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Basic Dyes Analysis

By HPLC

Multi-Charged Dyes

The analysis of dyes is an important analytical process during dye production and in real-world dye applications. Many dyes are produced as a mixture of several structural and constitutional isomers. After production, their purity level then needs to be established. Many dyes especially are used as food additives and therefore need to be free of possible harmful contaminants. Thus, it presents a significant problem if dyes are not properly tested. Also often a counterfeited dyes appears on the market and their testing is not trivial.

Basic dyes are important class of dye chemistry. They have at least 1 (and in many cases, more than 1) basic amine group. The strong ionic interactions make these compounds difficult to analyse utilizing traditional reverse phase HPLC mode.

To address these needs, SIELC Technologies has developed a new HPLC separation mode called BIST™ (Bridge Ion Separation Technology) that can easily separate multi-charged molecules such as these basic dye compounds.

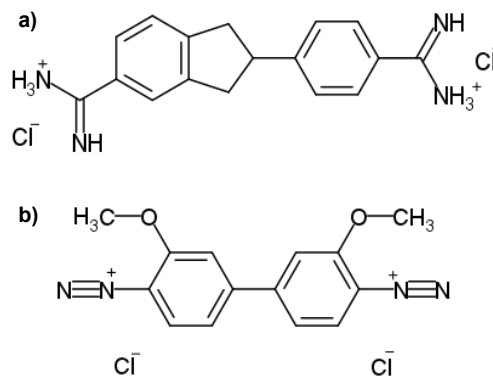
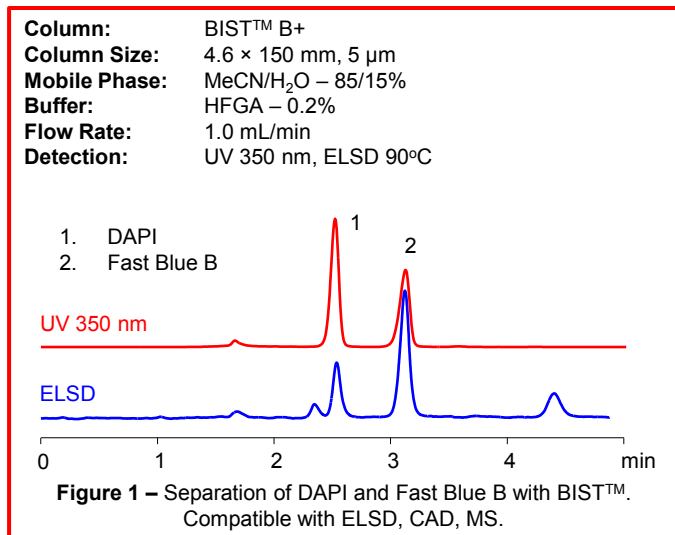
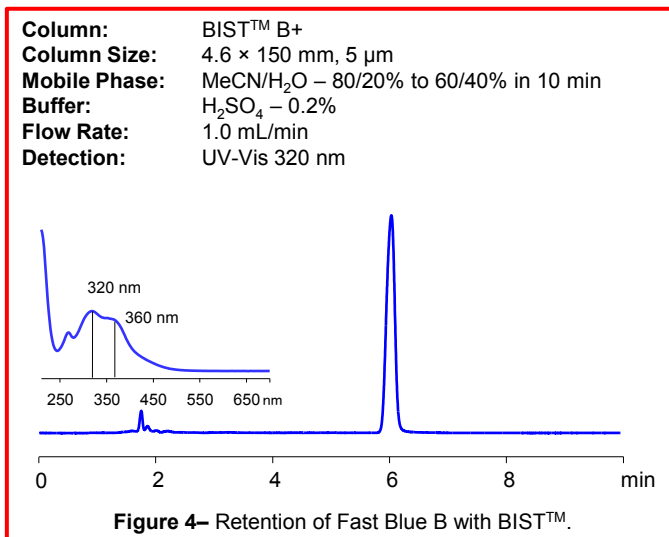
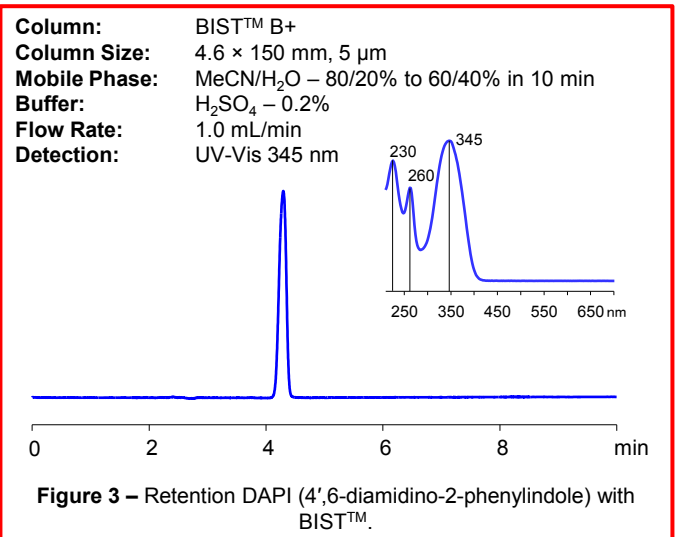


Figure 2 – Chemical structures of multi-charged dyes: a) DAPI and b) Fast Blue B salt

BIST™ in the study depicted in Figure 1 was used to separate and analyze the dye Fast Blue B and the fluorescent stain 4',6-diamidino-2-phenylindole (DAPI).

Typically, BIST™ methods rely on Sulfuric acid as the multi-charged ionic modifier on positively charged BIST™ columns; however, Sulfuric acid is not compatible with evaporative detection methods such as Evaporative Light Scattering Detection (ELSD), Charged Aerosol Detection (CAD), and Electrospray Ionization (ESI) for Mass Spectrometry (MS). Instead, we utilized Hexafluoroglutaric acid (HFGA), which also has a minus-2 charge in solution, but is volatile enough to be compatible with these detection methods.



