A Generic Approach to the Extraction of Basic Drugs using Polymeric Mixed-mode Cation Exchange SPE and Analysis by LC-MS/MS

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Introduction

The use of non-polar resin-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 resin-based SPE can mainly be 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 non-polar resin-based SPE. Resin-based mixed-mode cation exchange SPE sorbents provide a dual retention mechanism, allowing the use of rigorous interference elution regime (as illustrated in **Figure 1**), therefore significantly improving extract cleanliness. Resin-based 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.

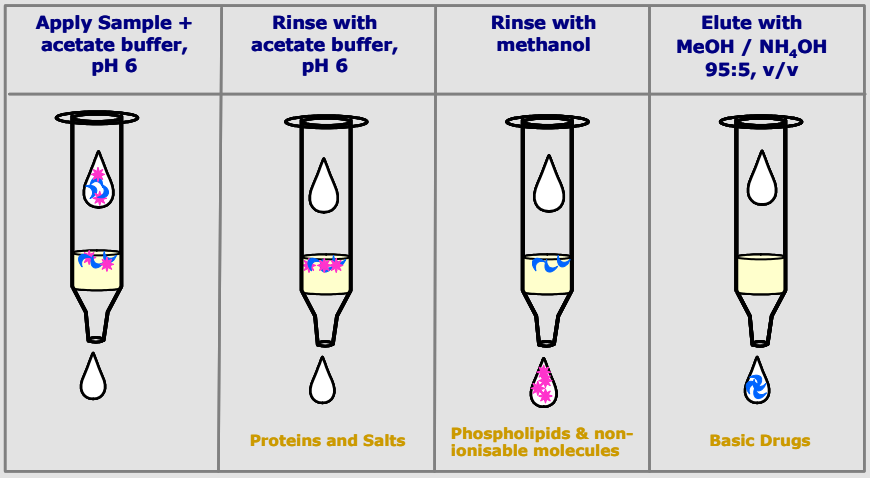


Figure 1. Representation of a mixed-mode SPE approach. The dual retention mechanism allows the use of 100% organic solvent in the interference wash, removing problem interferences and producing clean extracts.

This poster describes the use of EVOLUTE® CX, a new resin-based mixed-mode cation exchange SPE sorbent, for the extraction of various basic drugs from biological fluids. The analyte suite incorporates basic analytes with differing pK and logP values. See **Table 3** for full details.

Experimental Procedure

Reagents

All analytes (see **Table 3**, **page 3-4**) 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 50 ng/mL.

Sample Pre-treatment: Plasma sample diluted with 50 mM ammonium acetate buffer at pH 6 (1:3, v/v)
Column Conditioning: Methanol (1 mL)
Column Equilibration: 50 mM ammonium acetate buffer at pH 6 (1 mL)
Sample Loading: Pre-treated plasma sample (400 µL)
Interference Elution: 50 mM ammonium acetate buffer at pH 6 (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 1 mL of 80:20 (v/v) H_2O/MeOH 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)
Guard Column: C8 guard column (both 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: 15 µL
Temperature: Ambient temperature

Table 1. HPLC Gradient

Time (mins)	0.1% Formic acid aq (%)	MeCN (%)
0	90	10
6.2	20	80
6.3	90	10

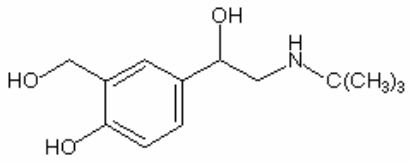
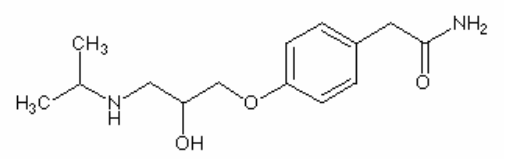
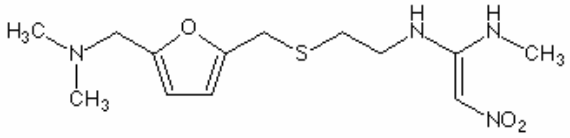
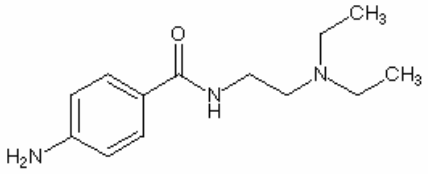
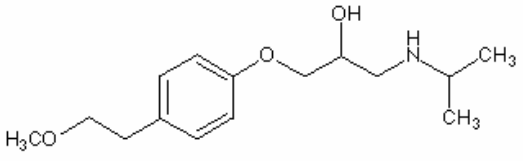
Mass Spectrometry Conditions
Instrument: Ultima Pt triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK) equipped with an electrospray interface for mass analysis. Positive ions were acquired in the multiple reaction monitoring mode (MRM)
Desolvation Temperature: 350 °C
Ion Source Temperature: 100 °C
Collision Gas Pressure: 2.4×10^{-3} mbar.

