EVOLUTE™ ABN for Extraction of Drugs from Biological Fluids

Chemistry Data Sheet

EVOLUTE™ Sample Preparation Products are a new generation of advanced polymeric solid phase extraction sorbents for the high throughput extraction of drugs from biological fluids. Designed to maximize analyte recovery, while reducing the concentration of matrix components in the final extract, EVOLUTE products provide improved extract cleanliness that minimizes ion suppression effects during LC-MS/MS analysis.

EVOLUTE ABN for 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 or neutral functionality. This methodology (described fully in **Appendix 1** of this Chemistry Data Sheet) has been used to successfully extract a large number of test compounds from biological fluid samples at low concentration levels (typically 5–50 pg/µL). To demonstrate the wide applicability of EVOLUTE ABN, typical results are shown below (**Figure 1**) for an analyte set selected for diverse functionality and polarity.

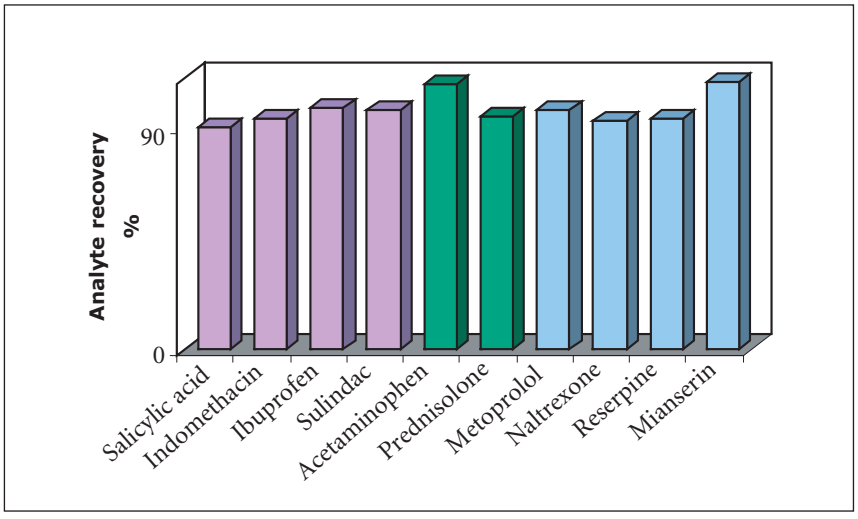
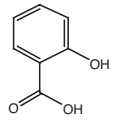
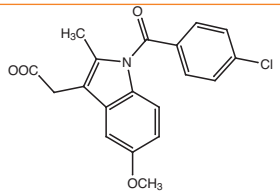
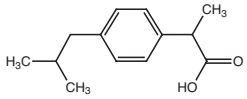
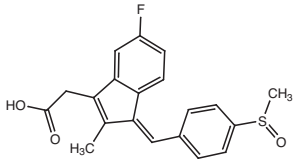
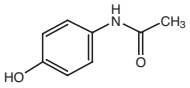
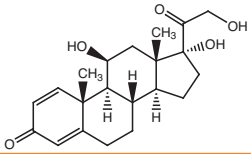
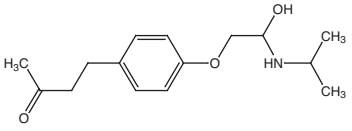
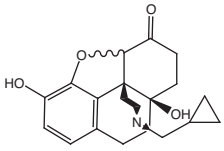
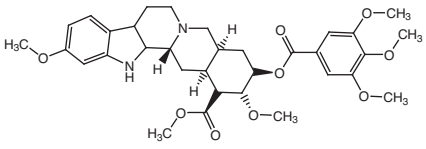
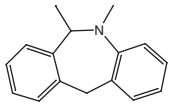


Figure 1. EVOLUTE ABN delivers high absolute recoveries (>90 %) with excellent reproducibility (0.75–7.9 % rsd, n=5) for a wide range of compounds.

