A Generic Approach to the Extraction of Multi-functional Drugs using Mixed-mode SPE with LC-MS/MS Analysis

Matthew Cleeve, Scott Merriman, Lee Williams, Steve Jordan, Richard Calverley, Joanna Smith & Steve Plant
Biotage GB Limited, Dyffryn Industrial Estate, Ystrad Mynach, Mid Glamorgan, CF82 7RJ, UK

Introduction

The use of non-polar polymer-based Solid Phase Extraction (SPE) prior to LC-MS/MS analysis of drugs in biological fluid samples is a well documented technique. The limitations of non-polar polymer-based SPE can be mainly attributed to lack of selectivity leading to increased ion suppression, and lower recoveries of very polar compounds. The use of mixed-mode SPE gives significant selectivity advantages over hydrophobic polymer-based SPE. Polymer-based mixed-mode cation exchange SPE sorbents provide a dual retention mechanism, allowing the use of rigorous interference elution regime, therefore significantly improving extract cleanliness. Polymeric mixed-mode cation exchange sorbents can also be used for extraction of very polar bases when retention on traditional non-polar polymers and silica-based mixed-mode sorbents is not sufficient.

This poster describes the use of a new polymeric mixed-mode cation exchange SPE sorbent, for the extraction of multi-functional drugs from biological fluids. The analyte suite incorporates acidic, neutral and basic analytes, selected for a wide variation of pK_a and logP values. Full details are available (see **Table 3**, **page 3**).

Recoveries were observed above 80% for all analytes studied with corresponding RSD's below 10%. Since cation exchange SPE is used to extract basic analytes, the purpose of the acidic and neutral analytes was to show correct fractionation in the final extraction procedure. The use of a quaternary amine in the test mix was also necessary to demonstrate the strong cation exchange functionality of the polymer.

Experimental Procedure

Reagents

All analytes (see **Table 3**, **page 3**) were purchased from Sigma Chemical Co. (Poole, UK). Blank human plasma was obtained through the Welsh Blood Service (Pontyclun, UK). All solvents were HPLC grade from Fisher Scientific (Loughborough, UK).

Sample Preparation Procedure

Extractions were performed in the 25 mg 96-well format using blank human plasma (100 µL) spiked at a concentration of 5 pg/µL.
Sample Pre-treatment: Plasma sample diluted with 1% (v/v) aqueous formic acid (1:3, v/v)
Column Conditioning: Methanol (1 mL)
Column Equilibration: 0.1% (v/v) aqueous formic acid (1 mL)
Sample Loading: Pre-treated plasma sample (400 µL)
Interference Elution: 0.1% aqueous formic acid (1 mL), followed by methanol (1 mL)
Analyte Elution: 5% (v/v) NH_4OH in methanol (1 mL)
Post Extraction: The extracts were evaporated to dryness and reconstituted in 200 µL of 90:10 (v/v) 0.1% aqueous formic acid/methanol for subsequent LC-MS/MS analysis.

HPLC Conditons

Instrument: Waters 2795 Liquid Handling System (Waters Assoc., Milford, MA, USA)
Column: Zorbax Eclipse XDB C18 3.5 µm analytical column (100 x 2.1 mm ID) (Agilent Technologies, Berkshire, UK)
Guard Column: C8 guard column (Agilent Technologies, Berkshire, UK)
Mobile Phase: 0.1% formic acid aq and MeCN at a flow rate of 0.25 mL/min. See **Table 1** for full gradient conditions.
Injection Volume: 25 µL
Temperature: Ambient temperature

***Table 1**. HPLC Gradient*

Time (mins)	0.1% Formic acid aq (%)	MeCN (%)
0	98	2
2.5	85	15
5.5	85	15
10	30	70
12	30	70
12.01	98	2
15	98	2

Mass Spectrometry Conditions

Analyte Recoveries

Instrument: Ultima Pt triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK) equipped with an electrospray interface for mass analysis. Positive ions were acquired in the multiple reaction monitoring mode (MRM)
Desolvation Temperature: 350 °C
Ion Source Temperature: 100 °C
Collision Gas Pressure: 2.9 x 10^{-3} mbar
The base peak in each compound spectrum was attributed to the protonated molecular ion $[M+H]^+$ and were subsequently used as the precursor ions in the resulting MRM transitions. Due to the large number of analytes present in this suite the MRM transitions were split into 5 scan funtions. Full MRM transitions and ionization conditions are shown below in **Table 2**.