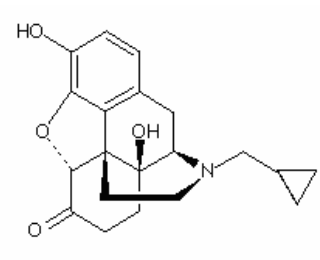
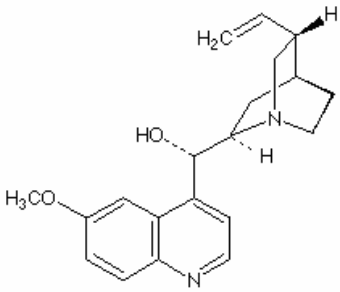
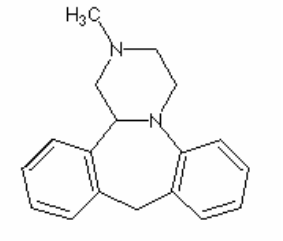
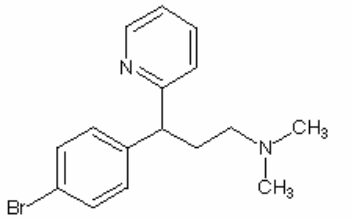
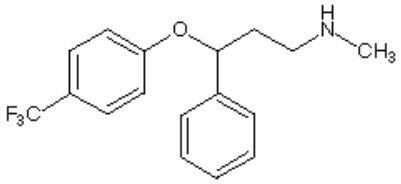
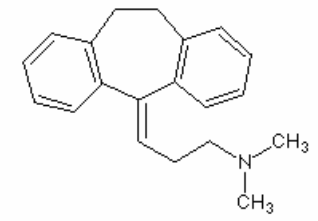
The base peak in each compound spectrum was attributed to the protonated molecular ion $[M+H]^+$ and were subsequently used as the precursor ions in the resulting MRM transitions. Due to the large number of analytes present in this suite the MRM transitions were split into 5 scan funtions. See **Table 2**, **page 3** for details on the full MRM transitions and ionization conditions.

Table 2. MRM Conditions

Scan Function	Analyte	MRM Transition	Cone Voltage (V)	Collision Energy (eV)
1	Procainamide	236.1 > 163.1	35	15
	Salbutamol	240.0 > 148.0	35	15
	Atenolol	267.2 > 190.2	55	18
	Ranitidine	315.1 > 176.0	35	16
2	Naltrexone	342.1 > 324.1	40	19
	Quinidine	325.1 > 160.0	35	25
3	Metoprolol	268.1 > 116.1	35	17
	Brompheniramine	319.1 > 274.0	35	15
4	Mianserin	265.0 > 208.0	35	19
5	Amitriptyline	278.1 > 233.0	35	15
	Fluoxetine	310.0 > 148.0	35	8

Table 3. Basic drug properties and structures.

Analyte	**LogP***	**pK_a***	**Structure**
Salbutamol	1.31	9.8	
Atenolol	0.16	9.1	
Ranitidine	0.27	8.8	
Procainamide	0.88	9.4	
Metoprolol	1.88	10.8	

Analyte	LogP*	pK_a*	Structure
Naltrexone	1.92	9.2	
Quinidine	3.44	8.56	
Mianserin	3.67	8.3	
Brompheniramine	4.06	3.59 and 9.2	
Fluoxetine	4.05	8.7	
Amitriptyline	4.92	9.4	

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

Results

The analyte suite was selected to incorporate common basic drugs showing wide ranging polarities. The mass chromatograms obtained from the analyte suite are shown in **Figure 2** below.

