SIELC

BIST™

A New Mode of LC Separation

THEORY
METHODS
COLUMNS
APPLICATIONS

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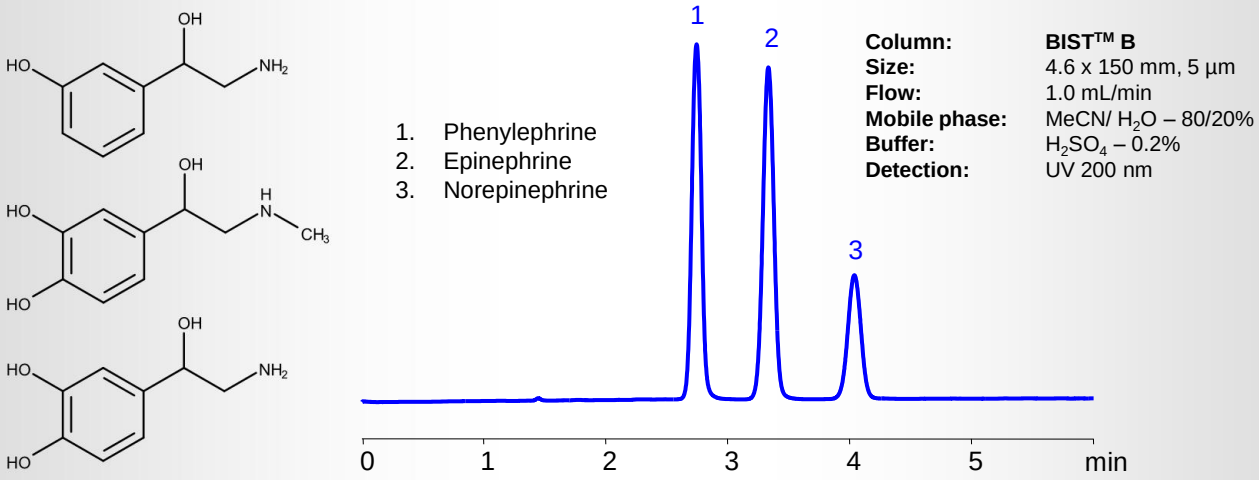
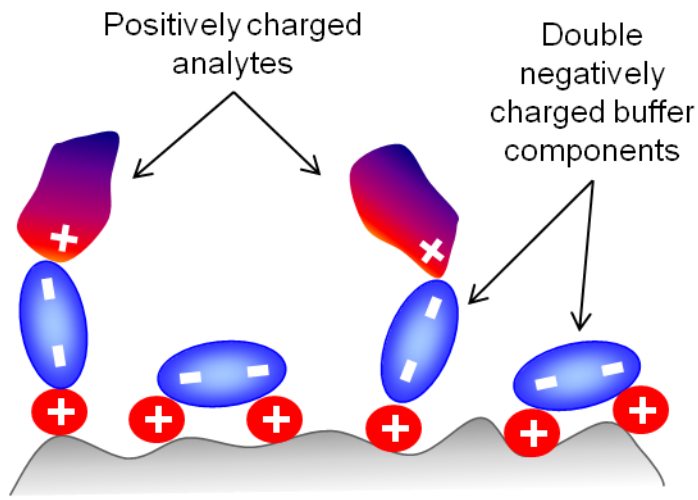
Have you been looking for a simple, efficient way to retain and separate charged molecules in your HPLC application? Are you tired of developing intricate methods that require multiple columns and complex gradients to separate compounds that too often co-elute? Have you had problems retaining or eluting polar ionic molecules with multiple charges?
With the revolutionary new Bridge Ion Separation Technology (BIST™), you can put those concerns to rest!
SIELC Technologies' BIST™ is a new and simple way to achieve many separations that are difficult or impossible to achieve with any other HPLC columns currently on the market.



What kind of beast is BIST?



Stationary phase with positively charged surface

BIST™ Mechanism and properties:

- Double-charged anions of the buffer in the mobile phase form a bridge between the positive charges of the stationary phase surface and the positively-charged analyte.
- Bridge formation is possible when molecules of polar solvent (water) do not solvate ions sufficiently and, therefore, do not separate the ions in solution.
- The more water the mobile phase has, the less retention of BIST™ type is observed.
- While BIST™ is based on the ionic electrostatic interaction, the concentration of ions in the mobile phase does not affect retention significantly (as opposed to the concentration of water).
- BIST™ is different from Ion-Exchange, HILIC, or any other common separation technique.

