Extraction of Basic Drugs from Plasma using EVOLUTE® CX 96-well Plates and Columns

Application Note

Introduction

This Application Note describes the extraction of a variety of basic drugs from plasma using EVOLUTE® CX mixed-mode resin-based SPE. The analyte suite includes basic drugs with wide ranging pK_a and logP values.

The sample preparation method and suggested analytical procedures are detailed on page 1. Recoveries and % RSD are shown on page 3.

Sample Preparation Procedure

Analytes

Procainamide, Salbutamol, Atenolol, Ranitidine, Naltrexone, Quinidine, Metoprolol, Brompheniramine, Mianserin, Amitriptyline and Fluoxetine

EVOLUTE CX Configuration

EVOLUTE Array CX 25 mg fixed-well plate (part number 601-0025-P01)

EVOLUTE CX Procedure

Sample:	Plasma spiked at 50 pg/µL concentration with the compounds listed above
Sample Pre-treatment:	Dilute plasma sample (1:3 (v/v), 100 µL) with 0.05 M NH_4OAc, pH 6 (total analyte load 5 ng)
Column Conditioning:	Methanol (1 mL)
Column Equilibration:	0.05 M NH_4OAc, pH 6 (1 mL)
Sample Application:	Apply diluted sample (400 µL)
Interference Elution:	Wash 1. Rinse with 0.05 M NH_4OAc, pH 6 (1 mL) Wash 2. Rinse with methanol (1 mL)
Analyte Elution:	Methanol / NH_4OH (95:5 (v/v), 1 mL)
Post Extraction:	Evaporate to dryness and reconstitute in water/methanol (80:20, 1mL) for subsequent LC-MS/MS analysis

For general guidelines on the use of EVOLUTE CX SPE plates and columns, request Chemistry Data Sheet **TN139** EVOLUTE CX Columns for Solid Phase Extraction in Bioanalysis.

HPLC Conditions

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
Gradient:	90:10 increasing to 20:80 (v/v) over 6.2 minutes. At 6.3 minutes initial starting conditions were applied.
Injection Volume:	15 µL
Temperature:	Ambient temperature

Biotage

Mass Spectrometry

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 Tempurature:	350 °C
Ion Source Tempurature:	100 °C
Collision Gas Pressure:	2.3 x 10^{-3} mbar.

The base peak in each compound spectrum was attributed to the protonated molecular ions, $[M+H]^+$ and were subsequently used as the precursor ions in the resulting MRM transitions. Full MRM transitions and ionization conditions are shown in **Table 1.**

Table 1. Quattro Ultima Pt mass spectrometer parameters

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	Quinidine	325.1 > 160.0	35	25
	Naltrexone	342.1 > 324.1	40	19
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

Results

Table 2. % Average Recovery and RSD of basic drugs from plasma

Analyte	% Recovery	% RSD
Procainamide	99	2
Salbutamol	99	3
Atenolol	98	1
Ranitidine	98	7
Naltrexone	96	2
Quinidine	95	3
Metoprolol	97	3
Brompheniramine	98	2
Mianserin	94	5
Amitriptyline	95	4
Fluoxetine	98	4

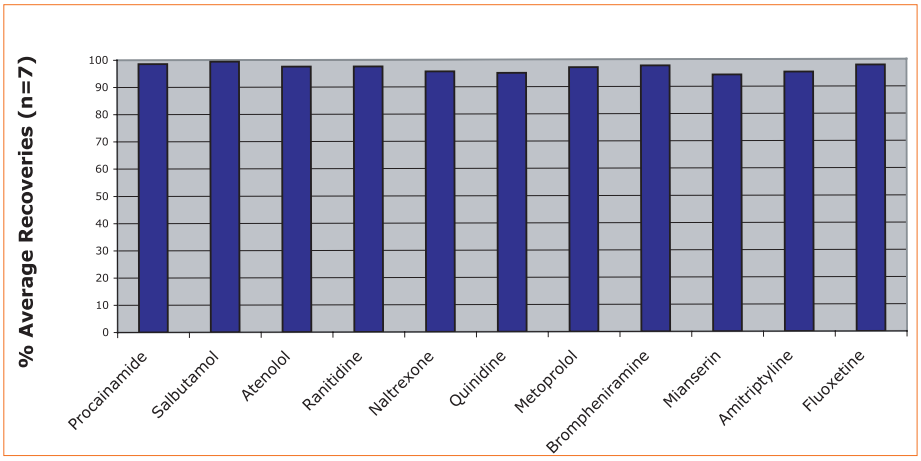

Figure 1. Recoveries (>90%, <7% RSD, n=7) for basic drugs from plasma using EVOLUTE CX mixed-mode SPE

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