EVOLUTE ABN SPE Columns for Solid Phase Extraction of Environmental Samples

Chemistry Data Sheet

This Chemistry Data Sheet provides guidelines for the simultaneous extraction of acidic, basic and neutral compounds from aqueous environmental samples using polymer-based non-polar SPE. The generic method is on page 1, with processing and method optimization guidelines on pages 2-4.

An example application showing the trace enrichment of pharmaceuticals in water illustrates the versatility of EVOLUTE ABN for the extraction of a wide range of analytes (see Appendix).

EVOLUTE ABN (Acidic Basic Neutral) has been developed for the extraction of acidic, neutral or basic compounds from a variety of aqueous matrices. The polystyrene based polymer is surface modified with well defined hydroxyl-functional oligomers, imparting excellent water-wettability. An optimized combination of non-polar (hydrophobic) and polar (hydrophilic) interactions allows efficient extraction of analytes of wide ranging polarities. These characteristics result in a versatile sorbent for extraction of the broad range of analytes encountered in routine environmental analysis.

SECTION 1: GENERIC METHOD FOR EVOLUTE ABN SPE COLUMNS

This method is optimized for 200 mg/6 mL EVOLUTE ABN 50 µm SPE columns. This method can be scaled to the appropriate column configuration. See Section 4 for Optimizing the SPE Method.

1.	**Sample Pre-treatment:**	If sample preservation is necessary, acidify the sample using HCl or other suitable acid. Controlling the pH of the sample may be necessary to minimize retention of ionizable interferences. Remove particulates from the sample using a filter or depth filter, see Section 4. For solid samples see Section 4.
2.	**Column Conditioning:**	Condition each column with methanol (6 mL)
3.	**Column Equilibration:**	Equilibrate each column with water (6 mL)
4.	**Sample Application:**	500 mL at a flow rate of ~15 mL/min (-5 "Hg)
5.	**Interference Elution:**	Elute interferences with water/methanol (95:5, v/v, 6 mL)
6.	**Analyte Elution:**	Elute analytes with methanol (6 mL)
7.	**Post-extraction:**	If desired, evaporate extract to dryness and reconstitute in mobile phase or other suitable solvent for analysis.

See **Application Note AN701 Extraction of Pharmaceuticals from Water using EVOLUTE ABN SPE Columns** for full details of the SPE and Analytical methodologies.

SECTION 2: PROCESSING CONDITIONS

Before processing, set the vacuum to the desired level to achieve the required flow rate. Use this vacuum setting for each subsequent step. For the 200 mg/6 mL format, a vacuum level of -5 "Hg produces a flow rate of approximately 15 mL/minute.

For large volume samples, the flow rate can be increased to reduce processing time. This should be optimized for a given application.

Biotage

SECTION 3: MAXIMUM SAMPLE LOAD

The volume of sample that can be extracted using EVOLUTE ABN 50 µm SPE columns may be restricted by liquid handing considerations.

However, the high capacity of EVOLUTE ABN allows higher sample volumes to be loaded without loss of analyte. Exact volumes should be determined on an application specific basis.

SECTION 4: OPTIMIZING THE SPE METHOD

a. Remove particulates from the sample using an ISOLUTE Depth Filter or other suitable filtration device. Contact Biotage for details.

b. Extract solid samples with water miscible organic solvent, Accelerated Solvent Extraction (ASE), etc. Dilute to less than 5% (v/v) extraction solvent to ensure correct analyte retention.

c. Recommended solvent volumes and flow rates are listed in Tables 2 and 3 respectively. These should be optimized for a particular application, to provide the most efficient extraction conditions.

Table 2: Typical volumes for each step

Step	Bed Volumes
Column Solvation	2-4 bed volumes
Column Equilibration	2-4 bed volumes
Sample Application	Application specific, based on analyte concentration in sample
Interference Elution	2-4 bed volumes
Analyte Elution	2-8 – dependant on choice of elution solvent. To minimize elution volume, apply in two aliquots, including a soak step, rather than one aliquot.