Complex Chromatography without the Hassle

SIELC developed a new separation technique to expand the scope of HPLC applications. BIST™ provides an additional separation tool to a whole class of organic and inorganic charged molecules. BIST™ columns, simple mobile phases, and an array of applications provide a whole new continent in the world of chromatography.

Separation mode useful for different compounds

Compound type	RP	HILIC	IE	BIST
Neutral hydrophobic	yes	no	no	no
Neutral hydrophilic	no	yes	yes	yes
Charged hydrophobic	yes	no	yes	yes
Charged hydrophilic	no	yes	yes	yes
Multi-charged hydrophobic	yes	no	no	yes
Multi-charged hydrophilic	no	yes	no	yes

How exactly does BIST™ work and how can it improve your HPLC separations? To answer these questions, we first need to understand the electrostatic interactions of charged molecules.

In traditional Ion Exchange Chromatography (IEC), analyte ions are usually separated by how they interact electrostatically with the column's charged surface. If the ions and the surface are charged oppositely (i.e. a negatively-charged surface and positively-charged ions), then the ions will be attracted to the surface and retained to a degree based on the number of charges, ion size, and other factors. However, if the ions and the surface share the same charge (i.e. if both are positively charged), the ions will be repelled by the surface charge to the point where they don't even enter the pores of the stationary phase particles. This will result in extremely quick elution and is referred to as pre-void elution.
This difference in same- and different-charges between ions and stationary phase is shown to the right, where a positively-charged ion was injected onto a positively-charged column (Anion Exchange) and onto a negatively-charged column (Cation Exchange).

The void time, or how long an analyte would take to travel through the column without any net interactions, is just under 1.5 minutes for the column dimensions given in the figure below. Notice that the red peak appears just a bit earlier, indicating the analyte eluted pre-void.

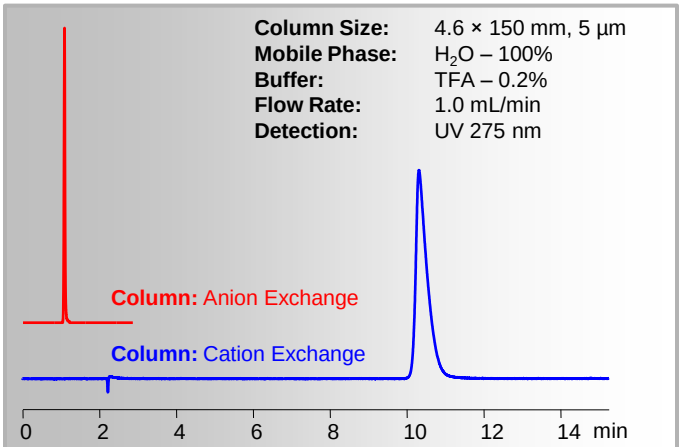


Figure 1 - Column surface charge effect on protonated Dopamine retention in Ion-Exclusion and Ion-Exchange modes.

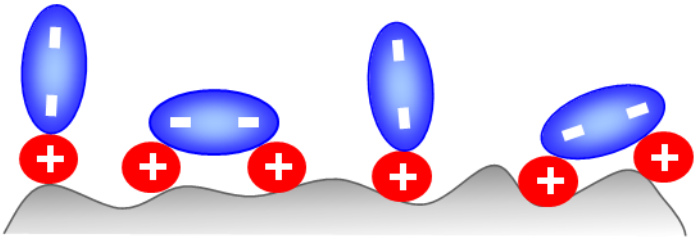
Analyze Ions Differently

BIST™ works differently. Three conditions need to be met:

- *A double-charged buffer ion present in the mobile phase*
- *Buffer's double-charged ions should be opposite in charge to that of the stationary phase surface*
- *Reduced water in the mobile phase to minimize ion solvation*

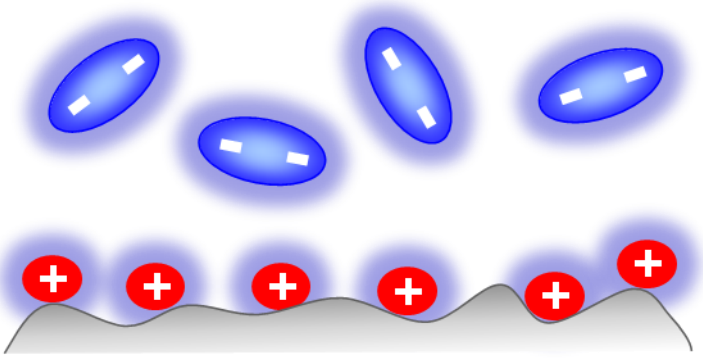
BIST™ explained

When double-charged ions are present in the mobile phase (MP), the surface of the stationary phase can switch its polarity. For example: if 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), then the surface can became negatively-charged.



