Chemistry Data Sheet

EVOLUTE® WCX Columns for Solid Phase Extraction of Quaternary Amines and Strongly Basic Compounds from Aqueous Samples

This Chemistry Data Sheet provides guidelines for the extraction of quaternary amines and strongly basic analytes from aqueous samples using EVOLUTE WCX (Method A).

A method optimized for extraction of a wide range of basic compounds, allowing elution under mildly acidic conditions (Method B) is also detailed. This approach is recommended for the extraction of basic compounds when basic elution conditions are unsuitable, or acidic elution conditions are preferred due to LC-MS/MS compatibility.

The procedures are described on page 1–2, with processing and optimisation guidelines on page 3.

An example application showing the extraction of quaternary amines from urine and plasma illustrates the use of EVOLUTE WCX (see Appendix 1) for clean-up of biological fluid samples.

EVOLUTE WCX 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 weak cation exchange interactions allows efficient extraction of basic analytes of wide ranging polarities. The non-polar/weak 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.

EVOLUTE WCX is available in two particle size ranges. The 30 µm mean particle size sorbent is optimised for extraction of low volume (up to 1 mL) biological fluid samples. 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 optimized 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.

Biotage

Method A:

1. Sample Pre-treatment:	Dilute sample 1:3 with aqueous ammonium hydroxide (5% 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 aqueous ammonium hydroxide (5% v/v, 2 mL)
4. Sample Loading:	Load sample at 3 mL/min
5. Interference Elution:	**Wash 1.** Elute polar/ionic interferences with aqueous ammonium hydroxide (5% v/v, 2 mL) **Wash 2.** Elute non-polar interferences with methanol (2 mL)
6. Analyte Elution:	Elute basic analytes with methanol/formic acid (98:2, v/v, 2 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.

Reagents: Method A

5% ammonium hydroxide

Take 14 mL of 28% ammonium hydroxide and add 88 mL water. Mix thoroughly.

98: 2 (v/v) Methanol/formic acid Solution

Used for analyte elution. Take 2 mL of 98% formic acid and add 98 mL methanol. Mix thoroughly.

Method B:

1. Sample Pre-treatment:	Dilute sample 1:3 with aqueous ammonium acetate (50mM, pH7) 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 aqueous ammonium acetate (50mM, pH7, 2 mL)
4. Sample Loading:	Load sample at 3 mL/min
5. Interference Elution:	**Wash 1.** Elute polar/ionic interferences with aqueous ammonium acetate (50mM, pH7, 2 mL) **Wash 2.** Elute non-polar interferences with methanol (2 mL)
6. Analyte Elution:	Elute basic analytes with methanol/formic acid (98:2, v/v, 2 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.

Reagents: Method B

Aqueous ammonium acetate (50mM, pH7)

Take 3.854 g ammonium acetate and dissolve in 1L water. Adjust pH to 7.0 with ammonium hydroxide.

98: 2 (v/v) Methanol/formic acid Solution

Used for analyte elution. Take 2 mL of 98% formic acid and add 98 mL methanol. Mix thoroughly.

Section 2: Processing Conditions

The well defined particle size distribution of EVOLUTE WCX 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	Fixed well plates, Array and 1 mL columns	3 mL and 10 mL 'H' columns	6 mL columns
Flow rate	-	3 mL/min	7 mL/min
Vacuum setting	Low (1-2" Hg). Increase vacuum when loading more viscous samples	-	-

Table 2: Typical volumes for each step

Step	**Bed mass**				
	25 mg[1]	**50 mg**	**100 mg**	**200 mg**	**500 mg**
Column conditioning	500 µL	1–2 mL	2–3 mL	3–4 mL	6 mL
Column equilibration	500 µL	1–2 mL	2–3 mL	3–4 mL	6 mL
Sample loading	400 µL	Application specific, based on analyte concentration in sample			
Interference elution (1 and 2)	500 µL	1–2 mL	2–3 mL	3–4 mL	6 mL
Analyte elution	500 µL	Dependant on analyte and choice of elution solvent. Minimum elution volume = 2 bed volumes[2]			

[1] For 10 mg products, scale down volumes appropriately .[2]1 bed volume is approximately 200 µL/100 mg of sorbent.

Section 3: Optimising the SPE method

a) For particularly viscous samples, increased sample dilution will normally improve flow rates

b) EVOLUTE WCX is a water wettable resin-based sorbent. Analyte recovery will be unaffected if the columns run dry after conditioning.

c) To minimise elution volume, apply 2 separate aliquots (X ÷ 2 mL), including a soak step, rather than a single aliquot of X mL.

