A Generic Approach to Extraction of Pharmaceuticals from Environmental Water Samples

Matthew Cleeve, Lee Williams, Elena Gairloch, Scott Merriman, Helen Lodder, Claire Desbrow, Steve Jordan, Richard Calverley

Argonaut Technologies, now a Biotage company, Dyffryn Industrial Estate, Ystrad Mynach, Mid Glamorgan, CF82 7RJ, UK

Introduction

There is growing concern over the presence of pharmaceutical residues in environmental water samples. The presence of a significant number of such contaminants, with widely differing polarity and functionality has been reported, with many present at extremely low concentrations.

LC-MS/MS techniques are increasingly used for such analyses, as they are applicable to a wide range of compounds, offer increased selectivity and can minimize run times compared to more traditional techniques such as GC-MS or LC-UV.

However, sample preparation approaches which can simultaneously extract and concentrate such a diverse range of analytes are required to match the productivity of LC-MS based analyses. Solid phase extraction is already an important tool in this area. Recent years have seen resurgence in the use of resin-based SPE sorbents as they generally show excellent recoveries of polar analytes and can simultaneously extract large numbers of analytes with differing functionalities.

A novel polymer based sorbent, EVOLUTE™ ABN, capable of extracting a wide range of compounds from water samples has recently been developed. The particle size and flow characteristics of the sorbent have been optimized for rapid processing of water samples. In this presentation, a generic approach to the extraction of a broad range of pharmaceuticals is described and forms the basis of a generic approach to analyte extraction to which additional compounds can be included with minimal method development time.

Pharmaceuticals in this investigation

A suite of pharmaceutical compounds were identified as potential environmental contaminants by the UK Environment Agency. These compounds exhibit a wide range of functionality and polarity (logP), as listed in **Table 1**.

Experimental Procedure

Sample Preparation

Water samples (500 mL) were spiked at 100 ng/L concentration of the compounds listed above.

Generic SPE Method

SPE Column: EVOLUTE ABN 50µm, 200 mg/6 mL
Sample Pre-treatment: None
Column Solvation: methanol (6 mL)
Column Equilibration: water (6 mL)
Sample Load: 500 mL at a flow rate of approx 15 mL/min (-10” Hg)
Interference Elution: Water (6 mL)
Elution: Methanol containing 5% NH4OH (6 mL)
Evaporate to dryness. Reconstitute in 200 µL methanol, add 800 µL water prior to injection.

Biotage

Table 1. Analyte functionality, pK value(s) and polarity (logP)

Analyte	Therapeutic Class	Functionality	pK	logP
Atenolol	Beta blocker	Basic	9.6	0.3
Sotalol	Beta blocker	Basic	8.2, 9.8	0.5
Trimethoprim	Antibacterial	Basic	6.6	1.3
Metoprolol	Beta blocker	Basic	13.3	1.8
Oxprenolol	Beta blocker	Basic	9.2	2.2
Labetalol	Beta blocker	Basic	7.4	2.7
Propranolol	Beta blocker	Basic	9.5	3.1
Erythromycin	Antibacterial	Basic	8.8	2.9
Citalopram	SSRI	Basic	9.5	1.5
Paroxetine	SSRI	Basic	9.9	5.0
Fluvoxamine	SSRI	Basic	8.7	1.3
Carbamazepine	Anticonvulsant	Basic	9.1	1.3
Fluoxetine	SSRI	Basic	9.5	4.2
Thioridazine	Antipsychotic	Basic	9.5	5.9
Tamoxifen	Antiestrogen, antineoplastic	Basic	8.9	6.2
Diclofenac	Anti-inflammatory	Acidic	4.0	4.4
Sulfamethoxazole	Antibacterial	Acid	6.0	2.4
Ibuprofen	Anti-inflammatory	Acidic	4.8	3.5
Mefenamic Acid	Anti-inflammatory	Acidic	4.2	4.9

HPLC Conditions

Instrument: Waters Alliance 2795 Separations Module.
Column: Zorbax Eclipse XDB-C18. 100 x 2.1 mm, 3.5 µm) Agilent
Guard Column: Zorbax Eclipse XDB-C8. (12.5 x 2.1 mm, 5 µm) Agilent
Injection Volume: 10 µL
Flow Rate: 0.25 mL/min. Entire column effluent directed into the MS

Table 2. HPLC Gradient

Time	A= 0.1% Formic in water	B=acetonitrile
0	88	12
9	53	47
12	10	90
14	10	90
14.10	88	12

MS Conditions

Instrument: Waters Quattro Ultima Pt triple quadrupole MS equipped with an electrospray source.
Source Temp: 100°C
Desolvation Temp: 350°C
Collision cell pressure: 2.23 e^{-3} mbar.