Biotage

Classification	Compound	Structure	logP[1]	pK[1]
Acidic, polar	Salicylic Acid[2]		2.24	2.9
Acidic, medium polarity	Indomethacin		3.10	4.3
Acidic, medium polarity	Ibuprofen		3.51	5.2
Acidic, non-polar	Sulindac		3.59	3.6
Neutral, very polar	Acetaminophen		0.34	N/A
Neutral, polar	Prednisolone		1.49	N/A
Basic, polar	Metoprolol		1.34	10.8
Basic, polar	Naltrexone		1.80	9.2
Basic, non-polar	Reserpine		3.11	6.6
Basic, non-polar	Mianserin		3.67	8.3

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

Test compounds spiked into pooled human plasma sample at concentrations of 5–50 pg/µL ([2]Salicylic acid extracted from buffer, due to its presence in blank plasma samples). 100 µL plasma sample extracted using EVOLUTE ABN 25 mg/1 mL columns and SPE method as described in **Appendix 1**. Analysis by LC-MS/MS.

Reproducible Extraction Performance

EVOLUTE ABN has a tightly controlled particle size distribution ensuring consistent flow rates and high, reproducible analyte recoveries. The material has a mean particle size of 30 µm, optimized to give high analyte recoveries and low elution volumes for small bed masses (10 and 25 mg).

Predictable, reproducible flow rates through EVOLUTE ABN 96-well plates and columns lead to optimum performance from automated liquid handling systems. Plasma samples should be diluted to minimize variation in flow rate. Generally, less variation is observed for higher dilution factors. During testing, an optimum dilution of 1:3 v/v was found to give less than 2% rsd during loading onto a 96-well plate. For viscous samples, additional dilution may be required to achieve similar performance.

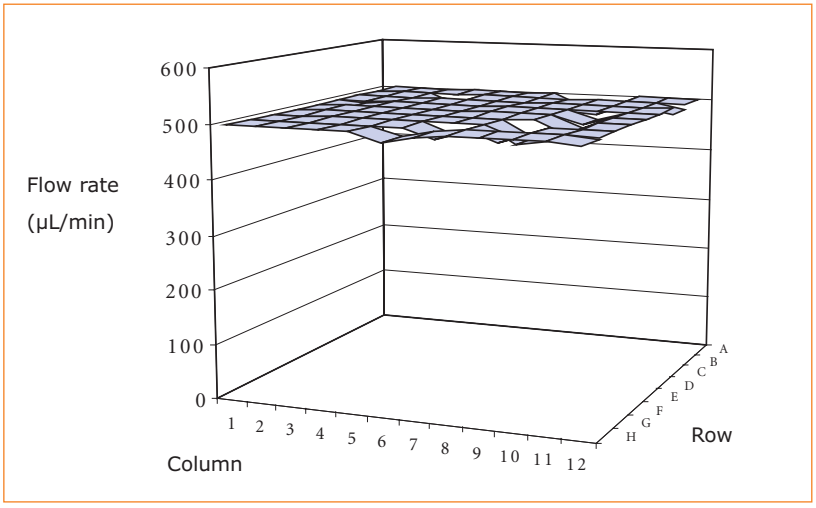


Figure 2. Flow reproducibility for plasma samples through an EVOLUTE ABN 25 mg fixed well plate. (Sample: 500 µL plasma at 1:3 v/v dilution - total sample volume loaded onto each well was 2 mL). No well blockage was observed.

Biotages' optimized packing techniques eliminate well or column blockage, and the fines-free sorbent provides maximum capacity without channelling or analyte breakthrough.

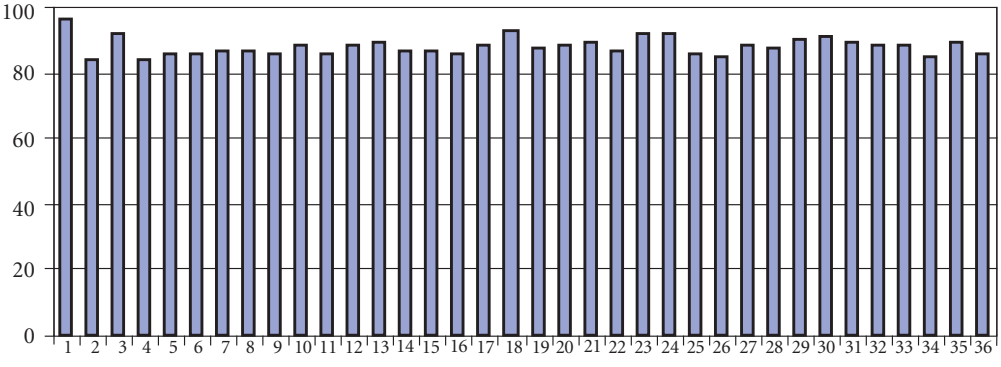


Figure 3. Reproducible well-to-well recovery using EVOLUTE ABN 25 mg fixed well plate. Sample: plasma spiked with propranolol at 100 ng/mL. 500 µL plama loaded, diluted 1:1. 36 sample extracts (3 wells each from columns 1–12) were randomly selected and analyzed by HPLC. Mean recovery 88%, 2.3% rsd.

