





EVOLUTE Sample Preparation Products

Optimized for Reduced Ion
Suppression in LC-MS
Applications



EVOLUTE ABN

ADVANCED WATER WETTABLE POLYMERIC SORBENT

EVOLUTE™ Sample Preparation Products

EVOLUTE™ Sample Preparation Products are advanced water-wettable polymeric sorbents for extracting analytes from biological fluid samples. Developed specifically for bioanalytical sample preparation, EVOLUTE products provide an effective solution to the problems of matrix effects associated with LC-MS. The product is supported by a generic procedure, minimizing method development time whilst providing excellent extract cleanliness for accurate analyte quantitation.



EVOLUTE ABN

Features and Benefits

- Cleaner extracts providing reduced matrix effects
- Improved signal-to-noise in LC-MS for lower detection levels
- Fast, simple method development using a single generic method
- High reproducible recoveries for a wide range of analytes

EVOLUTE ABN has been specifically developed for the isolation of **A**cidic, **B**asic and **N**eutral drugs from biological fluids. An optimized combination of polar (hydrophilic) and non-polar (hydrophobic) interactions allow efficient extraction of both parent drugs and their metabolites. The sorbent has an optimized pore structure that minimizes the retention of endogenous interferences from the original sample, reducing matrix effects during LC-MS analysis. Optimized particle size distribution and product cleanliness ensure reliable performance.

Cleaner Extracts

EVOLUTE ABN has a mean pore diameter of approximately 40Å. Along with its narrow pore size distribution, this ensures retention of small molecules routinely quantified in bioanalytical procedures, but minimizes the retention of endogenous materials present in the original biological fluid. The resulting cleaner extracts show reduced ion suppression and matrix effects leading to more accurate analyte quantitation, and an improvement in the signal to noise ratio in LC-MS analysis (see **figure 1**).

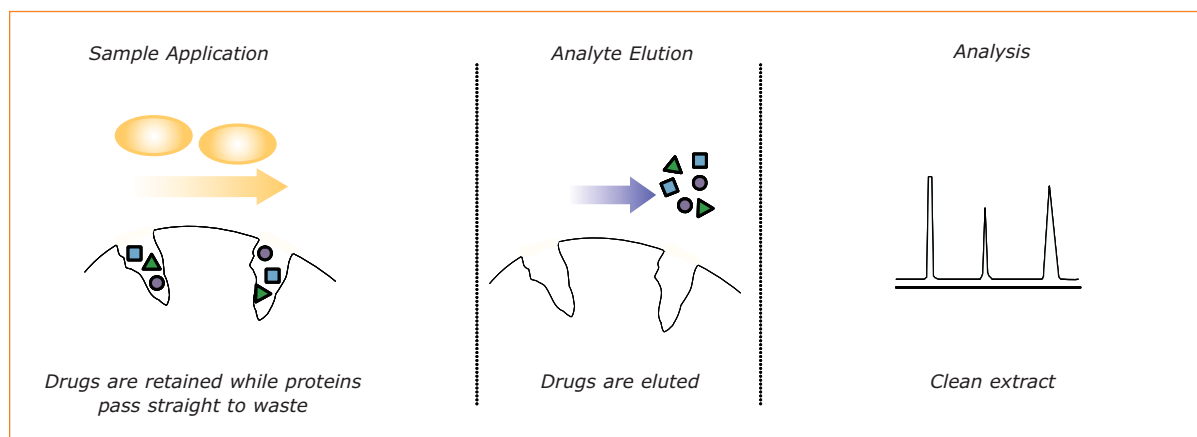


Figure 1. EVOLUTE ABN's optimized pore size eliminates proteins and delivers cleaner extracts.

Single Generic Method

The use of a single generic method for the extraction of acidic, basic and neutral analytes minimizes method development time and increases productivity. Analytes can be eluted in pure organic solvent, minimizing the use of modifiers that could adversely affect the analytical technique.