Table 3. MRM Conditions (analytes listed in order of elution)

Analyte	MRM Transition	Collision Energy (eV)
Atenolol	267.2 > 190.2	18
Sotalol	273.1 > 213.1	17
Trimethoprim	291.2 > 123.1	22
Metoprolol	268.2 > 116.1	18
Oxprenolol	266.2 > 72.1	18
Labetalol	329.2 > 311.1	12
Sulfamethoxazole	254.1 > 156.0	15
Propranolol	260.1 > 116.1	17
Erythromycin	734.5 > 158.2	31
Citalopram	325.1 > 109.1	23
Paroxetine	330.1 > 192.2	19
Fluvoxamine	319.2 > 71.0	15
Carbamazepine	237.1 > 194.1	17
Fluoxetine	310.2 > 148.2	7
Thioridazine	371.1 > 126.1	22
Tamoxifen	372.2 > 72.1	21
Diclofenac*	294.1 > 250.1	11
Ibuprofen*	205.2 > 159.2	7
Mefenamic Acid*	240.2 > 196.2	16

**All positive ion mode except Diclofenac, Ibuprofen, Mefenamic acid*
Dwell time 0.08-0.15 s; Cone Voltage 35-60 V. Details available on request

Biotage

Results

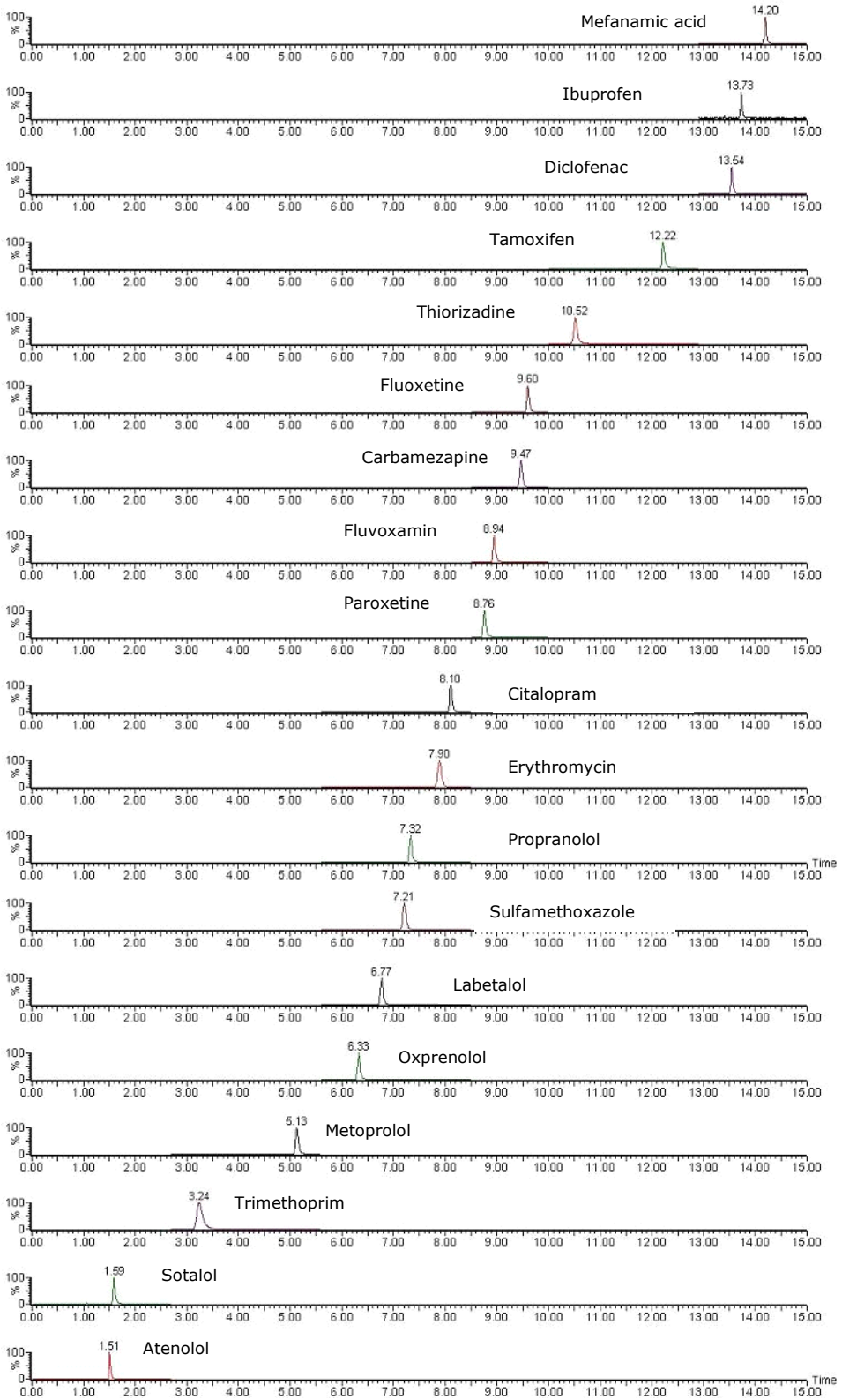

Figure 1. Mass chromatograms for all analytes

Table 4. Analyte recovery

Analyte	Analyte recovery (%)	% rsd (n=4)
Atenolol	63.2	10.8
Sotalol	86.4	7.3
Trimethoprim	97.7	0.8
Metoprolol	99.7	5.7
Oxprenolol	95.5	7.4
Labetalol	88.5	5.4
Sulfamethoxazole	93.9	3.8
Propranolol	92.6	6.4
Erythromycin	84.7	3.7
Citalopram	91.3	7.5
Paroxetine	76.9	4.4
Fluvoxamine	75.0	4.7
Carbamazepine	98.5	1.6
Fluoxetine	76.0	8.8
Thioridazine*	47.4	11.6
Tamoxifen*	48.9	22.2
Diclofenac	96.7	1.0
Ibuprofen	100.6	2.5
Mefenamic Acid	97.0	1.6

