EVOLUTE ABN Columns for Solid Phase Extraction of Forensic and Clinical Samples

Chemistry Data Sheet

This Chemistry Data Sheet provides guidelines for the simultaneous extraction of acidic, basic and neutral compounds from biological fluid samples using polymer-based non-polar SPE. The generic method is on page 1, with processing and method optimization guidelines on pages 2-3.

An example application involving the extraction of diuretics from urine 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. This results in a versatile sorbent for extraction of the broad range of analytes encountered in routine forensic and clinical analysis.

Section 1: Methodology

This method is optimized for 100 mg/3 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:	Dilute sample (typically 1-2 mL urine) 1:1 (v/v) with aqueous formic acid (1%, v/v). Add internal standard (if used) and mix thoroughly.
2. Column Conditioning:	Condition each column with methanol (3 mL)
3. Column Equilibration:	Equilibrate each column with aqueous formic acid (0.1%, v/v) (3 mL)
4. Sample Application:	Load the diluted sample
5. Interference Elution:	Elute interferences with water/methanol (95:5, v/v, 3 mL)
6. Analyte Elution:	Elute analytes with methanol (3 mL)
7. Post-extraction:	If desired, evaporate extract to dryness and reconstitute in mobile phase or other suitable solvent for analysis.

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 100 mg/3 mL format, a vacuum level of -1 "Hg produces a flow rate of 2 mL/minute.

Section 3: Maximum Sample Load

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

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.

Biotage

Section 4: Optimizing the SPE Method

a. Higher dilutions may be required to improve flow characteristics of particularly viscous samples.

b. For biological fluid samples containing high levels of endogenous proteins (such as plasma), the use of formic acid in the sample pre-treatment step may improve recovery of strongly protein bound analytes. Care should be taken when extracting acid labile compounds (e.g. lactones) if using the formic acid pre-treatment approach.

c. If buffering of urine samples is necessary, use a low ionic strength buffer (20-50 mM) at sample pre-treatment and column equilibration.

d. EVOLUTE ABN is a water-wettable resin. Analyte recovery will be unaffected if the columns run dry after conditioning.

e. 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.

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 bed volumes – dependant on choice of elution solvent. To minimize elution volume, apply 2 aliquots, including a soak step, rather than a single aliquot of the elution solvent

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.

f. 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.

g. 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.

h. Elution of the analytes depends on the solubility of a particular analyte in the elution solvent. Addition of a volatile acid or base to the elution solvent can improve solubility of the analytes to maximize analyte recovery.

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

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 1

Simultaneous Extraction of Acidic, Neutral and Basic 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 diuretics from urine at concentration levels of 50 ng/mL. **Figure 1** shows typical results of an analyte set selected for diverse functionality and polarity. **See Table 1** for analyte structures, logP and pK_a data.

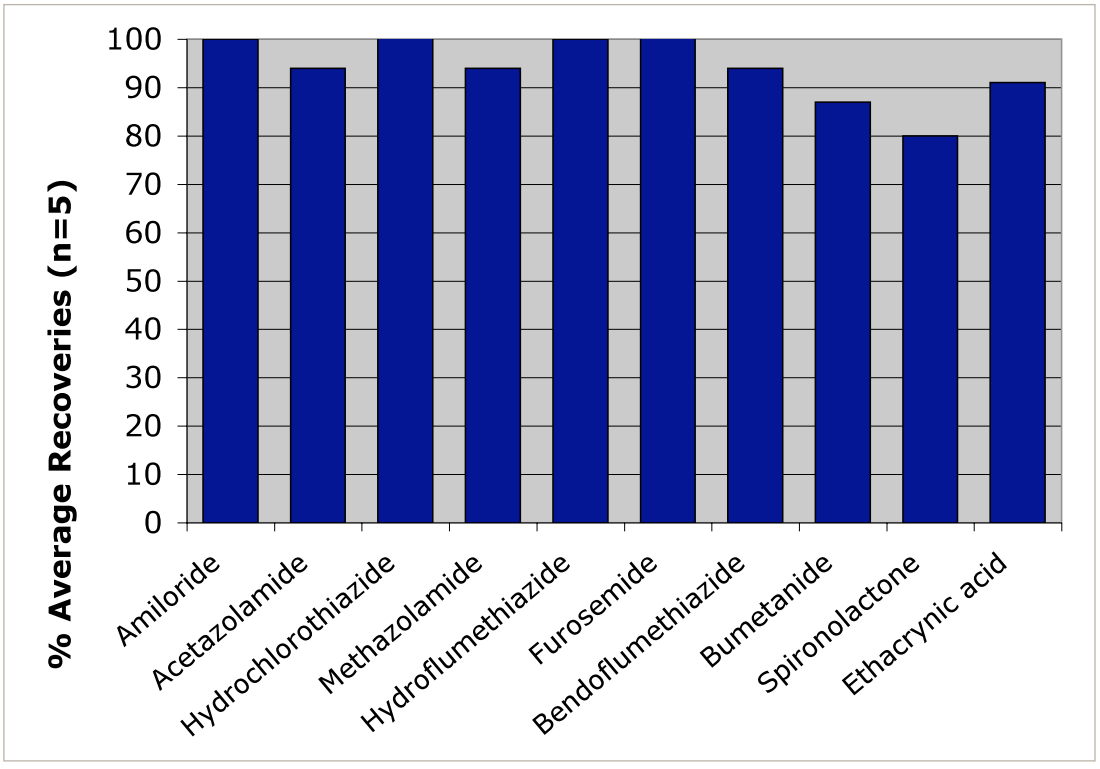
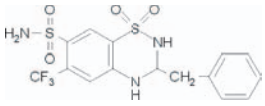
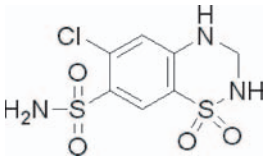
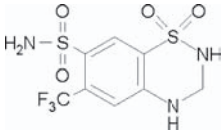
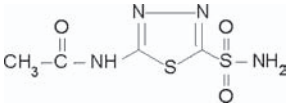
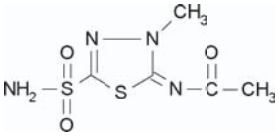


