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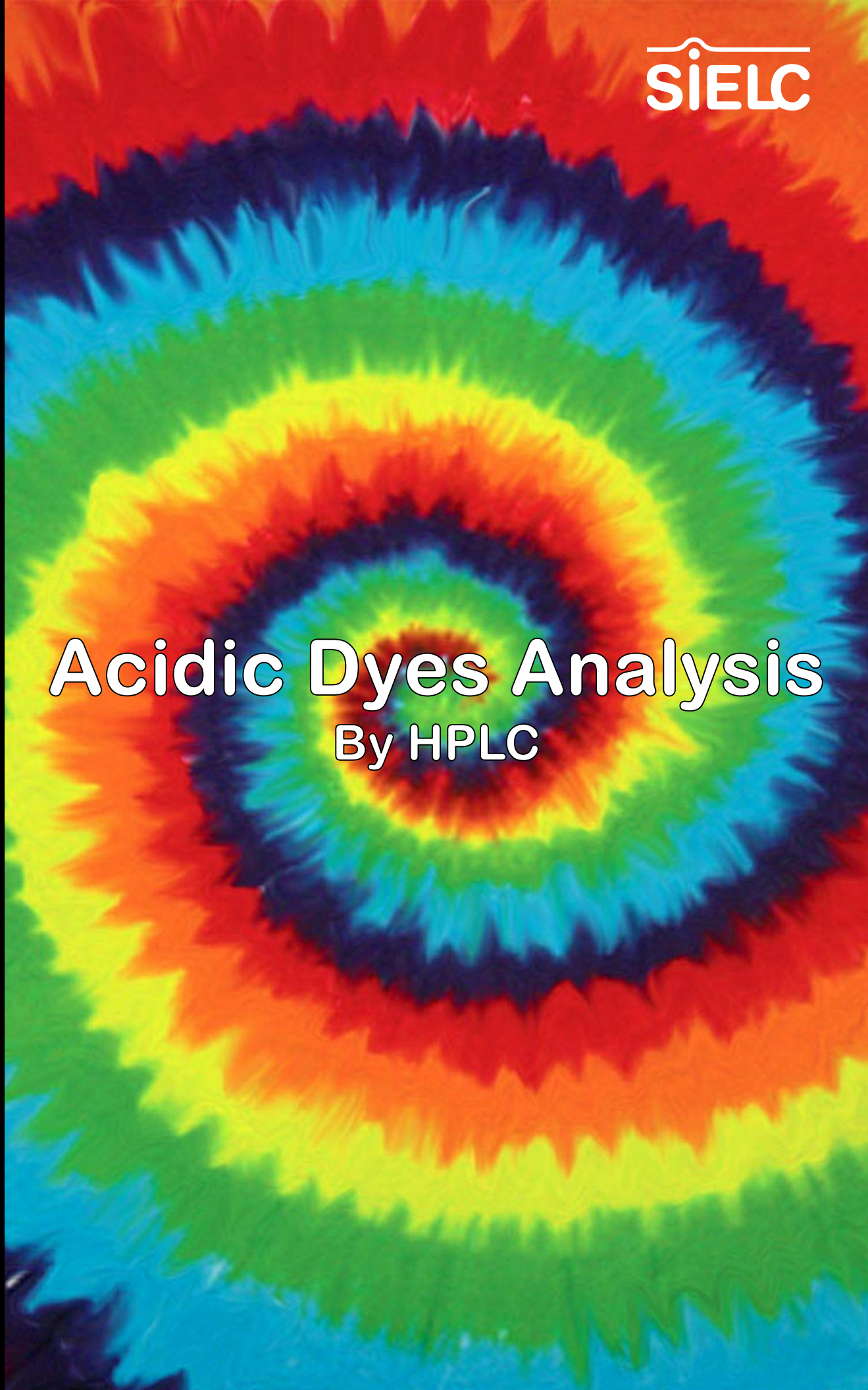

Acidic Dyes Analysis

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.

