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SIELC

ANALYZING PHMB
BY HPLC

Introduction: PHMB Separation Problem

Polyhexanide (PHMB, polyhexamethylene biguanide) has multiple industrial, medical, biological and research applications. It has strong antiseptic properties that make this compound very useful for materials that could be a possible source of bacterial contamination.

Typically, however, several colluding factors make this compound quite difficult to analyze via HPLC:

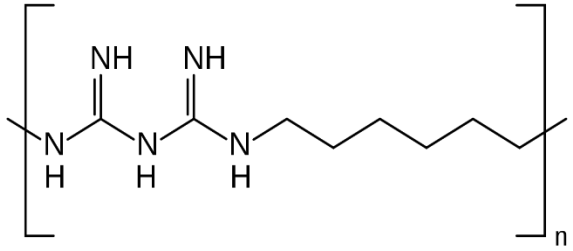

Figure 1 – Chemical Structure of the Repeating Hexamethylene Unit of PHMB.

- Interactions with silanol groups on silica-based columns, which are the most common for HPLC
- Multiple charges in acidic and neutral pH environments, which are the most common for HPLC
- Low UV absorption above 230 nm
- Strong chelating properties often lead to retention by the metal components of HPLC system
- Wide molecular weight distribution produces broad signal in typical separation methods
- Often part of a formulation including other excipients that complicate separation and detection

SIELC has developed a new selective set of methods to solve these difficulties. SIELC's recently announced Bridge Ion Separation Technology (BIST™) offers a simple and reliable method for PHMB analysis and quantitation in liquid samples.

In this brief brochure, SIELC now offers three different methods for PHMB analysis, each with its own unique benefits that can be used according to the specific analysis requirements your lab needs.

Method #1

The first method offered is quite useful when a group analysis of the entire range of molecular weights (MWs) of a sample of PHMB is required. This could be useful to acquire a general understanding of the total amount of PHMB in a particular sample.

This method, shown in Figure 2, relies on a simple isocratic method of Acetonitrile (MeCN), Water (H_2O), and Ammonium formate (AmFm), and can be completed in under 6 minutes. This is a fairly simple method that will give researchers a quick understanding of how much PHMB is prevalent in the solutions they are working with. It relies on size-ion-exclusion chromatography with positive-to-positive electrostatic interactions between the analyte and the stationary phase surface.

Column: BIST™ B+
Column Size: 4.6 × 250 mm, 5 µm
Mobile Phase: MeCN/H_2O – 40/60%
Buffer: AmFm – 20mM, pH 3.0
Flow Rate: 0.5 mL/min
Detection: UV 236 nm

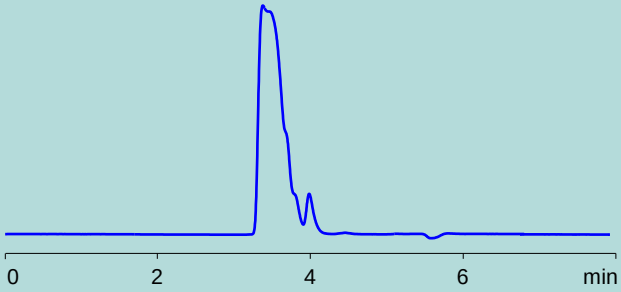

Figure 2 – Typical Retention of PHMB on a BISTB+ Column.