***Table 2**. MRM Conditions*

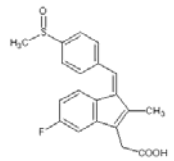
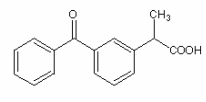
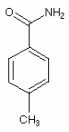
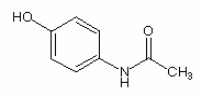
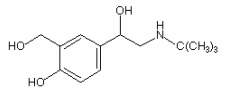
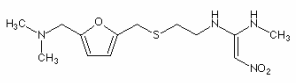
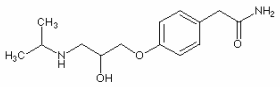
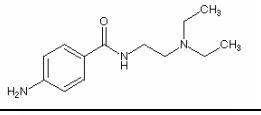
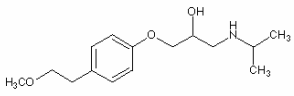
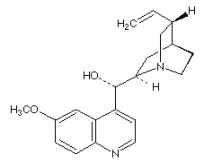
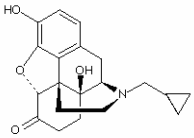
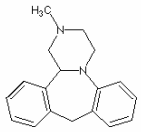
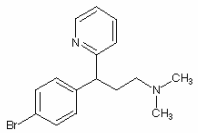
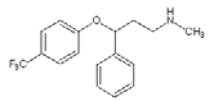
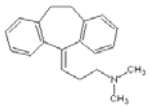
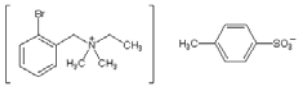
Scan Function	Analyte	MRM Transition	Cone Voltage (V)	Collision Energy (eV)
1	Procainamide	236.1 > 163.1	35	15
2	Acetaminophen	152.05 >110.05	40	12
	Salbutamol	240.0 > 148.0	35	15
	Atenolol	267.0 > 190.0	35	18
	Ranitidine	315.1 > 176.0	35	16
3	Bretylium Tosylate	242.05 > 168.95	35	15
	Quinidine	325.1 > 160.05	35	25
	Naltrexone	342.1 > 324.1	40	19
4	p-toluamide	136.0 > 93.0	35	10
	Metoprolol	268.1 > 116.1	35	17
5	Ketoprofen	255.05 > 209.05	35	11
	Mianserin	265.0 > 208.0	35	19
	Amitriptyline	278.05 > 233.0	35	15
	Fluoxetine	310.0 > 148.0	35	8
	Brompheniramine	319.05 > 273.95	35	15
	Sulindac	357.0 > 233.0	50	25

Ion Suppression FIA-MS/MS

Blank plasma samples (100 µL) were extracted as in the generic method listed above. MeOH interference wash steps were collected, evaporated to dryness and reconstituted in 1 mL of 50:45:5:0.1% H_2O/ACN/MeOH/Formic acid (v/v) mobile phase spiked with 1 µg/mL caffeine concentration. The 2795 liquid handling unit delivered an isocratic mobile phase of 50:45:5:0.1% H_2O/ACN/MeOH/Formic acid (v/v) at 0.25 mL/min.

Injection volumes were set to 5 µL and the MS set to monitor the MRM transition for caffeine (195>138, cone voltage 45 V and collision energy 16 eV).

***Table 3**. Analyte properties and structures*

Analyte	LogP	pK	Structure	Analyte	LogP	pK	Structure
Acidic Analytes							
Sulindac	3.42	4.7		Ketoprofen	3.12	4.45	
Neutral Analytes							
p-toluamide	1.18	N/A		Acetaminophen	0.46	9.38	
Basic Analytes							
Salbutamol	1.31	9.8		Ranitidine	0.27	8.8	
Atenolol	0.16	9.1		Procainamide	0.88	9.4	
Metoprolol	1.88	10.8		Quinidine	3.44	8.56	
Naltrexone	1.92	9.2		Mianserin	3.67	8.3	
Brompheniramine	4.06	3.59 and 9.2		Fluoxetine	4.05	8.7	
Amitriptyline	4.92	9.4					
Quaternary Amine							
Bretylium tosylate							

** logP and pK_a values were taken from literature or calculated values if not available*

Results

The analyte suite was selected to incorporate acidic, basic and neutral functionalities with varying polarity, in order to demonstrate the retention and elution characteristics of the mixed-mode sorbent. The mass chromatograms obtained from the analyte suite are shown in **Figure 1**. The extraction results (n=6) listed in **Table 4, page 5** shows recoveries greater than 80% with RSD's less than 10% for all analytes at a concentration of 5 ng/mL of plasma.

