20–240 pmol/µL for cetirizine, diphenhydramine and 20-140 pmol/µL for diclofenac. The concentrations used for the internal standards acetaminophen-(methyl-d3) and 4((4-chlorophenyl) phenylmethyl)-1-piperazine ethanol dihydrochloride were 402 and 29 pmol/µL respectively.

Conclusions

Compared to existing clean-up methods, the QuEChERS was found to be quick and simple to perform.

Although not optimised for clean-up of the target matrices, the method described in this application note achieves reasonable recoveries of all four compounds in both water and sludge cake. Despite significant matrix effects, limits of detection of between 3 and 12 µg/mL were obtained using a selected-reaction monitoring method and targeted precursor ion scan, indicating sufficient selectivity and sensitivity for detection in an environmental matrix using multiple acquisition modes.

This study suggests that a modified form of QuEChERS may be suitable for extraction and clean-up of pharmaceuticals in complex environmental matrices. Further work should investigate the use of alternative sorbent mixes in the d-SPE step to enhance clean-up and further reduce matrix effects.

Additional Notes

The extraction with the sludge samples resulted in a final extract that ranged between an orange to dark yellow in colour (as shown below). An oily, lattice-type film was also observed on the surface of the sample, which remained in the clean-up tube after aliquoting a sample for the LC-MS/MS analysis.

Figure 5. *Left:* Sludge extract before d-SPE. *Right:* Sludge extract after d-SPE

Ordering Information

Part Number	Description	Quantity
Q0020-15V	QuEChERS EN Extraction Tube	25
Q0035-15V	QuEChERS EN Fruit and Vegetable	25
Q0060-15V	QuEChERS EN Waxed Fruit and Vegetable	25
Q0080-15V	QuEChERS EN Pigmented Fruit and Vegetable	25
Q0090-15V	QuEChERS EN Highly Pigmented Fruit and Vegetable	25
Q0000-50V	QuEChERS 50 mL Centrifuge Tube With Rack	25

For the latest application notes and more information about ISOLUTE® QuEChERS visit **www.biotage.com**

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