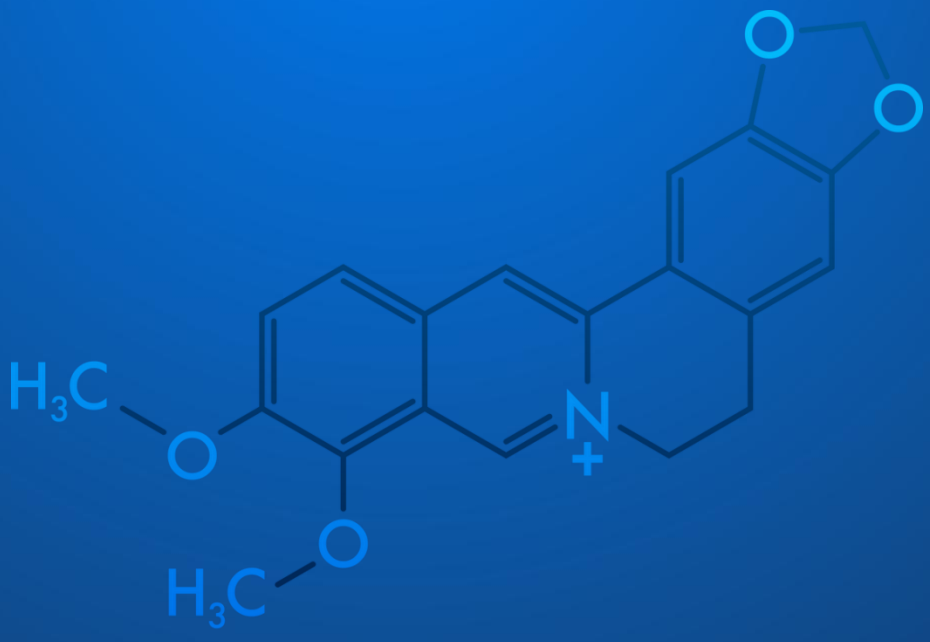
SIELC Technologies, Inc.
Wheeling, IL 60090 USA
P. 847-229-2629 **F. 847-655-6079**
mail@sielc.com www.sielc.com

SIELC

CHROMATOGRAPHY OF QUATERNARY AMINES



Quaternary amines can have very diverse structures based on the nature of the groups attached to the nitrogen atom. These compounds seem like good candidates for reverse phase (RP) HPLC separation based on their chemical structure. However, there are a few limitations. Quaternary amines, in fact, can be quite difficult for RP chromatographic analysis.

First, hydrophilic quaternary amines can not be retained on RP columns (Figure 1). While the pH of the mobile phase affects the charge state of primary, secondary, and tertiary amines, which influences their polarity, this does not apply to quaternary amines.

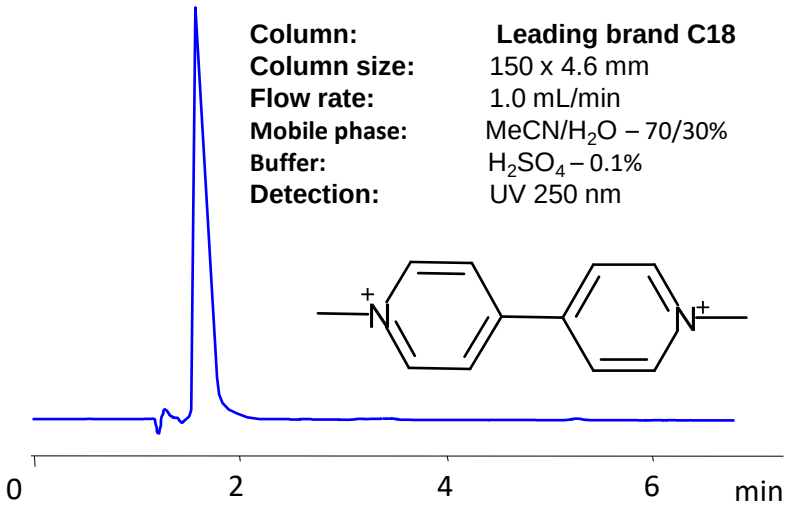


Figure 1. Retention of Paraquat on RP column

Second, due to ionic interactions with residual silanol groups, inadequate peak shape, and peak tailing often occur when hydrophobic quaternary amines are analyzed on RP columns (Figure 2), even with the use of end-capping or base-deactivation.