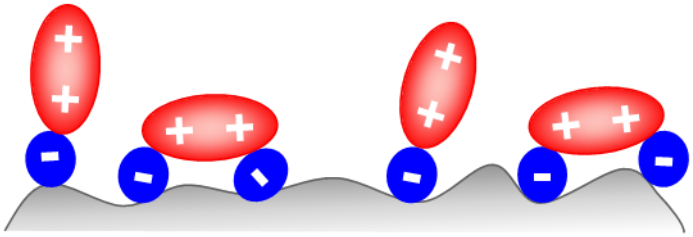
Stationary phase with positively charged surface with negative net charge

However, this can occur only when the mobile phase has a relatively low concentration of water. When water is the main component of the mobile phase, it forms a solvation shell around each ion and prevents the surface charge from switching.



Stationary phase with positively charged surface with positive net charge

The same surface switch is observed when a negatively-charged surface interacts with double-charged positive ions such as diamines, and some inorganic ions such as Mg^{2+} and Ca^{2+}.



Stationary phase with negatively charged surface with positive net charge

The essence of this phenomena is that the net charge of the surface can be switched from positive to negative (or vice versa) by only changing the concentration of water in the mobile phase. This can be done with either a negatively-charged surface or a positively-charged surface as long as the mobile phase contains a buffer with double-charged ions. When the surface switches polarity, an analyte with a charge similar to the initial surface charge can now be retained on this phase and the double-charged buffer component serves as a bridge between the surface and the analyte.
This phenomenon has opened up a new way of retention control of the charged molecules' interaction with the column's stationary phase by changing the relative amount of water in the mobile phase:
- If the analyte has the same charge as the column surface, then decreasing the relative amount of water will increase its retention
- If the analyte has the opposite charge of the column surface, then decreasing the relative amount of water will decrease the retention.

Double vs. Singly Charged Buffer Ions

Typically, when a positively-charged analyte is injected onto a column with a positively-charged surface, the analyte has very little interaction with the stationary phase. In fact, the analyte is repelled and does not even enter the particle pores, resulting in pre-void elution, as shown in blue in Fig. 2. However, when a buffer with double-charged ions (H_2SO_4) replaces a buffer with single-charged ions (TFA), retention occurs, as shown in red in Fig. 2. Conventional chromatographic wisdom tells us this shouldn't be happening, yet positively-charged Dopamine is retained when a H_2SO_4 buffer and a high MeCN concentration are employed. Apparently, something unique and exciting is occurring!

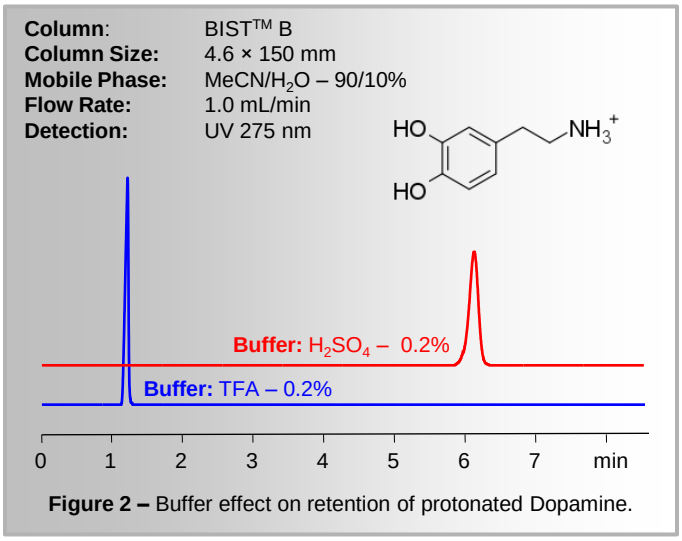


Figure 2 – Buffer effect on retention of protonated Dopamine.

BIST™ is not limited to just positively charged analytes and columns; it can work with negatively charged compounds and a negatively charged column surface. This can be seen in Fig. 3, where negatively charged Tartrazine (with three acidic groups) was analyzed on a cation-exchange column. The key to BIST™ chromatography is a double-charged cation of the buffer, such as magnesium acetate, calcium acetate, or N,N,N',N'-tetramethyldiaminopropane (TMDAP) and a high concentration of MeCN. If a buffer with a single-charged cation such as Sodium Acetate (NaAcO) is used, no retention occurs for Tartrazine at the same high MeCN concentration.

