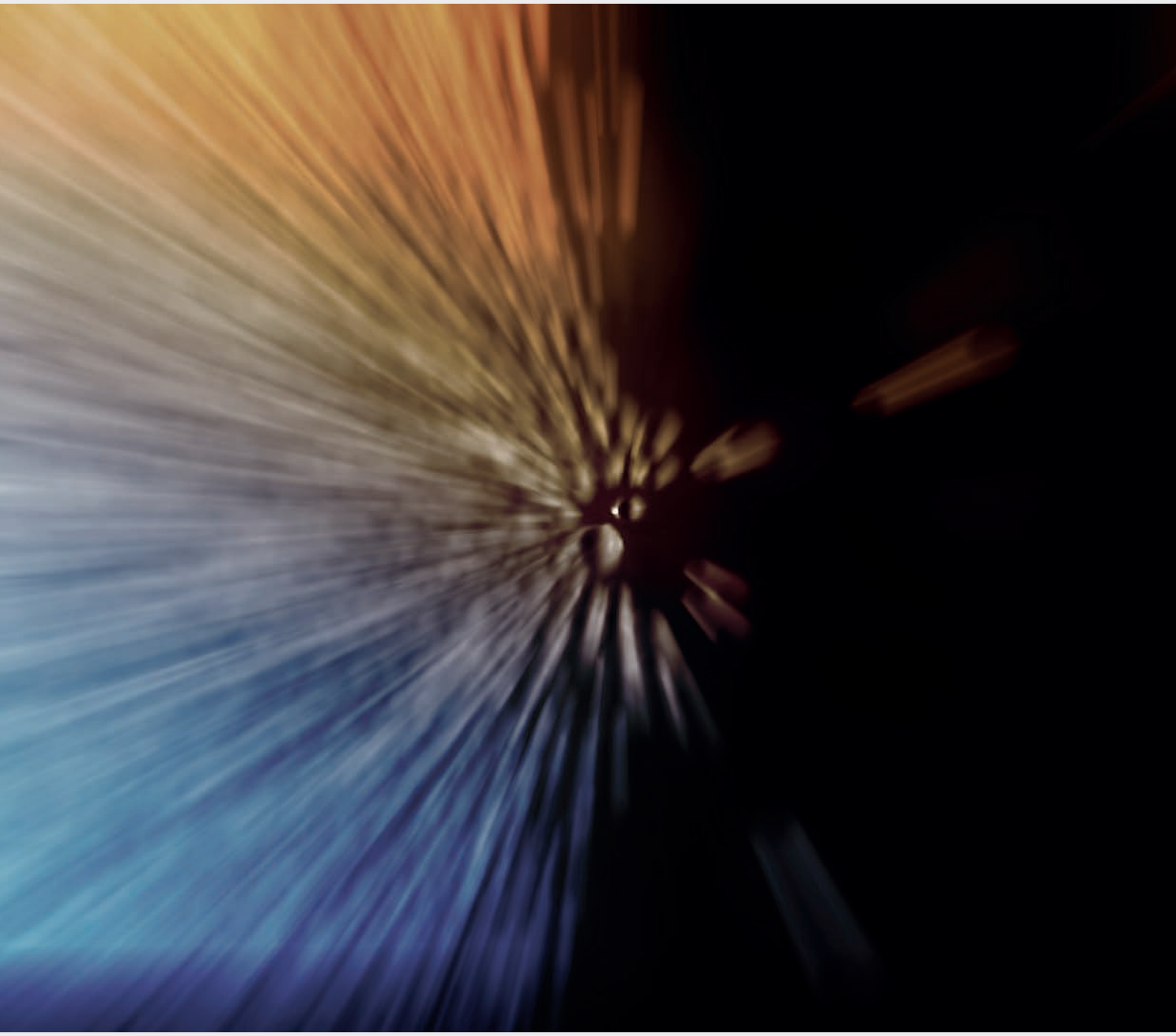
EVOLUTE® EXPRESS User Guide

Load-Wash-Elute SPE



Biotage®

EVOLUTE® EXPRESS User Guide

CONTENTS

EVOLUTE® EXPRESS

SPE Products from Biotage

EVOLUTE® EXPRESS Solid Phase Extraction products from Biotage are designed for all your sample preparation challenges.

Whether you are working in the field of bio-analysis during drug development, clinical or forensic toxicology, or with the diversity of samples in food safety and environmental applications, the tools and methods used for sample preparation have a clear impact on productivity.

In developing the EVOLUTE EXPRESS SPE family, Biotage's goal has been to improve productivity during method development, validation and routine analysis with a combination of sorbents, components and methods that can be applied to as wide a range of analytes as possible, and at the same time reduce or eliminate unwanted matrix components from the final extracts.

The EVOLUTE® EXPRESS product family is versatile, available in five sorbent chemistries to accommodate all of your extraction demands. Choose from a variety of formats including multiple tube sizes, tabless tubes, 96-well plates, and on-line cartridges* to find the best format for your overall objective.

Extractions are worry free. Unlike silica-based sorbents, EVOLUTE EXPRESS products are deconditioning resistant, meaning you will never experience poor analyte-sorbent interactions due to phase collapse. In addition they further benefit from Biotage's Load-Wash-Elute protocols.

Traditional silica-based SPE sorbents cannot perform under extremely high or low pH which can limit your analysis. EVOLUTE EXPRESS products are pH resistant so you can work without limitations.

*EVOLUTE® EXPRESS ABN only.

The Advantages

Faster Methods
We've eliminated the need to condition and equilibrate with Load-Wash-Elute protocols.

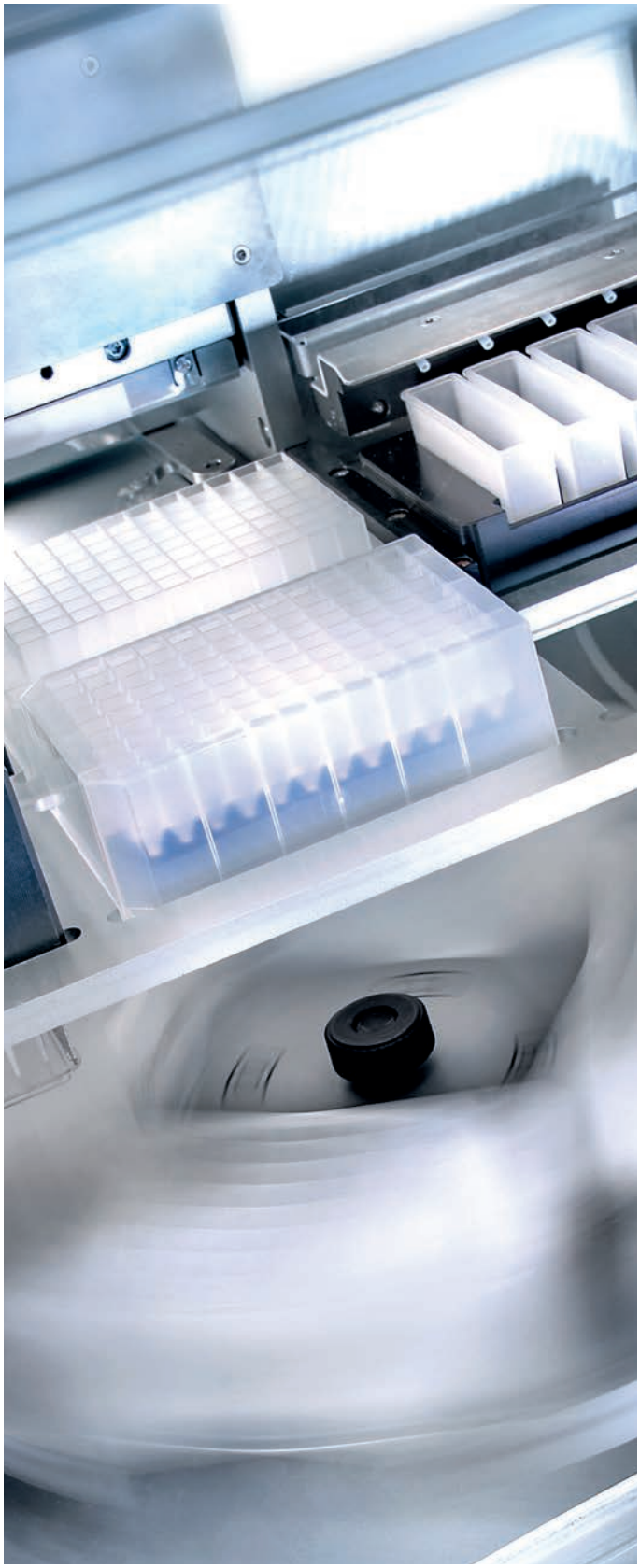
Faster and Consistent Flow Rates
We use frit components that enhance flows even for viscous samples.

Faster Method Development
We've developed proven generic methods and optimization tips that really work.

Cleaner Extracts
EVOLUTE EXPRESS sorbents have protein exclusion built in by design.

No Phospholipids
Our wash and elution protocols reduce or eliminate matrix interferences.

Cleaner Components
We make sure EVOLUTE EXPRESS products won't contaminate your samples.



EVOLUTE® EXPRESS Family Chemistry

EVOLUTE® EXPRESS ABN

Water wettable sorbent with non-polar interactions for simultaneous extraction of acidic, basic and neutral analytes.

EVOLUTE® EXPRESS CX

Mixed-mode sorbent combining non-polar with strong cation exchange interactions for extraction of basic analytes.

EVOLUTE® EXPRESS WCX

Mixed-mode sorbent combining non-polar with weak cation exchange interactions for extraction of strongly basic analytes.

EVOLUTE® EXPRESS AX

Mixed-mode sorbent combining non-polar with strong anion exchange interactions for extraction of acidic analytes.

EVOLUTE® EXPRESS WAX

Mixed-mode sorbent combining non-polar with weak anion exchange interactions for extraction of strongly acidic analytes.

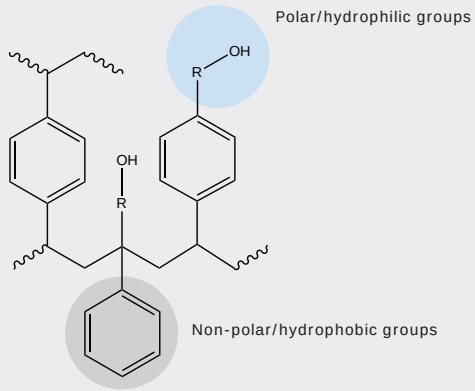


Figure 1. Polymeric backbone of EVOLUTE® EXPRESS sorbents.

EVOLUTE® EXPRESS sorbents provide robust, reliable SPE. They are based on a modified non-polar polystyrene-divinylbenzene polymer which incorporates polar hydroxyl groups (Figure 1). These non-ionizable hydroxyl groups ensure that the polymer is both highly water wettable, and also able to extract a diverse range of analytes through non-polar (van der Waals) interactions. No secondary interactions exist, so EVOLUTE EXPRESS retention and elution characteristics are completely predictable and extractions on these robust sorbents are not adversely affected by drying of the sorbent bed during sample processing.

EVOLUTE EXPRESS mixed-mode sorbents consist of the EVOLUTE EXPRESS polymer ‘backbone’ modified with ionic functional groups.