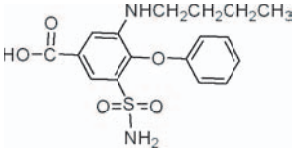
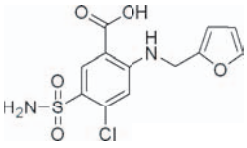
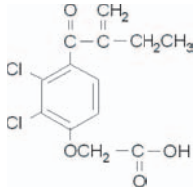
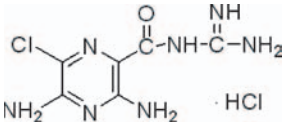
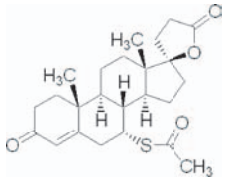
Figure 1. EVOLUTE ABN delivers high absolute recoveries (>80%) with excellent reproducibility (<10 % RSD, n=5) for a selection of diuretics from urine

Table 1. A number of diuretics have been selected to show the wide applicability of EVOLUTE ABN

Analyte	Structure	Therapeutic Class	Functionality	logP	pKa
Bendroflumethiazide		Thiazide	Basic, polar	2.09	8.5
Hydrochlorothiazide		Thiazide	Basic, very polar	-0.27	7.9, 9.2
Hydroflumethiazide		Thiazide	Basic, polar	0.11	8.9, 10.7
Acetazolamide		Carbonic Anhydrase Inhibitor	Basic, polar	0.25	7.2, 9.0
Methazolamide		Carbonic Anhydrase Inhibitor	Neutral, polar	0.23	7.3

(Table 1 continued on next page.)

Table 1. (Continued)

Analyte	Structure	Therapeutic Class	Functionality	logP	pK_a
Bumetanide		Loop Diuretic	Amphoteric, medium polarity	3.26	4.0, 10.0
Furosemide		Loop Diuretic	Acidic, polar	1.51	3.52, 3.04, 0.48
Ethacrynic Acid		Loop Diuretic	Acidic, non-polar	3.41	3.5
Amiloride		Potassium Sparing Diuretics	Basic, very polar	-1.25	8.7
Spironolactone		Potassium Sparing Diuretics	Acidic, non-polar	4.31	N/A

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

Diuretics spiked in urine (1 mL) at concentrations of 50 ng/L, using EVOLUTE ABN 50 µm 100 mg/3 mL columns and SPE method as described in **Appendix 1**. Analysis was by LC-MS/MS. Request **Application Note (APN700) Extraction of Diuretics from Urine using EVOLUTE ABN SPE Columns** for full sample preparation and analytical methodologies.

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.