Reduced Ion Suppression in LC-MS/MS Analysis using EVOLUTE ABN

Improved sample clean-up is possible due to EVOLUTE ABN's optimized pore size and distribution. This minimizes retention of endogenous plasma components during the solid phase extraction process (see **Figure 4**), while still efficiently retaining drugs of different functionalities and polarities. Drugs are eluted using low volumes of pure organic solvent for maximum compatibility with LC-MS/MS procedures – no ionic modifiers are required. The resulting extracts contain minimal biological interferences (see **Figure 5**), and can be evaporated quickly for maximum productivity.

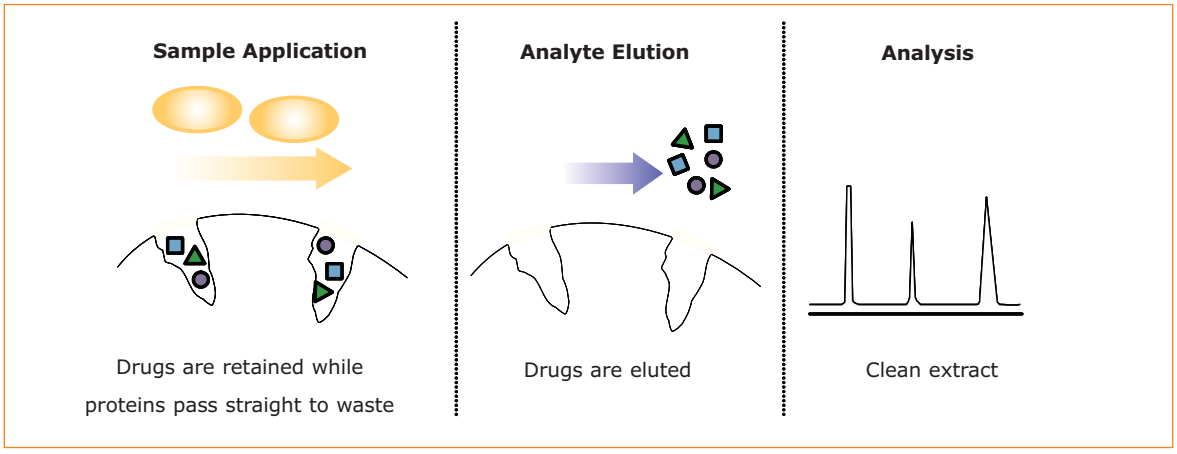


Figure 4. The optimized pore structure prevents retention of endogenous proteins, allowing drugs to be eluted in extremely clean extracts.