Reducing Matrix Effects By Eliminating Proteins From Sample Extracts

All EVOLUTE® EXPRESS SPE sorbents are manufactured with a very narrow pore size distribution (Figure 3), tailored to eliminate the co-extraction of plasma proteins and provide cleaner sample extracts for analysis. This pore size optimization dramatically reduces protein concentrations in biological fluid sample extracts, when compared to competitor SPE products.

The effect of this pore size optimization can be demonstrated using gel electrophoresis (Figure 2). Native serum contains many protein bands when stained with Coomassie blue. When a sample of native serum is extracted using an EVOLUTE EXPRESS product and the serum extract is separated by gel electrophoresis, these protein bands are virtually eliminated.

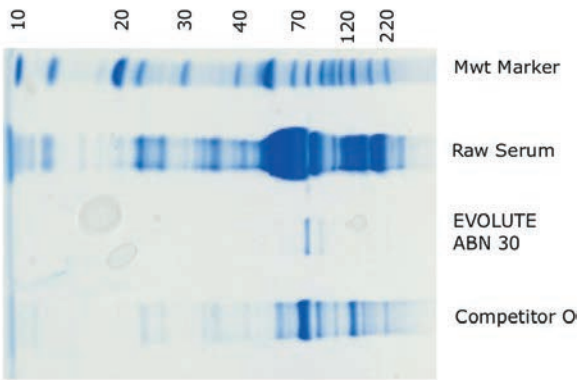


Figure 2. Gel electrophoresis showing protein removal from serum EVOLUTE® EXPRESS sorbents.

Removal of proteins from sample extracts avoids subsequent transfer into the analytical system, reducing matrix effects in LC-MS/MS during analysis. Increased backpressure in UPLC systems due to protein contamination is eliminated, avoiding the need for column back flushing and frequent replacement of guard columns. This is illustrated in figure 4.

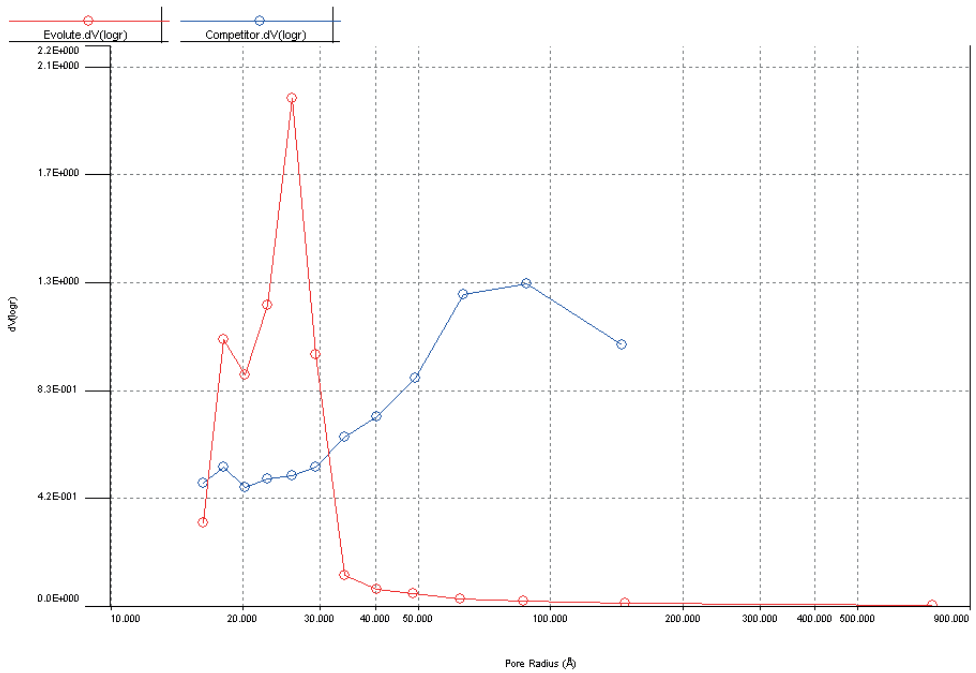


Figure 3. Pore size distribution of EVOLUTE® EXPRESS sorbents compared with a competitor polymeric SPE column.

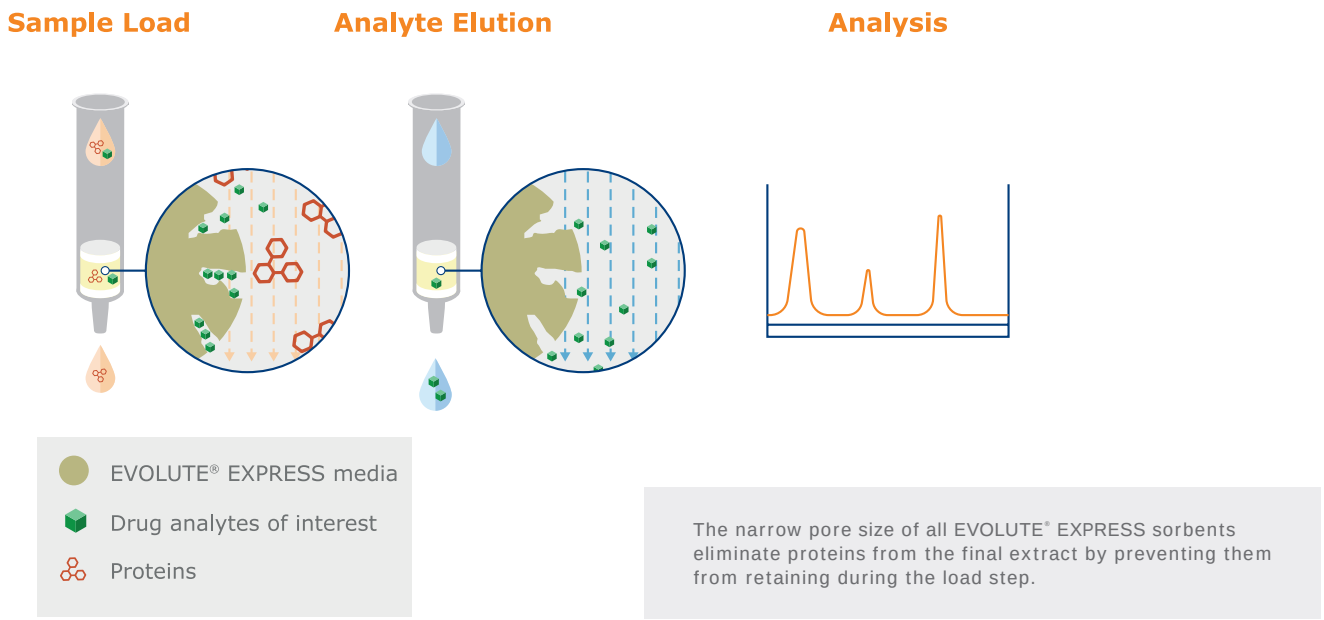


Figure 4. Exclusion of proteins from EVOLUTE® EXPRESS media pores leading to cleaner extracts.

Mixed-Mode SPE for Additional Selectivity and Extract Cleanliness

In addition to the 'built in advantage' of optimized pore size, which eliminates larger molecular weight matrix components, the EVOLUTE® EXPRESS SPE range includes mixed-mode sorbents to further enhance selectivity during extraction. Mixed-mode chemistries allow more vigorous wash procedures, while maintaining high analyte recovery, leading to increased analytical sensitivity for acidic and basic analytes.

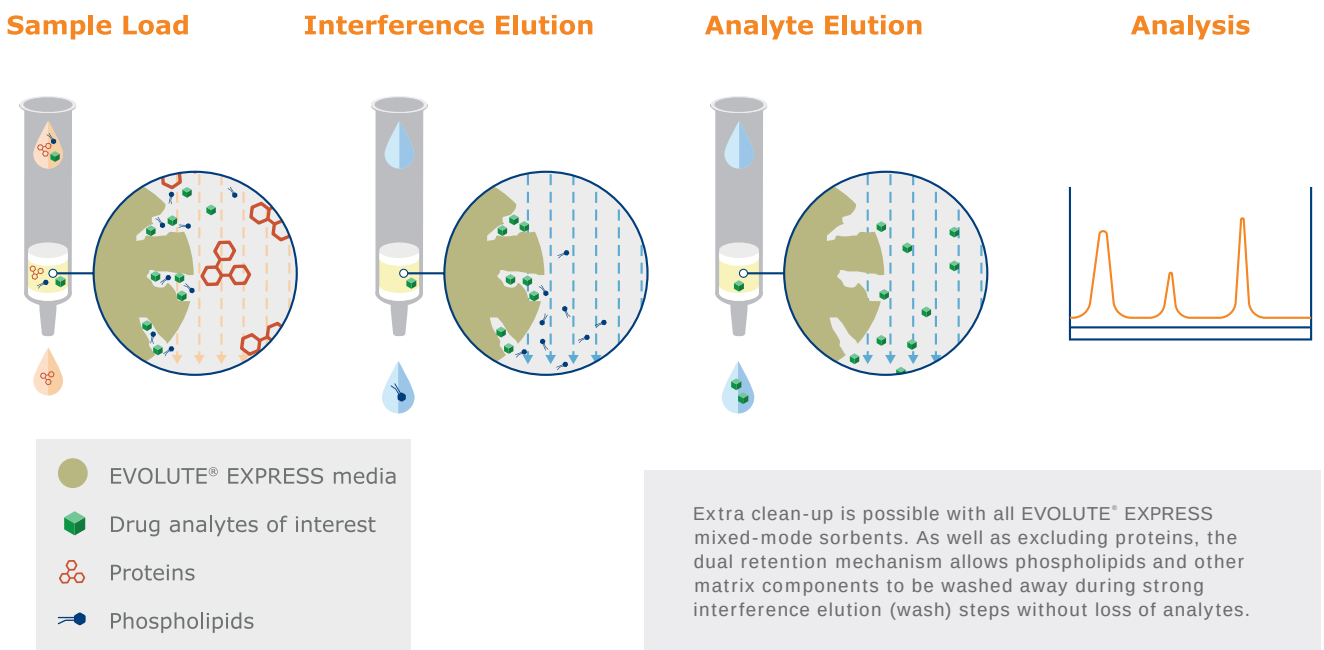


Figure 5. Exclusion of proteins is combined with additional wash steps in EVOLUTE® EXPRESS mixed-mode sorbents.

Load-Wash-Elute Procedure

Optimized design features of EVOLUTE® EXPRESS columns and 96-well plates dramatically improve flow characteristics, and enhance sample preparation productivity. By truly eliminating the need for media conditioning and equilibration, samples can be prepared using a simple, fast, three step 'load-wash-elute' procedure (Figure 6).

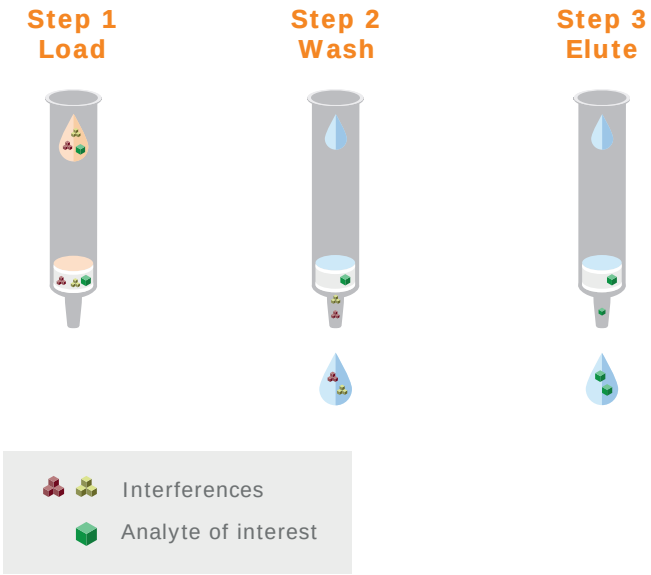


Figure 6. EVOLUTE® EXPRESS Load-wash-elute SPE.