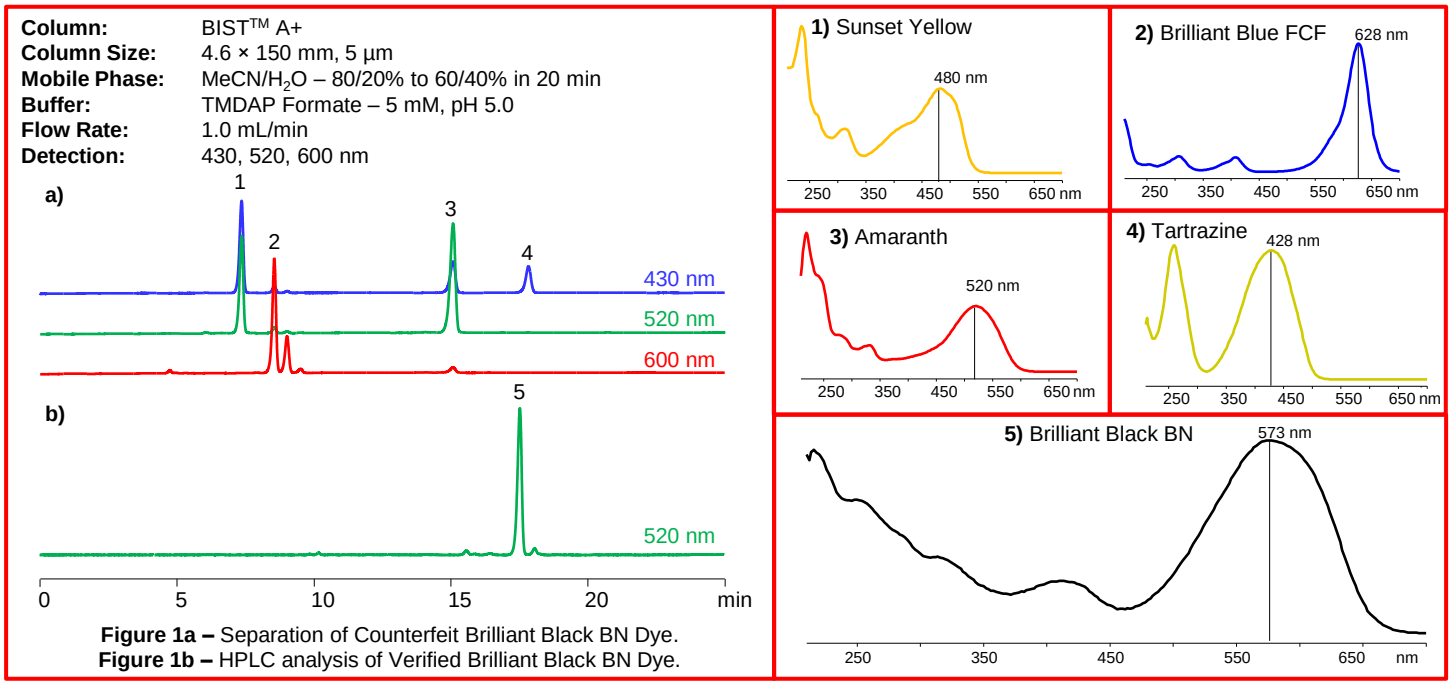

Figure 1a – Separation of Counterfeit Brilliant Black BN Dye.
Figure 1b – HPLC analysis of Verified Brilliant Black BN Dye.