BIST™ Explained

Bridge Ion Separation Technique, or BIST™, is based on the retention of charged molecules on a stationary phase of like polarity via a bridge formed between the analyte and stationary phase surface by means of a doubly-charged ionic component of the mobile phase. This doubly charged mobile phase component, which we call a bridge ion, provides the necessary electrostatic forces to retain ions on a stationary phase of the same charge polarity. This retention mechanism is expressed more profoundly in a mobile phase with reduced water content.

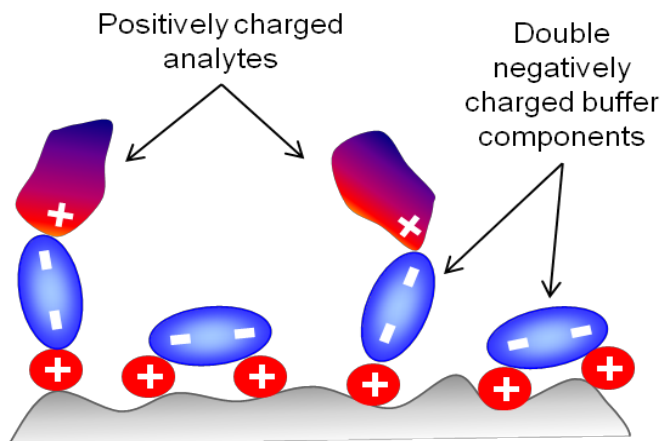


Figure 5 – Diagram of Bridge Formation with a Positively-Charged Analyte and Positively-Charged Surface.

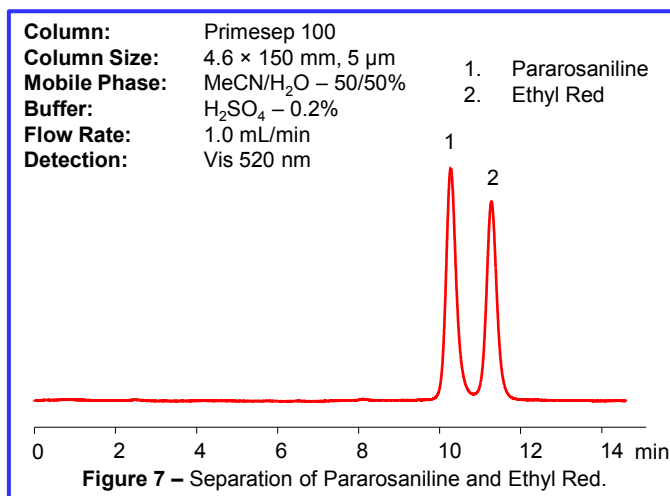
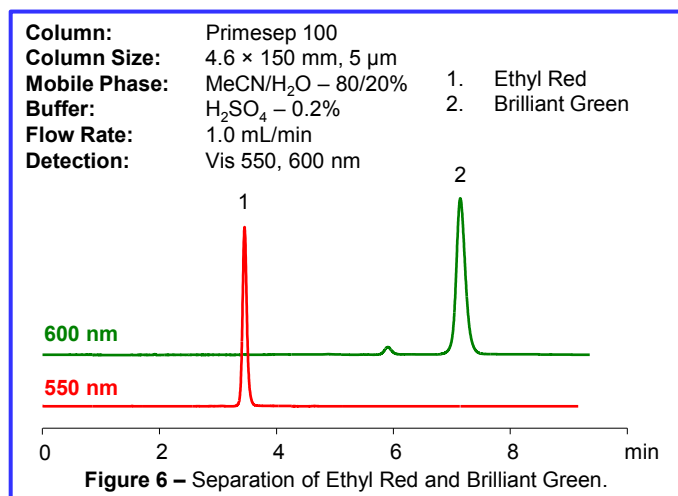
For BIST™ to work, 3 conditions need to be met:

- *A double-charged ionic modifier is present in the mobile phase*
- *The Ionic modifier's double-charged ions should be opposite in charge to that of the stationary phase surface*
- *Reduced water content in the mobile phase to minimize ion solvation*

Single-Charged Dyes

SIELC has also developed HPLC methods based on mixed-mode Primesep 100 columns to analyze single-charged basic dyes. The single charge of these compounds is not sufficient to retain these compounds on BIST™ columns, so they require a different analysis method in order to be analyzed, as show in Figs. 6-9.

Mixed-mode chromatography works differently compared to BIST™ in that it provides multiple modes of interaction for charged analytes. The presence of these interactions allows chemists to use mobile phase modifications with greater flexibility than in traditional reversed-phase chromatography. Ionizable compounds can interact with the stationary phase by reverse-phase (RP), ion-exchange (IE), or ion-exclusion mechanisms. The amount of the ionic modifier in the mobile phase (i.e. buffer strength) influences the retention characteristics to the ion-exchange interaction just as the organic component affects the retention in reverse-phase separation (i.e. hydrophobic interactions). Thus, the amounts of organic and ionic MP modifiers are both important for controlling the retention of ionizable analytes on these mixed-mode columns.



