

# EVOLUTE® AX Columns and Plates for Solid Phase Extraction of Acidic Compounds from Aqueous Samples

This Chemistry Data Sheet provides guidelines for the extraction of acidic compounds from plasma (method A) and urine (Method B) using EVOLUTE® AX.

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

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

EVOLUTE AX has been developed for extraction of acidic 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 strong anion exchange interactions allows efficient extraction of acidic analytes with a wide range of polarities. The anion exchange retention mechanism allows for the use of a rigorous interference wash regime, providing extremely clean extracts and reducing matrix effects associated with LC-MS/MS analysis.

EVOLUTE AX is available in two particle sizes. 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

These procedures are 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

### Method A: Extraction from Plasma Samples

1. **Sample Pre-treatment:** Dilute sample 1:3 with aqueous formic acid (2% 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 column with water (2 mL)
4. **Sample Loading:** Load sample at 1 mL/min, load volume will be application and analyte specific.
5. **Wash 1.** Ammonium acetate buffer (pH 7.0, 50 mM): methanol (95:5, v/v, 2 mL)
6. **Wash 2.** Methanol (2 mL)
7. **Analyte Elution:** Elute acidic analytes with methanol/formic acid (98:2, v/v, 2 mL). For strongly retained analytes, use an additional aliquot of elution solvent.
8. **Post-extraction:** If desired, evaporate extract to dryness and re-constitute in mobile phase or other suitable solvent for analysis.

## Method B: Extraction from Urine Samples

- 1. Sample Pre-treatment:** Dilute sample 1:3 with aqueous ammonium acetate (50 mM, pH 7.0)
  - 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 column with water (2 mL)
- 4. Sample Loading:** Load sample at 1 mL/min, load volume will be application and analyte specific
- 5. Wash 1.** Ammonium acetate buffer (pH 7.0, 50 mM): methanol (95:5 v/v, 2 mL)
- 6. Wash 2.** Methanol (2 mL)
- 7. 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.
- 8. Post-extraction:** If desired, evaporate extract to dryness and re-constitute in mobile phase or other suitable solvent for analysis.

### Reagents:

#### Aqueous ammonium acetate (50 mM, pH7.0)

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 AX 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	1 mL/min	1 mL/min	1 mL/min
Vacuum setting	Low (-1" Hg ). Increase vacuum when loading more viscous samples	Low (-1" Hg ). Increase vacuum when loading more viscous samples	Low (-1" Hg ). Increase vacuum when loading more viscous samples

**Table 2: Typical volumes for each step**

Step	Bed mass				
	25 mg <sup>1</sup>	50 mg	100 mg	200 mg	500 mg
Column conditioning	1 mL	2 mL	3 mL	6 mL	6 mL
Column equilibration	1 mL	2 mL	3 mL	6 mL	6 mL
Sample loading	400 µL	Application specific, based on analyte concentration in sample			
Wash Steps	1 mL	2 mL	3 mL	6 mL	6 mL
Analyte elution	1 mL	Dependant on analyte and choice of elution solvent. Minimum elution volume = 2 bed volumes <sup>2</sup>			

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

### Section 3: Optimising the SPE method

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

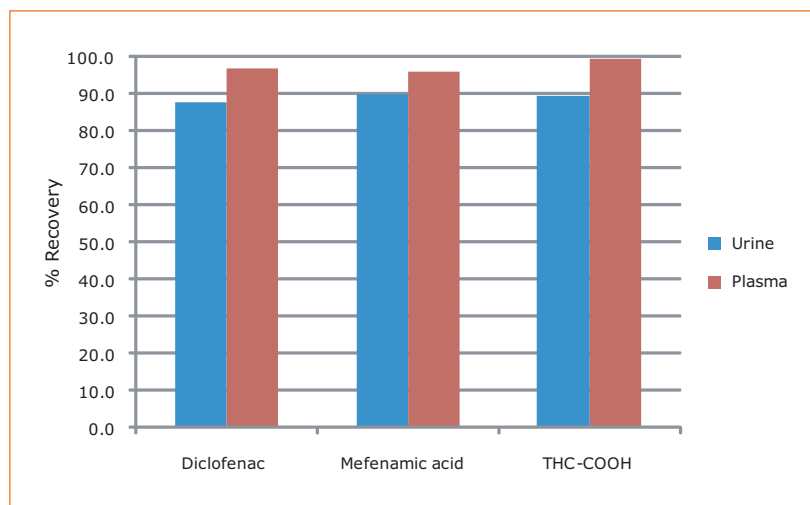
#### Using EVOLUTE AX with alternative SPE Procedures

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

### APPENDIX 1

#### Extraction of acidic drugs from plasma and urine

Using methods A and B described on pages 1-2, with sample volumes scaled to 400 µL for a 25 mg sorbent bed in a 96 well plate, EVOLUTE AX demonstrates recoveries in excess of 85% (RSD <10%) for the analytes shown from both plasma and urine.



**Figure 1.** Recovery of acidic analytes from urine and plasma samples using EVOLUTE AX. Extraction column: 25 mg fixed well plate, analytes spiked at a concentration of 500 ng/mL in urine and plasma. Urine total sample load of 400 µL diluted 1:3 (v/v) with 50 mM Ammonium acetate pH 7. Plasma total sample load 400 µL diluted 1:3 (v/v) in 2% formic acid.

## Processing Options

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

EVOLUTE AX 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 AX 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 and RapidTrace instruments for column-based SPE automation.

Due to the well defined particle size distribution of the EVOLUTE AX 50 µm polymer, many sample matrices can be processed using gravity.

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

Description	Quantity	Part number
<b>EVOLUTE AX Fixed Well Plate</b>		
EVOLUTE AX 10 mg Fixed Well Plate	1	603-0010-P01
EVOLUTE AX 25 mg Fixed Well Plate	1	603-0025-P01
<b>EVOLUTE Array AX Plates and Wells</b>		
EVOLUTE Array AX 25 mg/1 mL wells	100	603-0025-R
Pre-assembled EVOLUTE Array plates are available. To order, add the suffix P to the equivalent loose well part number. e.g. 603-0025-RP.		
<b>EVOLUTE Array Accessories</b>		
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
*Required when processing a partially populated EVOLUTE AX Array plate.		
<b>EVOLUTE AX 1 mL SPE Columns</b>		
EVOLUTE AX 25 mg/1 mL SPE Columns	100	603-0001-A

### Ordering Information for EVOLUTE AX 50 µm columns

Description	Quantity	Part number
EVOLUTE AX 50 µm 50 mg/3 mL SPE Columns	50	613-0005-B
EVOLUTE AX 50 µm 100 mg/3 mL SPE Columns	50	613-0010-B
EVOLUTE AX 50 µm 100 mg/10 mL SPE Columns	50	613-0010-H
EVOLUTE AX 50 µm 200 mg/3 mL SPE Columns	50	613-0020-B
EVOLUTE AX 50 µm 500 mg/6 mL SPE Columns	30	613-0050-C

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