

Primesep SB Column: a New Tool for Separation of Small Molecules

By SIELC, Inc. Prospect Heights, IL, USA
www.sielc.com

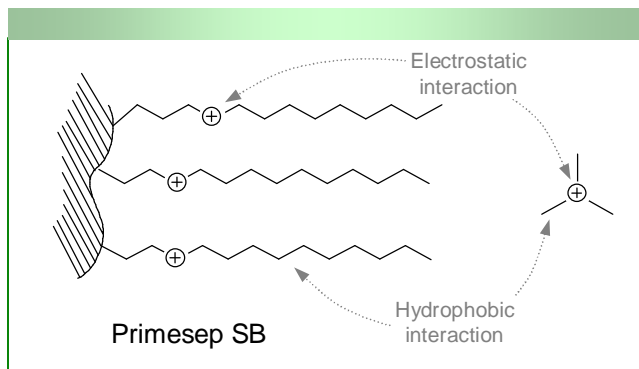


Figure 1. Simplified structure of stationary phase with mixed-mode ligand attached to silica support and two main interactions occurred with charged analytes.

Table 1. Type of molecules most suitable for separation by Primesep SB column in RP conditions

	Hydrophobic molecules	Hydrophilic molecules
Strong acidic	n/a	Fig. 2, 5
Weak acidic	Fig. 15	Fig. 3, 4, 9-11, 13-15
Neutral	Fig. 6, 7	Fig. 10,14
Zwitterionic	yes	Fig. 4,11,12
Weak basic	Fig. 15	n/a
Strong basic	Fig. 8	n/a

The liquid chromatography process involves the interaction of analytes with a stationary phase. Hydrophobic interaction and electrostatic interaction are the two most commonly used liquid chromatography forces. Primesep SB column, a new addition to Primesep column family, utilizes both of these interactions at the same time. The column has proprietary stationary phase with strong basic properties due to embedded strongly basic functionality and strong hydrophobic properties due to a long carbon chain (Fig. 1). This column demonstrates significant improvements in retention and selectivity for separation of weak acidic analytes with or without hydrophobic characteristics (Fig. 3, 4, 9-11, 13-15). Primesep SB column provides good retention for zwitterionic compounds with a strong acidic fragment (Fig. 12). These molecules are unretained on traditional reverse phase columns. Basic hydrophobic compounds are usually retained less on Primesep SB column than on similar RP due to charge repulsion effect.

Primesep SB Application Examples

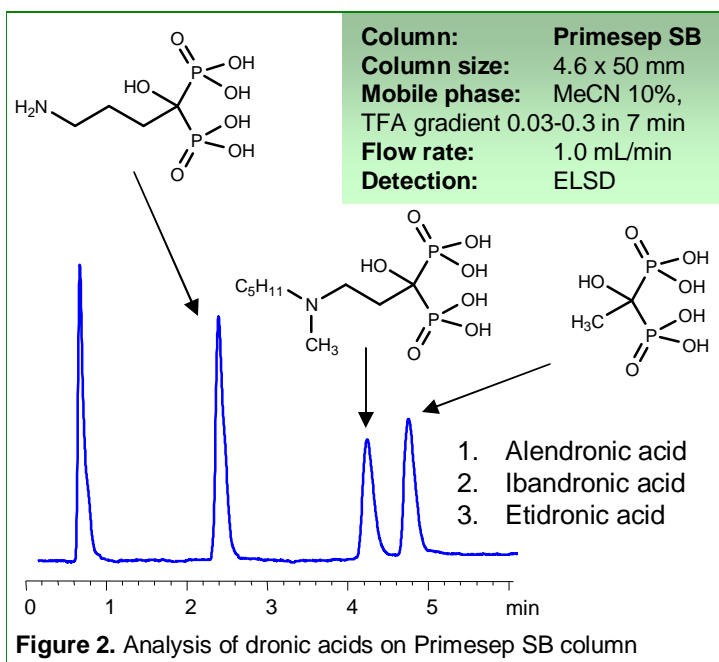


Figure 2. Analysis of dronic acids on Primesep SB column

Very symmetrical peak is usually observed due to masking effect by positively charged ligand, which eliminates silica interaction.

Primesep SB column is one of the best separation media for natural product extracts. High selectivity and high resolution are observed with complex mixtures (Fig. 4).

Primesep SB is the ideal column for separation of neutral compounds due to strong hydrophobic interaction of C18 carbon chain. Common solvents such as MeOH, MeCN, and THF can be used as organic modifier giving different selectivity (Fig. 6, 7). The column offers alternative selectivity compared with other silica-based columns.