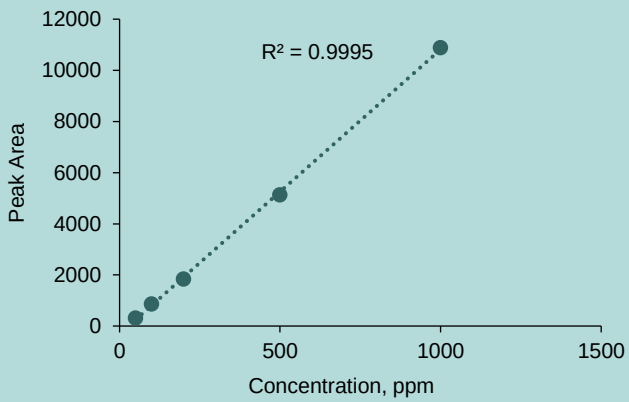

Figure 3 – PHMB Response at 236 nm vs Concentration

Method #2

In situations where you'll need to separate PHMB from other unwanted excipients, a stronger retention mode is required. This is where BIST™ can be fully utilized. Due to the multi-charged ionic bridge that allows BIST™ to function, the retention of charged analytes is highly dependent on the relative water content of the mobile phase, as we will see on page 4. This dependence can be exploited with respect to PHMB by using a step-gradient. The step-gradient allows researchers to modify the retention of PHMB by simply changing the step timing.

In figure 4 below, the retention of PHMB is shown with a step-gradient that starts at 60% ACN, 40% H_2O, and 0.2% H_2SO_4, and then switches to 100% H_2O after 2 minutes. The conditions of this gradient method can be tuned in order to maximize the separation efficiency of this method for your particular sample.

The following are a couple of tips on how to alter the retention and separation characteristics of PHMB while using BIST™:

- **Increase** [MeCN]/**Decrease** [H_2O] **in the 1st step:** Increasing the ratio of MeCN to H_2O will not increase the retention of PHMB but can change retention of other charged or neutral species.

- **Increase the length of the 1st step:** Increasing the length of the 1st step (i.e. longer than 2 minutes) will increase the retention of PHMB but decrease retention of other charge and neutral species.

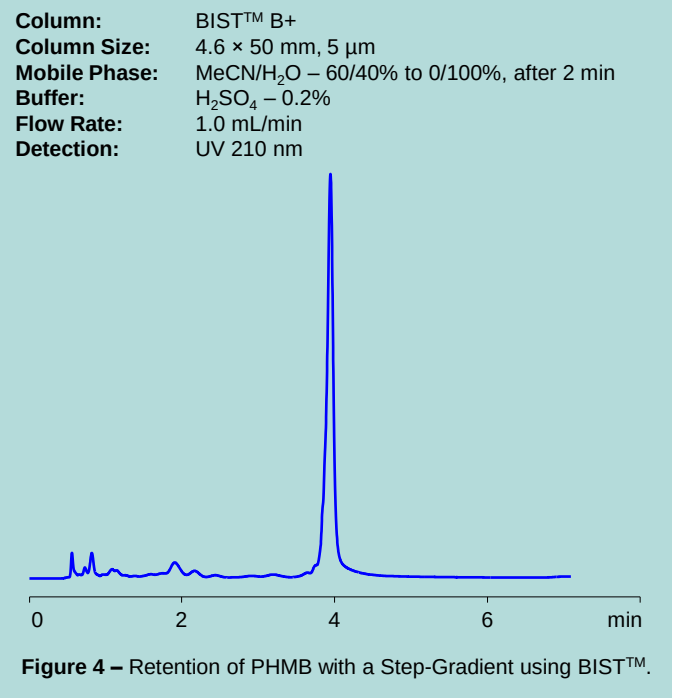

Figure 4 – Retention of PHMB with a Step-Gradient using BIST™.

Method #3

The third method is able to separate PHMB into its constituent oligomers based on the molecular weight, charge, and possibly even the geometry of each fraction.