**Increased recoveries and reduced RSDs for these specific analytes have been achieved with optimized elution solvents. This is thought to be due to solubility of the specific compounds, and will be investigated in further work*

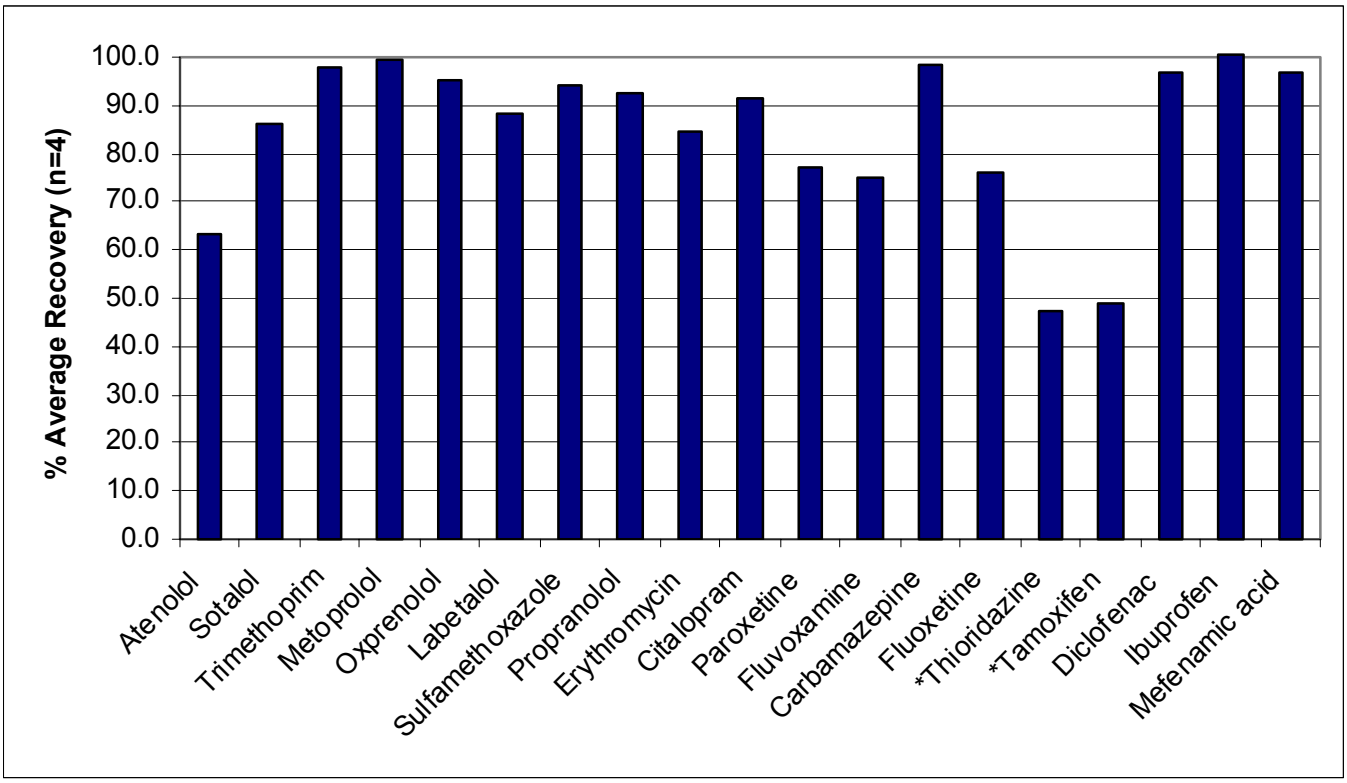

Figure 2. Summary of recovery results from Table 4.
**Increased recoveries and reduced RSDs for these specific analytes have been achieved with optimized elution solvents. This is thought to be due to solubility of the specific compounds, and will be investigated in further work*

Biotage

Overall Conclusions

A generic method for to the solid phase extraction of a broad range of pharmaceuticals has been developed, and forms the basis of a generic approach to analyte extraction to which additional compounds can be included with minimal method development time.

www.biotage.com

United States and Canada
Tel: +1 434 979 2319
Toll-Free: +1 800 446 4752
ordermailbox@biotage.com

United Kingdom, EIRE
Biotage
Tel: +44 1992 501535
order@eu.biotage.com

Sweden
Biotage
Tel: +46 18 56 59 00
order@eu.biotage.com

Japan
Biotage
Tel: +81 422 281233
order@biotage.co.jp

Biotage