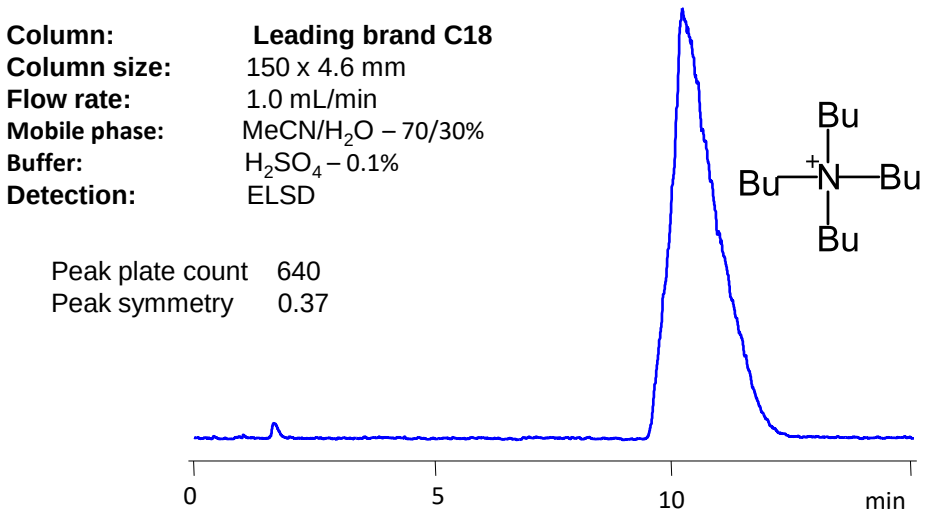


Figure 2. Retention of Tetrabutylammonium on RP column

Mixed-mode columns offer additional interactions to help retain hydrophilic quaternary amines and prevent unwanted effects such as asymmetrical peak shape. Depending on the hydrophobic properties of these compounds, different methods and columns can be selected to create the desired results (Figure 3).

Additional interactions, such as electrostatic attraction and electrostatic repulsion due to ionic functionality embedded in the stationary phase, offer higher efficiency, improved retention, better peak shape, and limit unwanted interactions with silica compared to traditional RP columns.