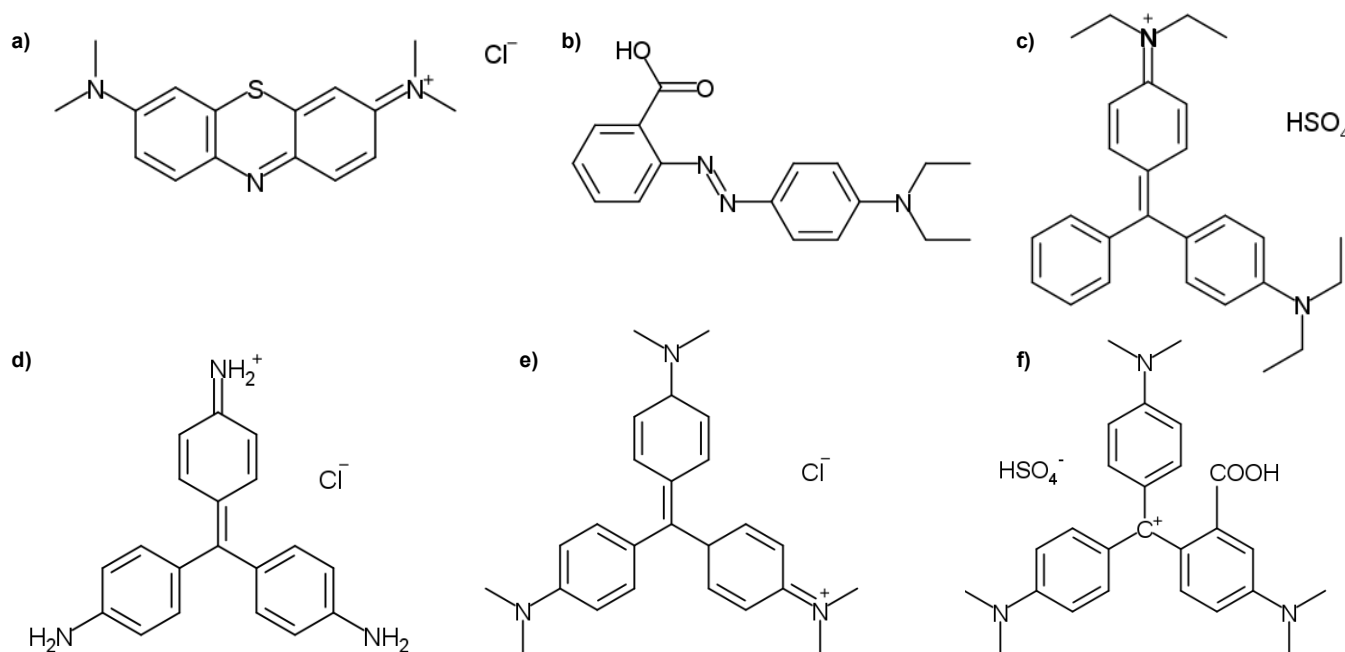
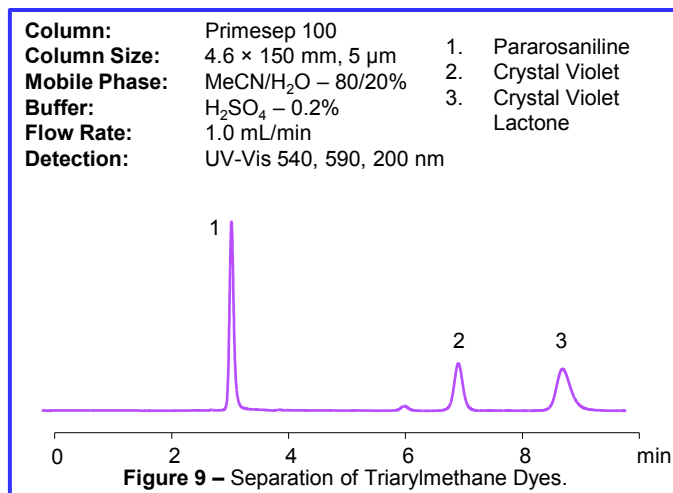
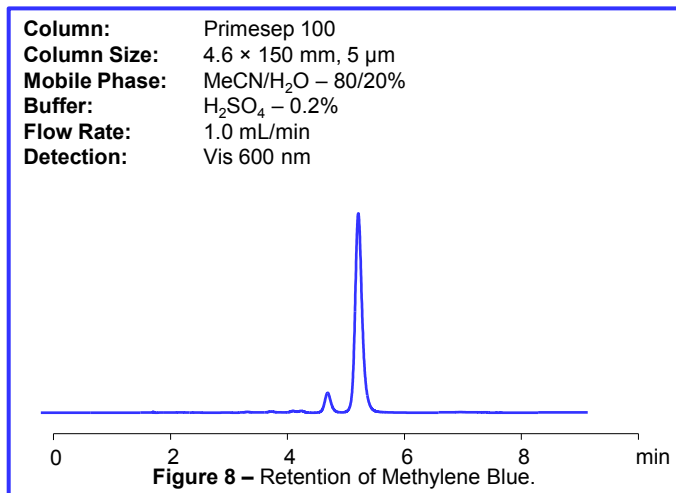



Figure 10 – Chemical structures of single-charged dyes: a) Methylene Blue b) Ethyl Red c) Brilliant Green d) Pararosaniline e) Crystal Violet f) Crystal Violet Lactone



SIELC

Column Part Number Generator

TBP – 32 . 100 . 0210

To order a column or ask a question send your message to sales@sielc.com or call us at: **+1 (847) 229-2629**

Type of Packing	
Primesep 100	100
BIST B+	TBP

Column ID	
50 mm	500
30 mm	300
22 mm	220
10 mm	100
4.6 mm	46
3.2 mm	32
2.1 mm	21
1.0 mm	10
0.5 mm	05

Column Length	
250 mm	250
150 mm	150
100 mm	100
50 mm	50
25 mm	25
10 mm	10
Guard	G

Particle Size	
2.7 μm	02
3 μm	03
5 μm	05
10 μm	10

Pore Size	
100 Å	10

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

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Acidic Dyes Analysis

By HPLC

Introduction - Dyes

The analysis of dyes is an important analytical process during dye production and in real-world applications. Many dyes are produced as a mixture of several structural and constitutional isomers. After production, their purity level then needs to be established. Many dyes especially are used as food additives and therefore need to be free of any possible harmful contaminants. Thus, it presents a significant problem if dyes are not at maximum purity or are actually composed of counterfeit compounds.