By eliminating the need for media conditioning and equilibration steps, time saving for processing 96 samples on EVOLUTE EXPRESS columns using load-wash-elute methodology is up to 30%.

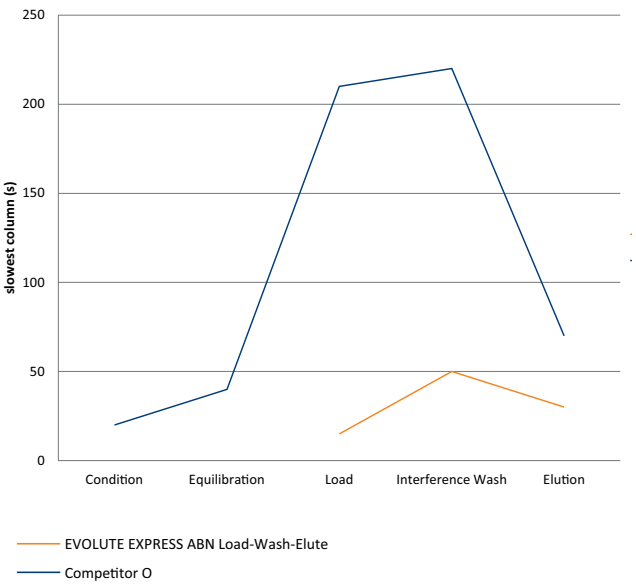


Figure 7. Time taken at each step: standard SPE vs. EVOLUTE® EXPRESS load-wash-elute SPE.

Processing Time Comparison

	Standard SPE Procedure	EVOLUTE® EXPRESS Load-Wash-Elute Procedure
Sample Pre-Treatment	2% Formic acid, 1:1, mix	2% Formic acid, 1:1, mix
Conditioning	Methanol, 1 mL	Not required
Equilibrate	0.1% Formic acid, 1 mL	Not required
Load Sample	Aqueous sample, 1 mL	Aqueous sample, 1 mL
Wash	H_2O/Methanol, 60/40 (v/v), 1 mL	H_2O/Methanol, 60/40 (v/v), 1 mL
Elute	Methanol, 500 µL	Methanol, 500 µL
Total time for 96 samples	33.15 minutes	**24.02 minutes**

Instrument: Biotage® Extrahera™ processing EVOLUTE® EXPRESS ABN 30 mg/1 mL (tabless)

Time saving for automated processing of 96 samples = **9.13 minutes (~30% reduction)**

As well as reducing overall processing time by eliminating two steps from the method, EVOLUTE EXPRESS flow rates are faster. This is particularly noticeable when loading aqueous/biological fluid samples, and for the subsequent wash step, as shown in Figure 7. This is because the EXPRESS frits encourage fast, even flow of aqueous liquids. They are also much less prone to blocking.

Which Sorbent Should I Choose?

Successful sample preparation depends on the correct choice of a particular sorbent and methodology for the application. There is often more than one option for a particular analyte or group of analytes. Biotage provide simple guidelines based on analyte and sample clean up needs to allow selection of the correct sorbent and methodology to meet your sample preparation requirements.

When characterizing a sample preparation problem, it is worth considering both the analyte(s) and matrix factors as well as other practical components such as the volume of sample and number of samples to be run.

For analytes with some **hydrophobic (non-polar)** functionality (whether acidic, basic or neutral), a sorbent with a single non-polar retention mechanism such as **EVOLUTE® EXPRESS ABN** is ideal for extraction from aqueous samples. Depending on the degree of hydrophobic character, and complexity of the matrix, the method can be optimized to reduce the amount of co-extracted matrix components.

For **acidic** or **basic** analytes containing ionizable functional groups, **mixed-mode** sorbents combining non-polar and ion exchange retention mechanisms can provide additional selectivity and clean up. This approach is particularly useful for extraction of analytes from biological fluids, as the dual retention mechanism allows strong interference elution solvents to be used, which eliminate phospholipids and other unwanted co-extracted species from the final extract.

Basic Analytes

For **basic analytes**, two mixed-mode (non-polar plus cation exchange mechanism) options are available:

- For extraction of basic analytes with pK_a 2–10, **EVOLUTE EXPRESS CX** combines non-polar with strong cation exchange retention mechanisms. **EVOLUTE EXPRESS CX** is also recommended for extraction of amphoteric species, and fractionation of complex mixtures.
- **For strongly basic analytes** (quaternary amines or pK_a >10) **EVOLUTE EXPRESS WCX** should be used.

Acidic Analytes

For **acidic analytes**, two mixed-mode (non-polar plus anion exchange mechanism) options are available:

- For acidic analytes with pK_a 2–8, **EVOLUTE EXPRESS AX** combines non-polar with strong anion exchange retention mechanisms.
- For strongly acidic analytes (pK_a <2) **EVOLUTE EXPRESS WAX** should be used.

EVOLUTE® EXPRESS Sorbent Choices Based on Analyte Functionality

Analyte Functionality	ABN	CX	WCX	AX	WAX
Neutral	Alternative				
Weakly acidic pK_a 2–8	Alternative			First Choice	
Strongly acidic pK_a <2	Alternative				First Choice
Weakly basic pK_a 2–10	Alternative	First Choice			
Strongly basic pK_a >10	Alternative		First Choice		
Acidic, neutral, basic mix	First Choice				
Amphoteric	Alternative	First Choice		First Choice	
Fractionation acidic/neutral/basic		First Choice			

Table 1. EVOLUTE® Sorbent Selection

First Choice

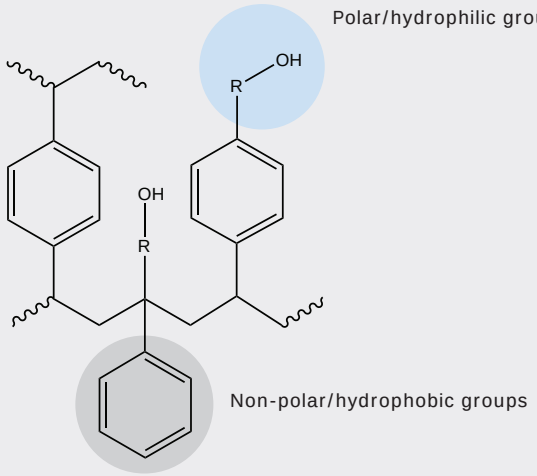
Alternative

Once the most appropriate sorbent chemistry is selected, methods can be further optimized using the practical hints and tips described in this guide for each sorbent type.

EVOLUTE® EXPRESS ABN

for Clean Extracts of Acidic, Basic and Neutral Analytes

EVOLUTE® EXPRESS ABN consists of a modified polystyrene-divinylbenzene polymer for reversed phase (hydrophobic) retention, incorporating non-ionizable hydroxyl groups which impart excellent wettability without secondary interactions. EVOLUTE EXPRESS ABN can be used to extract a wide range of acidic, basic and neutral analytes from aqueous matrices including biological fluids.



When To Use EVOLUTE® EXPRESS ABN

- When a simple, fast, robust SPE method is required
- For complex samples (e.g. biological fluids)
- Neutral analytes
- Mixtures of acids, bases and neutrals
- Clean samples (e.g. drinking water)
- Multi analyte suites
- When analyte stability issues mean it is necessary to avoid high or low pH conditions
- For simple solvent exchange procedures (from aqueous to organic solvent)
- Desalting applications

EVOLUTE® EXPRESS ABN Generic Method for Combined Extraction of Acidic, Basic and Neutral Analytes From Biological Fluids

1 Pre-treatment

Dilute sample 1:3 (v/v) with 2% formic acid.

» Reduces or eliminates protein binding.

2 Load

400 µL–2 mL diluted plasma.

» Analyte is retained by hydrophobic interactions. Large proteins cannot enter the pores of the sorbent, and are washed to waste during loading.

3 Wash (Interference Elution)

Water/methanol, 95/5 (v/v), 1 mL.

» Wash removes polar (water soluble) interferences (salts, small proteins and larger phospholipids).

4 Elute

Methanol, 500 µL.

» Elutes analytes by breaking hydrophobic (non-polar) retention.

Evaporate and re-constitute as necessary for analysis.

Volumes suitable for 30 mg format, see table 3 on page 23 for guidelines for other formats.

Optimizing Phospholipid Removal Using EVOLUTE® EXPRESS ABN

Whilst polymer-based SPE products that utilize a single non-polar (hydrophobic) retention mechanism are extensively used for the extraction of drugs from biological fluids, the relatively non-selective nature of these polymers can lead to co-extraction of high levels of unwanted endogenous sample components such as phospholipids.

The retentive nature of phospholipids makes them a common challenge in analytical sample preparation for LC–MS/MS. Unless they are removed during the sample preparation stage, phospholipid species can co-elute with analytes of interest, causing ion suppression or enhancement in the analytical system. Compared with simple protein precipitation, the EVOLUTE® EXPRESS ABN generic method removes greater than 90% of larger molecular weight phospholipids (PLs) but only reduces lysophospholipids (Lyso PLs) levels by approximately 35%.

Modification of the generic method, depending on analyte properties, can significantly improve removal of both types of phospholipids and result in cleaner extracts and more reliable quantification.

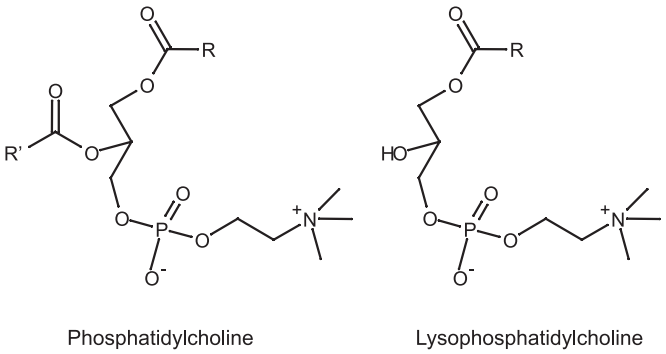


Figure 8. Typical Phospholipid structures.

Strategies for Enhanced Phospholipid Removal

Case A

For Strongly Retained (Hydrophobic or Non-polar) Analytes

When the analytes of interest are strongly retained on the SPE sorbent, phospholipid levels in the final extract can be reduced by increasing the wash (interference elution) solvent strength, without impacting analyte recovery.

Using an interference wash of 60% water/40% acetonitrile (v/v) will remove approximately 95% of both phospholipids and lysophospholipids from the final extract (see figure 9).