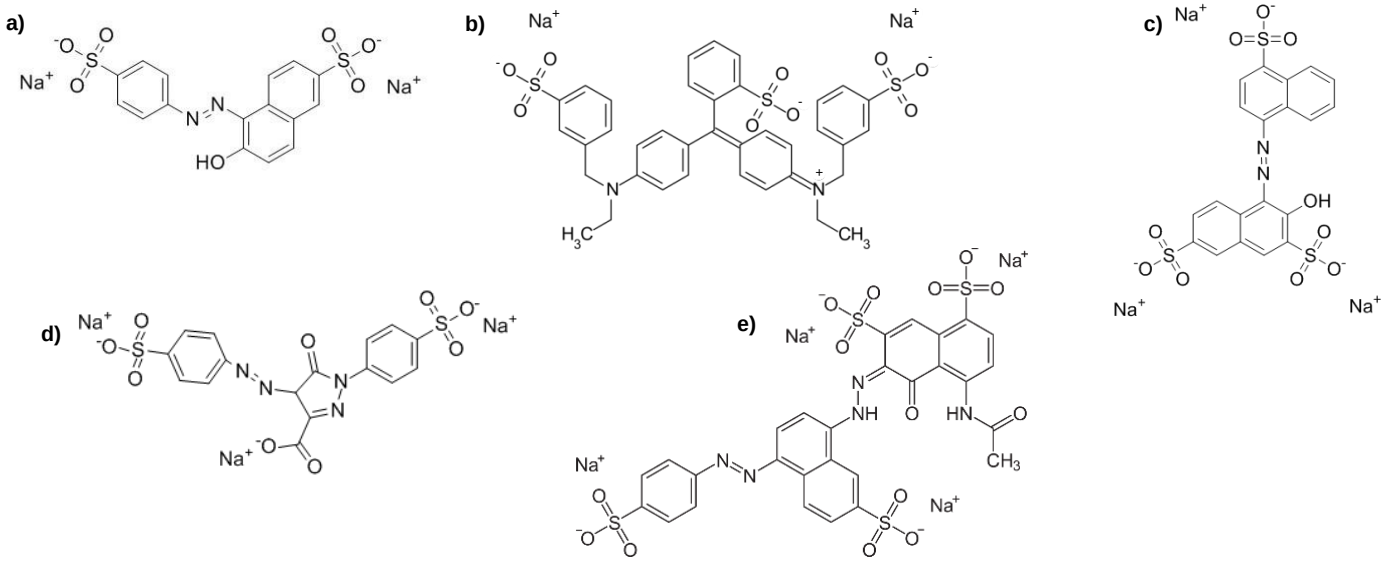

Figure 2 – Chemical structures of **a)** Sunset Yellow, **b)** Brilliant Blue FCF, **c)** Amaranth, **d)** Tartrazine, and **e)** Brilliant Black BN

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 nessesary 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.

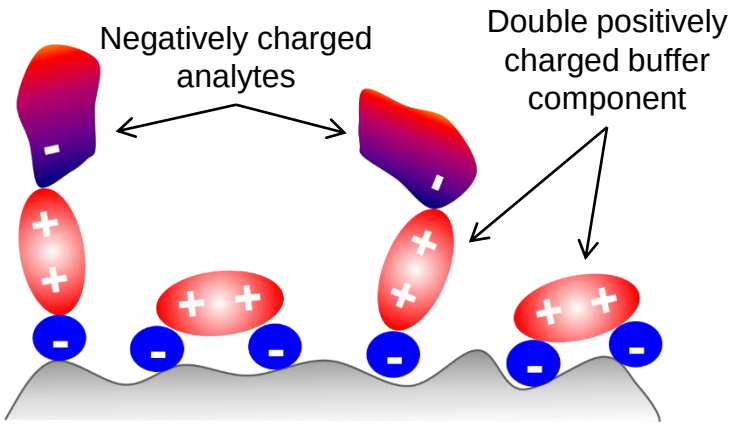

Figure 3 – Diagram of Bridge Formation with a Negatively-Charged Analyte and Negatively-Charged Surface.

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™!

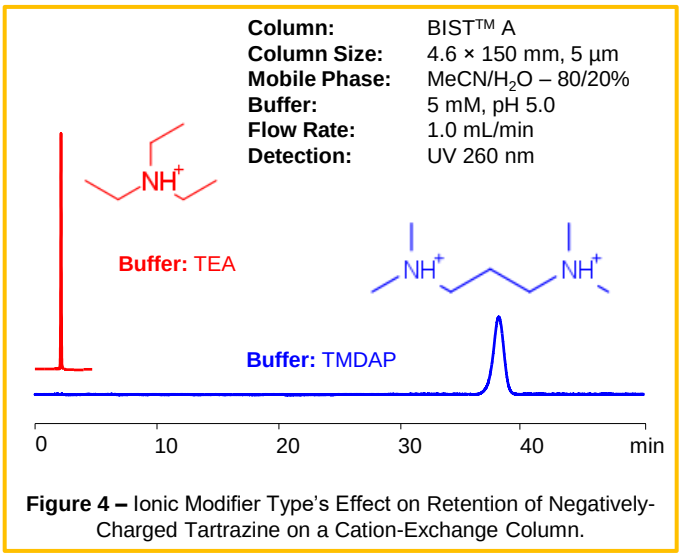

Figure 4 – Ionic Modifier Type's Effect on Retention of Negatively-Charged Tartrazine on a Cation-Exchange Column.