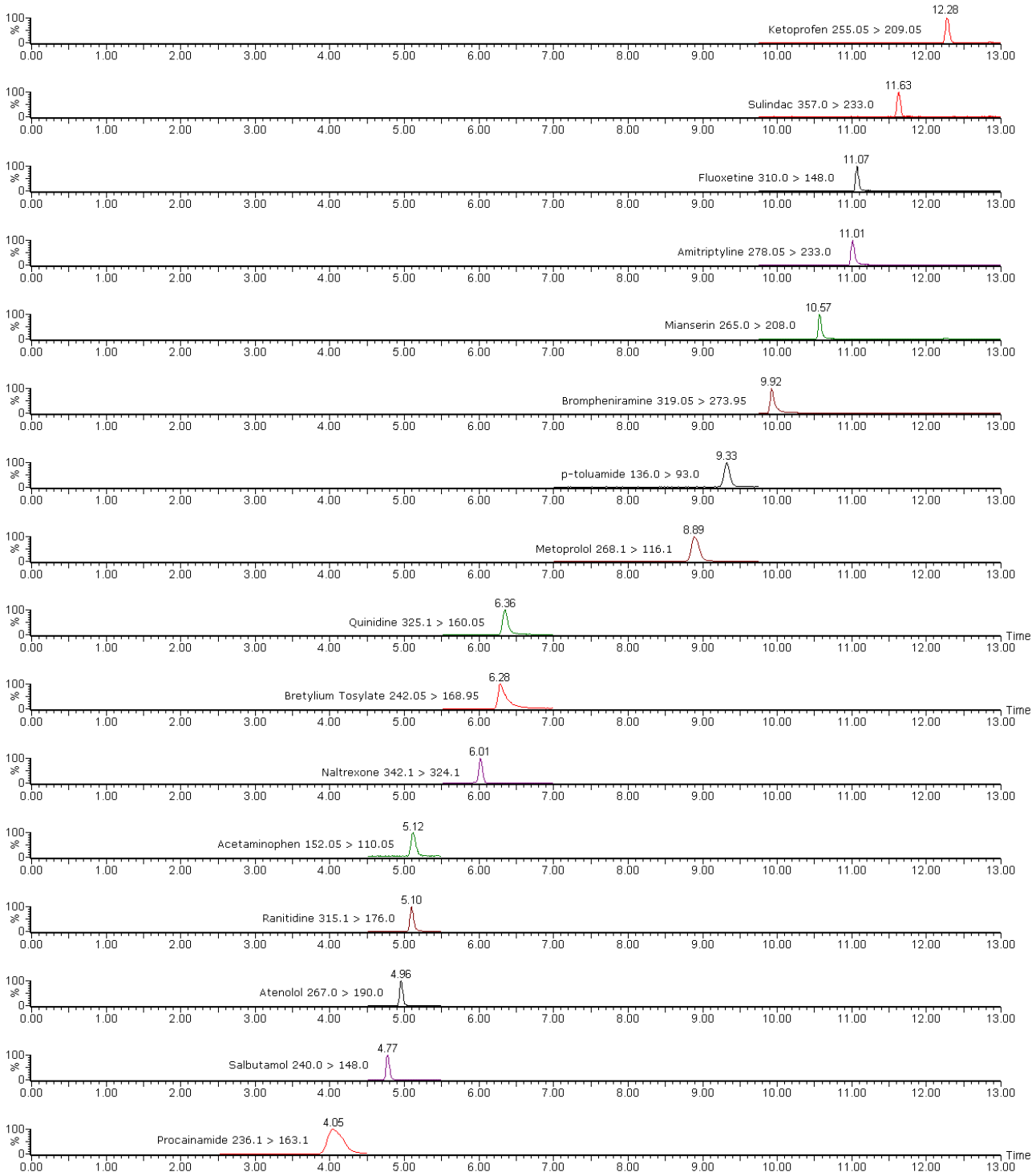


***Figure 1**. Mass chromatograms produced by a 25 µL injection of a standard at a concentration of 2.5 pg/µL*

***Table 4**. Full analyte recoveries (n=6). RSD's in parenthesis*

Analyte	% Recovery in MeOH wash (% RSD)	% Recovery in Elution (% RSD)
Sulindac	83 (4)	0
Ketoprofen	90 (5)	0
p-toluamide	94 (5)	0
Acetaminophen	91 (2)	0
Salbutamol	0	91 (2)
Atenolol	0	91 (4)
Ranitidine	0	92 (3)
Procainamide	0	89 (3)
Metoprolol	0	98 (3)
Naltrexone	0	85 (4)
Quinidine	0	92 (7)
Mianserin	0	87 (4)
Brompheniramine	0	88 (9)
Fluoxetine	0	89 (7)
Amitriptyline	0	90 (4)
Bretylium tosylate	0	0

All acidic and neutral analytes were successfully eluted in the methanol interference wash step, while the basic analytes were retained until the final elution step. No cross contamination was observed between the interference wash and elution steps. The quaternary amine, bretylium tosylate, did not elute from the sorbent in any step.