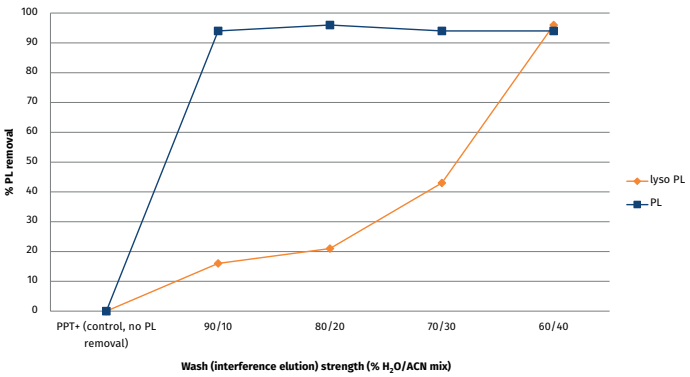


Figure 9. Effect of increasing wash (interference elution) solvent strength on phospholipid removal.

Case A: EVOLUTE® EXPRESS ABN Method Modified for Reduction of Phospholipids in the Final Extract When Analytes are Strongly Retained (i.e. Non-Polar Analytes)

1 Pre-treatment

Dilute sample 1:3 (v/v) with 2% formic acid.

» Reduces or eliminates protein binding.

2 Load

400 µL–2 mL diluted plasma.

» Large proteins cannot enter the pores of the sorbent, and are washed to waste during loading.

3 Wash (Interference Elution)

Water/Acetonitrile, 60/40 (v/v), 1 mL.

» Wash removes polar interferences (salts, small proteins plus 94% of PLs and 96% of LysoPLs).

4 Elute

Methanol, 500 µL.

» Elutes analytes by breaking hydrophobic (non-polar) retention.

Evaporate and re-constitute as necessary for analysis.

Volumes suitable for 30 mg format, see table 3 on page 23 for guidelines for other formats.

Case B

For Polar Analytes That are Weakly Retained

When the analytes of interest are weakly retained on the SPE sorbent, phospholipid concentration in the final extract can be reduced by modifying the strength of the elution solvent. Using a weaker elution solvent, analytes are still efficiently eluted, but phospholipids remain on the column, and do not contaminate the extract.

Using an elution solvent of 20% water/80% methanol (v/v) can reduce lysophospholipid levels in the final extract by more than 50%, and phospholipid levels by more than 95% (see figure 10).

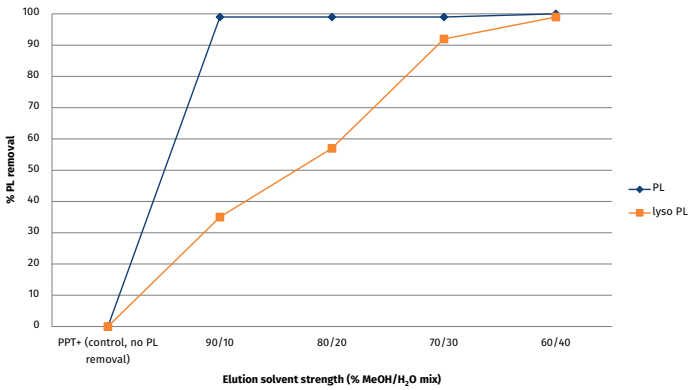


Figure 10. Effect of decreasing elution solvent strength on phospholipid removal.

Case B: EVOLUTE® EXPRESS ABN Method Modified for Reduction of Phospholipids in the Final Extract When Analytes are Weakly Retained (i.e. Polar Analytes)

1 Pre-treatment

Dilute sample 1:3 (v/v) with 2% formic acid.

» Reduces or eliminates protein binding.

2 Load

400 µL–2 mL diluted plasma.

» Large proteins cannot enter the pores of the sorbent, and are washed to waste during loading.

3 Wash (Interference Elution)

Water/methanol, 95/5 (v/v), 1 mL.

» Removes polar interferences (salts, small proteins and larger phospholipids).

4 Elute

Methanol/water, 80/20 (v/v), 500 µL.

» Elutes analytes, reduces LysoPLs in the final extract by leaving them on the column.

Evaporate and re-constitute as necessary for analysis.

Volumes suitable for 30 mg format, see table 3 on page 23 for guidelines for other formats.

Method Optimization Tips for EVOLUTE® EXPRESS ABN

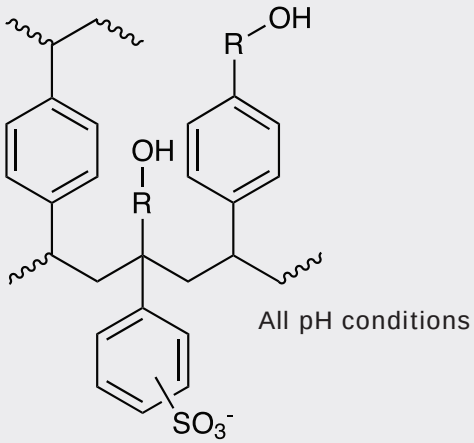
Low analyte recovery can be caused by low retention of analytes during load or wash steps, or inefficient retention disruption during the elution step.

To improve retention of polar or weakly retained acidic analytes during sample loading and interference elution steps.	Ensure sample pH is 2 pH units below the pK_a of the analyte, to ensure that any ionizable functional groups are uncharged. Evaluate the use of buffer at this pH for equilibration and interference elution steps.
To improve elution of acidic analytes.	Analyte elution depends on analyte solubility in the elution solvent. Addition of a volatile acid can maximize analyte recovery. Acidic analytes will have greater solubility at 2 pH units below the pK_a of the analyte. Evaluate the use of up to 0.1% formic acid in methanol.
To improve retention of polar or weakly retained basic compounds during the sample loading and interference elution steps.	Ensure sample pH is 2 pH units above the pK_a of the analyte, to ensure that any ionizable functional groups are uncharged. Evaluate the use of buffer at this pH for equilibration and interference elution steps.
To improve elution of basic analytes.	Analyte elution depends on analyte solubility in the elution solvent. Addition of a volatile base to the elution solvent can maximize analyte recovery. Basic analytes will have greater solubility at 2 pH units above the pK_a of the analyte. Evaluate the use of up to 5% ammonia in methanol.
Viscous samples.	Additional dilution may be required to improve flow characteristics of particularly viscous samples.
Acid labile compounds.	For biological fluid samples containing high levels of endogenous proteins, such as plasma, the use of formic acid in the pre-treatment can improve recovery of strongly protein bound analytes. **However, care should be taken when extracting acid labile compounds (e.g. lactones) if using this approach.** If desired, a neutral sample pH can also be used. We recommend 0.1 M ammonium acetate for sample dilution. If buffering of urine samples is necessary, use a lower ionic strength buffer (20–50 mM) for sample pre-treatment.

EVOLUTE® EXPRESS CX

Water wettable EVOLUTE® EXPRESS CX combines non-polar (hydrophobic) and strong cation exchange functionality for extraction of extract a wide range of positively charged basic/cationic species from aqueous samples.

EVOLUTE EXPRESS CX is modified with a sulfonic acid group. This negatively charged sorbent retains basic (positively charged) analytes through non-polar and strong **C**ation e**X**change retention mechanisms. It has an exchange capacity of ~0.5 mM/g. The dual ('mixed-mode') retention mechanism exhibited by mixed-mode sorbents allows the use of 100% organic solvents in the interference wash step thus removing problem interferences, without compromising analyte recovery. Analyte elution is achieved at high pH by eliminating the positive charge on the **analyte**.



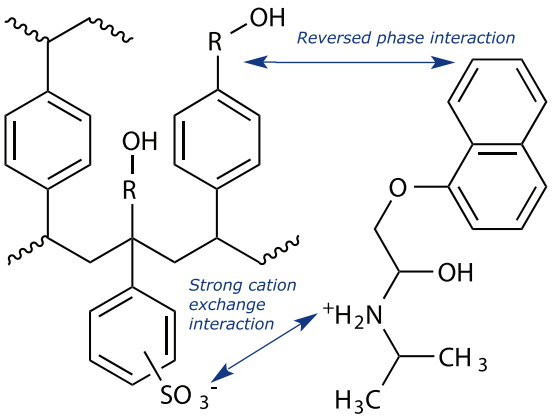


Figure 11. Mixed-mode (hydrophobic and strong cation exchange) interactions with EVOLUTE® EXPRESS CX sorbent.

EVOLUTE EXPRESS CX can also be used to fractionate complex mixtures. For example, for a sample containing a mixture of acidic, neutral and basic analytes, extracted on EVOLUTE EXPRESS CX, wash 2 can be used to elute acidic and neutral compounds, with basic analytes eluted in the analyte elution step.

When To Use EVOLUTE® EXPRESS CX

- Basic analytes (pK_a 2–10)
- Basic compounds that are too polar for a single non-polar retention mechanism using EVOLUTE EXPRESS ABN
- Amphoteric analytes
- Fractionation of complex mixtures (separates acidic and neutrals from bases)
- For analytes stable in high pH elution solvent
- To selectively retain basic interferences

Optimized Method Development for Basic Analytes Using EVOLUTE® EXPRESS CX

EVOLUTE EXPRESS CX is designed to extract basic analytes from biological fluids and other aqueous samples using mixed-mode non-polar/strong cation exchange retention mechanisms. The sorbent consists of the water wettable EVOLUTE EXPRESS surface modified with a sulfonic acid functional group. By using both non-polar and strong cation exchange retention mechanisms, basic analytes are selectively retained. The simple wash steps in the EVOLUTE EXPRESS CX generic method remove matrix components such as salts, non-ionizable interferences, proteins and phospholipids. In fact EVOLUTE EXPRESS CX removes greater than 98% of both proteins and phospholipids from plasma samples.

EVOLUTE EXPRESS CX combines non-polar and strong cation exchange in a mixed-mode retention mechanism for improved recovery of basic compounds and cleaner extracts. Optimal retention is obtained at least 2 pH units below the pK_a of the analyte, to ensure that any ionizable functional groups are charged. Analyte elution is achieved by eliminating ANALYTE charge i.e. by using a high pH.

EVOLUTE® EXPRESS CX Method for Extraction of Basic Analytes From Biological Fluids.

1 Pre-treatment

Dilute sample, 1:3 (v/v) with 0.05 M ammonium acetate pH 6.0.

***For protein bound drugs in plasma or serum, dilute with 2% formic acid.**

» Ensures positively charged analyte.
» Reduces or eliminates protein binding.

2 Load

400 µL–2 mL diluted plasma.

» Analyte is retained by ionic and hydrophobic interactions.
» Large proteins cannot enter the pores of the sorbent, and are washed to waste during loading.

3 Wash 1 (Interference Elution)

0.05 M ammonium acetate pH 6.0, 1 mL.

» Maintains analyte charge. Wash removes polar (water soluble) interferences (salts, small proteins and larger phospholipids).

4 Wash 2 (Interference Elution)

Methanol, 1 mL.

» Analyte is retained by cation exchange interactions. Wash removes remaining phospholipids along with neutral and acidic interferences. Alternatively, collect this wash to analyze acidic and neutral compounds of interest.

5 Elute

Methanol /ammonium hydroxide, 95/5 (v/v), 500 µL–1 mL.

» Elutes basic analytes by eliminating ANALYTE charge.
Evaporate and re-constitute as necessary for analysis.

Volumes suitable for 30 mg format, see table 3 on page 23 for guidelines for other formats.

Reagents

» 0.05 M ammonium acetate pH 6.0. Dissolve 3.854 g of ammonium acetate in 950 mL of deionized water. Adjust to pH 6.0 with acetic acid (ACS reagent grade). Make up to 1 L with deionized water and mix thoroughly.