Ordering Information

EVOLUTE ABN 1 mL SPE Columns

Description	Quantity	Part number
EVOLUTE ABN 10 mg/1mL columns	100	600-0001-A
EVOLUTE ABN 25 mg/1mL columns	100	600-0002-A

EVOLUTE ABN Fixed Well Plate

Description	Quantity	Part number
EVOLUTE ABN 10 mg plate	1	600-0010-P01
EVOLUTE ABN 25 mg plate	1	600-0025-P01

EVOLUTE Array ABN Loose Wells

Description	Quantity	Part number
EVOLUTE Array ABN 10 mg/1 mL wells	100	600-0010-R
EVOLUTE Array ABN 25 mg/1 mL wells	100	600-0025-R

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

EVOLUTE Array Accessories

Description	Quantity	Part number
EVOLUTE Array base plate	1	120-6000-P01
Strip of 8 base plate sealing plugs*	50	120-1200
Luer adaptors (to fit any standard sample processing manifold)	25	120-1201
Well removing tool	1	120-1202

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

For support documentation please request Chemistry Data Sheet **TN131**: EVOLUTE ABN for the Extraction of Drugs from Biological Fluids.

Please contact your local Biotage representative or Distributor for products that are available through our **Custom Manufacturing Service** (see page 152-153).





Protein Precipitation
Products

Fast, Simple Protein
Removal

ISOLUTE PPT+

ISOLUTE PPT+ Protein Precipitation Plates

Protein precipitation is a routinely used sample preparation technique for removal of proteins from biological fluid samples prior to drug analysis. Protein precipitation by filtration in the 96-well format is a high throughput alternative to the traditional centrifugation based technique using collection plates or micro tubes.

ISOLUTE PPT+ Protein Precipitation Plates have revolutionized this sample preparation technique. The optimized filtration system in ISOLUTE PPT+ plates provides an easy to automate solution for efficient protein removal. The procedure is streamlined, with no off-line steps.



The proprietary filtration system holds up acetonitrile allowing the plasma sample to be applied to a pool of solvent. This results in very efficient protein precipitation. Unlike membrane based devices, the system has an optimized porosity distribution and acts as a depth filter, holding up the precipitated protein without well blockage whilst still allowing rapid transfer of the filtrate to the collection plate (see **figure 1**).

Features and Benefits

- 'Solvent first' methodology produces efficient protein removal, eliminating cloudy extracts
- No well blockage, due to optimized filtration system
- Optimized for automation without off-line vortex mixing
- Fixed well plates for routine high throughput applications
- Modular Array plate format for method development and variable sample numbers

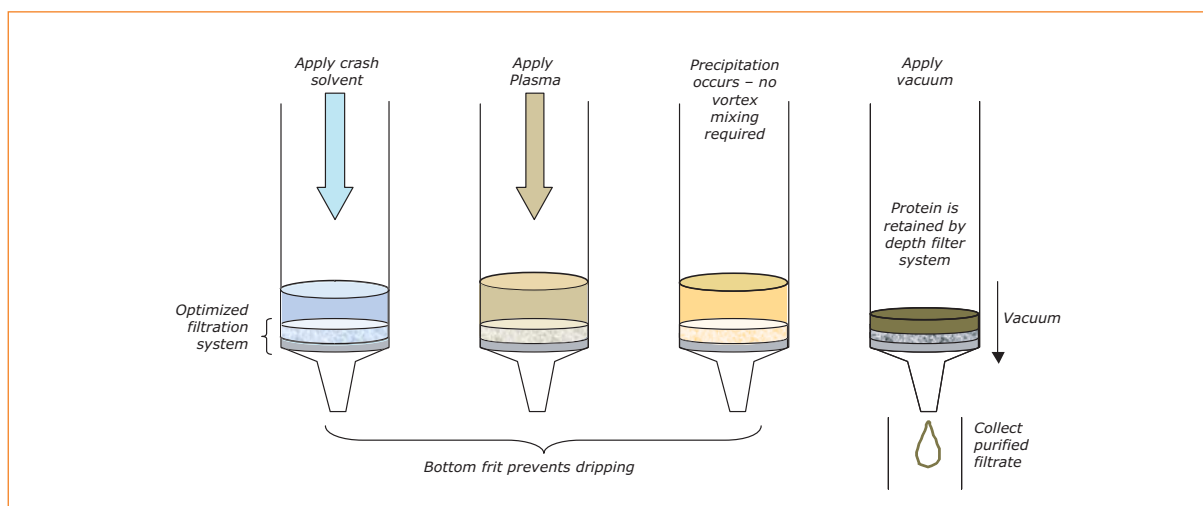


Figure 1. 'Solvent first' protein precipitation procedure

Ordering Information

Fixed Well Plate

Description	Quantity	Part number
ISOLUTE PPT+ fixed well plate, 2 mL	1	120-2040-P01