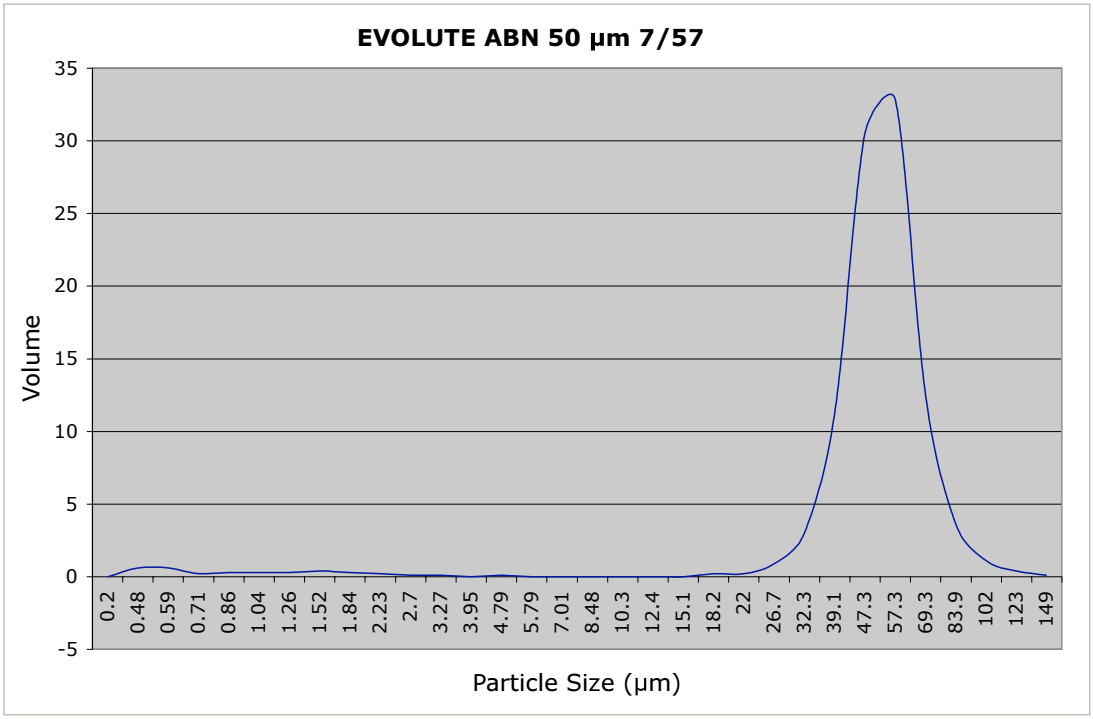


Figure 2. Particle size distribution of a batch of EVOLUTE ABN 50 µm

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.

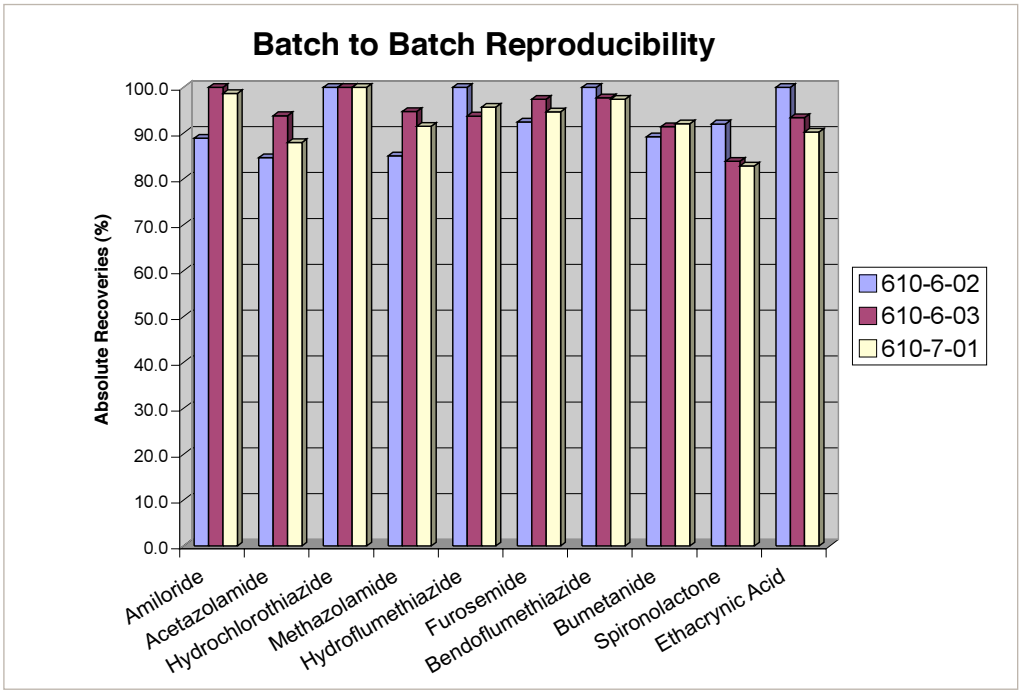


Figure 3. Extraction of diuretics from urine from EVOLUTE ABN 50 µm 100 mg/3 mL columns made from three different batches of sorbent.

Improved Productivity
EVOLUTE ABN 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 impact the analytical technique.

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.

APPENDIX 2

ORDERING INFORMATION

Item	Description	Quantity	Part Number
EVOLUTE ABN	10 mg/1 mL	100	600-0001-A
EVOLUTE ABN	10 mg/1 mL (tab-less)	100	600-0001-AG
EVOLUTE ABN	25 mg/1 mL	100	600-0002-A
EVOLUTE ABN	25 mg/1 mL (tab-less)	100	600-0002-AG
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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