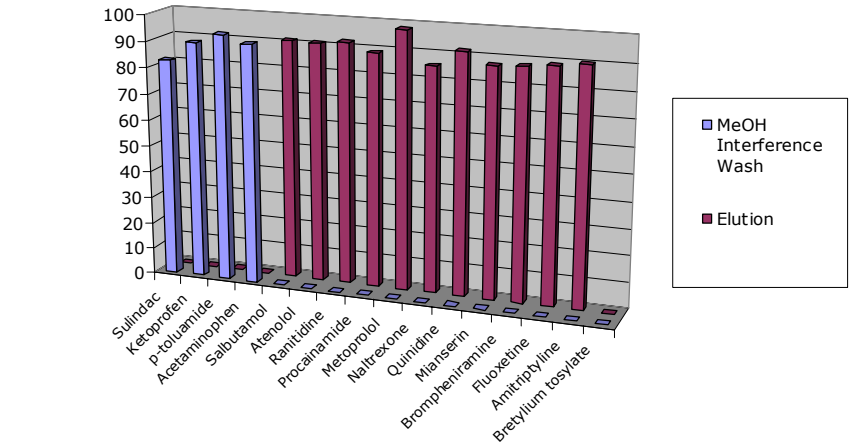


***Figure 2**. Analyte elution profile showing recoveries in both the methanol interference wash and elution steps (n=6).*

Figure 2 shows graphically the removal of the acidic and neutral analytes in the methanol wash step, and recovery of the basic compounds in the analyte elution step. The RSD's observed for the acidic and neutral analytes show that the methanol interference wash step was sufficiently clean to provide good quantitation results for the analytes tested in this suite. From this, the ion suppression on the methanol interference wash step was tested.

Figure 3 shows the FIA-MS/MS TIC obtained when comparing the ion suppression from the MeOH interference wash step with pure spiked mobile phase (caffeine spiked at 1 µg/mL). The overall suppression from this wash step (n=3) was 28% with an RSD of 5% between replicate extractions.

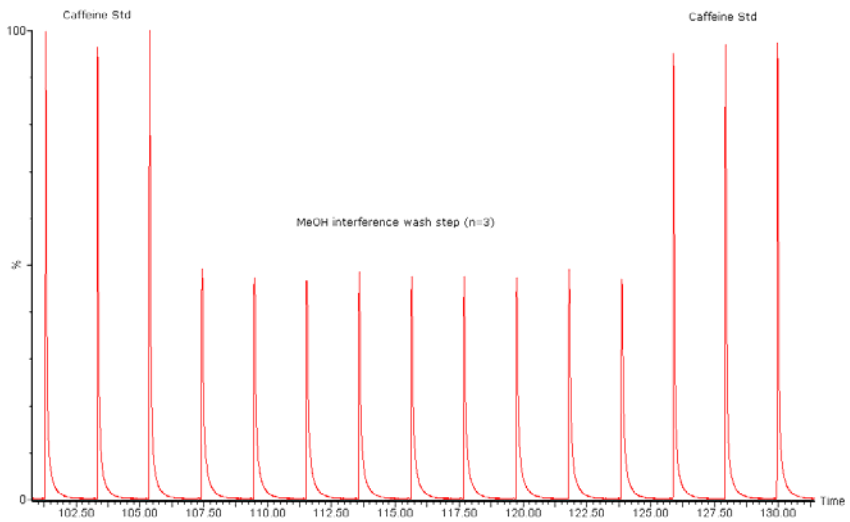


***Figure 3**. FIA-MS/MS TIC showing MeOH interference wash suppression compared to 1 µg/mL caffeine standard injections.*

Conclusions

- Excellent recoveries were obtained for all basic analytes.
- Acidic and neutral analytes were recovered in the methanol interference wash step, demonstrating the selectivity of the mixed-mode approach.
- Good RSD's were obtained for analytes in both the methanol interference wash step and the elution step.
- Only 28% suppression was observed from the methanol wash step. This along with good RSD's show that it is possible to accurately quantitate acidic and neutral analytes from this wash step.
- No elution of the quaternary amine indicates that the SPE polymer exhibits strong cation exchange functionality.

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UK, Germany, Switzerland, Austria
Service and Support
Telephone: +46 18 56 59 11
E-mail: 1-PointSupport@eu.biotage.com

United States
Service and Support
Telephone: 1 800 446 4752
press (3) at the auto attendant
E-mail: 1-PointSupport@biotage.com

Japan
Service and Support
Telephone: +81 422 28 1233
E-mail: 1-PointSupport@biotage.co.jp

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