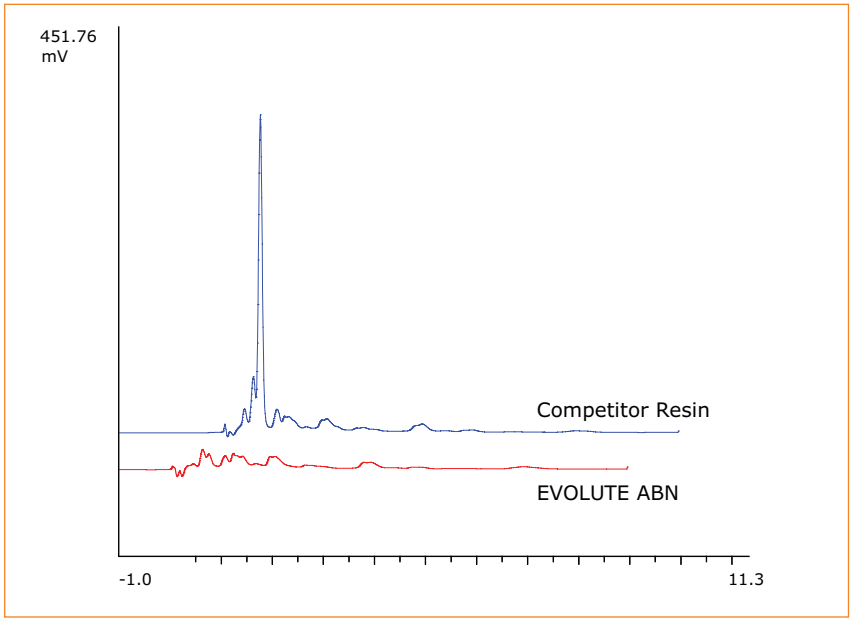


Figure 5. Comparison of extract cleanliness using HPLC-UV (220 nm). Blank plasma samples (500 µL diluted to 2 mL) extracted using EVOLUTE ABN (25 mg/1 mL) contain significantly fewer interferences than those extracted using a competitor resin-based product (30 mg /1 mL).

Using EVOLUTE ABN for plasma extraction, ion suppression in LC-MS/MS analysis can be reduced, leading to a fourfold increase in sensitivity[3] (see **Figure 6**) and more reliable quantification.

([3]compared to a competitor resin)

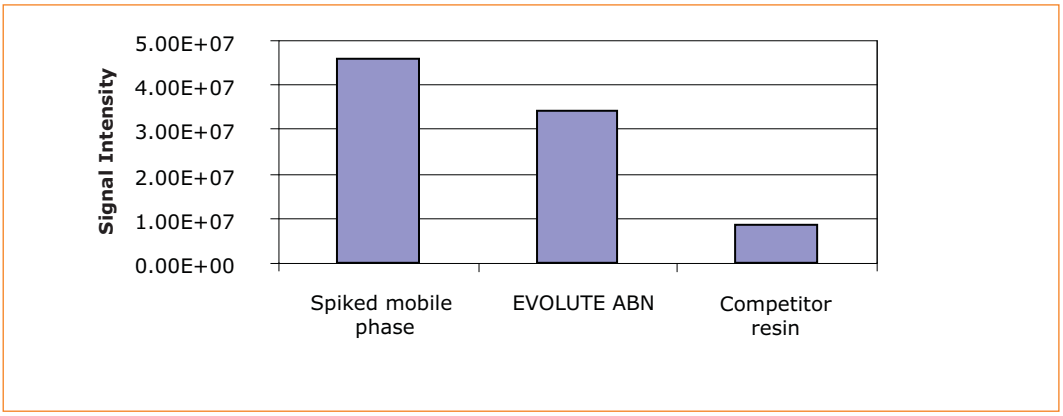


Figure 6. LC-MS/MS signal (m/z 195>138) for caffeine spiked plasma extracts, compared to spiked mobile phase (flow injection analysis). EVOLUTE gives approximately four times higher s/n than a competitor resin-based product.

EVOLUTE Sample Preparation Products are manufactured using ultra-clean sorbents and components, which will not contaminate sample extracts. Every EVOLUTE column and plate meets Biotage's stringent quality assurance standards.

Processing Options

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

EVOLUTE 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 Array plates and wells are ideal for method development or for small batches of samples, and are a cost effective format when variable sample numbers need to be extracted. The versatile Array plate can be populated with the required number of wells, with unused positions simply capped to avoid wastage.

EVOLUTE SPE columns are suited to both automated and manual sample processing, while the tab-less format is compatible with certain automated processing systems.

Format	EVOLUTE ABN fixed well plate	EVOLUTE Array ABN 1 mL well or plate	EVOLUTE ABN SPE column	EVOLUTE ABN tab-less SPE column
10 mg	✓	✓	✓	✓
25 mg	✓	✓	✓	✓

Appendix 1

Simple Methodology for Simultaneous Extraction of Acidic, Basic and Neutral Drugs from Biological Fluid

Section 1: Methodology

This method is optimized for 25 mg EVOLUTE ABN SPE columns and 96-well plates. The method can readily be transferred to the 10 mg sorbent mass (see **Section 4, part g**).

1. **Sample Pre-treatment:** Dilute sample (typically plasma) 1:3 (v/v) with aqueous formic acid (1 %, v/v). Add internal standard and mix.
2. **Conditioning:** Condition each column or well with methanol (1 mL).
3. **Equilibration:** Equilibrate each column or well with aqueous formic acid (0.1%, v/v, 1 mL).
4. **Sample Load:** Load sample (typically 400 µL diluted plasma).
5. **Interference Wash:** Elute interferences with water/methanol (95:5, v/v, 1 mL).
6. **Analyte Elution:** Elute analytes with methanol (500 µL).
7. **Post-extraction:** If desired, evaporate extracts to dryness and reconstitute in mobile phase or other suitable solvent prior to analysis.