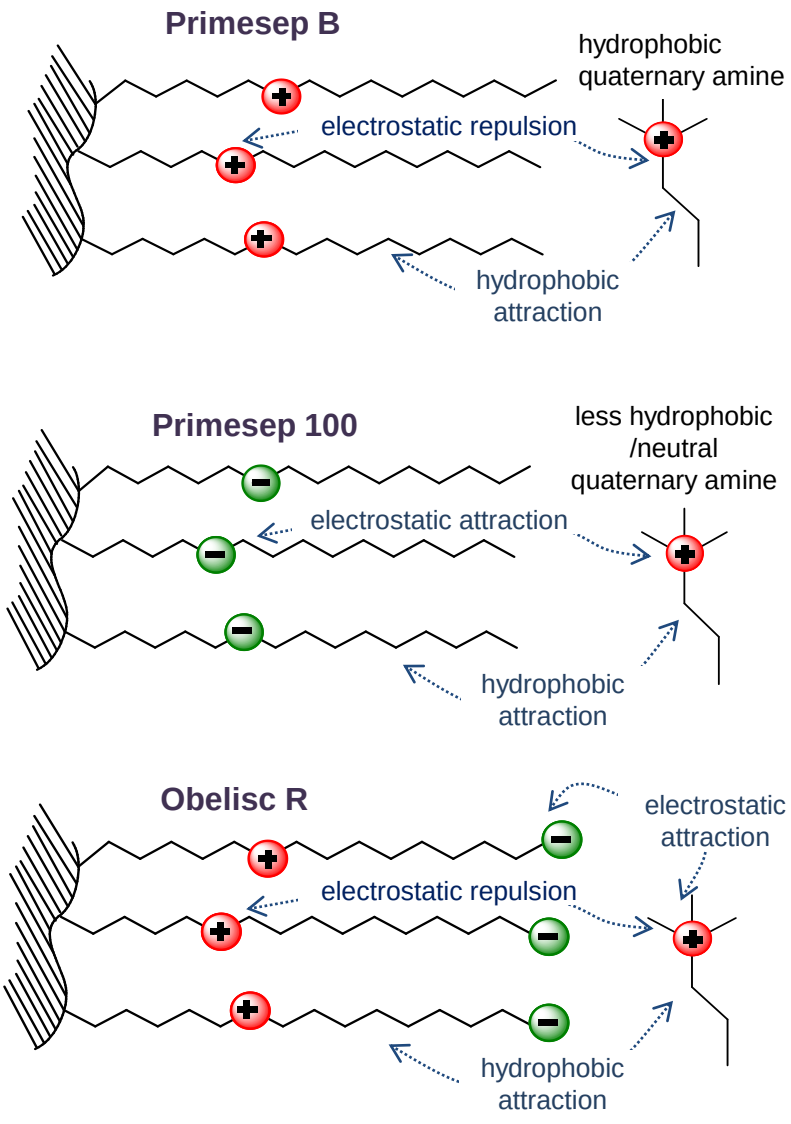


Figure 3. Different types of interactions of quaternary amines with Mixed-Mode columns

Primesep mixed-mode columns are designed to provide controllable secondary ionic interaction as an additional mechanism for retaining charged compounds while eliminating non-controllable silica effects.

Obeslic R columns have two oppositely – charged ions in a ligand, which can effectively attract different polar molecules while still providing a hydrophobic attraction for analyzing different hydrophobic molecules (Figure 4).

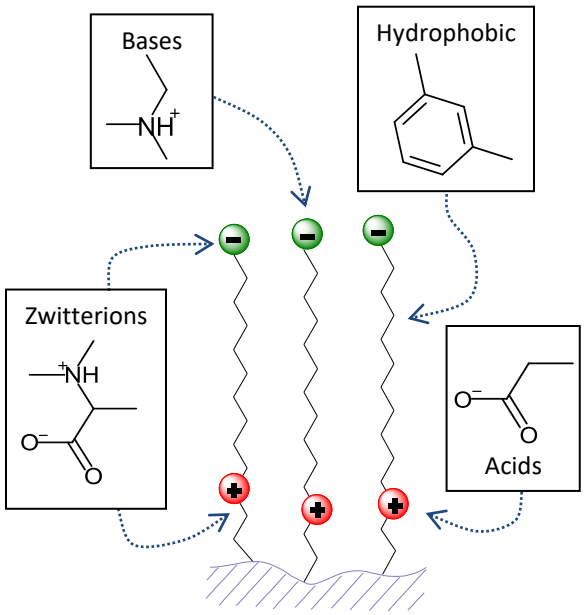


Figure 4. Capability of Obelisc R in retaining different analytes

Many quaternary amine compounds have multiple charges or are present in large polymer structures. Multi-charged compounds are very difficult to analyze effectively. Usually, if multi-charged quaternary amines are analyzed on the negatively charged column due to strong interaction with opposite charges, the elution can be quite challenging to perform. Moreover, pre-void elution results if these compounds are analyzed on the column that has a positive charge.

Traditionally, if the ionic compound and the stationary phase share the same charge, the ions will be repelled by the surface charge, which results in rapid elution. SIELC Technologies developed a new separation technique, Bridge Ion Separation Technique (BIST), which takes advantage of same-charges to separate multi-charged compounds effectively.