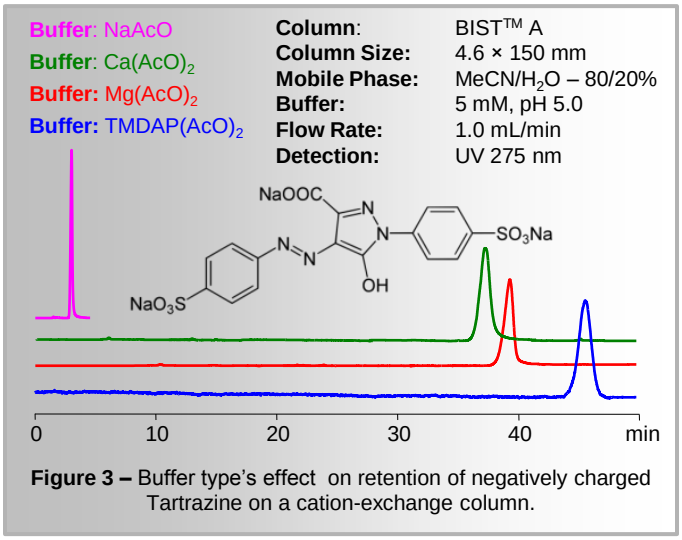


Figure 3 – Buffer type's effect on retention of negatively charged Tartrazine on a cation-exchange column.

BIST™ provides not only great retention of charged analytes, but also offers good selectivity. When a bridge forms between the stationary phase and analyte, the structure of the analyte plays an important role in the bridge stability. Since this interaction happens close to the surface of the solid stationary phase, even small differences in the charge position within the solute molecule or presence of other functional groups may significantly influence the analyte's retention time and enhance the column's selectivity. It is clear in Fig. 4 that a single-charged buffer can provide neither retention nor selectivity.

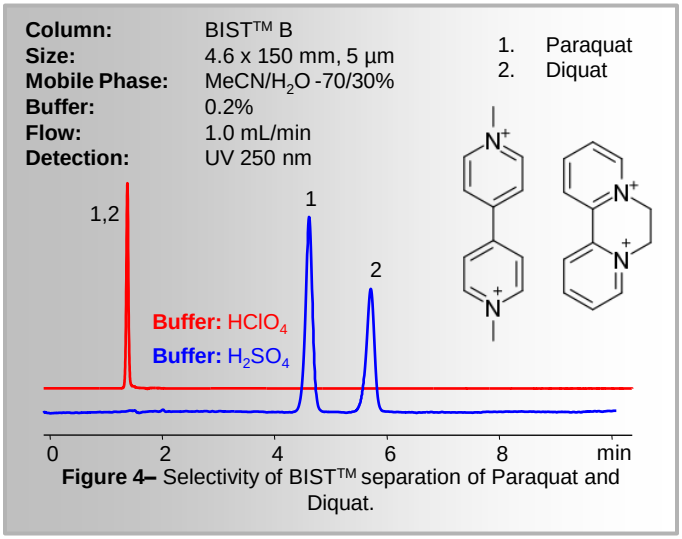


Figure 4– Selectivity of BIST™ separation of Paraquat and Diquat.

BIST™ Application Examples

Here we demonstrate three examples of separation of basic polar and non-polar molecules that, in typical reverse phase chromatography settings, are either difficult to retain or produce poor peak shape and efficiency. BIST™ conditions can, instead, produce high efficiency, retention and symmetrical peak shape.

Column: BIST™ B
Size: 4.6 x 150 mm
Flow: 1.0 mL/min
Mobile phase: MeCN/ H_2O – 70/30% to10/90% in10 min
Buffer: H_2SO_4 – 0.2%
Detection: UV 210 nm

1. Lisinopril
2. Trimetazidine
3. m – Xylylenediamine
4. 2,4,6-Tris(dimethylaminomethyl)phenol
5. Desmosine

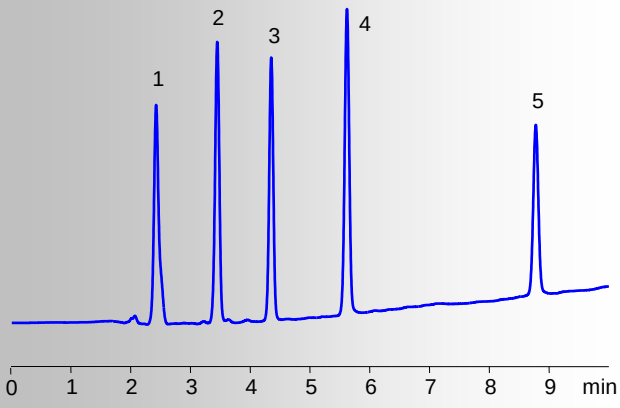
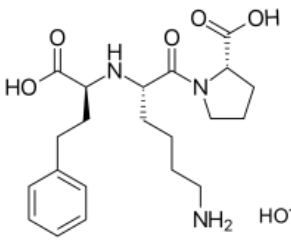
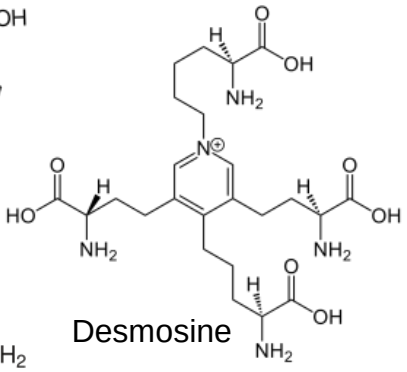