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

Table 3: Recommended flow rates for method development

Column size	1 mL and 10 mL 'G' columns	3 mL and 10 mL 'H' columns	6 mL
Flow rate	1 mL/min	3 mL/min	7 mL/min

Once optimum chemistry has been established, optimize flow rate to maximise productivity. Increase flow until breakthrough is observed. Final flow rate should be set at 10-20% lower than the breakthrough limit.

d. Unlike silica-based non-polar sorbents, EVOLUTE ABN 50 µm is water-wettable, and therefore does not require addition of a water miscible solvent (e.g. 2% (v/v) methanol) to the sample during large volume sample loading. This eliminates the need to carry additional solvents when collecting samples "in the field", but also ensures the more polar analytes are fully retained on the SPE column.

e. For particularly polar ionizable analytes, evaluate the use of aqueous acid or base in the sample prior to sample loading, and during interference elution, to improve analyte retention.

Acidic analytes will have greater retention at 2 pH units below the pK_a of the analyte.
Basic analytes will have greater retention at 2 pH units above the pK_a of the analyte.

f. Extract cleanliness can be optimized by the use of methanol in the interference elution step. As a guide, this can be up to 40% (v/v) methanol/water but should be optimized for each application. The optimal ratio will give the best extract cleanliness without analyte breakthrough. Starting at 5% (v/v) methanol/water, increase the organic content at increments of 5% up to 40%, or until breakthrough of analyte is observed. Use the greatest ratio of methanol/water that does not cause analyte breakthrough.

g. Thorough drying of the SPE column is necessary prior to elution with a water immiscible solvent, such as ethyl acetate and DCM. **See Figure 4, page 7** for the drying curve of a 200 mg/6 mL column.

h. Elution of the analytes depends on the solubility of a particular analyte in the elution solvent. Typical solvents used are water miscible solvents such as methanol and acetonitrile.

   Addition of a volatile acid or base to the elution solvent can improve solubility of the analytes to maximize analyte recovery. Basic compounds will have greater solubility at high pH, while acidic compounds will have greater solubility at low pH.

   Acidic analytes: Evaluate the use of up to 0.1% (v/v) formic or other suitable volatile acid in methanol

   Basic analytes: Evaluate the use of up to 5% (v/v) ammonia or other suitable volatile base in methanol

i. Elution volumes can be minimized by successive aliquot of elution solvent instead of a single volume (e.g. 2 x 1 mL instead of 1 x 2 mL)

Using EVOLUTE ABN with Alternative SPE Procedures
EVOLUTE ABN is a versatile solid phase extraction sorbent and can be used with other manufacturers non-polar polymer based SPE procedures, although further optimization may be required because of the subtle differences in retention and elution characteristics.

Extraction of Highly Polar Compounds
For extraction of challenging polar, water soluble compounds that do not retain well on EVOLUTE ABN, contact Biotage to evaluate ISOLUTE ENV+ SPE Columns.

APPENDIX

Simultaneous Extraction of Acidic, Basic and Neutral Analytes
Using a single, generic methodology, EVOLUTE ABN is suitable for extraction of compounds with wide-ranging polarity and acidic, basic and neutral functionality. This methodology (described in Appendix 1) has been successfully used in the analysis of pharmaceuticals from water at low concentration levels (100 ng). **Figure 1** shows typical results of an analyte suite selected for diverse functionality and polarity. See **Table 1** for analyte structures, logP and pK_a data.