» Methanol /ammonium hydroxide, 95/5 (v/v) solution. Take 5 mL of ammonium hydroxide (28%) and add 95 mL methanol. Mix thoroughly.

Method Optimization Tips for EVOLUTE® EXPRESS CX

Amphoteric compounds	For optimum cleanliness without compromising analyte recovery of amphoteric compounds with dual carboxylic acid/amine functionality (e.g. benzoylecgonine): Use an additional wash using 2% formic acid after interference wash 1. This step ionizes the amine while neutralizing the acid functionality ensuring a true cation exchange effect occurs.
Polar basic drugs	For optimum cleanliness without compromising analyte recovery of polar basic drugs with pK_a ~8 and above: Use an additional wash using 2% formic acid after interference wash 1. This step removes lysophospholipids and ensures that the amine functionality is “locked on” such that analyte/s are not eluted in the subsequent methanol wash.
For whole blood samples	Pre-treat the sample by dilution with 0.05 M ammonium acetate, pH6, followed by sonication and centrifugation, to denature (lyse) the blood. Do not use 2% formic acid, as this can cause discoloration of the sample.
For very polar basic analytes	To enhance recoveries of very polar basic analytes, where 2% formic acid pre-treatment cannot be used due to the nature of the matrix (e.g. milk or whole blood), a buffer of intermediate pH (e.g. pH 5) can be used for sample pre-treatment and interference wash 1 (interference elution).
For strongly basic compounds	Such as quaternary amines. which are strongly/irreversibly bound to the EVOLUTE® EXPRESS CX sorbent, and cannot be eluted using pH control. We recommend the use of EVOLUTE EXPRESS WCX for extraction of strongly basic analytes.
For basic analytes that are not stable at high pH	Basic conditions are needed to elute analytes from EVOLUTE EXPRESS CX. For extraction of basic analytes that are unstable under basic pH conditions, the use of EVOLUTE EXPRESS WCX, which can be eluted at acidic pH, is recommended.

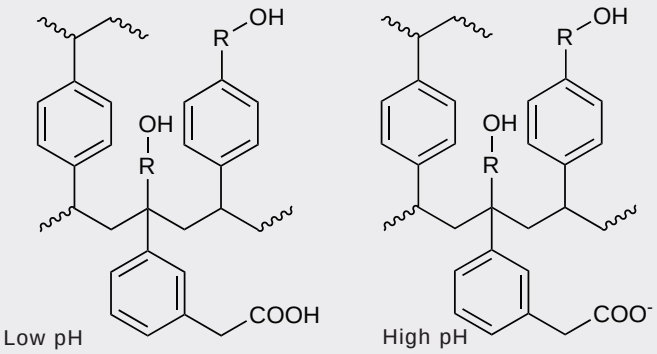
EVOLUTE® EXPRESS WCX

Water wettable EVOLUTE® EXPRESS WCX combines non-polar (hydrophobic) and weak cation exchange functionality for extraction of strongly basic/cationic species from aqueous samples.

EVOLUTE EXPRESS WCX is modified with a carboxylic acid group. This negatively charged sorbent retains strongly basic (positively charged) analytes through non-polar and **W**eak **C**ation e**X**change retention mechanisms. It has an exchange capacity of ~0.4 mM/g.

The carboxylic acid group has a pK_a of ~5. This means that analyte retention is maximized at pH of 7 and above, when 100% of the WCX sorbent is negatively charged. At pH 3 and below, 100% of the sorbent is neutralized, thus analyte elution is achieved at low pH by eliminating the negative charge on the SORBENT.

The dual ('mixed-mode') retention mechanism exhibited by mixed-mode sorbents allows the use of 100% organic solvents in the interference wash step thus removing problem interferences, without compromising analyte recovery.



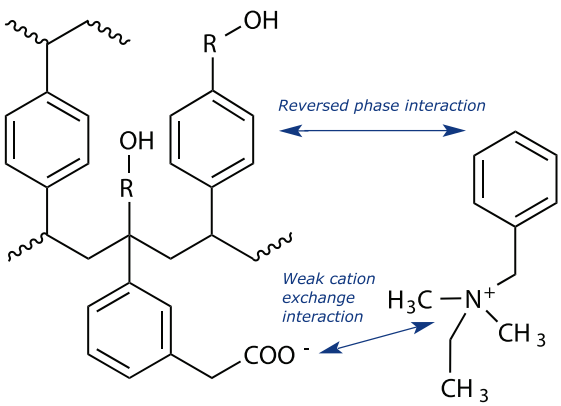


Figure 12. Mixed-mode (hydrophobic and weak cation exchange) interactions with EVOLUTE EXPRESS WCX sorbent.

When To Use EVOLUTE® EXPRESS WCX

- Quaternary amines
- Strong bases (pK_a >10) (use method 1)
- Mixtures of bases including strong bases (use method 2)
- Bases unstable at high pH (use method 2)
- Bases, when acidic elution conditions are preferred due to LC-MS/MS compatibility
- When basic compounds are difficult to elute from EVOLUTE EXPRESS CX

Optimized Method Development for Strongly Basic Analytes Using EVOLUTE® EXPRESS WCX

EVOLUTE EXPRESS WCX is designed to extract strongly basic analytes (including quaternary amines) from biological fluids and other aqueous samples using mixed-mode non-polar/weak cation exchange retention mechanisms. The sorbent consists of the water wettable EVOLUTE EXPRESS surface modified with a carboxylic acid functional group (~0.4 mM/g capacity). By using both non-polar and weak cation exchange retention mechanisms, strongly basic analytes are selectively retained. The simple wash steps in the EVOLUTE EXPRESS WCX generic method remove matrix components such as salts, non-ionizable interferences, proteins and phospholipids delivering cleaner extracts with reproducible recoveries for reliable, accurate quantification. The ability to elute using acidic conditions makes EVOLUTE EXPRESS WCX invaluable for the extraction of bases which are unstable under basic elution conditions.

EVOLUTE® EXPRESS WCX Generic Method 1: For Extraction of Strong Bases pK_a >10 (Including Quaternary Amines) From Biological Fluids

1 Pre-treatment

Dilute sample 1:3 (v/v) with water/ammonium hydroxide, 95/5 (v/v).

***For protein bound drugs in plasma or serum, dilute with 2% formic acid.**

» Reduces or eliminates protein binding.

2 Load

400 µL–2 mL diluted plasma.

» Analyte is retained by ionic and hydrophobic interactions.
» Large proteins cannot enter the pores of the sorbent, and are washed to waste.

3 Wash 1 (Interference Elution)

Water/ammonium hydroxide, 95/5 (v/v), 1 mL.

» High pH prevents loss of sorbent charge. Wash removes polar (water soluble) interferences (salts, small proteins and larger phospholipids).

4 Wash 2 (Interference Elution)

Methanol, 1 mL.

» Analyte is retained by cation exchange interactions. Wash removes remaining phospholipids along with neutral, acidic and weakly basic interferences.

5 Elute

Methanol/formic acid, 98/2 (v/v), 500 µL–1 mL

» Elutes strongly basic analytes by eliminating SORBENT charge.

Evaporate and re-constitute as necessary for analysis.

Volumes suitable for 30 mg format, see table 3 on page 23 for guidelines for other formats.

Reagents

» Water/ammonium hydroxide, 95/5 (v/v). Take 5 mL of 28% ammonium hydroxide solution, and make up to 100 mL with water. Mix thoroughly.

» Methanol /formic acid, 98/2 (v/v). Take 2 mL of 98% formic acid solution, and add 98 mL methanol. Mix thoroughly.

EVOLUTE® EXPRESS WCX Generic Method 2: For Extraction of Mixtures of Weak and Strong Bases, or Basic Analytes Unstable at High pH, From Biological Fluids

1 Pre-treatment

Dilute sample, 1:3 (v/v) with 0.05 M ammonium acetate pH 7.0.

***For protein bound drugs in plasma or serum, dilute with 2% formic acid.**

» Reduces or eliminates protein binding.

2 Load

400 µL–2 mL diluted plasma.

» Analyte is retained by hydrophobic and ionic interactions.
» Large proteins cannot enter the pores of the sorbent, and are washed to waste.

3 Wash 1 (Interference Elution)

0.05 M ammonium acetate pH 7.0, 1 mL.

» Removes polar (water soluble) interferences (salts, small proteins and larger phospholipids).

4 Wash 2 (Interference Elution)

Methanol, 1 mL.

» Analyte is retained by cation exchange interactions. Wash removes remaining phospholipids along with neutral and acidic interferences.

5 Elute

Methanol /formic acid, 98/2 (v/v), 500 µL–1 mL

» Elutes strongly basic analytes by eliminating SORBENT charge.

Evaporate and re-constitute as necessary for analysis.

Volumes suitable for 30 mg format, see table 3 on page 23 for guidelines for other formats.

Reagents

» 0.05M ammonium acetate pH 7.0. Dissolve 3.854 g of ammonium acetate in 950 mL of deionized water. Make up to 1 L with deionized water and mix thoroughly. Adjust to pH 7.0 with ammonium hydroxide. Mix thoroughly.

» Methanol /formic acid, 98/2 (v/v). Take 2 mL of 98% formic acid solution, and add 98 mL methanol. Mix thoroughly.

Method Optimization Tips for EVOLUTE® EXPRESS WCX

For samples containing weakly basic interferences

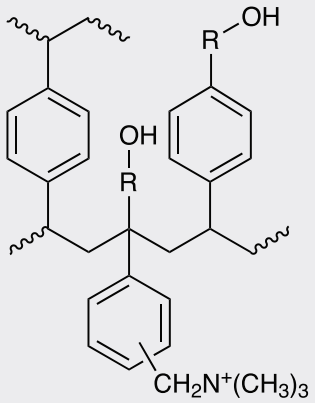
Use EVOLUTE® EXPRESS WCX generic method 1. This allows elution of weak bases in interference wash 2, so they do not contaminate the final extract.

For analytes with pK_a ~9 and below, elution can be achieved using high pH solvent (e.g. methanol/ammonium hydroxide, 95/5 (v/v). This may improve extract cleanliness where interfering species elute using acidic elution conditions.

EVOLUTE® EXPRESS AX

Water wettable EVOLUTE® EXPRESS AX combines non-polar (hydrophobic) and strong anion exchange functionality for extraction of extract a wide range of negatively charged acidic/anionic species from aqueous samples.

EVOLUTE EXPRESS AX is modified with a quaternary amine group with a chloride counter ion. This positively charged sorbent retains acidic (negatively charged) analytes through non-polar and strong **A**nion e**X**change retention mechanisms. It has an exchange capacity of ~0.7 mM/g. The dual ('mixed-mode') retention mechanism exhibited by mixed-mode sorbents allows the use of 100% organic solvents in the interference wash step thus removing problem interferences, without compromising analyte recovery. Analyte elution is achieved at low pH by eliminating the negative charge on the **analyte**.