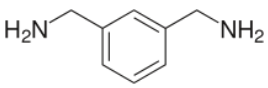
Figure 5 – Separation of drugs with BIST™ .



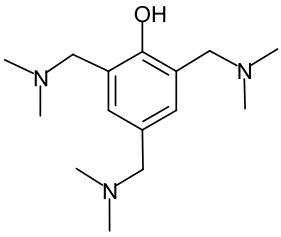
Lisinopril



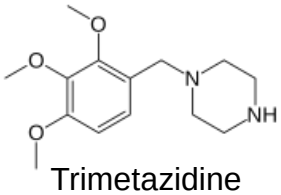
Desmosine



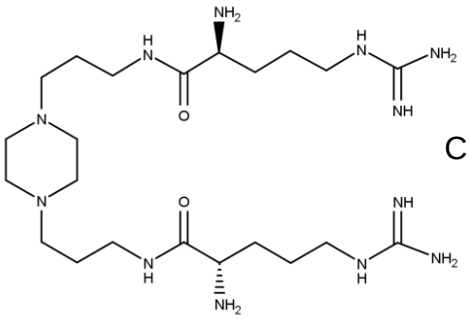
m – Xylylenediamine



2,4,6-Tris(dimethyl-aminomethyl)phenol



Trimetazidine



Ciraparantag

Column: BIST™ B
Size: 4.6 x 150 mm
Mobile phase: MeCN/H2O – 70/30%
Buffer: H_2SO_4 – 0.2%
Flow: 1.0 mL/min
Detection: UV 210 nm

1. Arginine
2. Lysine
3. Histidine

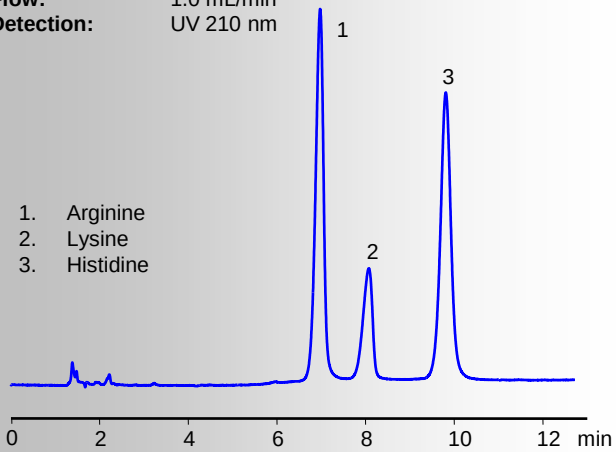


Figure 6 – Separation of basic amino acids with BIST™.

Column: BIST™ B
Size: 4.6 x 150mm, 5 µm
Flow: 1.0 mL/min
Mobile phase: MeCN/ H_2O 80/20% to 30/70% in10 min
Buffer: H_2SO_4– 0.2%
Detection: UV 200 nm

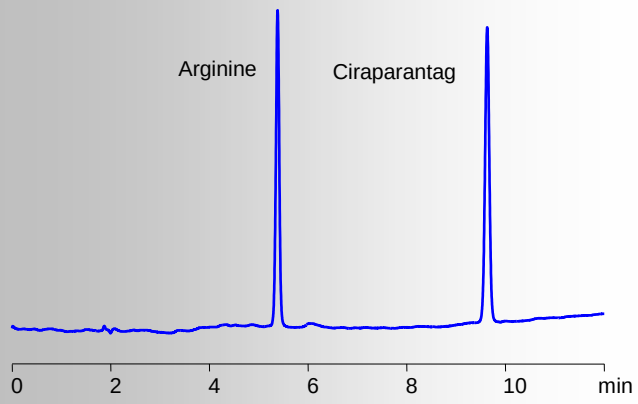


Figure 7 – Separation of basic molecules with BIST™.