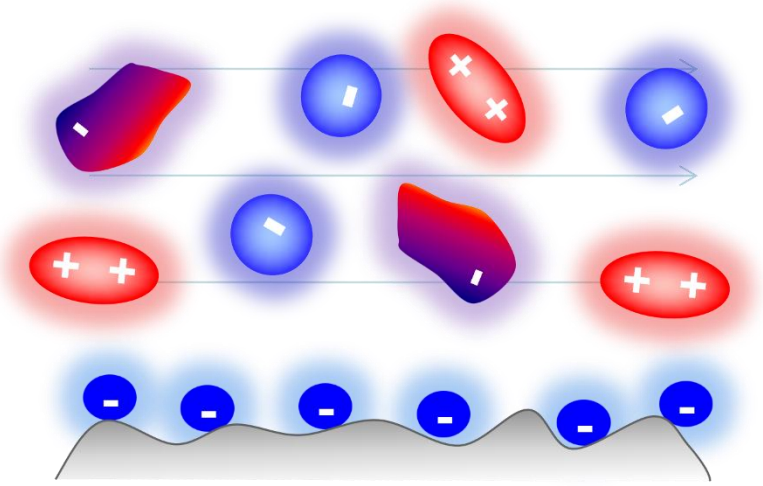

Figure 5 – Diagram of a Fully Aqueous Mobile Phase with a Negatively-Charged Analyte and Negatively-Charged Surface.

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.

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.

Column: BIST™ A+
Column Size: 4.6 × 150 mm, 5 µm
Mobile Phase: MeCN/H_2O – 80/20%
Buffer: 5 mM pH 5.0
Flow Rate: 1.0 mL/min
Detection: UV 275 nm

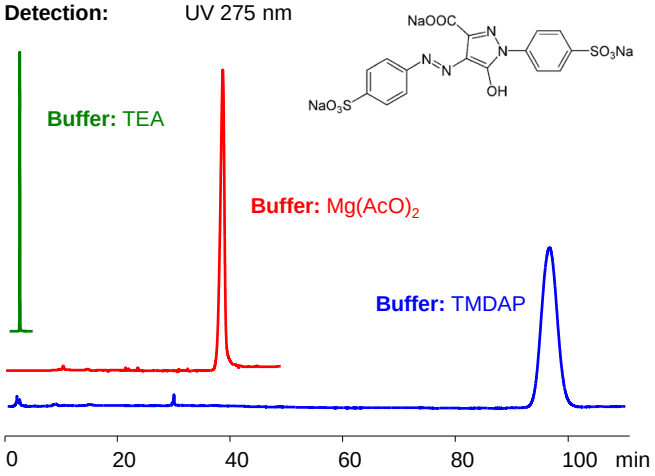

Figure 4 – Effect of Buffer Type on Tartrazine Retention with BIST™.

Column: BIST™ A
Column Size: 4.6 × 150 mm, 5 µm
Mobile Phase: MeCN/H_2O – 80/20%
Buffer: TMDAP – 5 mM, pH 5.0
Flow Rate: 1.0 mL/min
Detection: UV-Vis

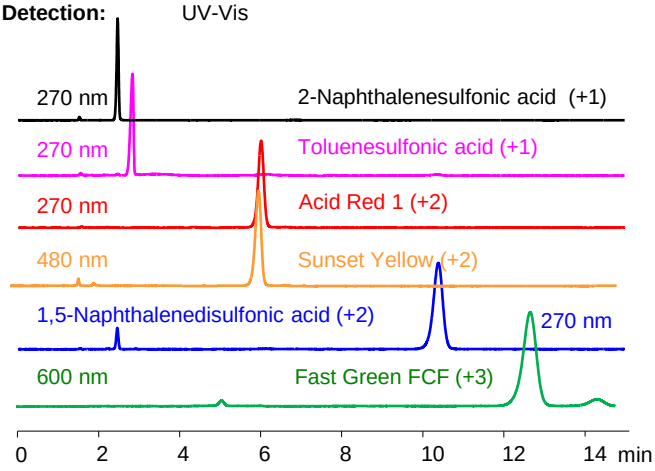

Figure 5 – Effect of Molecular Structure and Number of ionizable Groups on Retention with BIST™.

Column: BIST™ A
Column Size: 4.6 × 150 mm, 5 µm
Mobile Phase: MeCN/H_2O – 70/30%
Buffer: TMDAP – 5 mM, pH 4.0
Flow Rate: 1.0 mL/min
Detection: Vis 500 nm

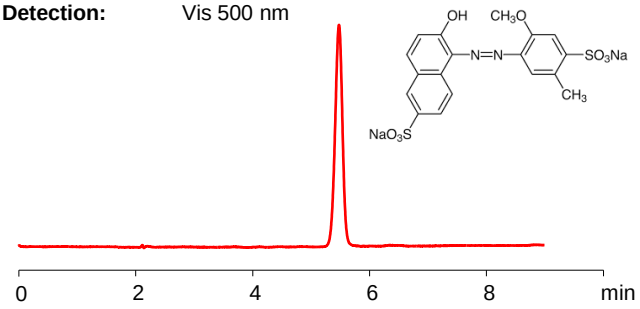

Figure 6 – HPLC Analysis of Allura Red with BIST™.