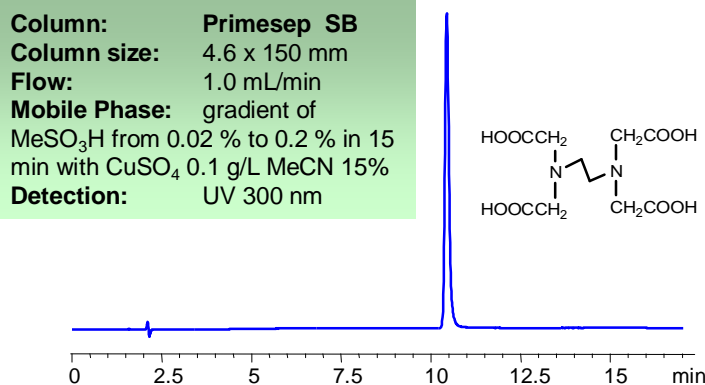
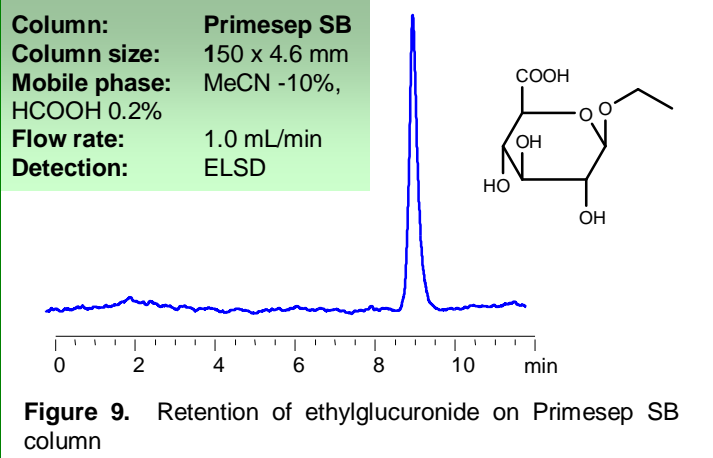
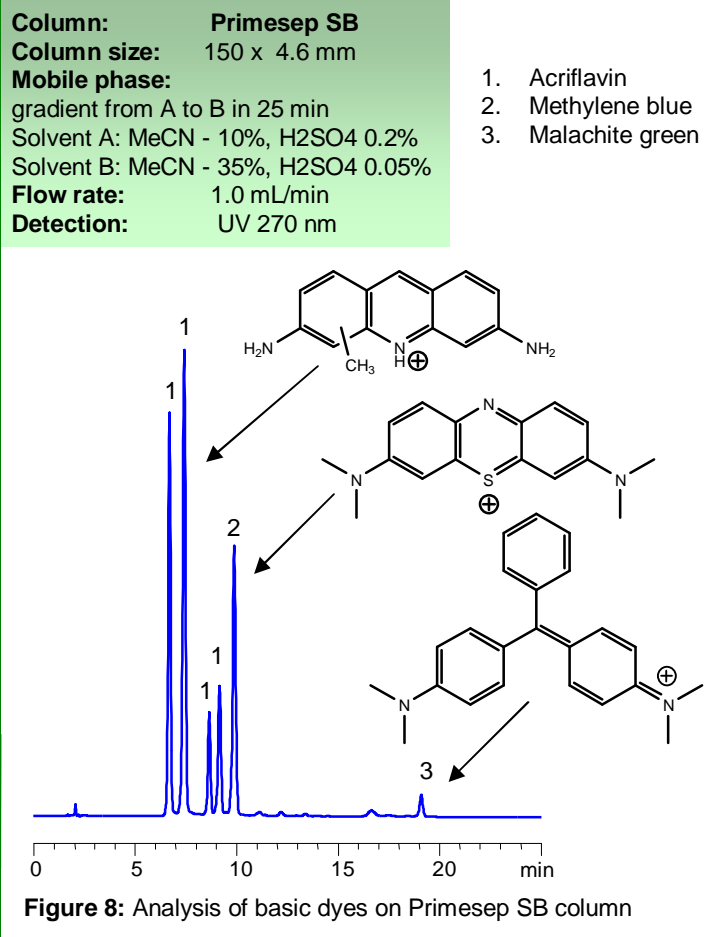
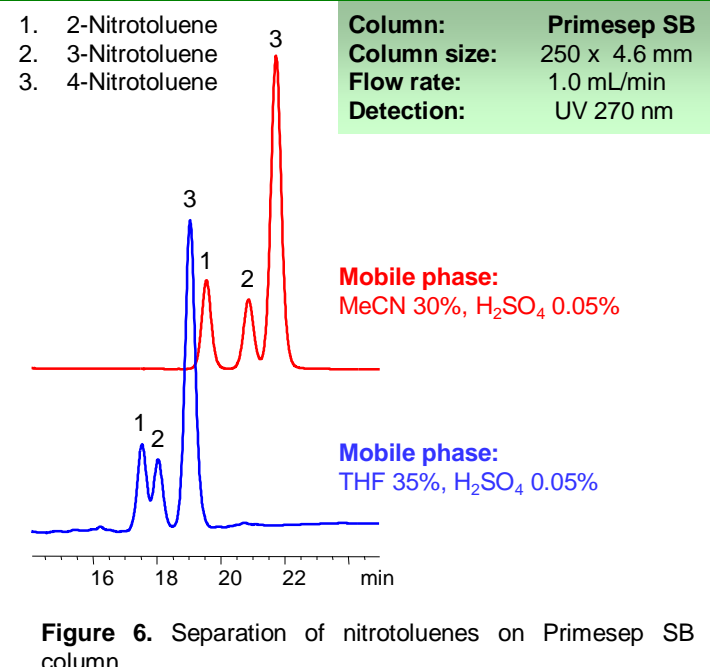