Many of these dyes, including Brilliant Blue FCF, Tartrazine, Sunset Yellow, and Amaranth are acid dyes since they have at least 1 (and in many cases, more than) acidic sulfate group. The strong ionic interactions make these compounds difficult to separate and purify utilizing traditional HPLC modes.

To address these needs, SIELC Technologies has developed a new HPLC separation mode called BIST™ (Bridge Ion Separation Technology) that can easily separate multi-charged species such as these acidic dye mixtures.

In the study below, BIST™ was used to analyze the azo dye Brilliant Black BN (Food Black 1, E151) from a reputable chemical supplier. It was discovered that this sample was actually composed of 4 different dyes: Sunset Yellow (Yellow 6, E110), Tartrazine (Yellow 5, E102), Brilliant Blue FCF (Blue 1, E133), and Amaranth (Acid Red 27, E123). There was no Brilliant Black BN present at all.

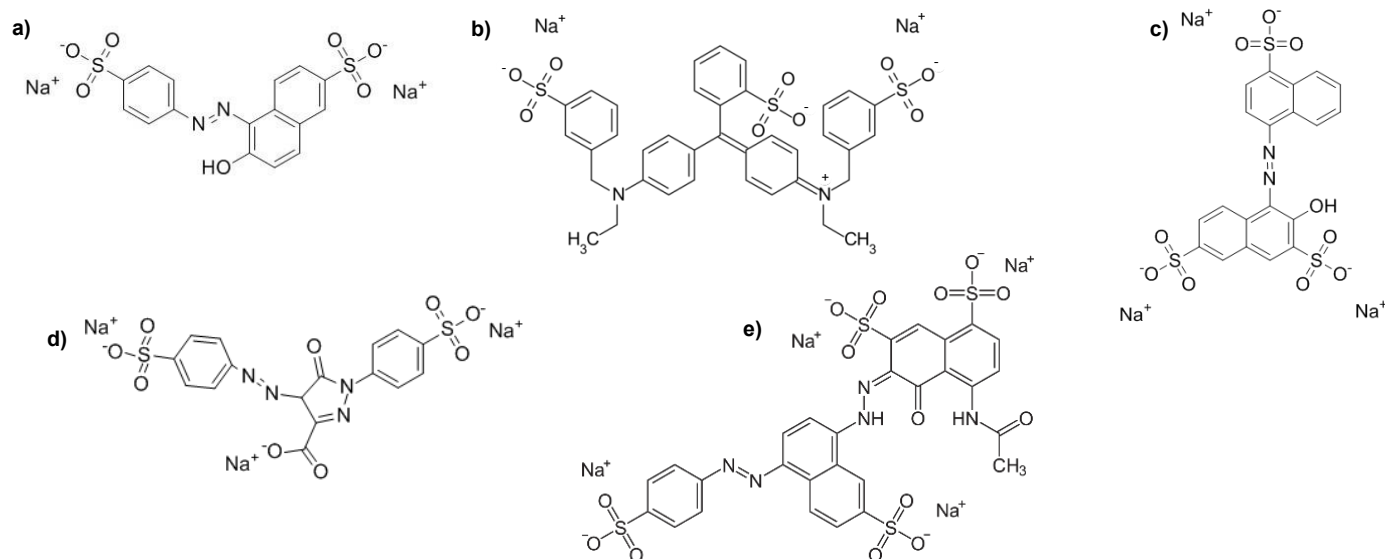
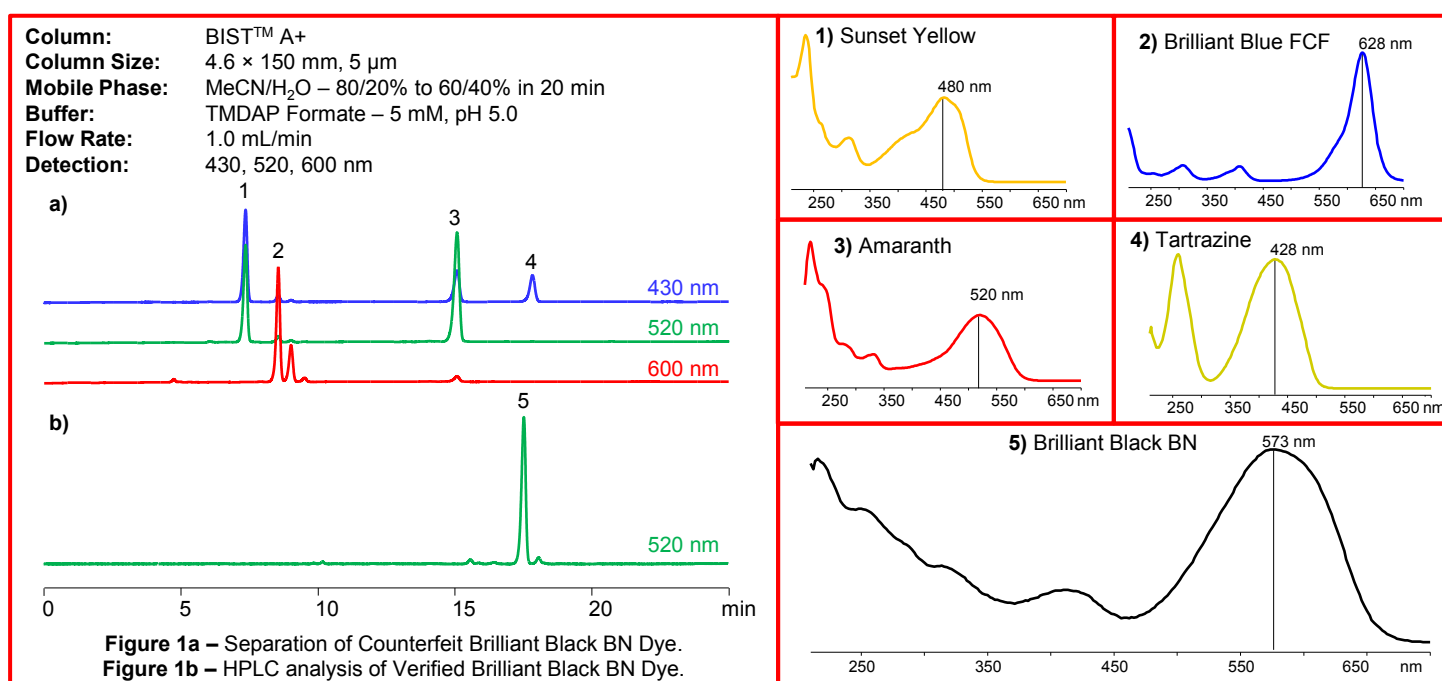