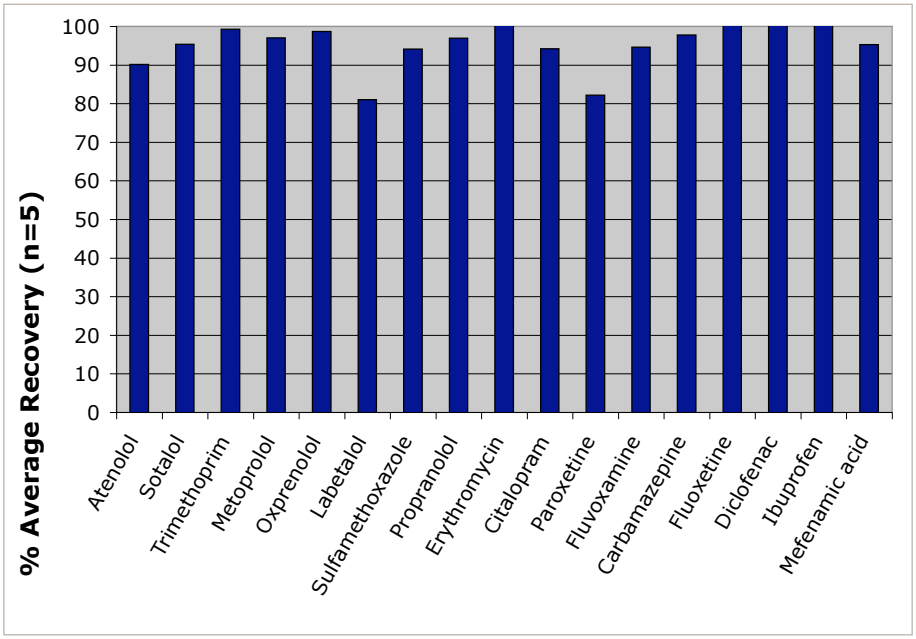
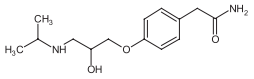
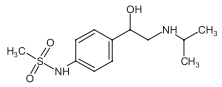
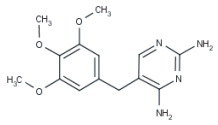
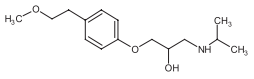
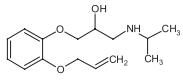
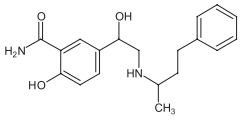
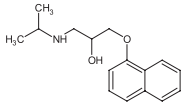
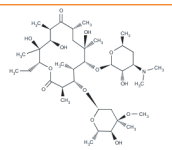
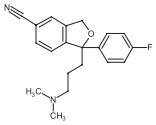
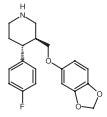
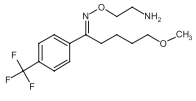


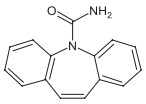
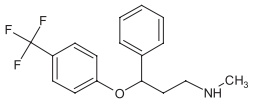
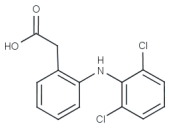
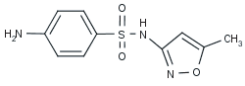
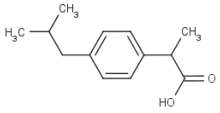
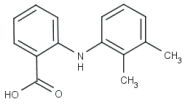
Figure 1. EVOLUTE ABN delivers high absolute recoveries (>80%) with excellent reproducibility (<10 % RSD, n=5) for a wide range of pharmaceuticals from water

Table 1. A number of pharmaceuticals have been identified as potential environmental contaminants. These have been selected to show the wide applicability of EVOLUTE ABN.

Analyte	Structure	Therapeutic Class	Functionality	logP	pK_a
Atenolol		Beta blocker	Basic, very polar	0.3	9.6
Sotalol		Beta blocker	Basic, very polar	0.5	8.2, 9.8
Trimethoprim		Antibacterial	Basic, polar	1.3	6.6
Metoprolol		Beta blocker	Basic, polar	1.8	13.3
Oxprenolol		Beta blocker	Basic, polar	2.2	9.2
Labetalol		Beta blocker	Basic, polar	2.7	7.4
Propranolol		Beta blocker	Basic, medium polarity	3.1	9.5
Erythromycin		Antibacterial	Basic, polar	2.9	8.8
Citalopram		SSRI	Basic, polar	1.5	9.5
Paroxetine		SSRI	Basic, non-polar	5.0	9.9
Fluvoxamine		SSRI	Basic, polar	1.3	8.7

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

Table 1. (Continued)

Analyte	Structure	Therapeutic Class	Functionality	logP	pK_a
Carbamazepine		Anticonvulsant	Basic, polar	1.3	9.1
Fluoxetine		SSRI	Basic, non-polar	4.2	9.5
Diclofenac		Anti-inflammatory	Acidic, non-polar	4.4	4.0
Sulfamethoxazole		Antibacterial	Acid, non-polar	2.4	6.0
Ibuprofen		Anti-inflammatory	Acidic, non-polar	3.5	4.8
Mefenamic Acid		Anti-inflammatory	Acidic, non-polar	4.9	4.2

Pharmaceuticals spiked in water samples at concentrations of 100 ng/L. 500 mL of river water extracted using EVOLUTE ABN 50 µm 200 mg/6 mL columns and SPE method as described on page 1. Analysis by LC-MS/MS. For full details request A**pplication Note AN701 Extraction of Pharmaceuticals from Water using EVOLUTE ABN SPE Columns.**

Reproducible Extraction Performance

Fines free sorbents are an important feature of a quality SPE product. The particle size distribution of EVOLUTE ABN is carefully controlled during manufacturing and quality controlled to ensure a narrow distribution optimal for SPE. This minimizes fines and maximizes the packing efficiency and performance of the SPE column. **Figure 2** shows the particle size distribution of EVOLUTE ABN 50 µm.