Using EVOLUTE WCX with alternative SPE Procedures

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

APPENDIX 1

Extraction of quaternary amines from plasma and urine

Using method A described on page 2, with all volumes scaled to 500 µL for a 25 mg sorbent bed, EVOLUTE WCX SPE columns are suitable for extraction of quaternary amine compounds from biological fluids. **Figure 1** shows typical results.

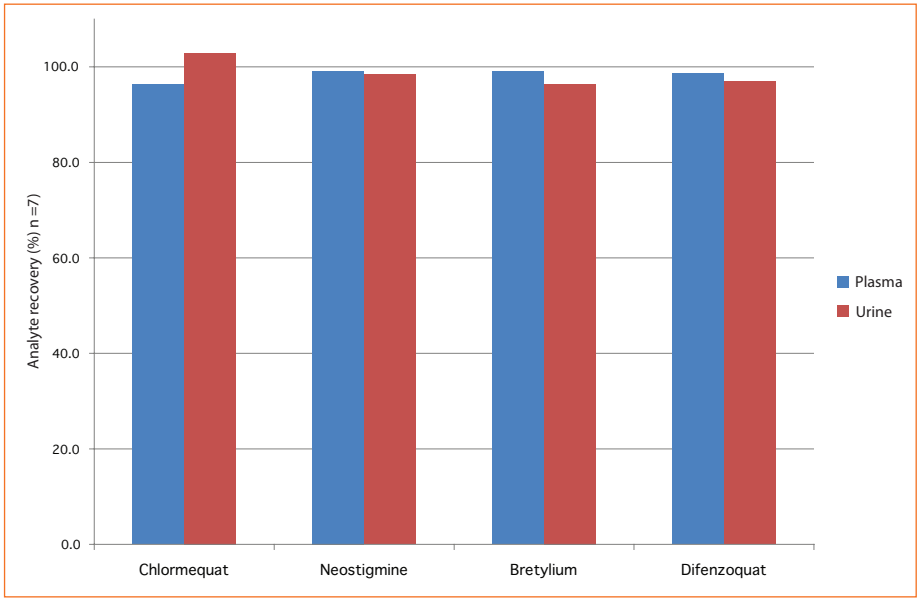

Figure 1. Recovery of quaternary amines from urine and plasma samples. Extraction column: 25 mg fixed well plate, analytes spiked at a concentration of 500 ng/mL in urine and plasma.

Processing Options

EVOLUTE WCX is available in individual SPE columns and 96-well plates (fixed well and versatile EVOLUTE Array formats) to match all processing requirements.

EVOLUTE WCX fixed well plates are suitable for high throughput extraction of drugs from biological fluids, and are particularly useful for automated sample processing due to their uniform flow characteristics.

EVOLUTE WCX 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 WCX 50 µm sorbents, many samples can be processed using gravity.

Ordering Information for EVOLUTE WCX 30 µm columns and 96-well plates

Description	Quantity	Part number
EVOLUTE CX Fixed Well Plate		
EVOLUTE WCX 10 mg Fixed Well Plate	1	602-0010-P01
EVOLUTE WCX 25 mg Fixed Well Plate	1	602-0025-P01
EVOLUTE WCX Array Plates and Wells		
EVOLUTE Array WCX 25 mg/1 mL wells	100	602-0025-R

Pre-assembled EVOLUTE Array plates are available. To order, add the suffix P to the equivalent loose well part number. e.g. 602-0025-RP.

EVOLUTE Array Accessories		
EVOLUTE Array base plate	1	120-6000-P01
Strip of 8 base plate sealing plugs*	50	120-1200
Luer adaptors (fits standard sample processing manifold)	25	120-1201
Well removing tool*	1	120-1202
EVOLUTE WCX 1 mL SPE Columns		
EVOLUTE WCX 25 mg/1 mL SPE Columns	100	602-0001-A

*Required when processing a partially populated EVOLUTE WCX Array plate.

Ordering Information for EVOLUTE WCX 50 µm columns

Description	Quantity	Part number
EVOLUTE WCX 50 µm 50 mg/3 mL SPE Columns	50	612-0005-B
EVOLUTE WCX 50 µm 100 mg/3 mL SPE Columns	50	612-0010-B
EVOLUTE WCX 50 µm 100 mg/10 mL SPE Columns	50	612-0010-H
EVOLUTE WCX 50 µm 200 mg/3 mL SPE Columns	50	612-0020-B
EVOLUTE WCX 50 µm 500 mg/6 mL SPE Columns	30	612-0050-C

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