EVOLUTE® CX Columns for Solid Phase Extraction of Basic Compounds from Aqueous Samples

Chemistry Data Sheet

This Chemistry Data Sheet provides guidelines for the extraction of basic analytes from aqueous samples using resin-based mixed-mode SPE. The procedure is described on page 1, with processing and optimisation guidelines on page 2.

An example application showing the extraction of basic drugs from plasma illustrates the versatility of EVOLUTE® CX when extracting a wide range of analytes (see Appendix 1).

EVOLUTE CX has been developed for extraction of basic analytes from aqueous samples. The resin-based mixed-mode sorbent is surface modified with well defined hydroxyl-functional oligomers, imparting excellent water wettability. An optimised combination of non-polar (hydrophobic), polar (hydrophilic) and cation exchange interactions allows efficient extraction of basic analytes of wide ranging polarities. The non-polar/cation exchange dual retention mechanism allows the use of a rigorous interference regime, providing extremely clean extracts and reducing matrix effects associated with LC-MS/MS analysis.

The 50 µm mean particle size sorbent is optimised to facilitate the processing of larger sample volumes and more viscous samples common in forensic, clinical, food and environmental applications.

Section 1: Methodology

This procedure is optimised for 50 mg/3 mL configuration SPE columns. The method can readily be transferred to other configurations using the information described in **Table 2** of this Chemistry Data Sheet.

1. Sample Pre-treatment:	Dilute sample with ammonium acetate buffer (50 mM, pH 5, 1:1, v/v) a. Particulate laden samples: filter to remove particulate material b. Viscous samples: viscous samples may require additional dilution
2. Column Conditioning:	Condition each column with methanol (2 mL)
3. Column Equilibration:	Equilibrate each column with ammonium acetate buffer (50 mM, pH 5, 2 mL)
4. Sample Loading:	Load sample at 3 mL/min
5. Interference Elution:	**Wash 1.** Elute polar/ionic interferences with ammonium acetate buffer (50 mM, pH 5, 2 mL) **Wash 2.** Elute non-polar interferences* with methanol (2 mL)
6. Analyte Elution:	Elute basic analytes with methanol/ammonia (95:5, v/v, 1 mL). For strongly retained analytes, use an additional aliquot of elution solvent.
7. Post-extraction:	If desired, evaporate extract to dryness and re-constitute in mobile phase or other suitable solvent for analysis.

Biotage

*EVOLUTE CX can also be used to fractionate complex mixtures of acidic, neutral and basic compounds. When used in this mode, acidic and neutral analytes are eluted in this step.

Section 2: Reagents

0.05 M Ammonium Acetate Buffer, pH 5

Used in sample pre-treatment, equilibration and interference wash 1. Dissolve 3.854 g ammonium acetate in 950 mL deionised water, adjust to pH 5 using acetic acid (ACS reagent grade), mix thoroughly and make up to 1L with deionised water.

95:5 (v/v) Methanol/Ammonia Solution

Used for analyte elution. Take 5 mL of 28% ammonium hydroxide, and add 95 mL methanol. Mix thoroughly.

Section 3: Processing Conditions

The well defined particle size distribution of EVOLUTE CX 50 µm allows many samples to flow under gravity. For samples which do not flow under gravity, the flow rates described in **Table 1** should be used for method development. For further optimisation, increase the vacuum until the desired flow rate is reached. If analyte breakthrough is observed, reduce flow rate.

For each step, load solvent or sample onto columns prior to applying vacuum. This will ensure even flow rates and improved analytical precision.

Table 1: Recommended flow rates for method development

Column size	3 mL and 10 mL 'H' columns	6 mL columns
Flow rate	3 mL/min	7 mL/min

Table 2: Typical volumes for each step

Step	**Bed mass**			
	50 mg	**100 mg**	**200 mg**	**500 mg**
Column conditioning	2 mL	2-3 mL	3-4 mL	6 mL
Column equilibration	2 mL	2-3 mL	3-4 mL	6 mL
Sample load	Application specific, based on analyte concentration in sample			
Interference wash (1 and 2)	2 mL	2-3 mL	3-4 mL	6 mL
Analyte elution	Dependant on analyte and choice of elution solvent. Minimum elution volume = 2 bed volumes.			