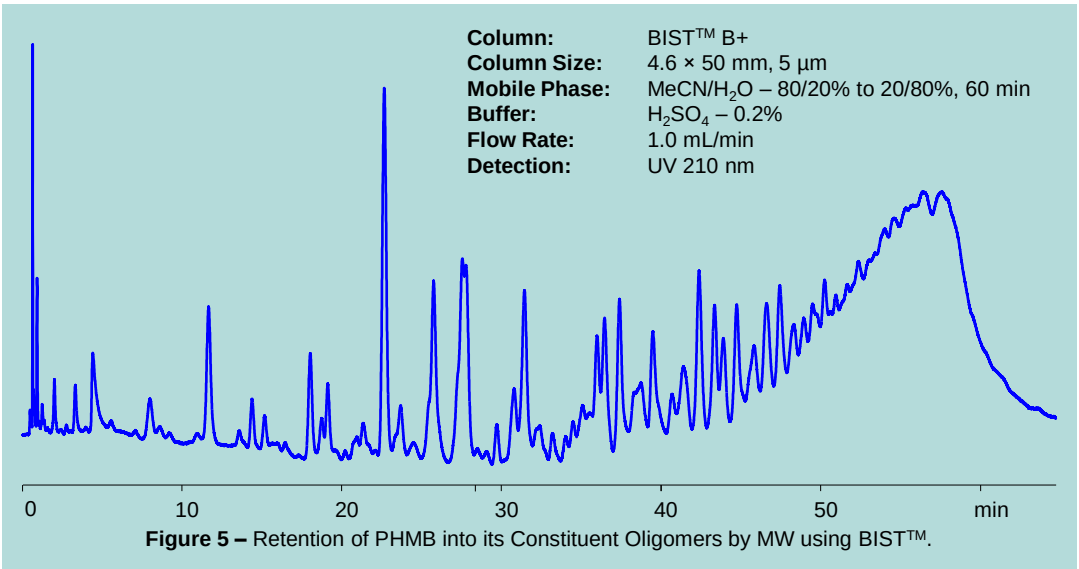

Figure 5 – Retention of PHMB into its Constituent Oligomers by MW using BIST™.

This new method shown in Figure 5 utilizes a reverse gradient of MeCN and H_2O with an H_2SO_4 ionic modifier and extends to over 60 minutes, providing a general profile of the PHMB composition.

Two last methods in this brochure utilizes a new separation mode developed by SIELC called Bridge Ion Separation Technology, or BIST™. This mode generates the retention of charged molecules on a stationary phase of the same polarity via forming a bridge between the analyte and stationary phase surface. This is done by means of a doubly charged ionic modifier in the mobile phase. This doubly charged ionic 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 most profoundly in a mobile phase with reduced water content.

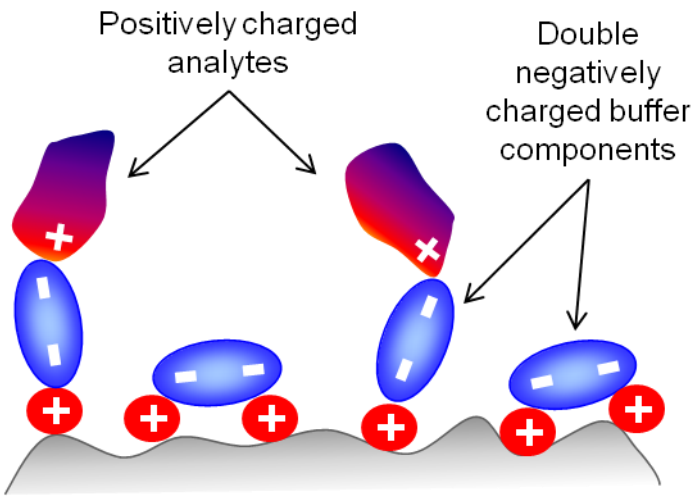

Figure 6 – Diagram of Bridge Formation with a Positively-Charged Analyte and Positively-Charged Surface.

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 and analytes*
- *Reduced water content in the mobile phase to minimize ion solvation*

When a single-charged ionic modifier, such as Trifluoroacetic Acid (TFA) is employed, almost no retention will occur; however, when a double-charged ionic modifier, like Sulfuric Acid (H_2SO_4), is employed, the retention time will increase significantly. In an aqueous mobile phase, a solvation layer formed by water molecules will surround and separate each ion. This solvation layer will increase mobility of the ions, preventing the formation of the ionic stationary bridge. As a result, in high-aqueous mobile phases, little to no retention is observed with either a single or double charged ionic modifier.

BIST™ Part Number Generator

TBP – 32 . 100 . 0210

Type of Packing

BIST B	TB
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**
300 Å*	30
800 Å*	80

*Only available with 5 µm particles

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