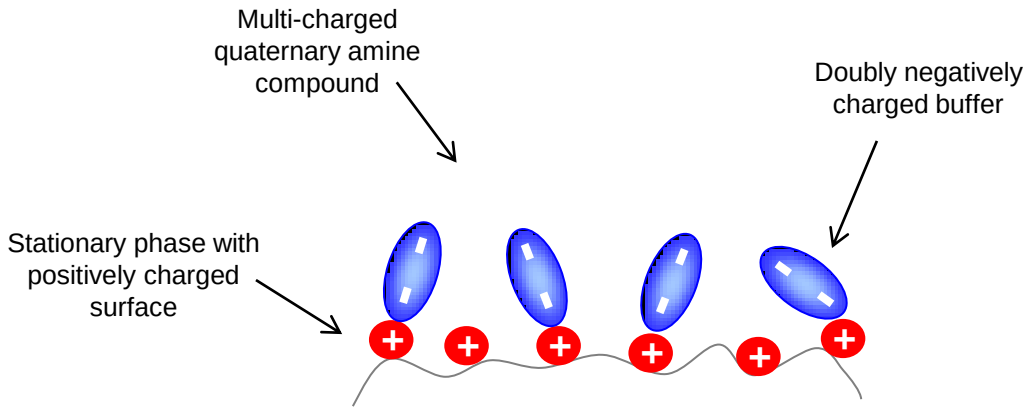


Figure 5. Interaction of multi-charged quaternary amine with positively charged stationary phase

When double-charged ions are present in the mobile phase (MP), the surface of the stationary phase can switch its polarity. For example, suppose the surface is positively charged, and the double-charged ions in the mobile phase are sulfate ions with a minus 2 charge (from ionized sulfuric acid). In that case, the surface can become negatively charged (Figure 5). This phenomenon becomes quite pronounced when there is a low water concentration in the mobile phase. Water can form a solvation shell, which prevents the formation of the “Bridge”. However, this is one of the crucial aspects of this technique. The overall net charge of the surface can be switched from positive to negative and vice versa by only changing the water concentration (Figure 6). Therefore, this phenomenon allows for effective retention of multi-charged compounds and eluting them without the usual issues.