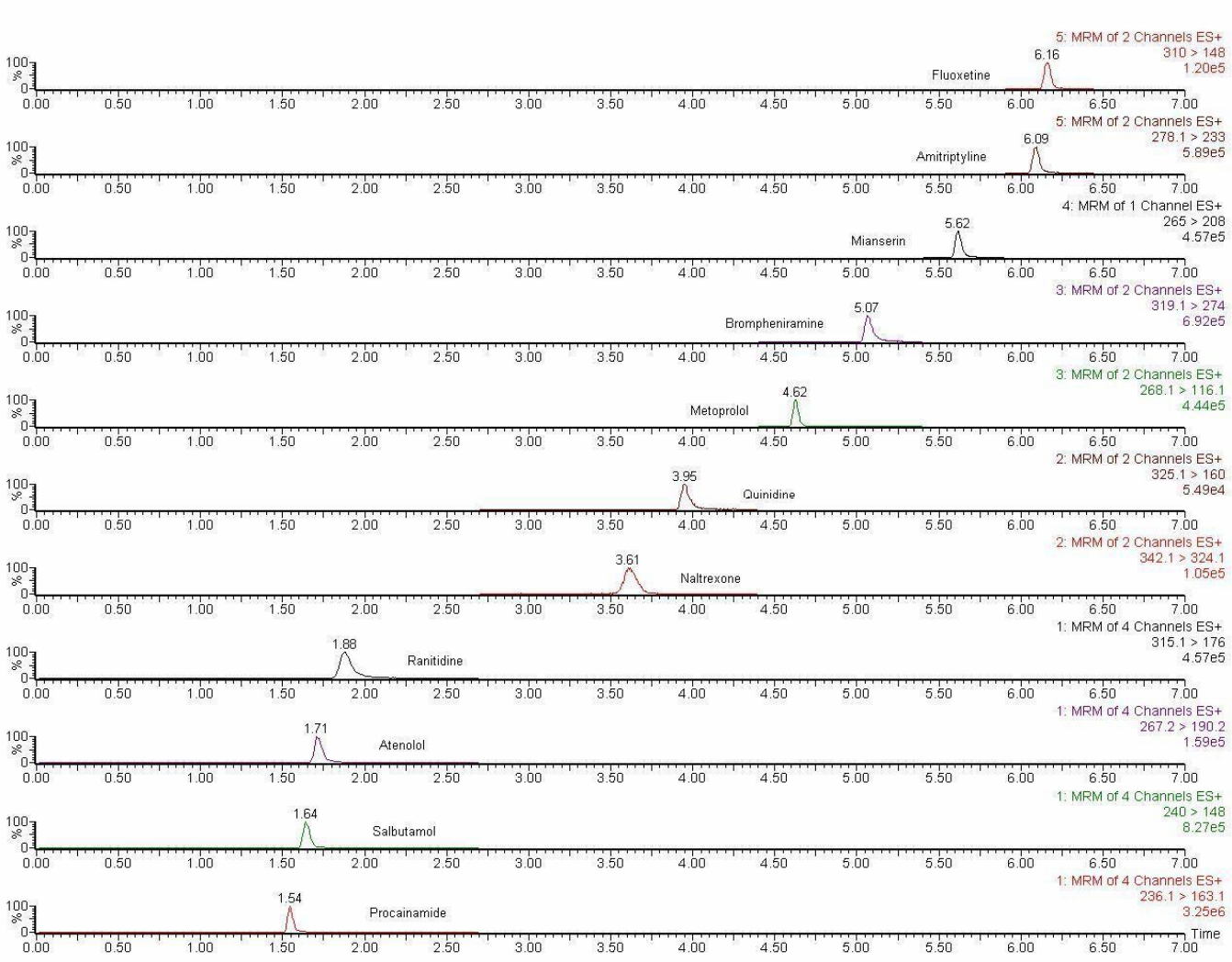


Figure 2. Mass chromatograms for the 11 basic analytes

The extraction results (n=7) listed in **Table 3** show recoveries greater than 90% with RSD's less than 10% for all analytes at a concentration of 50 ng/mL in plasma.

***Table 3**. Full analyte recoveries (n=7). RSD's in parenthesis*

Analyte	% Recovery in Elution (% RSD)
Procainamide	98.5 (1.5)
Salbutamol	99.4 (2.9)
Atenolol	97.6 (1.3)
Ranitidine	97.5 (7.1)
Naltrexone	95.7 (2.0)
Quinidine	95.1 (2.7)
Metoprolol	97.1 (2.5)
Brompheniramine	97.7 (2.0)
Mianserin	94.4 (5.1)
Amitriptyline	95.3 (3.5)
Fluoxetine	97.9 (3.9)

Figure 3 shows graphically the recovery of the basic analytes in the elution.