Note: *1 bed volume is approximately 200 µL/100 mg of sorbent.*

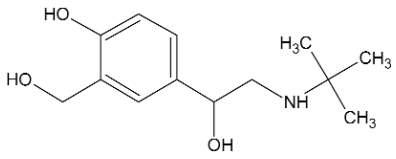
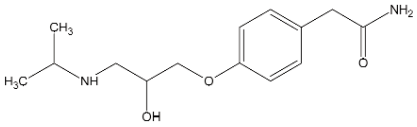
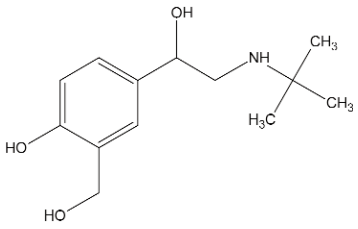
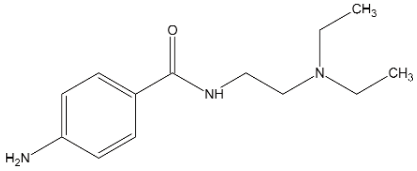
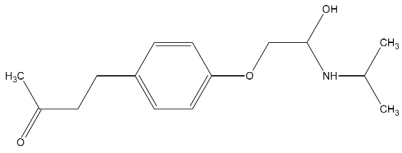
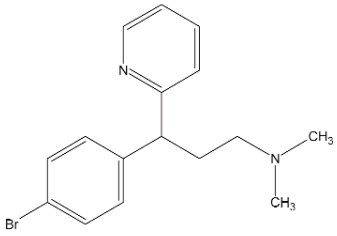
Section 4: Optimising the SPE method

a) For particularly viscous samples, increasing sample dilution will normally improve flow rates.
b) EVOLUTE CX is a water wettable resin-based sorbent. Analyte recovery will be unaffected if the columns run dry after conditioning.
c) To minimize elution volume, apply 2 separate aliquots (X ÷ 2 mL), including a soak step, rather than a single aliquot of X mL.

Using EVOLUTE CX with alternative SPE Procedures

EVOLUTE CX is a versatile solid phase extraction sorbent, and can be used with other manufacturers mixed-mode cation exchange polymer based SPE procedures. Further optimisation may be required because of the subtle differences in retention and elution characteristics.

Table 3: Structures of Basic Drug Analytes

Analyte	Structure	Functionality	logP	pK_a
Salbutamol		Basic, polar	1.31	9.8
Atenolol		Basic, highly polar	0.16	9.1
Ranitidine		Basic, highly polar	0.27	8.8
Procainamide		Basic, highly polar	0.88	9.4
Metoprolol		Basic, polar	1.88	10.8
Brompheniramine		Basic, non-polar	4.06	3.59 and 9.2

[1] pK and logP values were obtained from literature or values were calculated if not available

Basic drugs spiked in plasma (1 mL) at concentrations of 5 ng/ mL, extracted using EVOLUTE CX 50 µm 50 mg /3 mL SPE columns, and the extraction method described on page 1. Analysis was by LC-MS/MS.

APPENDIX 1

Extraction of Basic Drugs from Plasma

Using the generic method described on page 1, EVOLUTE CX SPE columns are suitable for extraction of basic drugs with wide ranging polarity. **Figure 1** shows typical results. See **Table 1** for analyte structures, logP and pK_a data.

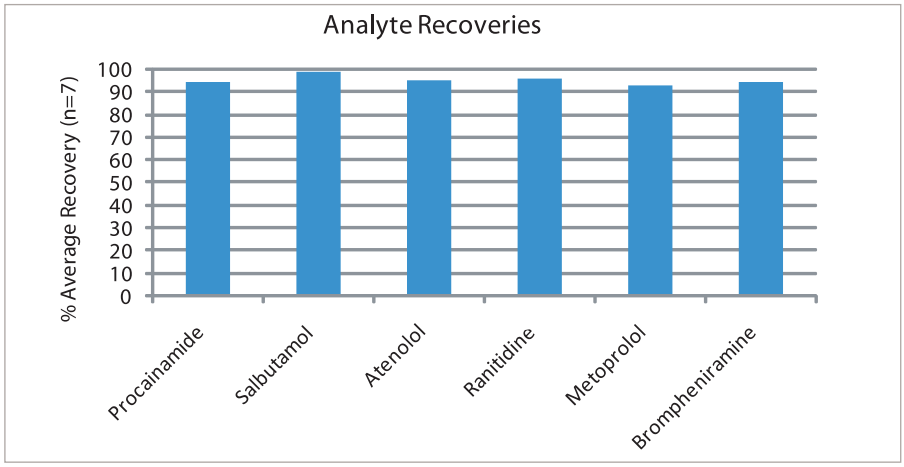


Figure 1. EVOLUTE CX provides high absolute recoveries (>85%) with excellent reproducibility (<10 % RSD, n=7) for a selection of basic drugs from plasma (1 mL sample volume at a concentration of 5 ng/mL).

Reproducible Extraction Performance

Demanding QC testing, including the use of LC-MS/MS analysis for a carefully selected suite of basic drugs ensures consistent performance from batch-to-batch. **Figure 2** shows the reproducibility across three batches of EVOLUTE CX 50 µm.