[4]Typical sample consists of 100 µL plasma diluted to a total volume of 400 µL. For larger volumes, see Section 3.

Section 2: Processing Conditions for EVOLUTE ABN SPE Columns and Plates

Set the vacuum levels for the format being used (-2" Hg for SPE columns and fixed well plates. -10" Hg for individual EVOLUTE Array wells, and both fully and partially populated EVOLUTE Array plates) before starting the extraction procedure. This produces flow rates of approximately ~1 mL/min for each format. For each step, load solvent or sample into all columns or wells prior to applying the vacuum. this will ensure even flow rates and improved analytical precision.

For optimum extraction efficiency, a short pulse of high vacuum can be used following the interference wash and analyte elution steps. This will ensure no aqueous solvent is transferred to the final extract, and that the elution solvent is fully recovered, maximizing analyte recovery.

Section 3: Maximum Sample Load

The volume of sample that can be extracted using EVOLUTE ABN SPE columns and plates may be restricted by liquid handling considerations. The maximum sample volume that can be applied to each format in a **single aliquot** is listed.

EVOLUTE ABN fixed well plate	2 mL (500 µL plasma at 1:3 v/v dilution)
EVOLUTE ABN Array 1 mL wells and plate	1 mL (250 µL plasma at 1:3 v/v dilution)
EVOLUTE ABN 1 mL columns (including tab-less)	1 mL (250 µL plasma at 1:3 v/v dilution)

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

Section 4: Optimizing the SPE Procedure

a) The use of formic acid in the sample pre-treatment step improves recovery of strongly protein bound analytes.

b) For particularly viscous samples, increased sample dilution may improve flow characteristics.

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

d) Specific interferences may be removed by increasing the methanol concentration in the interference wash step. However, care should be taken to avoid loss of more polar analytes.

e) For very polar compounds, recovery and/or reproducibility may be improved by substitution of water with aqueous formic acid (0.1%, v/v) in the interference wash step.

f) Elution volumes can be minimized by the use of successive aliquots of elution solvent (for example 2 x 200 µL instead of 1 x 500 µL).

g) For 10 mg columns and plates, solvent volumes can be reduced to 500 µL for conditioning, equilibration and interference wash steps. Elution solvent volume can be reduced to 200 µL.

Other SPE procedures

The EVOLUTE ABN generic method described in this Technical Note has been optimized during extensive testing under a wide variety of conditions to give optimum performance. However, EVOLUTE ABN is a versatile solid phase extraction sorbent, and other SPE procedures may provide similar results.

Appendix 2

EVOLUTE ABN Product Ordering Information

Part Number	Description	Quantity
Fixed Well Plate		
600-0010-P01	EVOLUTE ABN 10 mg fixed well plate	1
600-0025-P01	EVOLUTE ABN 25 mg fixed well plate	1
Versatile EVOLUTE Array Plate		
600-0010-R	EVOLUTE Array ABN 10 mg/1 mL wells	100
600-0010-RP	EVOLUTE Array ABN 10 mg/1 mL plate	1
600-0025-R	EVOLUTE Array ABN 25 mg/1 mL wells	100
600-0025-RP	EVOLUTE Array ABN 25 mg/1 mL plate	1
EVOLUTE Array Accessories		
120-6000-P01	EVOLUTE Array base plate	1
120-1200	Strip of 8 base plate sealing plugs[5]	50
120-1201	Luer adaptors (to fit any standard sample processing manifold)	25
120-1202	Well removing tool	1
[5]required when processing a partially populated EVOLUTE Array plate		
SPE Columns		
600-0001-A	EVOLUTE ABN 10 mg/1 mL	100
600-0002-A	EVOLUTE ABN 25 mg/1 mL	100
Tab-less SPE Columns		
600-0001-AG	EVOLUTE ABN 10 mg/1 mL (tab-less)	100
600-0002-AG	EVOLUTE ABN 25 mg/1 mL (tab-less)	100

For other configurations, contact Biotage.

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