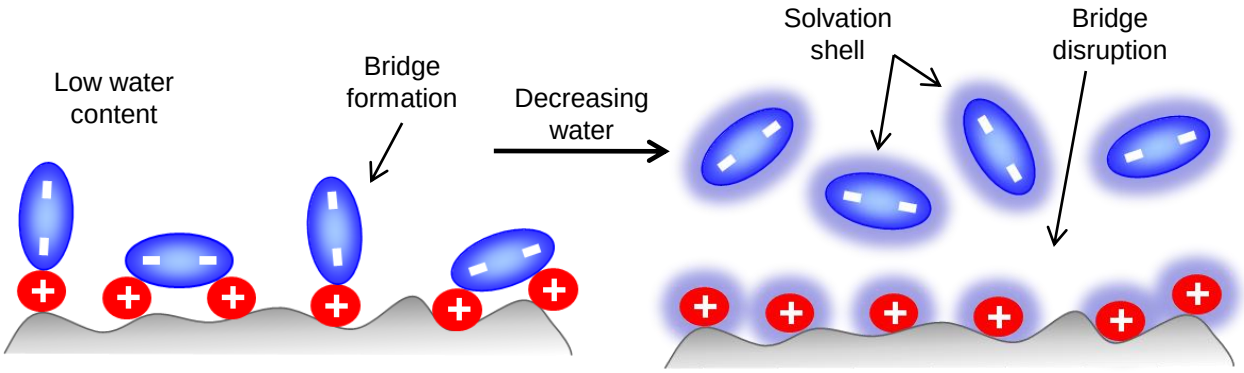
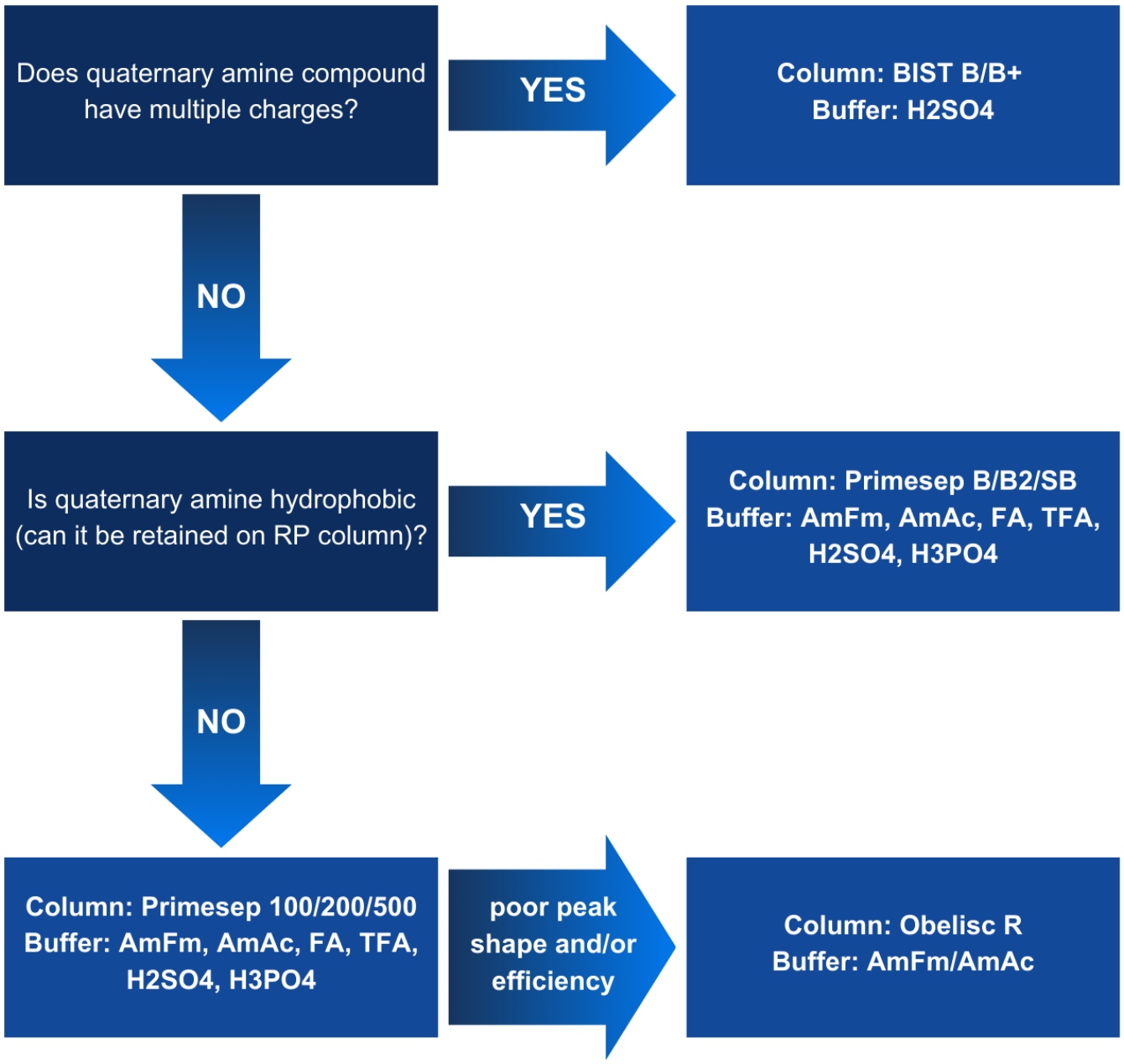