All pH conditions

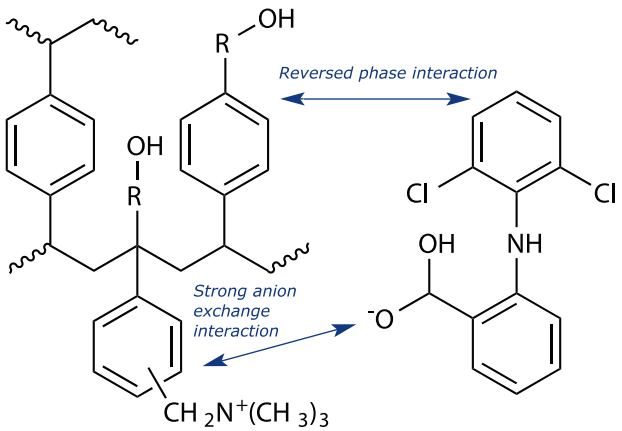


Figure 13. Mixed-mode (hydrophobic and strong anion exchange) interactions with EVOLUTE® EXPRESS AX.

When To Use EVOLUTE® EXPRESS AX

- For aqueous samples including biological fluids
- Acidic analytes (pK_a 2–8)

Optimized Method Development for Strongly Acidic Analytes Using EVOLUTE® EXPRESS AX

EVOLUTE EXPRESS AX is designed to extract acidic analytes from biological fluids and other aqueous samples using mixed-mode non-polar/strong anion exchange retention mechanisms. The sorbent consists of the water wettable EVOLUTE EXPRESS surface modified with a quaternary amine functional group. By using both non-polar and strong anion exchange retention mechanisms, acidic analytes are selectively retained. The simple wash steps in the EVOLUTE EXPRESS AX generic method remove matrix components such as salts, non-ionizable interferences, proteins and phospholipids delivering cleaner extracts with reproducible recoveries for reliable, accurate quantification.

EVOLUTE® EXPRESS AX Method for Extraction of Acidic Analytes From Biological Fluids

1 Pre-treatment

Dilute sample 1:3 (v/v) with 2% formic acid.

» Reduces or eliminates protein binding.

2 Load

400 µL–2 mL diluted plasma.

» Analyte is retained by hydrophobic and ionic interactions.
» Large proteins cannot enter the pores of the sorbent, and are washed to waste.

3 Wash 1 (Interference Elution)

Ammonium acetate (0.05M, pH 7.0)/methanol, 95/5 (v/v), 1 mL.

» Removes polar (water soluble) interferences (salts, small proteins and larger phospholipids).

4 Wash 2 (Interference Elution)

Methanol, 1 mL.

» Analyte is retained by anion exchange interactions. Wash removes remaining phospholipids along with neutral and basic interferences.

5 Elute

Methanol /formic acid, 98/2 (v/v), 500 µL–1 mL.

» Elutes acidic analytes by eliminating ANALYTE charge.
Evaporate and re-constitute as necessary for analysis.

Volumes suitable for 30 mg format, see table 3 on page 23 for guidelines for other formats.

Reagents

» 0.05 M ammonium acetate pH 6.0. Dissolve 3.854 g of ammonium acetate in 950 mL of deionized water. Adjust to pH 6.0 with acetic acid (ACS reagent grade). Make up to 1 L with deionized water and mix thoroughly.

» Methanol /formic acid, 98/2 (v/v) solution. Take 2 mL of 98% formic acid solution, and add 98 mL methanol. Mix thoroughly.

Method Optimization Tips for EVOLUTE® EXPRESS AX

For urine samples	»	Dilute sample with 50 mM ammonium acetate pH 7.0 (avoid acidification of sample). This prevents co-elution of polar urinary acids in the elution step, and can lead to a cleaner extract.
For very polar analytes	»	In extreme cases the use of a polar aprotic solvent such as ACN for sample pre-treatment can help retention of very polar analytes e.g. EtG, providing advanced cleanliness. See application note AN718 for further details. For very polar acids, reducing the salt and acid concentration in the loading and wash steps can provide better retention.

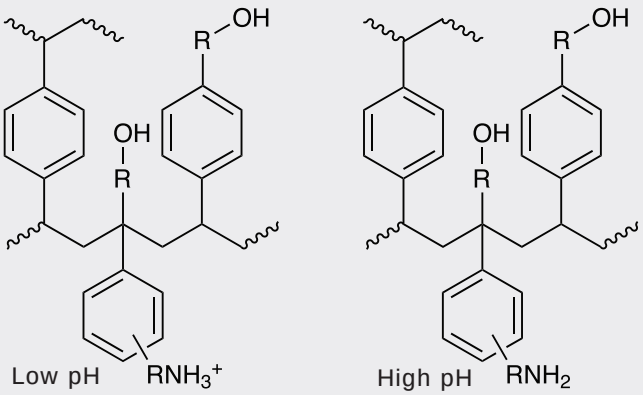
EVOLUTE® EXPRESS WAX

Water wettable EVOLUTE® EXPRESS WAX combines non-polar (hydrophobic) and weak anion exchange functionality for extraction of extract a wide range of negatively charged acidic/ anionic species, including strong acids, from aqueous samples.

EVOLUTE EXPRESS WAX is modified with amino groups. This positively charged sorbent retains acidic (negatively charged) analytes through non-polar and **W**eak **A**nion e**X**change retention mechanisms. It has an exchange capacity of ~0.3 mM/g for the 30 µm material and 0.7 mM/g for the 50 µm material.

The amino groups have a pK_a of ~10. This means that analyte retention is maximized at pH 8 and below, when 100% of the sorbent is positively charged. At high pH, the sorbent is neutralized, this analyte elution is achieved at high pH by eliminating the charge on the SORBENT.

The dual ('mixed-mode') retention mechanism exhibited by mixed-mode sorbents allows the use of 100% organic solvents in the interference wash step thus removing problem interferences, without compromising analyte recovery.



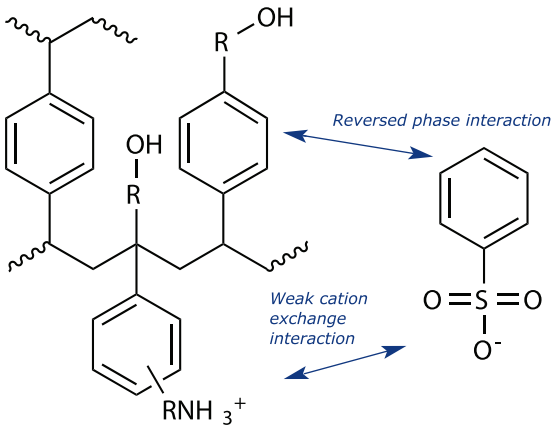


Figure 14. Mixed-mode (hydrophobic and weak anion exchange) interactions with EVOLUTE® EXPRESS WAX.

When To Use EVOLUTE® EXPRESS WAX

- For aqueous samples including biological fluids
- Strongly acidic analytes (pK_a <2) such as alkyl phosphates, alkyl sulfonates
- Acidic analytes unstable at the low pH used for EVOLUTE EXPRESS AX elution step but stable under basic pH conditions
- Acidic analytes difficult to elute from EVOLUTE EXPRESS AX

Optimized Method Development for Acidic Analytes Using EVOLUTE® EXPRESS WAX

EVOLUTE® EXPRESS WAX is designed to extract strongly acidic analytes (pK_a <2) from biological fluids and other aqueous samples using mixed-mode non-polar/weak anion exchange retention mechanisms. The phase consists of the EVOLUTE backbone surface modified with a primary-secondary amine functional group in the free base form 0.3 mM/g capacity for the 30 µm material and 0.7 mM/g for the 50 µm material. By using non-polar and weak anion exchange retention mechanisms, strongly acidic analytes are selectively retained. The simple interference elution steps in the EVOLUTE EXPRESS WAX generic method remove matrix components such as proteins, salts, non-ionizable interferences and phospholipids delivering cleaner extracts with reproducible recoveries for reliable, accurate quantification.

EVOLUTE® EXPRESS WAX Method for Extraction of Acidic Analytes From Biological Fluids

1 Pre-treatment

Dilute sample 1:3 (v/v) with 2% formic acid.

» Reduces or eliminates protein binding. Strong acids will retain their negative charge under these conditions.

2 Load

400 µL–2 mL diluted plasma.

» Analyte is retained by hydrophobic and ionic interactions.
» Large proteins cannot enter the pores of the sorbent, and are washed to waste.

3 Wash 1 (Interference Elution)

2% formic acid, 1 mL.

» Removes polar interferences (salts, proteins and larger phospholipids).

4 Wash 2 (Interference Elution)

Methanol, 1 mL.

» Analyte is retained by anion exchange interactions. Wash removes remaining phospholipids along with neutral and basic interferences.

5 Elute

Methanol /ammonium hydroxide, 95/5 (v/v), 500 µL–1 mL.

» Elutes acidic analytes by eliminating SORBENT charge.
Evaporate and re-constitute as necessary for analysis.

Volumes suitable for 30 mg format, see table 3 on page 23 for guidelines for other formats.

Reagents

» 2% formic acid. Take 2 mL of 98% formic acid, and make up to 100 mL with water. Mix thoroughly

» Methanol /ammonium hydroxide, 95/5 (v/v) solution. Take 5 mL of ammonium hydroxide (28%) and add 95 mL methanol. Mix thoroughly.

Method Optimization Tips for EVOLUTE® EXPRESS WAX

To maximize recoveries of low molecular weight, polar acids

» Evaluate the use of deionized water for sample pre-treatment, equilibration and/or wash steps to prevent analyte breakthrough.

Processing Options for EVOLUTE® EXPRESS 96-well Plates and Columns

EVOLUTE® EXPRESS 96-well plates and columns are compatible with all industry standard processing systems. We recommend the following processing options from Biotage:

Table 2. Processing Options.

Format		Automated	Positive Pressure	Vacuum	
96-well plate	Tabless Columns	Biotage® Extrahera™	Biotage® PRESSURE+ 48 and PRESSURE+ 96	Biotage® VacMaster™-96	Biotage® VacMaster™ -10 or -20
					
96-well plate		Biotage® Extrahera™ (96 position)	Biotage® PRESSURE+ 96	Biotage® VacMaster-96	
1 mL column (tabless) High throughput options		Biotage® Extrahera™ (96 position) *Process up to 96 samples with tabless 1 mL column rack*	Biotage® PRESSURE+ 96 *Process up to 96 samples with tabless 1 mL column holder**	Biotage® VacMaster-96 *Process up to 96 samples with tabless 1 mL column holder***	
1 mL column (tabless)		Biotage® Extrahera™ (24 position) *Process up to 24 samples with 1 mL column rack*	Biotage® PRESSURE+ 48 *Process up to 48 samples with 1 mL column rack*	Biotage® VacMaster-10 or -20 *Process up to 10 (or 20) samples*	
3 mL column (tabless)		Biotage® Extrahera™ (24 position) *Process up to 24 samples with 3 mL column rack*	Biotage® PRESSURE+ 48 *Process up to 48 samples with 3 mL column rack*	Biotage® VacMaster-10 or -20 *Process up to 10 (or 20) samples*	
6 mL column (tabless)		Biotage® Extrahera™ (24 position) *Process up to 24 samples with 6 mL column rack*	Biotage® PRESSURE+ 48 *Process up to 48 samples with 6 mL column rack*	Biotage® VacMaster-10 or -20 *Process up to 10 (or 20) samples*	

For high throughput applications that require the ability to process less than 96 samples simultaneously, 1 mL tabless columns can be processed using the Biotage® Extrahera™, Biotage® PRESSURE+ 96 or VacMaster-96 using appropriate racks/column holders.