Figure 2 – Chemical structures of **a)** Sunset Yellow, **b)** Brilliant Blue FCF, **c)** Amaranth, **d)** Tartrazine, and **e)** Brilliant Black BN

Introduction - BIST

This new mode, called Bridge Ion Separation Technique, or BIST™, is based on retention of the charged molecules on a stationary phase of the same polarity via forming a bridge between analyte and stationary phase surface by means of a doubly charged ionic component of the mobile phase. This doubly charged mobile phase component, which we call a bridge ion, provides the necessary electrostatic forces to retain ions on a stationary phase of the same charge polarity. This retention mechanism is expressed more profoundly in a mobile phase with reduced water content.

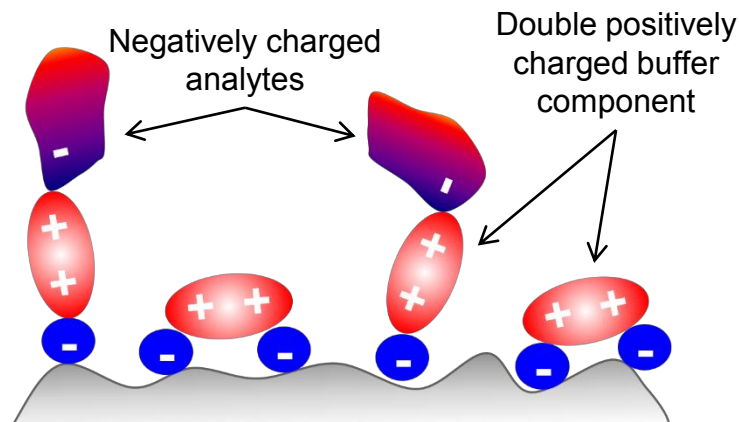


Figure 3 – Diagram of Bridge Formation with a Negatively-Charged Analyte and Negatively-Charged Surface.

For BIST to work, 3 conditions need to be met:

- *A double-charged ionic modifier is present in the mobile phase*
- *The ionic modifier's double-charged ions should be opposite in charge to that of the stationary phase surface*
- *Reduced water content in the mobile phase to minimize ion solvation*

With these conditions fulfilled, the power of BIST™ can be fully unlocked. Figure 4 to the right shows how BIST™ works with the common yellow dye Tartrazine (Yellow 5). When a single-charged Triethylamine (TEA) ionic modifier was employed, almost no retention occurred; however, when a double charged TMDAP (N,N,N',N'-tetramethyldiaminopropane) ionic modifier was employed, the retention time jumped to almost 40 minutes. This is the power of BIST™!

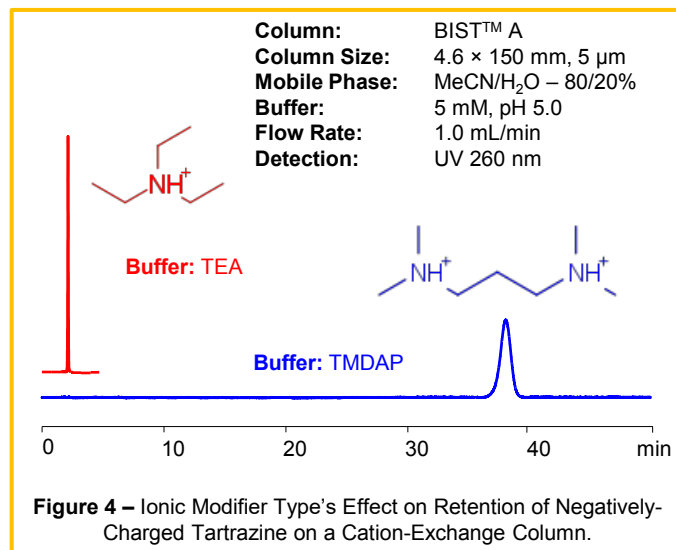


Figure 4 – Ionic Modifier Type's Effect on Retention of Negatively-Charged Tartrazine on a Cation-Exchange Column.