Modular ISOLUTE Array Wells

Description	Quantity	Part number
ISOLUTE Array PPT+ wells, 1 mL	100	120-2040-R
ISOLUTE Array PPT+ wells, 2 mL	100	120-2040-T

Pre-assembled ISOLUTE Array plates are available. To order, add the suffix P to the equivalent loose well part number.

ISOLUTE Array Accessories

Description	Quantity	Part number
ISOLUTE Array base plate	1	120-1000-P01
Strip of 8 base plate sealing plugs*	50	120-1200
Luer adaptors (to fit any standard sample processing manifold)	25	120-1201
Well removing tool	1	120-1202

*Required when processing a partially populated ISOLUTE Array PPT+ plate

For support documentation please request **PS424: ISOLUTE Protein Precipitation Plates** and **TN130: Sample Preparation using ISOLUTE PPT+ Protein Precipitation Plates**.

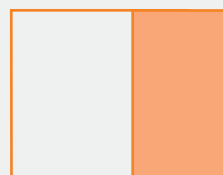
Please contact your local Biotage representative or Distributor for products that are available through our **Custom Manufacturing Service** (see **page 152-153**).





Supported Liquid Extraction

High Purity, Efficient
Alternative to
Liquid-Liquid Extraction



SUPPORTED LIQUID EXTRACTION

Supported liquid extraction (SLE) is a sample preparation technique that is analogous to liquid-liquid extraction. The technique uses columns or 96-well plates packed with an inert support material (a modified form of diatomaceous earth). When the aqueous sample is applied, it spreads over the surface of the support, and is absorbed. The analytes remain on the surface of the support, which forms the phase interface for the extraction (equivalent to the phase interface in LLE). When the water immiscible extraction solvent is applied, analytes are efficiently desorbed, and the extract is collected (see **figure 1**). Problems such as emulsion formation are eliminated.

Supported liquid extraction uses the same solvent systems as traditional liquid-liquid extraction, allowing direct method transfer.

Biotage offers both 96-well plates and traditional columns for sample preparation by supported liquid extraction.

ISOLUTE SLE+ Plates for Supported Liquid Extraction

ISOLUTE SLE+ plates are packed with a modified form of diatomaceous earth for optimized high throughput, automated bioanalytical SLE procedures. Each well contains 200 mg of support material, suitable for extraction of up to 200 μ L aqueous sample.



Features and Benefits

- Optimized for high throughput bioanalytical applications
- Automation friendly procedure improves productivity – no off-line steps required
- Efficient extraction process maximizes analyte recovery compared to LLE
- Clean extracts – reduced ion suppression leads to improved signal-to-noise
- Fixed well plate format is compatible with all common liquid handling systems

Process ISOLUTE SLE+ plates manually using the VacMaster-96 Sample Processing Manifold or automated liquid handling systems equipped with a vacuum manifold.

Ordering Information

Description	Quantity	Part number
ISOLUTE SLE+ 200 mg Supported Liquid Extraction Plate	1	820-0200-P01