Figure 6. Graphical representation of water influence over BIST mechanism

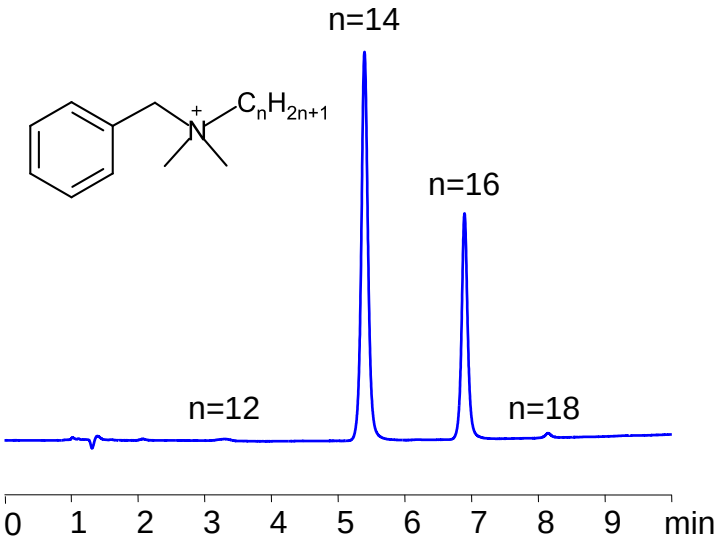
Method Selection

Quaternary amines constitute a diverse class of compounds characterized by distinct chemical properties. Mixed-mode columns present various interactions and functional combinations within their stationary phases. These functionalities can be selectively paired with the compounds under analysis, allowing for tailored chromatographic separations and optimized results.



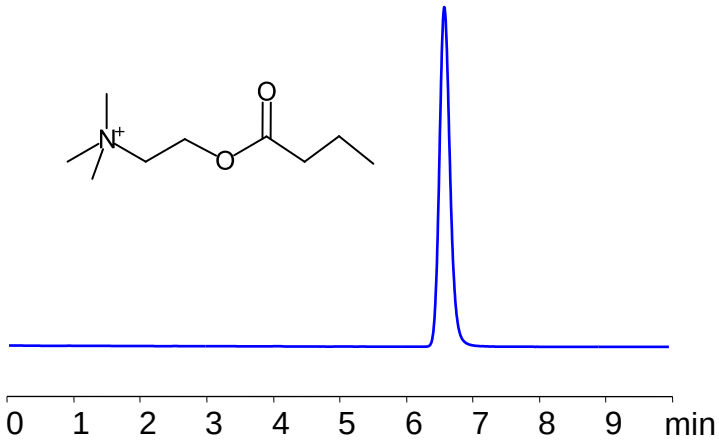
Benzalkonium Chloride on Primesep SB

Column:	**Primesep SB**
Column size:	100 x 3.2 mm
Flow rate:	0.5 mL/min
Mobile phase:	Gradient MeCN – 30-70%, 10min
Buffer:	H_2SO_4 – 0.2%
Detection:	UV 210 nm



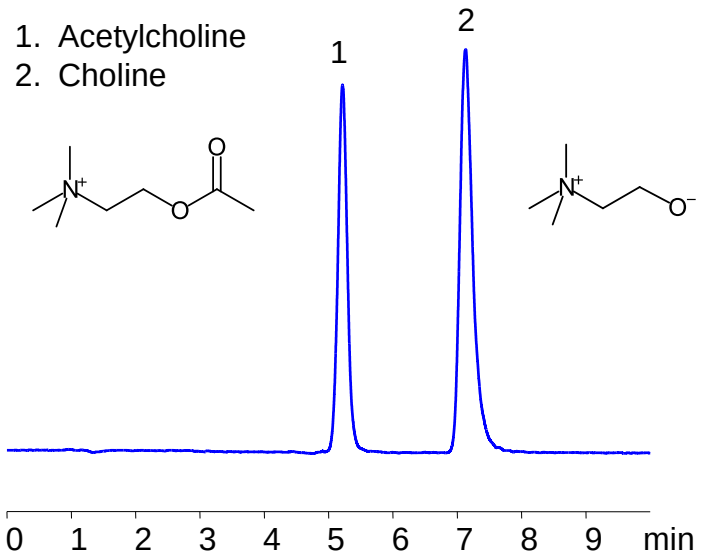
Betain on Primesep 100

Column:	**Primesep 100**
Column size:	150 x 4.6 mm
Flow rate:	1.0 mL/min
Mobile phase:	MeCN/H_2O – 10/90%
Buffer:	H_2SO_4 – 0.1%
Detection:	UV 200 nm