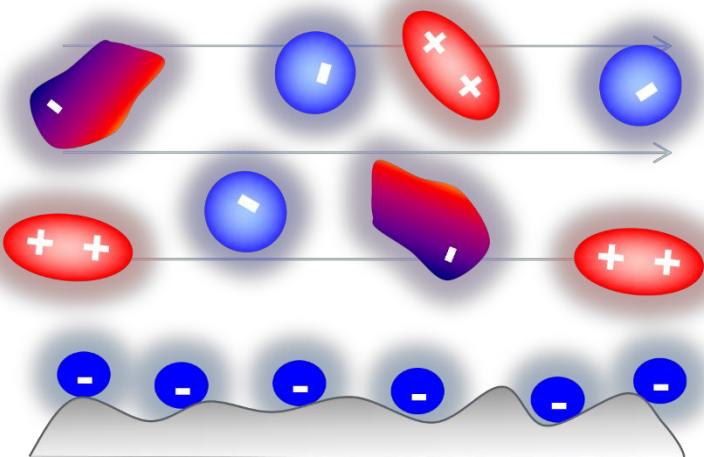
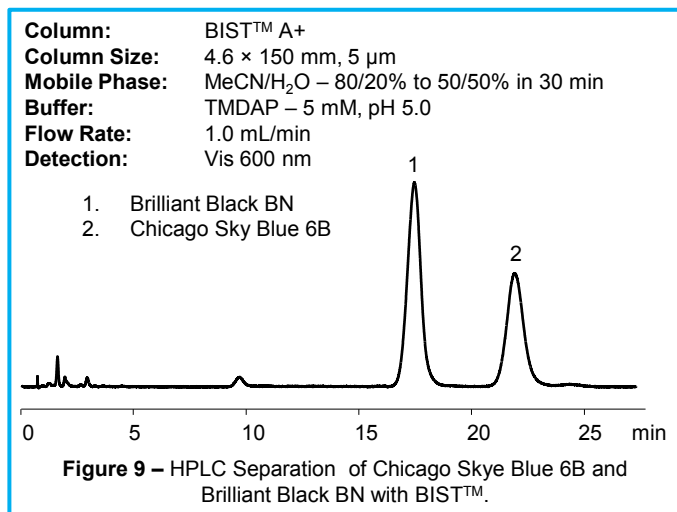
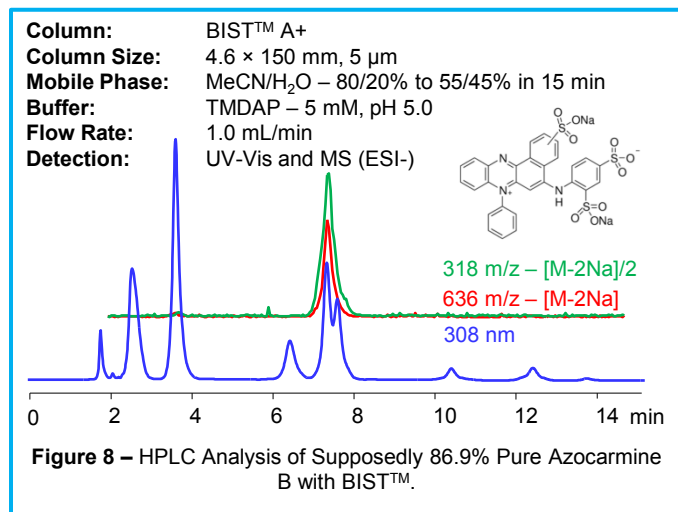
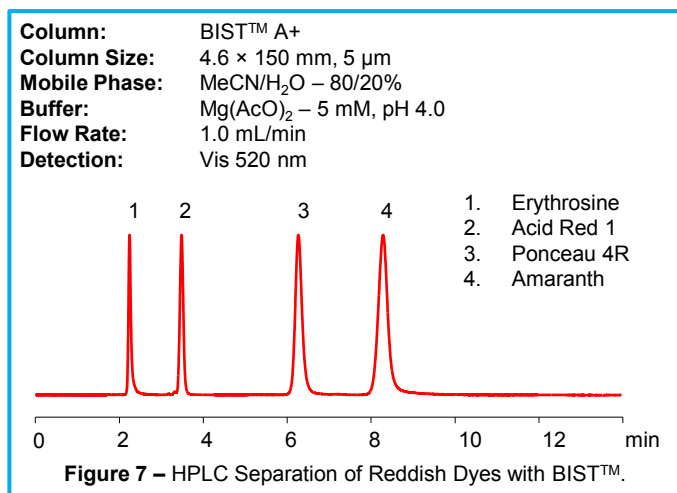
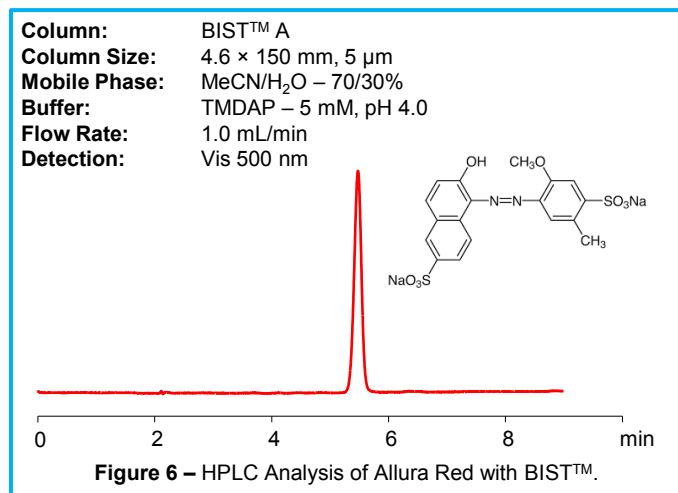
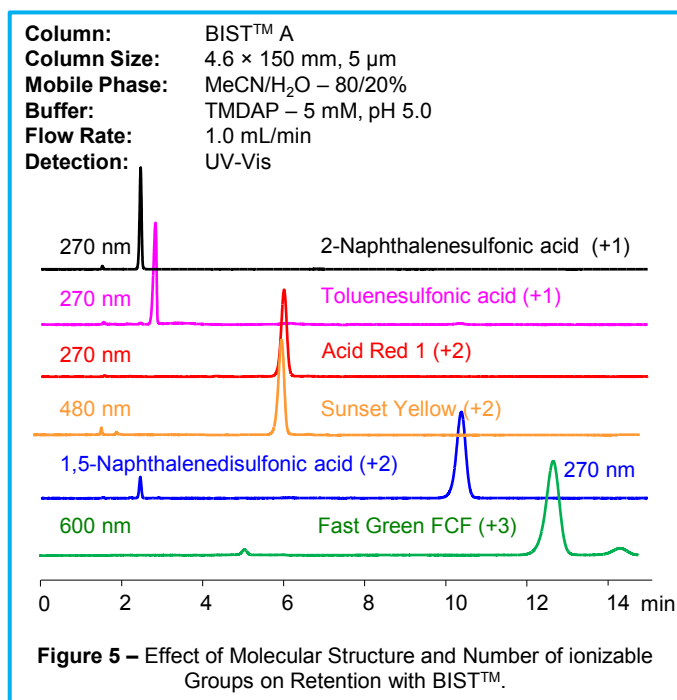
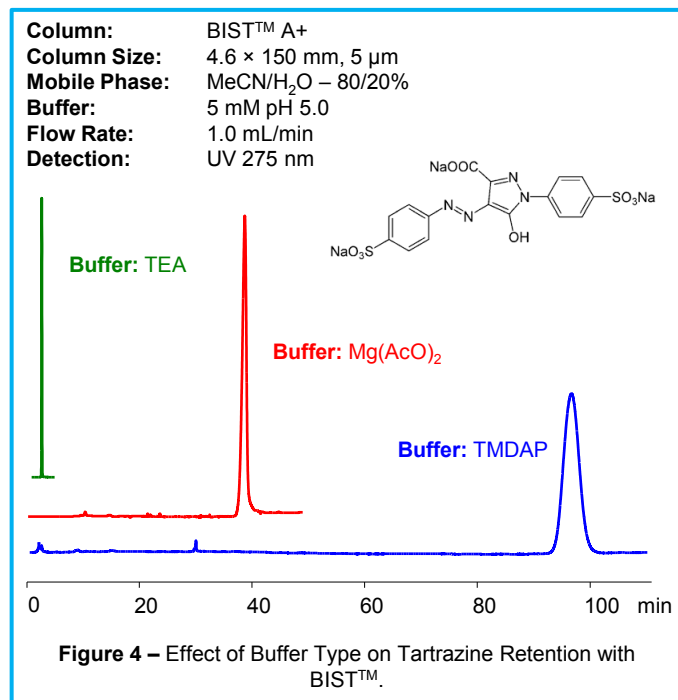