SUPPORTED LIQUID EXTRACTION

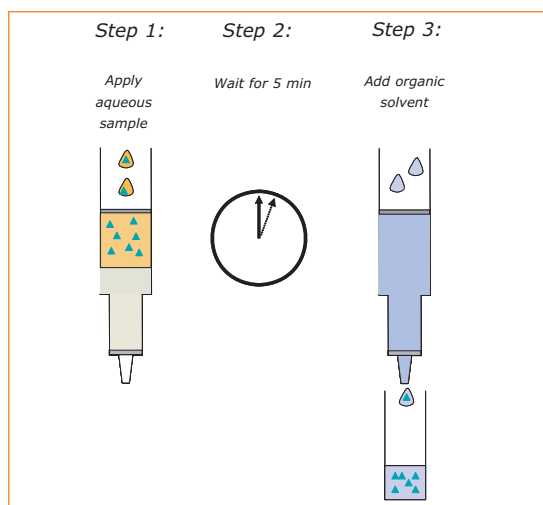


Figure 1. Schematic of Supported Liquid Extraction process

ISOLUTE HM-N Columns for Supported Liquid Extraction

Supported liquid extraction can be performed using the traditional column format with ISOLUTE HM-N columns. ISOLUTE HM-N columns contain an inert support material optimized for efficient extraction of aqueous samples. A range of column sizes is available to match the sample size.

Features and Benefits

- Column format for efficient liquid-liquid extraction
- Simple and quick for multiple extractions
- Extract aqueous samples from 300 μ L to 20 mL in volume
- Compatible with viscous matrices which may block SPE columns
- Eliminate emulsion formation
- Easy to transfer existing LLE methods to SLE column format
- Gravity processing with IST Gravity Rack (no vacuum required)

ISOLUTE HM-N columns are processed under gravity.
See **page 106** for further details on the IST Gravity Rack.



Ordering Information

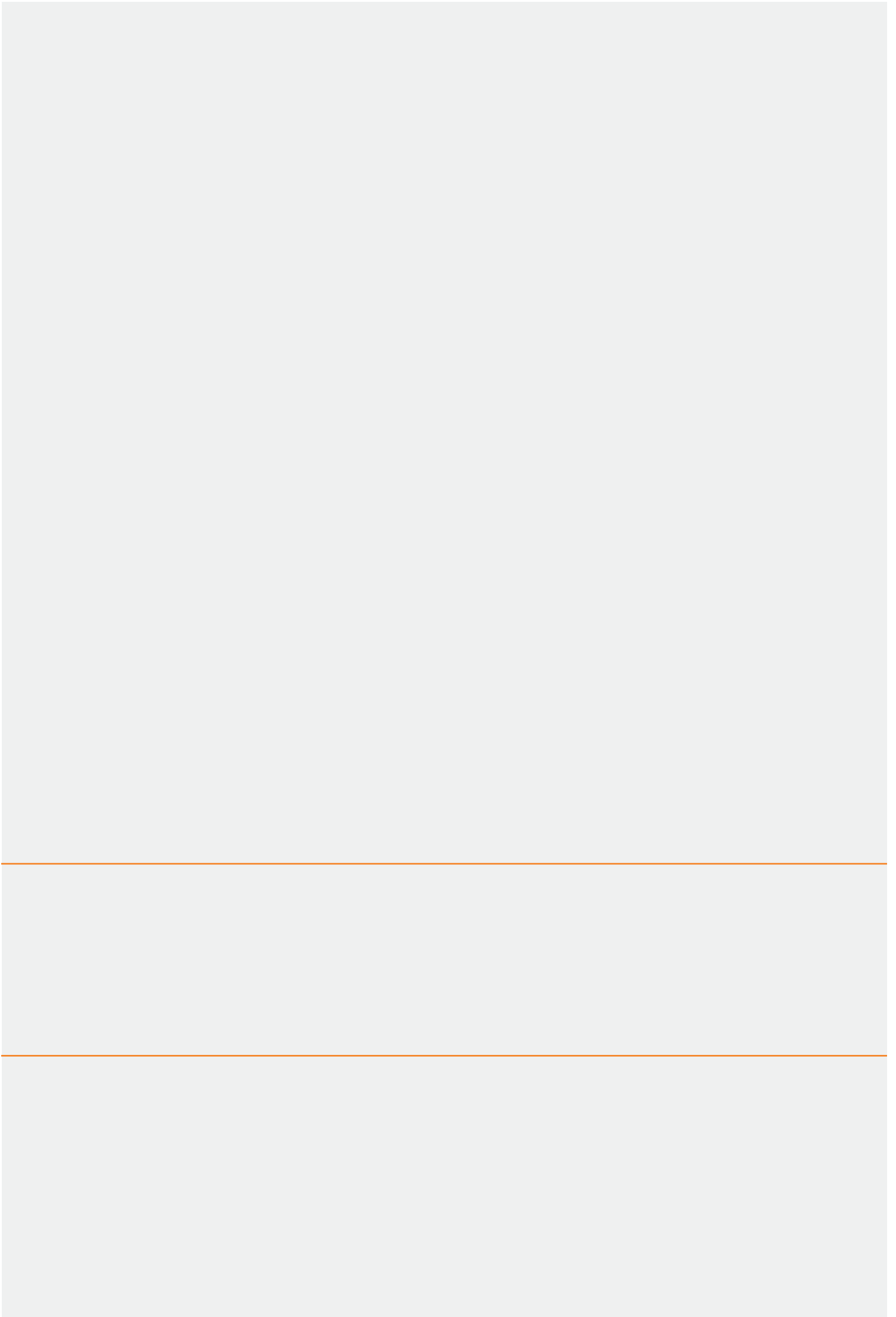
Description	Quantity	Part number
ISOLUTE HM-N (300 μ L sample)	100	800-0040-BM
ISOLUTE HM-N (1 mL sample)	100	800-0100-CM
ISOLUTE HM-N (3 mL sample)	100	800-0220-DM
ISOLUTE HM-N (5 mL sample)	100	800-0350-EM
ISOLUTE HM-N (10 mL sample)	50	800-0700-FM
ISOLUTE HM-N (20 mL sample)	50	800-1300-FM