*PPM-A96-CH - PRESSURE+ 96 Column Holder 96

**121-9620 - VacMaster-96 Tabless 1 mL column holder

EVOLUTE® EXPRESS SPE Formats

EVOLUTE® EXPRESS sorbents are available in a range of standard formats, to match throughput and application requirements. Typical solvent volumes for each stage of the time saving EVOLUTE EXPRESS Load-Wash-Elute procedure are shown below.

Table 3. EVOLUTE® EXPRESS SPE Formats.

Format	Bed Mass	Typical Sample Volume*	Wash (1 and 2) volume	Elution volume**
96-well plate	10 mg	100 µL–400 µL	500 µL	100–500 µL
	30 mg	200 µL–500 µL	1 mL	250 µL–1 mL
1 mL column (tabless)	10 mg	100 µL–500 µL	500 µL	100–500 µL
	30 mg	100 µL–500 µL	1 mL	250 µL–1 mL
3 mL column (tabless)	60 mg	1 mL–10 mL	2 mL	500 µL–2 mL
	100 mg	2 mL–20 mL	3 mL	1 mL–3 mL
6 mL column (tabless)	150 mg	5 mL–200 mL	4 mL	4 mL
	500 mg	50 mL–1 L	6 mL	6 mL

*Application specific, guideline only. The effective capacity of each bed mass will be affected by choice of extraction conditions. The sample volume extracted should be optimized based on the expected concentration range of analytes in the sample, the final extract volume and the sensitivity of the analytical method

** Guideline for method development. Optimize to minimize elution volume and eliminate (or minimize) post extraction evaporation

Note: no traditional conditioning and equilibration steps are necessary when using EVOLUTE EXPRESS products. However, if these steps are included, use wash volumes as stated above for the appropriate bed mass.

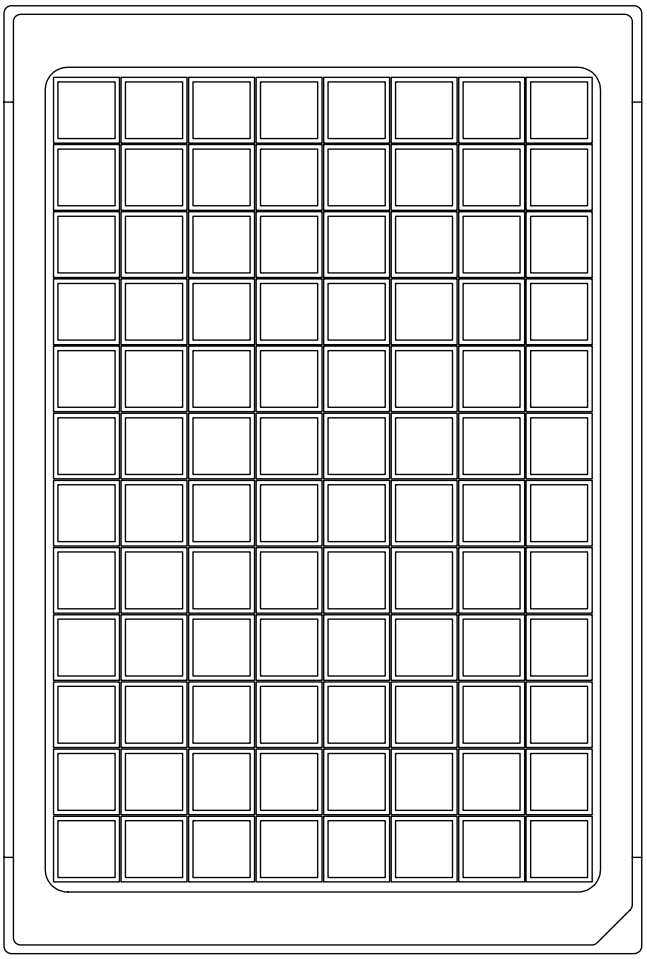


Figure 16. Actual size diagram of 96-well plate.

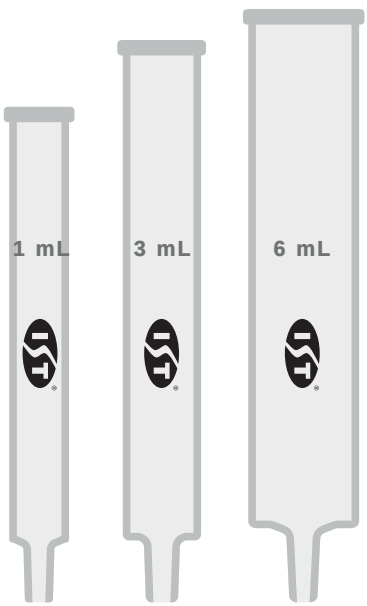


Figure 15. Actual size diagram of 1 mL, 3 mL and 6 mL tabless columns.

Flow Guidelines for Plates and Columns

EVOLUTE® EXPRESS 96-well plates and columns incorporate wettable sorbents and frits providing fast, reproducible flow characteristics for all aqueous samples including biological fluids without the need for traditional conditioning or equilibration steps.

During method development, processing equipment should be set to produce the following flow rates for each column format. Many samples will flow under gravity without the need to apply pressure or vacuum.

Table 4. Flow Guidelines.

Format	96-well plate	1 mL column	3 mL column	6 mL column
Initial flow rate	1 mL/min	1 mL/min	3 mL/min	7 mL/min

Flow Optimization Tips

- Due to the excellent flow characteristics of EVOLUTE® EXPRESS plates and columns, appropriate flow rates may be achieved using gravity or very low pressure (1 psi) or vacuum conditions. For particularly viscous samples, processing pressure or vacuum should be increased to achieve the minimum levels shown. Once chemistry is established flow rate optimization can proceed.
- Once optimum chemistry has been achieved, flow rate can be adjusted for maximum throughput. Final flow rate should be set at 10–20% lower than the breakthrough limit.
- For particularly viscous samples, consider additional dilution of the sample with water or low concentration buffer. This increased volume does not usually require a larger bed mass for equivalent analyte recovery.

Sample Preparation Instruments and Accessories

Biotage® Extrahera™

Biotage® Extrahera™ is a compact eight channel automation instrument, designed for speed, flexibility and with end user operation in mind. The system has been designed to automatically process methods using 96-well plate or column consumables. Ideal for processing EVOLUTE® EXPRESS based methods. The system benefits from a compact two level layout for solvent and sample pipette tips, extraction consumables and samples. The lower level features an innovative carousel based design. Switching between processing either well plates or columns can be achieved in less than five minutes. The system processes a full standard 96-well plate SPE method in approximately 30 minutes including sample pre-treatment, conditioning, equilibration, load, wash and elution steps – even when using volatile and low surface tension solvents.

Biotage® PRESSURE+

Biotage® PRESSURE+ manifolds offer positive pressure, parallel processing for 96-well plates, 1 mL, 3 mL and 6 mL EVOLUTE® EXPRESS column formats. The systems utilize a consistent, uniform flow of positive pressure to process both low and high viscosity liquids through EVOLUTE EXPRESS plates and columns. Each port of the PRESSURE+ manifold independently maintains constant pressure, increasing the overall reproducibility of analyte recoveries. This unique design allows for partially populated racks to be used without sacrificing extraction efficiency. The intuitive Biotage PRESSURE+ is easily incorporated into laboratory work flow regardless of SPE format.

Biotage® PRESSURE+ 96

The self-adjusting upper manifold of the PRESSURE+ 96 manifold is compatible with all 96-well plate formats, in addition to tabless 1 mL columns when used with the appropriate tabless 1 mL column holder. Biotage collection plates are recommended for the most consistent and reliable results.

Biotage® PRESSURE+ 48

The same self-adjusting technology utilized in the PRESSURE+ 96 manifold allows the PRESSURE+ 48 to process SPE columns up to 6 mL without the need to purchase supplementary gaskets. In addition, the unique design allows for between 1 and 48 columns to be processed in parallel without empty ports affecting flow rates. Tabless or flangeless columns should be used for full population and optimum sealing. The modular rack system accommodates most popular collection vessels.

Biotage® VacMaster™ Processing Stations

Biotage® VacMaster™96
The VacMaster™96 manifold is ideal for processing EVOLUTE® EXPRESS 96-well plates and additionally tabless 1 mL columns when used with the appropriate tabless 1 mL column holder. The compact design and lightweight construction make it suitable for manual processing.

Biotage® VacMaster™-10 and -20
The VacMaster -10 and -20 manifolds are ideal for processing up to 10 (VacMaster- 10) or 20 (VacMaster-20) samples in parallel using EVOLUTE EXPRESS SPE columns. Designed to meet the most demanding criteria for safety, extract purity, flexibility and ease-of-use the VacMaster range of vacuum manifolds can be readily incorporated into the laboratory workflow.

Two control units are available for use with either a vacuum source or for use with lab air to generate the vacuum. A range of stopcock options and spare parts for VacMaster manifolds are available.

Evaporation

Designed for high throughput laboratories, the SPE Dry 96 and SPE Dry 96 Dual sample concentrator systems provide efficient evaporation in microplate format.

Biotage® SPE Dry 96

The SPE Dry 96 utilizes heated gas flow both above and below the collection plate to rapidly dry both aqueous and organic solvent. PTFE-coated needles are available for applications that use volatile acids or bases. The SPE Dry 96 Dual benefits from all the features of the single product but offers double the evaporation capacity.

Use in combination with the Biotage® ACT (Anti Cross Talk) Plate Adapter, a novel (patent pending) solution to the phenomenon of hot spot cross contamination, or cross talk, during evaporation.

The 2pH Unit Rule

The pK_a of a molecular functional group can be described as the pH at which 50% of this group in solution is charged, and 50% is uncharged. Each pH unit change affects the percentage of charged or uncharged groups by a factor of 10, so it is sensible to perform extractions at a pH at least 2 pH units from the pK_a value, to ensure that 99.5% of the functional groups are in the desired state of ionization.

e.g. Effect of pH on the dissociation of a weak acid with a pK_a value of 4.0.