Multi-Charged Molecule Analysis

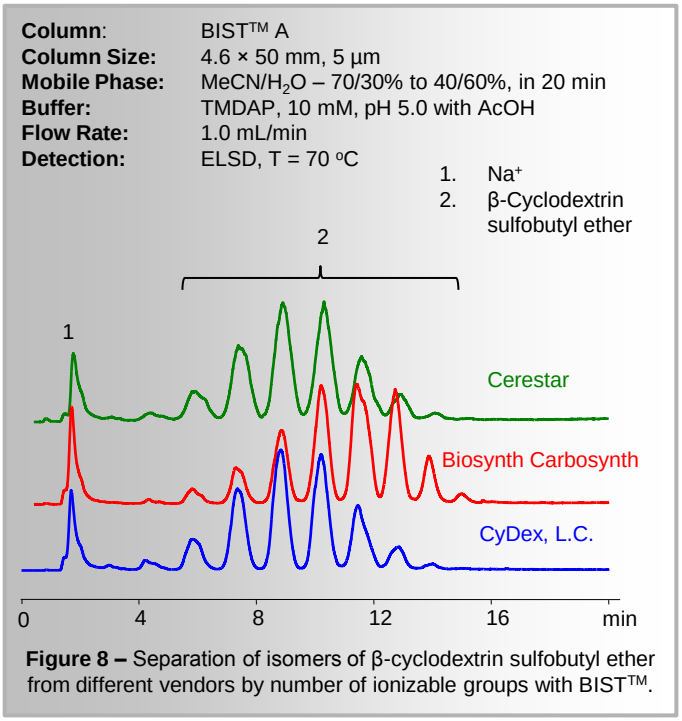
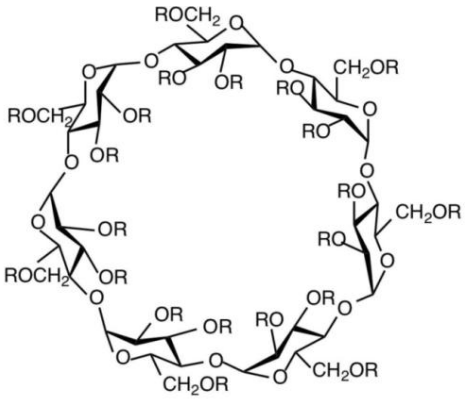


Figure 8 – Separation of isomers of β-cyclodextrin sulfobutyl ether from different vendors by number of ionizable groups with BIST™.

BIST™ can also separate structural isomers of multi-charged compounds, such as sulfated cyclodextrin, with a relatively simple gradient method on a short column. Previous methods for analysis of similar compounds can require much longer columns, higher concentration buffers, and do not provide complete separation of the constituent isomers.



R = - H or
R = - $CH_2CH_2CH_2CH_2SO_3H$

The power of BIST™ lies in its ability to do these kinds of separations on short columns with simple mobile phases and low concentration buffers. Polymers with charged units, like polylysine, are difficult to separate using ion-exchange (IE) chromatography due to very strong and often irreversible interactions with the oppositely-charged stationary phase of the column. Usually, an extremely high concentration of the buffer needs to be used to generate an ion-exchange process. This high buffer concentration isn't usually desirable because of the higher viscosity of the MP and the likely formation of salt in the system components. With BIST™, polymers, like these, can be retained and analyzed with a small concentration of the buffer in the mM range using a simple gradient of water and MeCN.