Stringent QC tests are carried out during manufacturing, ensuring the extracted sample is not contaminated with sorbent fines or impurities from the SPE column components.

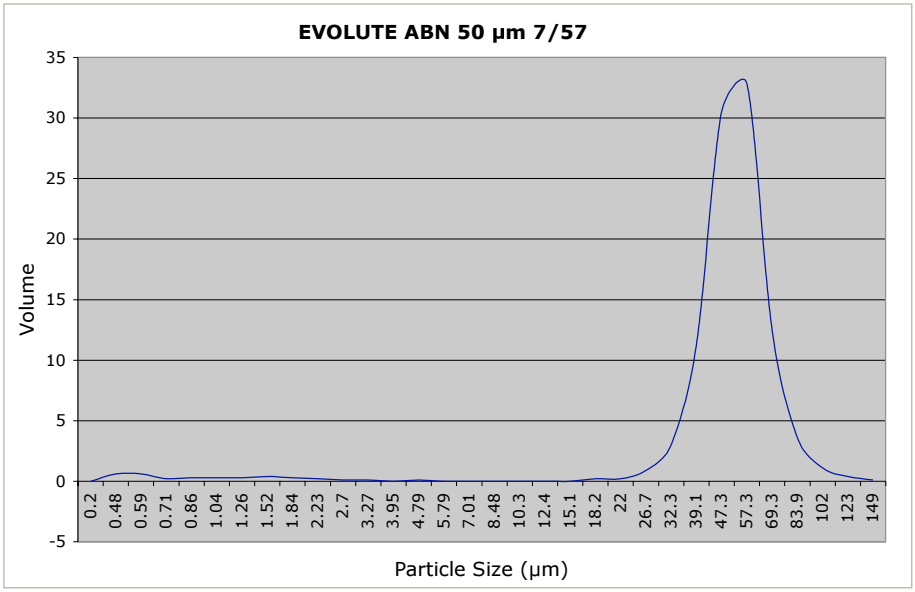


Figure 2. Particle size distribution of a batch of EVOLUTE ABN 50 µm

The surface characteristics of each batch of EVOLUTE ABN 50 µm sorbent are carefully controlled during the manufacturing process. An integral part of the quality control testing includes LC-MS/MS analysis of a carefully selected analyte test mix at low concentrations, ensuring no secondary interactions. These characteristics remain constant, ensuring reliable performance with high analyte recoveries from batch-to-batch. **Figure 3** shows the reproducibility across three batches of EVOLUTE ABN 50 µm.

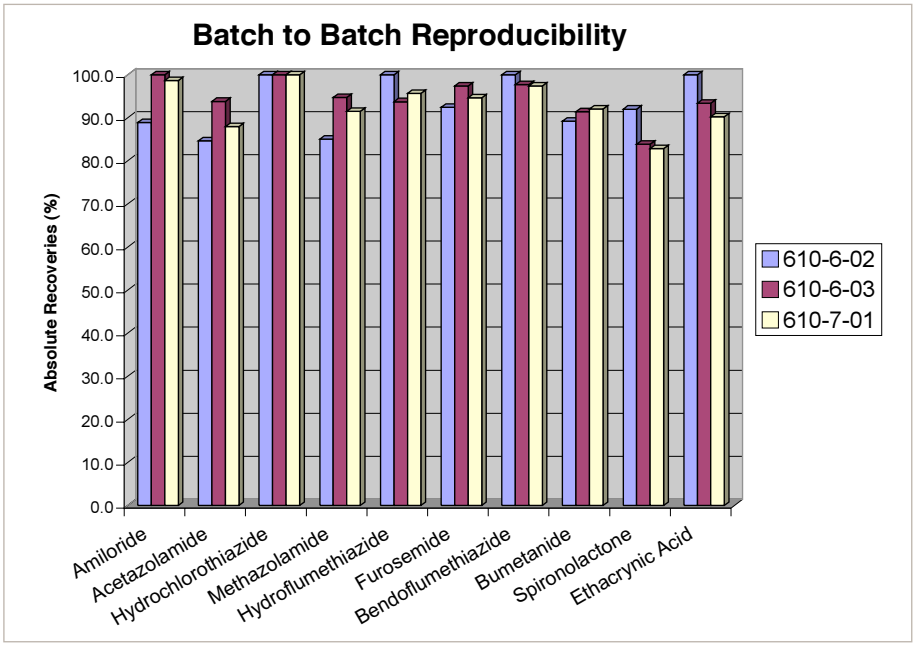


Figure 3. Extraction of pharmaceuticals from water from EVOLUTE ABN 50 µm 100 mg/3 mL columns made from three different batches of sorbent