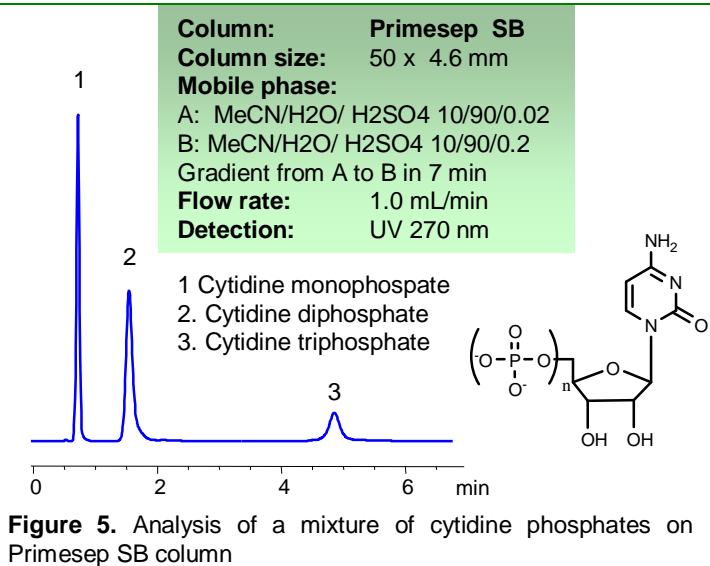
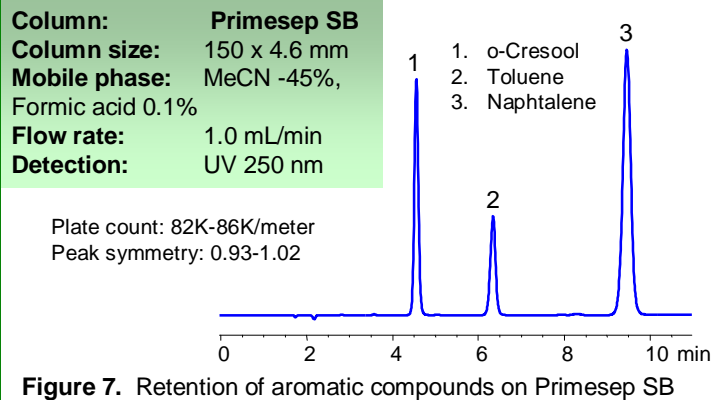
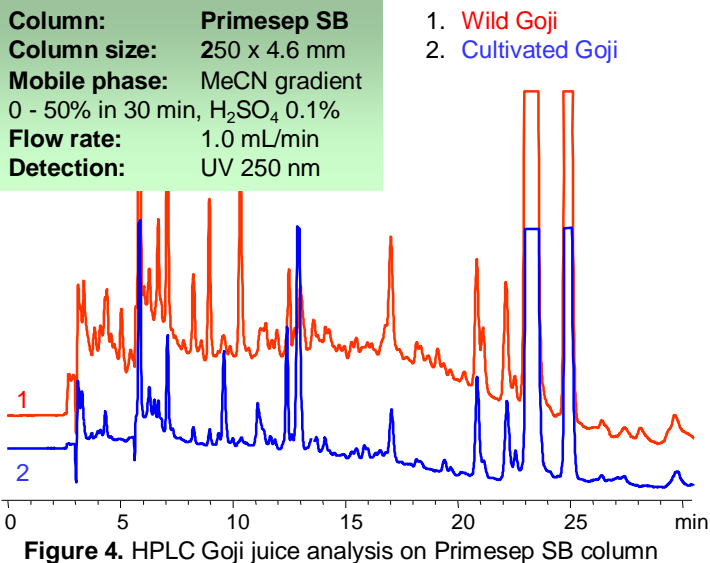