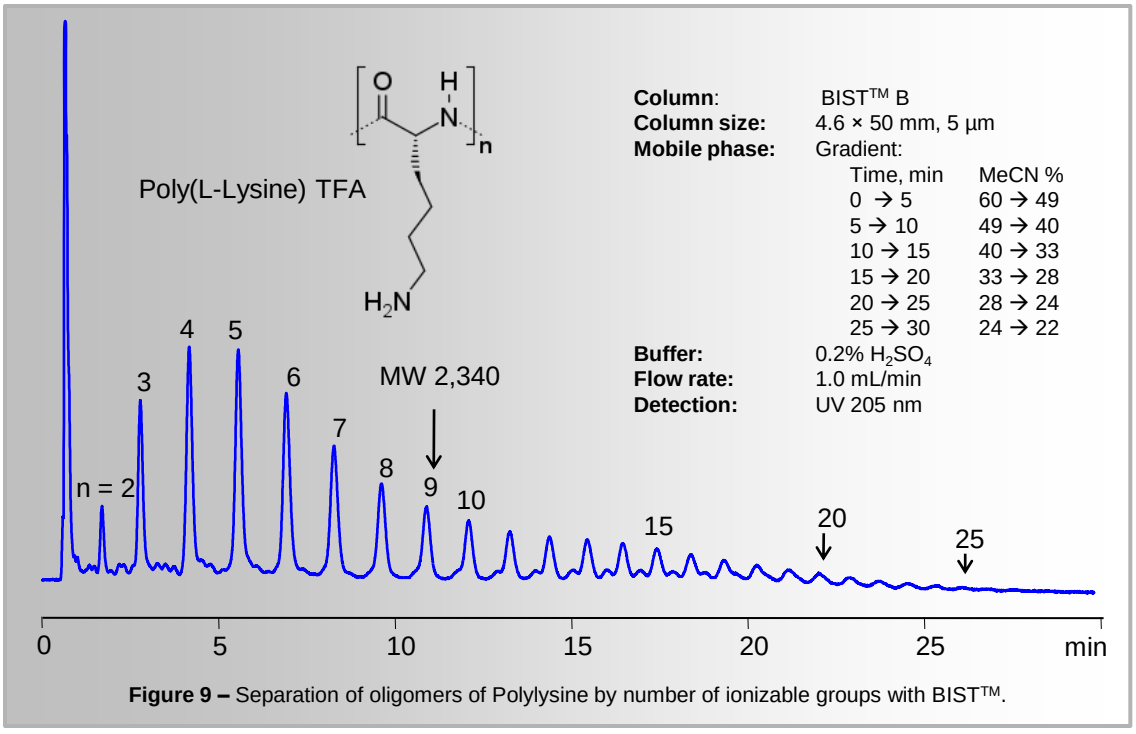


Figure 9 – Separation of oligomers of Polylysine by number of ionizable groups with BIST™.

Other large multi-charged molecules such as polyethyleneimine (PEI) can be very difficult to separate and retain on standard columns. PEI is a complex mixture of molecules of different sizes and geometries. A simple BIST™ method with a step gradient allows for the retention of a single peak for each PEI fraction (Fig. 10)

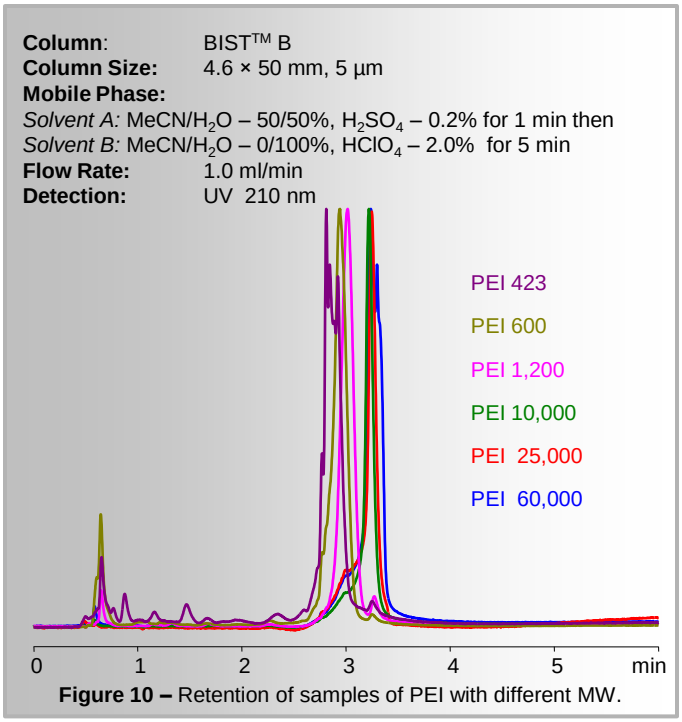
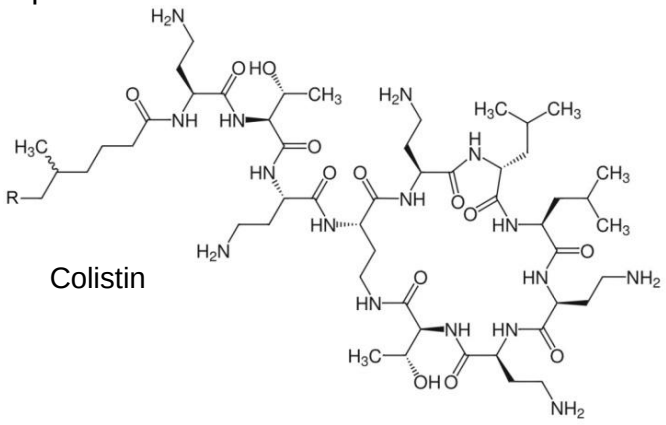


Figure 10 – Retention of samples of PEI with different MW.