Improved Productivity

This product is supported by a generic procedure, minimizing method development time and increasing productivity. The generic method is suitable for a wide range of analytes and matrices, ensuring reliable results for a many analytical applications. Analytes can be eluted in pure organic solvent, minimizing the use of modifiers that could affect the analytical technique.

Column drying is required prior to elution with water immiscible organic solvents. EVOLUTE ABN 50 µm columns dry rapidly, reducing the total processing time whilst ensuring maximum analyte recovery (see **Figure 4**).

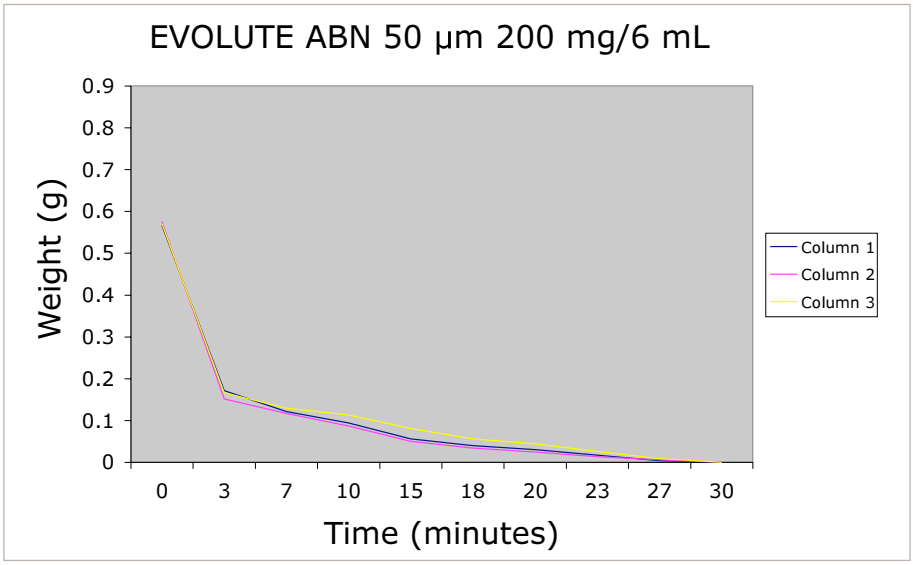


Figure 4. Column drying times of EVOLUTE ABN of 200 mg/6 mL SPE columns. Columns were conditioned with methanol and loaded with water (6 mL). Full vacuum was applied and columns weighed at specific time intervals to constant weight.

Processing Options
EVOLUTE ABN 50 µm SPE columns are compatible with manual and automated sample processing. Contact Biotage for details on VacMaster-10 and -20 Sample Processing Manifolds for manual processing.

ORDERING INFORMATION

Item	Description	Quantity	Part Number
EVOLUTE ABN	25 mg/10 mL (G)[1]	50	600-0002-G
EVOLUTE ABN 50 µm[3]	50 mg/3 mL	50	610-0005-B
EVOLUTE ABN 50 µm[3]	100 mg/3 mL	50	610-0010-B
EVOLUTE ABN 50 µm[3]	100 mg/10 mL (H)[2]	50	610-0010-H
EVOLUTE ABN 50 µm[3]	200 mg/3 mL	50	610-0020-B
EVOLUTE ABN 50 µm[3]	200 mg/6 mL	30	610-0020-C

[1] 25 mg/10 mL (G) columns have the same sorbent bed dimensions as a 1 mL SPE column, but with an expanded reservoir for loading sample volumes up to 10 mL in one aliquot
[2] 100 mg/10 mL (H) columns have the same sorbent bed dimensions as a 3 mL SPE column, but with an expanded reservoir for loading sample volumes up to 10 mL in one aliquot
[3] The particle size is optimized for SPE columns with sorbent masses greater than 25 mg.

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