Figure 5 – Diagram of a Fully Aqueous Mobile Phase with a Negatively-Charged Analyte and Negatively-Charged Surface.

In an aqueous mobile phase, as shown to the left in Figure 5, a solvation layer formed by water molecules surrounds each ion. This solvation layer severs any potential interaction between oppositely charged ions, preventing the formation of the ionic bridge key to BIST™. As a result, in high-aqueous mobile phases, little to no retention is observed with either a single or double charged ionic modifier.

Applications

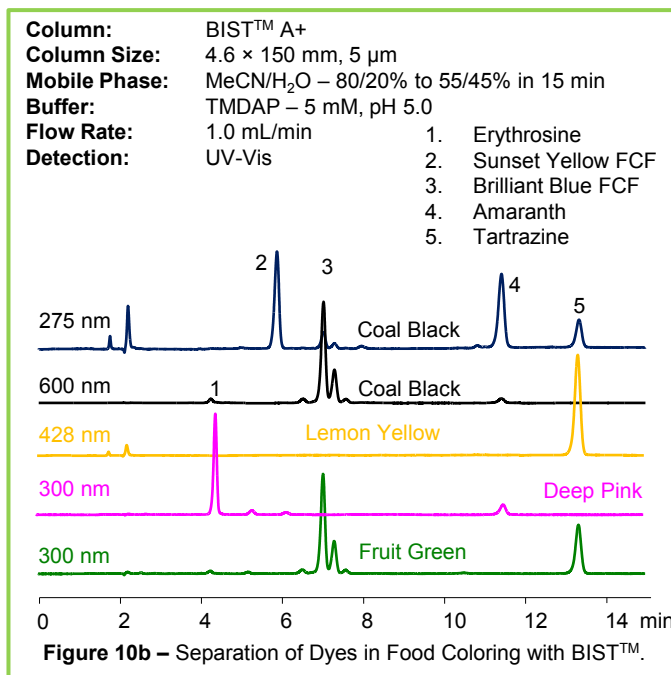
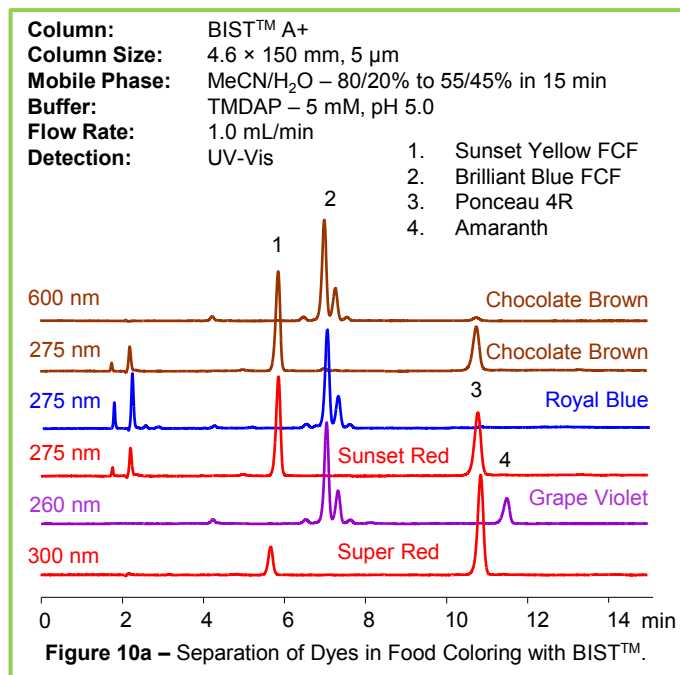
This new retention mode is not limited to just Tartrazine or TMDAP; it works with any charged analyte and can be of particular use for the analysis, retention, and separation of other charged acidic dyes. Here some examples of how other dyes and charged compounds behave under BIST™ conditions. Note that other multi-charged buffers, such as Magnesium Acetate or Calcium Acetate can generate BIST™ separation as well.