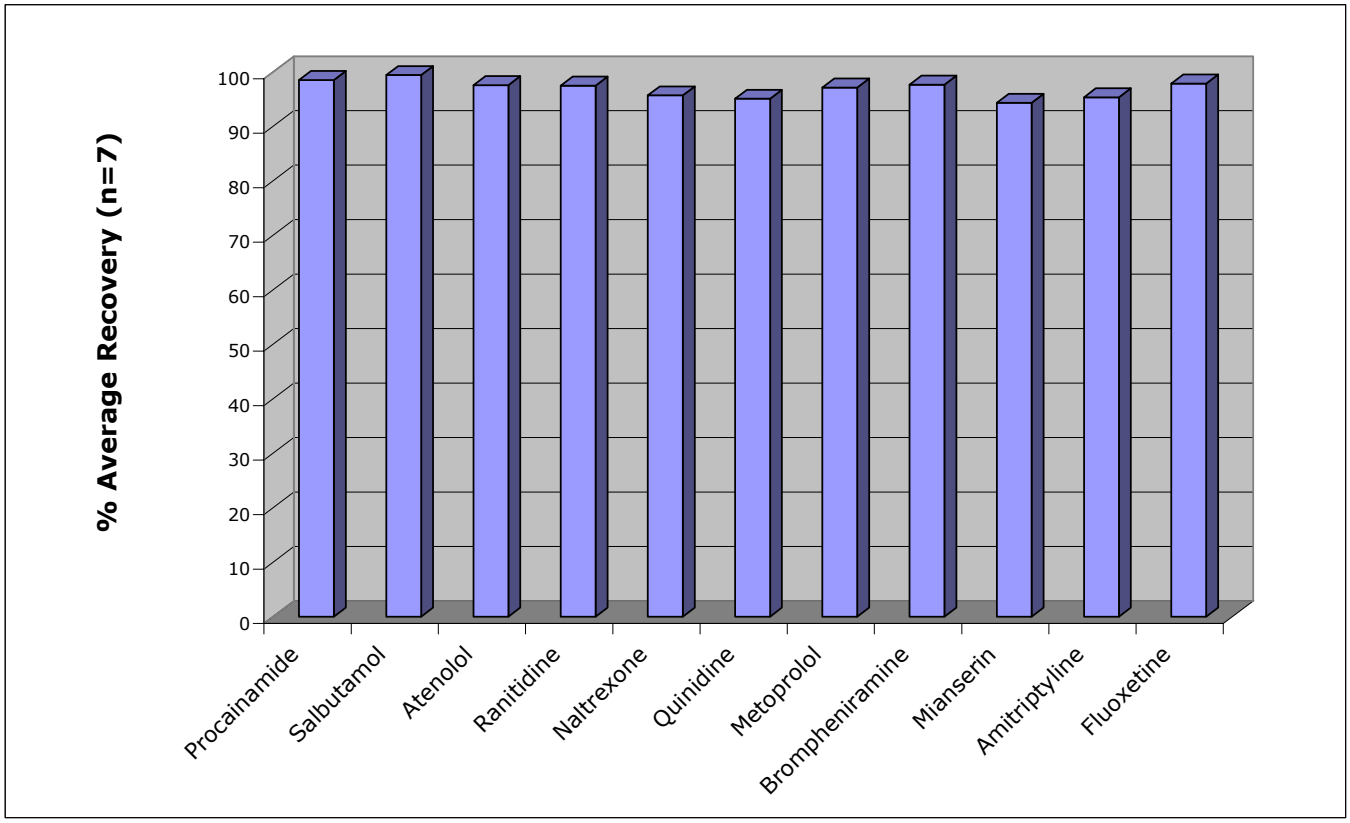


***Figure 3**. Basic analyte recovery profile (n=7).*

Conclusions

This poster has demonstrated that mixed-mode SPE with EVOLUTE CX provides a robust and reliable approach to extraction of basic drugs from plasma.

1. Consistent high recoveries were obtained for all basic analytes with varying LogP and pKa values
2. Low RSDs of less than 10 % were obtained for all analytes

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