Note: Always use a column of equal or greater capacity than the sample volume. The maximum capacity of the column is included in the column description listed in the ordering table.

Please contact your local Biotage representative or Distributor for alternative configurations that are available through our **Custom Manufacturing Service** (see **page 152-153**).

For support documentation please request **TN118** The use of ISOLUTE HM-N for Rapid Sample Preparation.







Products for
Bioanalytical
Applications



In today's bioanalytical laboratory, reliable, high throughput sample preparation techniques are essential. IST Sample Preparation Products from Biotage provide a range of solutions matching the requirements for throughput and sample clean-up throughout the drug development process.

Biotage is dedicated to developing sample preparation products that solve the daily sample preparation challenges faced by bioanalytical scientists. For example, our product development programme is strongly influenced by the need to reduce matrix effects in LC-MS, without compromising speed or simplicity of sample preparation.

Generic methodologies optimized for extraction of small volumes of biological fluids are available for most products, minimizing method development time and enhancing productivity.

With the increasing use of automation in sample preparation, another focus is the development of reliable, easily automated techniques and procedures, which can maximize the use of liquid handling systems. Biotage supplies a range of 96-well and conventional column products, compatible with all commonly used automated systems.

Products for Bioanalytical Sample Preparation

Technique	Products	Analyte functionality	Extract cleanliness	Features/benefits
Protein Precipitation (PPT)	ISOLUTE PPT+ Protein Precipitation Plates	Simultaneous extraction of acidic, basic or neutral compounds	Protein removal only.	A fast and simple technique for removal of proteins from biological fluid samples. Non-selective technique, suitable for extraction of a broad range of compounds; extracts can give relatively high levels of ion suppression.
Supported Liquid Extraction (SLE)	ISOLUTE SLE+ Supported Liquid Extraction Plates	<ul style="list-style-type: none"> • Acidic, basic and neutral* • Acidic and neutral* • Basic and neutral* 	Dependant on choice of extraction solvent.	An easily automated technique, analogous to liquid-liquid extraction.
Solid Phase Extraction (SPE)	ISOLUTE and EVOLUTE SPE Products	<ul style="list-style-type: none"> • Simultaneous extraction of acidic, basic or neutral compounds • Acidic only • Basic only 	Dependant on sorbent choice. Improved clean-up compared with protein precipitation.	Depending on sorbent choice, can provide high analyte recoveries of a broad range of compounds, or extremely selective extraction of specific compounds with very clean extracts.

*Analytes must be soluble in water immiscible solvents.