Colistin is another multi-charged molecule with structural isomers that BIST™ can retain. All that's needed is the typical high-MeCN concentration MP and a double-charged H_2SO_4 buffer. With this MP, the two isomers, which differ only by an additional methyl group, can be separated.



Colistin

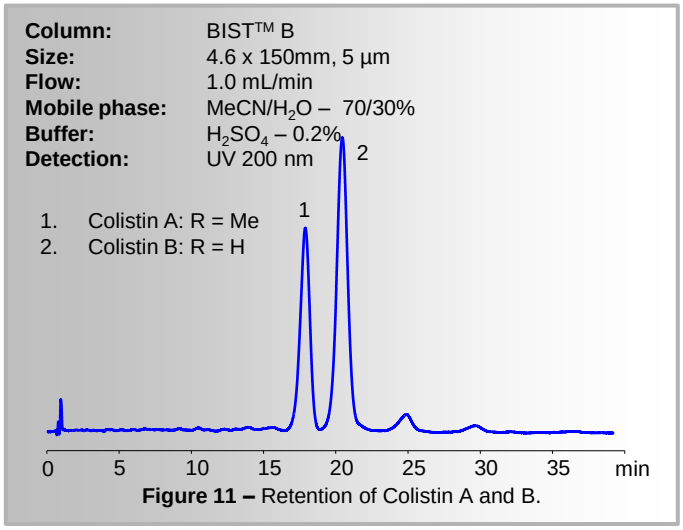


Figure 11 – Retention of Colistin A and B.

Carboxylic acids can also be retained and separated with BIST™ (Fig. 12).

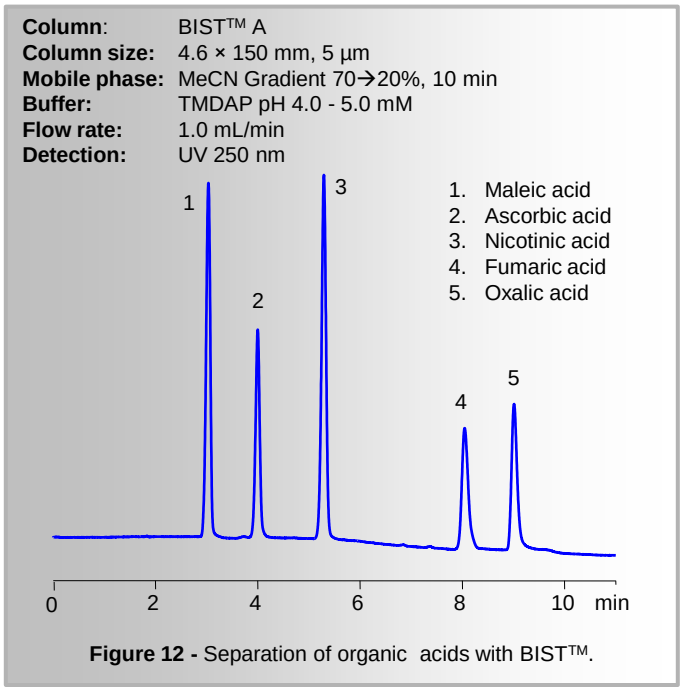


Figure 12 - Separation of organic acids with BIST™.

BIST™ can save you time, money, and provide peace-of-mind on complex separations.

Column	Surface Charge	Useful For
BIST A	Negative	Anions
BIST B	Positive	Cations

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U.S. Patents Pending. All data were obtained in SIELC Technologies labs.

SIELC

formerly Allsep Technologies

For decades liquid chromatography stationary phase design has been dominated by the goal to eliminate multiple, or “unwanted”, interactions and to obtain a simple and predictable retention mechanism. Unfortunately, the simplification of the retention process limits the ability to control elution order of the analytes and the scope of available applications this system can be used for. As a response to this limitation, hundreds of different reverse-phase columns were introduced in the last years to cover a variety of analytical situations.

In contrast, PrimesepTM stationary phases were intentionally designed with two major interactions offered on the same column. Both interactions are independently adjustable with mobile-phase composition producing unlimited states of the stationary phase. The hydrophobic interaction is controlled by the amount of organic modifier in the mobile phase (as in any reverse-phase column), while the ion-exchange interaction is controlled by the ion-strength and pH of the mobile phase (as in other ion-exchange columns). This unique property allows using one stationary phase for numerous applications, including analyses of polar and non-polar, ionizable and neutral, organic and inorganic compounds. The behavior of PrimesepTM columns is predictable and reproducible. The method development process is simple and versatile.

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