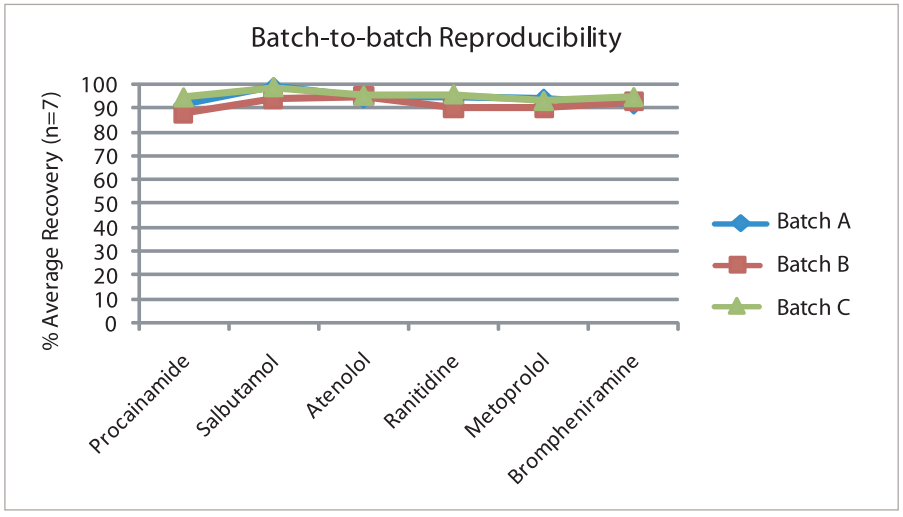


Figure 2. Extraction of basic drugs from plasma at a concentration of 5 ng/mL using EVOLUTE CX 50 mg/ 3 mL columns manufactured using three different batches of sorbent.

Processing Options

EVOLUTE CX 50 µm SPE columns are compatible with manual and automated sample processing. Contact Biotage for details on the range of VacMaster™ Sample Processing Manifolds for manual processing.

Due to the well defined particle size distribution of EVOLUTE CX 50 µm sorbents, many samples can be processed using gravity. **Figure 3** shows typical flow rates obtained when loading urine onto various column configurations of EVOLUTE CX 50 µm under gravity.

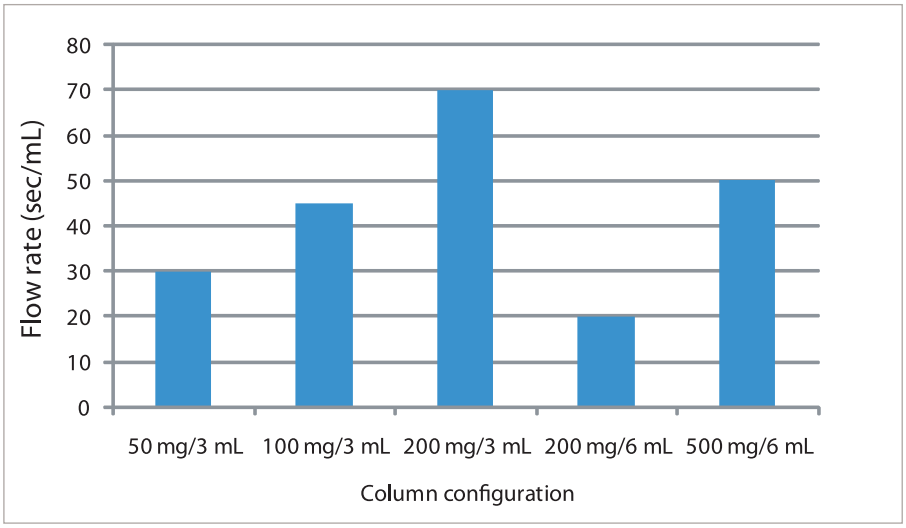


Figure 3. Flow rates though EVOLUTE CX 50 µm columns (configurations as shown) using **GRAVITY** processing. Sample: Urine. Sample load: 3 mL (3 mL columns), 6 mL (6 mL columns).

Ordering Information for EVOLUTE CX 50 µm columns

Description	Quantity	Part number
EVOLUTE CX SPE Columns		
EVOLUTE CX 50 µm 50 mg/3 mL SPE Columns	50	611-0005-B
EVOLUTE CX 50 µm 100 mg/3 mL SPE Columns	50	611-0010-B
EVOLUTE CX 50 µm 100 mg/10 mL SPE Columns (H)[1]	50	611-0010-H
EVOLUTE CX 50 µm 200 mg/3 mL SPE Columns	50	611-0020-B
EVOLUTE CX 50 µm 200 mg/6 mL SPE Columns	30	611-0020-C
EVOLUTE CX 50 µm 500 mg/6 mL SPE Columns	30	611-0050-C

[1] 100 mg/10 mL (H) columns have the same sorbent bed dimensions as a 3 mL column, but with an extended reservoir for loading sample volumes up to 10 mL in one aliquot.

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