ISOLUTE PPT+ Protein Precipitation Plates

ISOLUTE PPT+ Protein Precipitation Plates provide effective, high throughput, protein removal from plasma samples. The simple filtration based procedure is easily automated, and is applicable to a wide range of drugs and metabolites.

See **page 84-85** for more details and ordering information.



ISOLUTE SLE+ Supported Liquid Extraction Plates



ISOLUTE SLE+ Supported Liquid Extraction Plates are optimized for reliable high throughput extraction of 96-samples simultaneously using liquid handling systems. Up to 200 μL of aqueous sample can be extracted per extraction well. For larger sample volumes, ISOLUTE HM-N columns for supported liquid extraction are available.

See **page 88-89** for more details and ordering information.

Solid Phase Extraction (SPE) Products

Biotage manufactures a comprehensive range of solid phase extraction products. These include both EVOLUTE and ISOLUTE resin-(or polymer-) based and silica-based sorbents which have different but complementary extraction characteristics. The range includes sorbents offering a choice of non-polar, mixed-mode and ion exchange retention mechanisms for extraction of drugs and metabolites from biological fluids. Depending on the assay requirements, the SPE method can be tailored to deliver high recoveries of a range of acidic, basic and neutral drugs using a single generic method, or highly selective extraction of a single drug with a very clean extract that exhibits minimal ion suppression.

EVOLUTE ABN

EVOLUTE ABN SPE columns and plates are ideal for extraction of a broad range of compounds from biological fluid samples. The sorbent has been specifically designed for LC-MS assays, and has an optimized pore size distribution ensuring reduced ion suppression (improved signal to noise) for biological fluid extracts, compared to other resin based sample preparation products.

See **page 80-81** for more details and ordering information.

See Appendix 1a of the QuickStart Guide (**page 148**) for the EVOLUTE ABN generic method.



ISOLUTE ENV+

ISOLUTE ENV+ SPE columns and plates are ideal for extraction of extremely polar compounds which are not retained on EVOLUTE ABN. The sorbent is suitable for extraction of polar acidic, basic and neutral drugs and metabolites from biological fluids.

See **page 28** for more details and ordering information.

ISOLUTE Non-polar SPE Products

Available in a range of column and plate formats, ISOLUTE non-polar sorbents offer a range of choices for the extraction of acidic, basic and/or neutral compounds from biological fluid samples.

The hydrophobic character of the non-polar sorbent decreases with chain length. C18 and C8 are commonly used non-polar sorbents, capable of extracting compounds of wide ranging polarity. Extract cleanliness can be enhanced for compounds with significant non-polar character by using a shorter chain length non-polar sorbent (e.g. ISOLUTE C2). This will allow more polar interferences to be removed from the column prior to analyte elution. Request Chemistry Data Sheet **TN126** for more details.

The secondary interactions provided by non-encapped non-polar sorbents can enhance the extraction of basic drugs from biological fluids, giving higher recoveries and cleaner extracts than encapped non-polar sorbents. Request Chemistry Data Sheet **TN112** for more details.

See **pages 28-43** for more details and ordering information.

ISOLUTE Mixed-mode SPE Products

Mixed-mode SPE is a robust sample preparation approach that can lead to extremely selective isolation of ionizable compounds from complex biological fluid matrices. The dual retention mechanism allows the use of a rigorous interference elution regime, leading to extremely clean final extracts (see **figure 1**), which exhibit minimal ion suppression.