Table 5.

pH	% free acid (uncharged)	% dissociated (charged)
2.0	99.5	0.5
3.0	95	5.0
Acid with pK_a = 4.0	50	50
5.0	5.0	95
6.0	0.5	99.5

For strongest retention of a weakly acidic analyte with pK_a of 4.0, using a:

- Non-polar (hydrophobic) retention mechanism – adjust sample to pH 2.0 (2 pH units **BELOW** the pK_a)
- Anion exchange retention mechanism - adjust sample to pH 6.0 (2 pH units **ABOVE** the pK_a)

e.g. Effect of pH on the dissociation of the conjugate acid of a weak base with a pK_a value of 9.0

Table 6.

pH	% free base (uncharged)	% dissociated (charged)
11.0	99.5	0.5
10.0	95	5.0
Acid with pK_a = 9.0	50	50
8.0	5.0	95
7.0	0.5	99.5

For strongest retention of a weakly basic analyte with pK_a of 9.0, using a:

- Non-polar (hydrophobic) retention mechanism – adjust sample to pH 11.0 (2 pH units **ABOVE** the pK_a)
- Cation exchange retention mechanism - adjust sample to pH 7.0 (2 pH units **BELOW** the pK_a

Ordering Information

EVOLUTE® EXPRESS 96-Well Plates

Part Number	Product
EVOLUTE® EXPRESS ABN	
600-0010-PX01	EVOLUTE EXPRESS ABN 10 mg Fixed Well Plate
600-0030-PX01	EVOLUTE EXPRESS ABN 30 mg Fixed Well Plate
EVOLUTE® EXPRESS CX	
601-0010-PX01	EVOLUTE EXPRESS CX 10 mg Fixed Well Plate
601-0030-PX01	EVOLUTE EXPRESS CX 30 mg Fixed Well Plate
EVOLUTE® EXPRESS WCX	
602-0010-PX01	EVOLUTE EXPRESS WCX 10 mg Fixed Well Plate
602-0030-PX01	EVOLUTE EXPRESS WCX 30 mg Fixed Well Plate
EVOLUTE® EXPRESS AX	
603-0010-PX01	EVOLUTE EXPRESS AX 10 mg Fixed Well Plate
603-0030-PX01	EVOLUTE EXPRESS AX 30 mg Fixed Well Plate
EVOLUTE® EXPRESS WAX	
604-0010-PX01	EVOLUTE EXPRESS WAX 10 mg Fixed Well Plate
604-0030-PX01	EVOLUTE EXPRESS WAX 30 mg Fixed Well Plate
EVOLUTE® EXPRESS Sorbent Selection Plates	
650-0010-PX01	EVOLUTE EXPRESS Sorbent Selection 10 mg Fixed Well Plate
650-0030-PX01	EVOLUTE EXPRESS Sorbent Selection 30 mg Fixed Well Plate

EVOLUTE® EXPRESS Columns

Part Number	Product
EVOLUTE® EXPRESS ABN	
600-0001-AXG	EVOLUTE EXPRESS ABN 10 mg /1 mL (Tabless)
600-0003-AXG	EVOLUTE EXPRESS ABN 30 mg/1 mL (Tabless)
610-0006-BXG	EVOLUTE EXPRESS ABN 60 mg/3 mL (Tabless)
610-0010-BXG	EVOLUTE EXPRESS ABN 100 mg/3 mL (Tabless)
610-0015-CXG	EVOLUTE EXPRESS ABN 150 mg/6 mL (Tabless)
610-0050-CXG	EVOLUTE EXPRESS ABN 500 mg/6 mL (Tabless)
EVOLUTE® EXPRESS CX	
601-0001-AXG	EVOLUTE EXPRESS CX 10 mg/1 mL (Tabless)
601-0003-AXG	EVOLUTE EXPRESS CX 30 mg/1 mL (Tabless)
611-0006-BXG	EVOLUTE EXPRESS CX 60 mg/3 mL (Tabless)
611-0010-BXG	EVOLUTE EXPRESS CX 100 mg/3 mL (Tabless)
611-0015-CXG	EVOLUTE EXPRESS CX 150 mg/6 mL (Tabless)
611-0050-CXG	EVOLUTE EXPRESS CX 500 mg/6 mL (Tabless)
EVOLUTE® EXPRESS WCX	
602-0001-AXG	EVOLUTE EXPRESS WCX 10 mg/1 mL (Tabless)
602-0003-AXG	EVOLUTE EXPRESS WCX 30 mg/1 mL (Tabless)
612-0006-BXG	EVOLUTE EXPRESS WCX 60 mg/3 mL (Tabless)
612-0010-BXG	EVOLUTE EXPRESS WCX 100 mg/3 mL (Tabless)
612-0015-CXG	EVOLUTE EXPRESS WCX 150 mg/6 mL (Tabless)
612-0050-CXG	EVOLUTE EXPRESS WCX 500 mg/6 mL (Tabless)
EVOLUTE® EXPRESS AX	
603-0001-AXG	EVOLUTE EXPRESS AX 10 mg/1 mL (Tabless)
603-0003-AXG	EVOLUTE EXPRESS AX 30 mg/1 mL (Tabless)
613-0006-BXG	EVOLUTE EXPRESS AX 60 mg/3 mL (Tabless)
613-0010-BXG	EVOLUTE EXPRESS AX 100 mg/3 mL (Tabless)
613-0015-CXG	EVOLUTE EXPRESS AX 150 mg /6 mL (Tabless)
613-0050-CXG	EVOLUTE EXPRESS AX 500 mg /6 mL (Tabless)
EVOLUTE® EXPRESS WAX	
604-0001-AXG	EVOLUTE EXPRESS WAX 10 mg/1 mL (Tabless)
604-0003-AXG	EVOLUTE EXPRESS WAX 30 mg/1 mL (Tabless)
614-0006-BXG	EVOLUTE EXPRESS WAX 60 mg/3 mL (Tabless)
614-0010-BXG	EVOLUTE EXPRESS WAX 100 mg/3 mL (Tabless)
614-0015-CXG	EVOLUTE EXPRESS WAX 150 mg/6 mL (Tabless)
614-0050-CXG	EVOLUTE EXPRESS WAX 500 mg/6 mL (Tabless

Sample Processing Manifolds and Instruments

Part Number	Product
Biotage® Extrahera™	
414001	Biotage Extrahera
415040	Configuration Kit 96 Positions Dual Flow
415041	Configuration Kit 24 Positions Dual Flow
414253SP	Column Rack 96 x 1 mL (Tabless)
414169SP	Column Rack 24 x 1 mL
414174SP	Column Rack 24 x 3 mL
413640SP	Column Rack 24 x 6 mL (Tabless)
Biotage® PRESSURE+ 48 and 96	
PPM-96	PRESSURE+ 96 Positive Pressure Manifold
PPM-A96-CH	PRESSURE+ 96 Column Holder 96
PPM-48	PRESSURE+ 48 Positive Pressure Manifold
PPM-A48-1RCK	PRESSURE+ 48 SPE Column Rack 1 mL
PPM-A48-3RCK	PRESSURE+ 48 SPE Column Rack 3 mL
PPM-A48-6RCK	PRESSURE+ 48 SPE Column Rack 6 mL
PPM-A48-1232	PRESSURE+ 48 Sample Vial Rack 12 x 32 mm
PPM-A48-1275	PRESSURE+ 48 Collection Rack 12 X 75 mm
PPM-A48-13100	PRESSURE+ 48 Collection Rack 13 x 100 mm
PPM-A48-16100	PRESSURE+ 48 Collection Rack 16 x 100 mm
Biotage® VacMaster™-96	
121-9600	VacMaster-96 Sample Processing Manifold (without vacuum control)
121-9620	VacMaster-96 Tabless 1 mL Column Holder
121-9601	VacMaster VCU-1 Vacuum Control Unit
121-9602	VacMaster VCU-2 Vacuum Control and Generation Unit
Biotage® VacMaster™10 and 20	
121-1010	VacMaster-10 Sample Processing Manifold (with 10 mm rack)
121-1012	VacMaster-10 Sample Processing Manifold (with 12 mm rack)
121-1016	VacMaster-10 Sample Processing Manifold (with 16 mm rack)
121-2010	VacMaster-20 Sample Processing Manifold (with 10 mm rack)
121-2012	VacMaster-20 Sample Processing Manifold (with 12 mm rack)
121-2016	VacMaster-20 Sample Processing Manifold (with 16 mm rack)
Biotage® VacMaster™ Drying Adaptor	
Connect to laboratory air or nitrogen supply to dry 10 or 20 EVOLUTE EXPRESS columns simultaneously.	
124-1001	VacMaster-10 Drying Adaptor for 1, 3 & 6 mL Columns
124-2001	VacMaster-20 Drying Adaptor for 1, 3 & 6 mL Columns

Part Number	Product
Biotage® VacMaster™ Trap Kit	
Waste traps should be installed between the outlet of the VacMaster sample processing manifold and the vacuum source, trapping all waste liquids exiting the manifold. Compatible with VacMaster 10 and 20 and -96 processing manifolds, VacMaster Trap Kits are available with 1 L or 10 L capacity.	
121-2095	VacMaster Trap Kit 1 L
121-2195	VacMaster Trap Kit 10 L
Biotage® VacMaster™ Large Volume Extraction (LVE) Kit	
For unattended loading of large volume samples. Inert PTFE tubing prevents sample contamination.	
121-2090	VacMaster LVE Kit for 1, 3 and 6 mL Columns
Consumables	
121-5201	96-Well Collection Plate, 350 µL
121-5202	96-Well Collection Plate, 1 mL, Square
121-5203	96-Well Collection Plate, 2 mL, Square
121-5213	96-Well Collection Plate, 2 mL, Round
Biotage® SPE Dry 96 and 96 Dual	
SD-9600-DHS-EU	SPE Dry 96 Sample Concentrator System 220V
SD-9600-DHS-NA	SPE Dry 96 Sample Concentrator System 110V
SD-9600-DHS-T-EU	SPE Dry 96 Sample Concentrator System PTFE Needles (Top Head Only) 220V
SD-9600-DHS-T-NA	SPE Dry 96 Sample Concentrator System PTFE Needles (Top Head Only) 110V
SD2-9600-DHS-EU	SPE Dry 96 Dual Sample Concentrator System 220V
SD2-9600-DHS-NA	SPE Dry 96 Dual Sample Concentrator System 110V
SD2-9600-DHS-T-EU	SPE Dry 96 Dual Sample Concentrator System PTFE Needles (Top Head Only) 220V
SD2-9600-DHS-T-NA	SPE Dry 96 Dual Sample Concentrator System PTFE Needles (Top Head Only) 110V
SD2-9606	96-Channel Cap Plate for use on SPE Dry 96 Dual Head
414355SP	Biotage® ACT Plate Adapter

Biotage® 1-Point Support™

The Answer To All Your Questions

www.biotage.com

The Biotage website offers our customers easy access to current information on new products, applications, and events.

Europe
Service and Support
Telephone: +46 18 56 59 11
E-mail: eu-1-pointsupport@biotage.com

North & Latin America
Service and Support
Telephone: +1 800 446 4752
Outside the USA: +1 704 654 4900
E-mail: us-1-pointsupport@biotage.com

Japan
Service and Support
Telephone: +81 3 5627 3123
E-mail: jp-1-pointsupport@biotage.com

China
Service and Support
Telephone: +86 21 2898 6655
E-mail: cn-1-pointsupport@biotage.com

To locate a distributor
Please visit our website at
www.biotage.com

Your Complete Partner for Effective Chemistry

Biotage is a worldwide supplier of instruments and accessories designed to facilitate the work of laboratory and process chemists. With our deep knowledge of the industry, academic contacts and in-house R&D teams, we can deliver the best solutions to your challenges. We take great pride in our flexibility and ability to meet our customer's individual needs. With strong foundations in both analytical, organic and process chemistry, we can offer the widest range of solutions available on the market.

EUROPE
Main Office: +46 18 565900
Toll Free: +800 18 565710
Fax: +46 18 591922
Order Tel: +46 18 565710
Order Fax: +46 18 565705
order@biotage.com
Support Tel: +46 18 56 59 11
Support Fax: + 46 18 56 57 11
eu-1-pointsupport@biotage.com

NORTH & LATIN AMERICA
Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 704 654 4900
Order Fax: +1 434 296 8217
ordermailbox@biotage.com
Support Tel: +1 800 446 4752
Outside US: +1 704 654 4900
us-1-pointsupport@biotage.com

JAPAN
Tel: +81 3 5627 3123
Fax: +81 3 5627 3121
jp_order@biotage.com
jp-1-pointsupport@biotage.com

CHINA
Tel: +86 21 2898 6655
Fax: +86 21 2898 6153
cn_order@biotage.com
cn-1-pointsupport@biotage.com

To locate a distributor, please visit our website www.biotage.com

Part Number: UI330

Biotage®