Column: BIST™ A+
Column Size: 4.6 × 150 mm, 5 µm
Mobile Phase: MeCN/H_2O – 80/20%
Buffer: $Mg(AcO)_2$ – 5 mM, pH 4.0
Flow Rate: 1.0 mL/min
Detection: Vis 520 nm

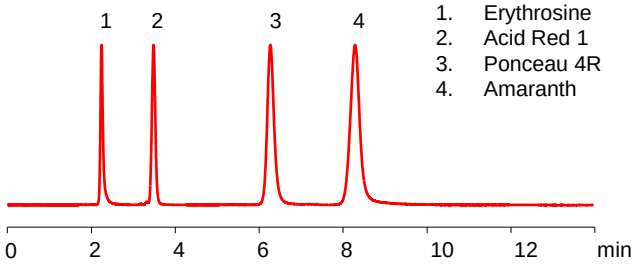

Figure 7 – HPLC Separation of Reddish Dyes with BIST™.

Column: BIST™ A+
Column Size: 4.6 × 150 mm, 5 µm
Mobile Phase: MeCN/H_2O – 80/20% to 55/45% in 15 min
Buffer: TMDAP – 5 mM, pH 5.0
Flow Rate: 1.0 mL/min
Detection: UV-Vis and MS (ESI-)

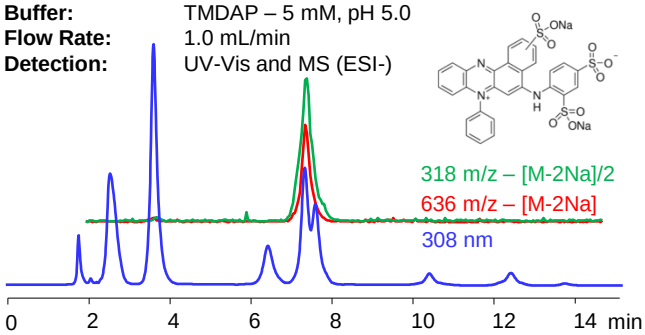

Figure 8 – HPLC Analysis of Supposedly 86.9% Pure Azocarmine B with BIST™.

Column: BIST™ A+
Column Size: 4.6 × 150 mm, 5 µm
Mobile Phase: MeCN/H_2O – 80/20% to 50/50% in 30 min
Buffer: TMDAP – 5 mM, pH 5.0
Flow Rate: 1.0 mL/min
Detection: Vis 600 nm

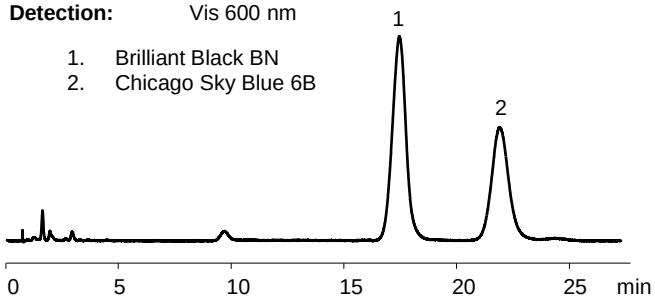

Figure 9 – HPLC Separation of Chicago Skye Blue 6B and Brilliant Black BN with BIST™.

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.

Column: BIST™ A+
Column Size: 4.6 × 150 mm, 5 µm
Mobile Phase: MeCN/H_2O – 80/20% to 55/45% in 15 min
Buffer: TMDAP – 5 mM, pH 5.0
Flow Rate: 1.0 mL/min
Detection: UV-Vis