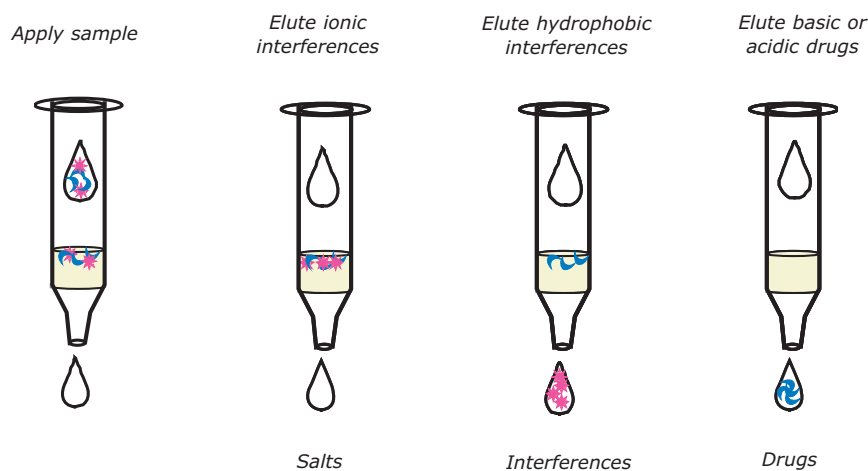


Figure 1. Schematic of mixed-mode SPE procedure

A range of ISOLUTE mixed-mode SPE sorbents are available, providing mixed-mode retention mechanisms suitable for extraction of most drug(s) and metabolites.

Selection of ISOLUTE mixed-mode sorbents by analyte class

Drug Class	HAX	HGX	HGX-3	HGX-5	HGX-Q ^a
Acidic	✓				
Basic		✓	✓	✓	
Basic / lipophilic		✓		✓	
Basic (pK > 11)					✓
Polybasic					✓
Quaternary Amine					✓

^aOptimum sorbent for basic drugs where it is difficult or impossible to eliminate positive charge(s) using pH.

See **pages 44-52** for more details and ordering information.

Refer to Appendix 1b of the QuickStart Guide (**page 148-149**) for generic methodology for use with each mixed-mode sorbent.

ISOLUTE Ion Exchange Sorbents

- ISOLUTE cation exchange sorbents for extraction of basic drugs
- ISOLUTE anion exchange sorbents for extraction of acidic drugs

For very polar, ionizable compounds that cannot be retained using a non-polar retention mechanism, ISOLUTE ion exchange sorbents offer an alternative that can provide selective extraction with very clean extracts.

See **pages 54-65** for more details and ordering information.

The QuickStart Guide to at the back of this catalog provides methodology details for these sorbents. Chemistry Data Sheets are also available, see relevant sorbent ordering pages.

25 mg SPE Columns and 96-well Plates

ISOLUTE SPE sorbents (non-polar, mixed-mode and ion exchange) are available in SPE columns and 96-well plates containing 25 mg of sorbent. This is the recommended sorbent mass for the extraction of biological fluid samples, and enables elution in 100 μ L or less (see **figure 2**).

Ordering information for 25 mg formats can be found on the relevant sorbent page.

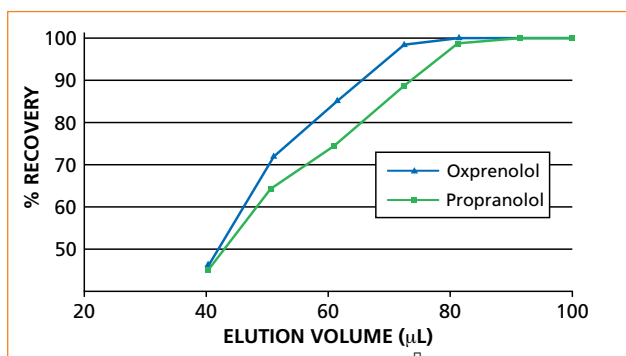


Figure 2. Elution profile for beta blockers using ISOLUTE C2 25 mg/1 mL columns, showing elution in less than 100 μ L.

Formats for High Throughput Sample Preparation

The 96-well plates from Biotage are available in two formats: fixed well plates for routine high throughput applications, and the modular Array format for routine processing of fewer than 96 samples and method development. Both formats are processed on the VacMaster-96 Sample Processing Manifold and are compatible with commonly used liquid handling systems. See **page 16-19** for more details.

Standard 1 and 3 mL SPE columns can be processed on liquid handling systems for automated sample preparation. Some systems, including the Gilson SPE 215, require tab-less columns held in a rack system that allows a microplate footprint. Examples of standard and tab-less 1 mL SPE columns are shown opposite. Tab-less columns are available from Biotage under the **Custom Manufacturing Service** (see **page 152-153** for more details).

Ordering information for high throughput formats can be found on the relevant sorbent page.



Tab-less and standard SPE columns