Applications

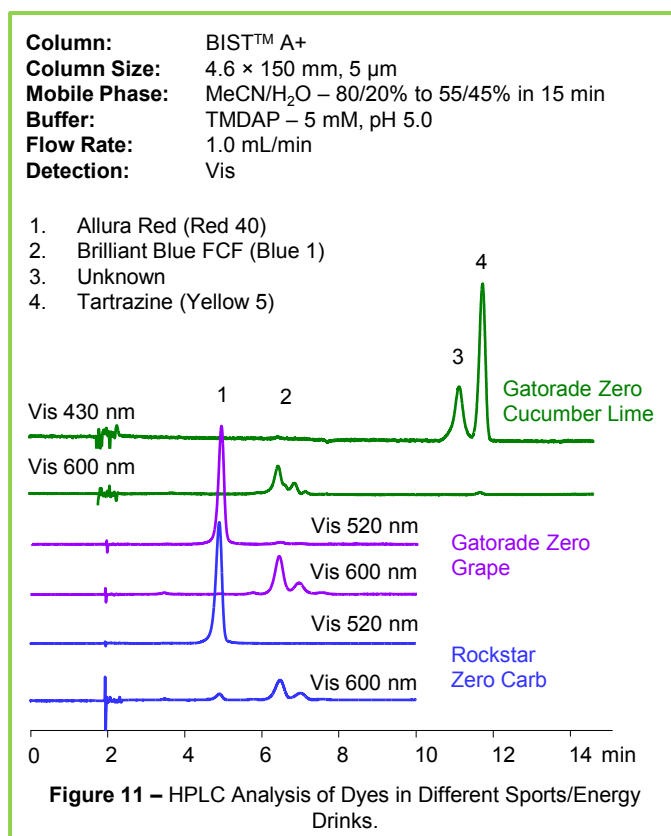
The ability of BIST™ to effectively retain charged acidic dyes can be used to separate multi-dye food colorings into their component dyes in order to verify what the manufacturers claim they used to make their food colorings. This verification can be extremely important for two reasons:

- **Food Safety:** Since only certain dyes are approved for use in foods, it is important for consumer health that the claimed ingredients are present and that manufacturers are not using other, less safe ingredients.
- **Fraud:** Certain dyes might be more expensive than others, and if consumers are paying extra for what they expect to be a better product, then it could be fraudulent and outright stealing if a manufacturer is using lower quality, cheaper ingredients and charging as if it is the better product.



Dye Name	Listed
Super Red	E110, E120, E124
Deep Pink	E127, E123
Sunset Red	E124, E110
Royal Blue	E133, E110, E123
Sunset Yellow	E102, E110
Grape Violet	E123, E133
Lemon Yellow	E102, E110
Chocolate Brown	E124, E110, E133
Coal Black	E123, E102, E110, E133
Fruit Green	E102, E133

Figure 10c – Table of Each Food Coloring and its Component Dyes. **Bolded Dyes were Detected via UV-Vis. Underlined Dyes are Listed as Ingredients.**



Columns and Methods

BIST can retain and separate nearly every type of compound, whether it's charged or not charged; large or small; hydrophilic or hydrophobic. While it cannot retain neutral hydrophobic compounds, these compounds can still be separated from charged or hydrophilic compounds that will retain using BIST conditions. The table below shows which types of compounds can be separated with BIST.

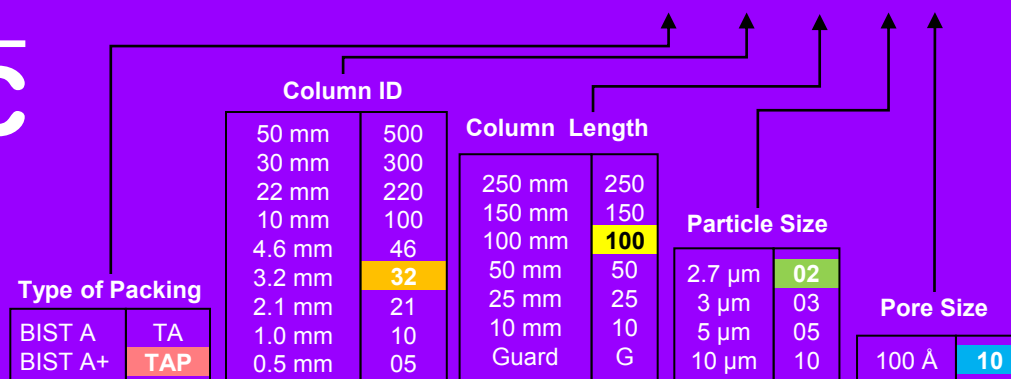
Compound type	RP	HILIC	IE	BIST
Neutral hydrophobic	yes	no	no	no
Neutral hydrophilic	no	yes	yes	yes
Charged hydrophobic	yes	no	yes	yes
Charged hydrophilic	no	yes	yes	yes
Multi-charged hydrophobic	yes	no	no	yes
Multi-charged hydrophilic	no	yes	no	yes

BIST can support a wide variety of detection modes for a wide variety of compounds and separations. However, not every compound can be separated and not every ionic modifier will work ideally with every detection mode. The table below shows our recommendations for which ionic modifier should be used depending on the detection mode and polarity (anion or cation) of the analyte of interest.

Detection Mode	Ionic Modifier	
	Anionic Analyte	Cationic Analyte
Low UV	TMDAP + H ₃ PO ₄	H ₂ SO ₄
Non-Suppressed Conductivity	TMDAP + HCOOH	H ₃ PO ₄
Suppressed Conductivity	N, N, N ,N',N',N'- hexamethyl- ethylenediamin	H ₂ SO ₄
RI	any	any
Fluorescent	any	any
MS	TMDAP + HCOOH	HFGA
ELSD	TMDAP + HCOOH	HFGA

BIST Part Number Generator

TAP – 32 . 100 . 0210



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