1. Sunset Yellow FCF
2. Brilliant Blue FCF
3. Ponceau 4R
4. Amaranth

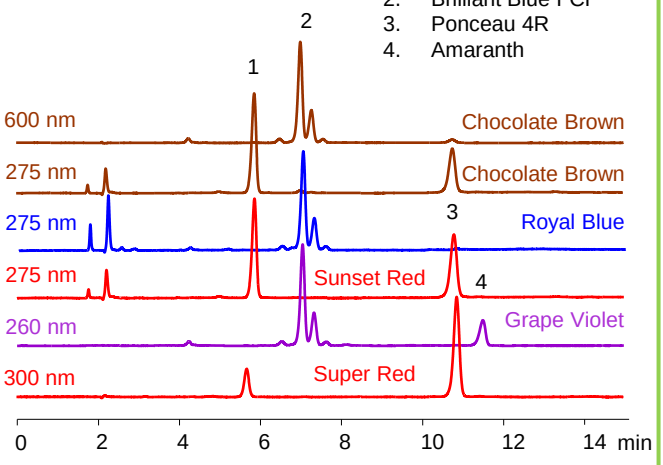

Figure 10a – Separation of Dyes in Food Coloring with BIST™.

Column: BIST™ A+
Column Size: 4.6 × 150 mm, 5 µm
Mobile Phase: MeCN/H_2O – 80/20% to 55/45% in 15 min
Buffer: TMDAP – 5 mM, pH 5.0
Flow Rate: 1.0 mL/min
Detection: UV-Vis

1. Erythrosine
2. Sunset Yellow FCF
3. Brilliant Blue FCF
4. Amaranth
5. Tartrazine

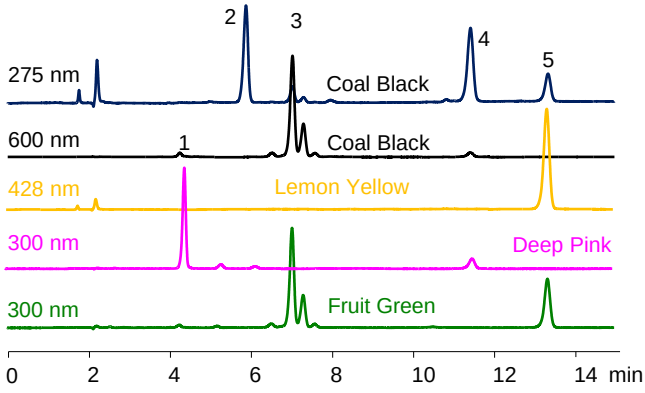

Figure 10b – Separation of Dyes in Food Coloring with BIST™.

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.

Column: BIST™ A+
Column Size: 4.6 × 150 mm, 5 µm
Mobile Phase: MeCN/H_2O – 80/20% to 55/45% in 15 min
Buffer: TMDAP – 5 mM, pH 5.0
Flow Rate: 1.0 mL/min
Detection: Vis

1. Allura Red (Red 40)
2. Brilliant Blue FCF (Blue 1)
3. Unknown
4. Tartrazine (Yellow 5)

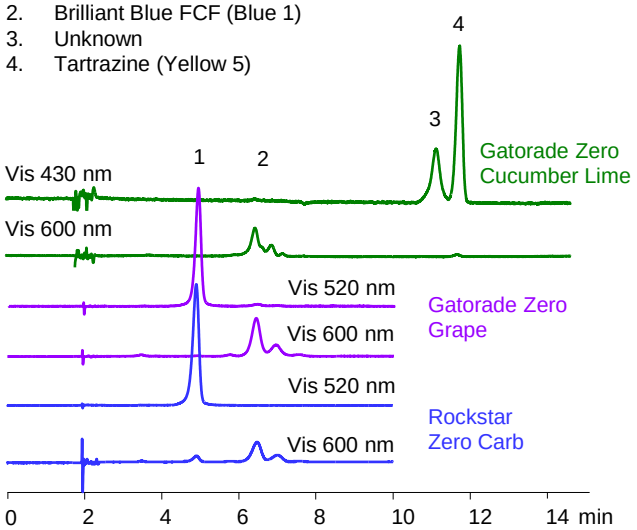

Figure 11 – HPLC Analysis of Dyes in Different Sports/Energy Drinks.

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_3PO_4	H_2SO_4
Non-Suppressed Conductivity	TMDAP + HCOOH	H_3PO_4
Suppressed Conductivity	N, N, N ,N',N',N'-hexamethyl-ethylenediamin	H_2SO_4
RI	any	any
Fluorescent	any	any
MS	TMDAP + HCOOH	HFGA
ELSD	TMDAP + HCOOH	HFGA

BIST Part Number Generator **TAP – 32 . 100 . 0210**