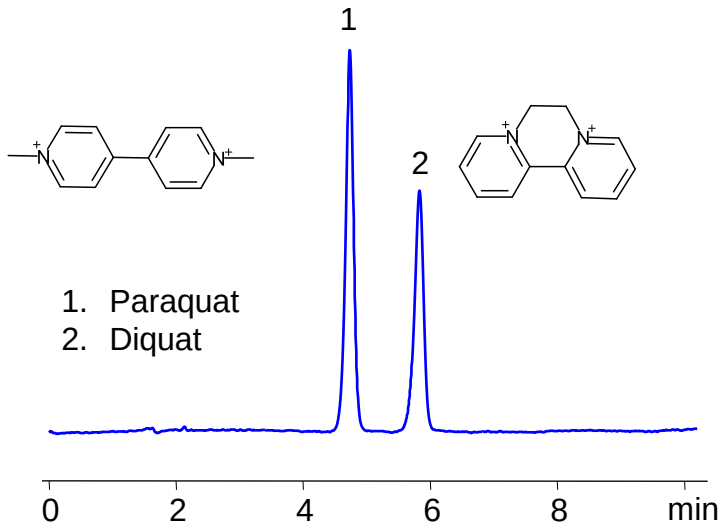
Acetylcholine and Choline on Obelisc R

Column:	**Obelisc R**
Column size:	100 x 2.1 mm
Flow rate:	0.2 mL/min
Mobile phase:	MeCN/H_2O – 60/40%
Buffer:	AmAc pH 5.0 – 10 mM
Detection:	ELSD



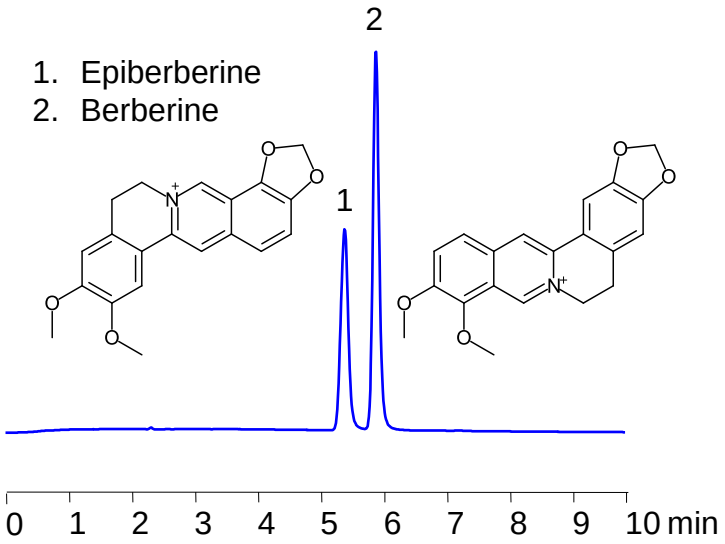
Paraquat and Diquat on BIST B+

Column:	**BIST B+**
Column size:	150 x 4.6 mm
Flow rate:	1mL/min
Mobile phase:	MeCN/H_2O - 70/30
Buffer:	H_2SO_4 – 0.2%
Detection:	UV 210 nm



Berberine and Epiberberine on Primesep B

Column:	**Primesep B**
Column size:	4.6 × 150 mm
Flow rate:	1.0 mL/min
Mobile phase:	Gradient MeCN 10-50%, 10 min
Buffer:	AmFm pH 3.0 – 40 mM
Detection:	UV 266 nm



Butyrylcholine and Benzyltrimethylammonium on Primsep 200

Column:	**Primesep 200**
Column size:	2.1 × 100 mm
Flow rate:	1.0 mL/min
Mobile phase:	MeCN/H_2O – 60/40%
Buffer:	AmFm – pH 3.0 – 20 mM
Detection:	ELSD