Figure 3. Analysis of EDTA as a Cu⁺⁺ complex on Primesep SB column



Column: Primesep SB
Column size: 150 x 4.6 mm
Mobile phase: MeCN -20%,
 HCOOH 0.1%
Flow rate: 1.0 mL/min
Detection: ELSD

1. Dehydroascorbic Acid
2. Ascorbic Acid

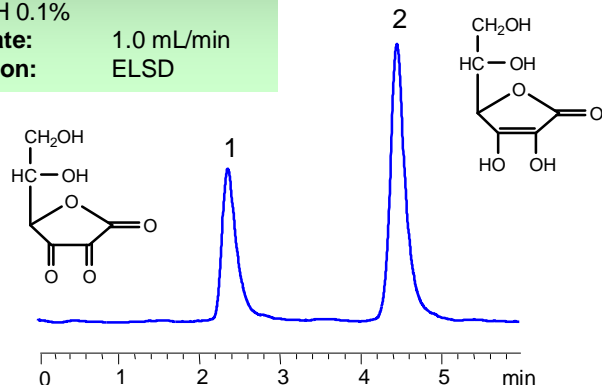


Figure 10. Retention and separation of ascorbic and dehydroascorbic acids on Primesep SB column

Column: Primesep SB
Column size: 250 x 4.6 mm
Mobile phase: MeCN -50%,
 H₂SO₄ 0.05%
Flow rate: 1.0 mL/min
Detection: UV 210 nm

1. Acetic Acid
2. Formic Acid

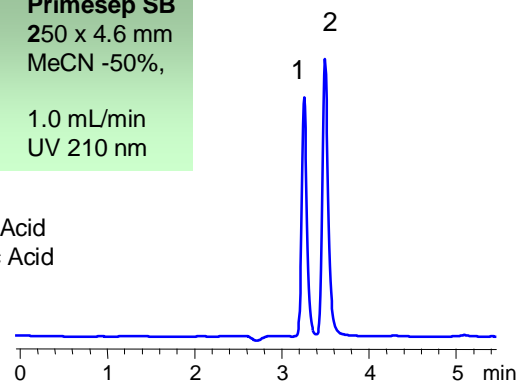


Figure 13. Separation and retention of acetic and formic acids on Primesep SB column

1. Glyphosate
2. PMDAA

Column: Primesep SB
Column size: 50 x 4.6 mm
Mobile phase: MeCN -10%,
 AmFm pH 3.1 gradient from 10
 to 50 mM in 5 min + 5 min hold
Flow rate: 1.0 mL/min
Detection: ELSD

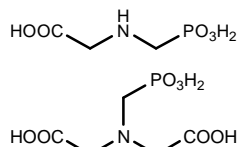
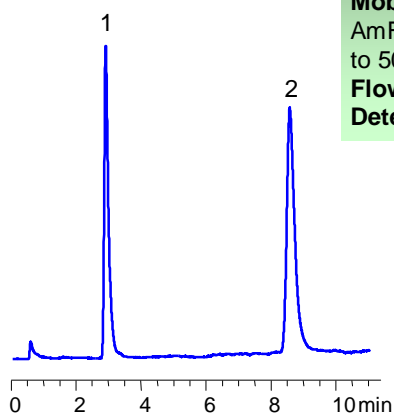


Figure 11. Retention of glyphosate and glyphosate's production intermediate (PMDAA) on Primesep SB column

Column: Primesep SB
Column size: 4.6 x 50 mm
Flow rate: 1.0 mL/min
Detection: ELSD

Mobile phase:
 MeCN -20%, AmFm
 10 mM pH 3.0

Mobile phase:
 MeCN -20%, AmFm
 5 mM pH 3.0

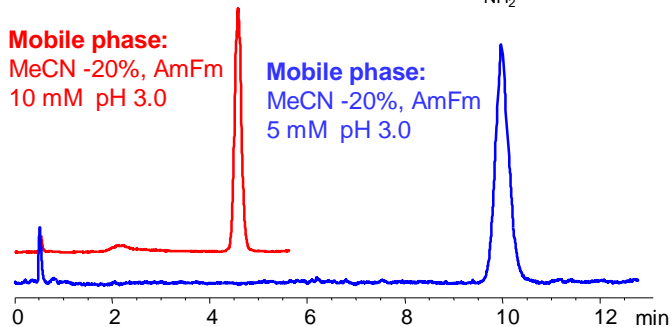
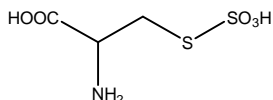
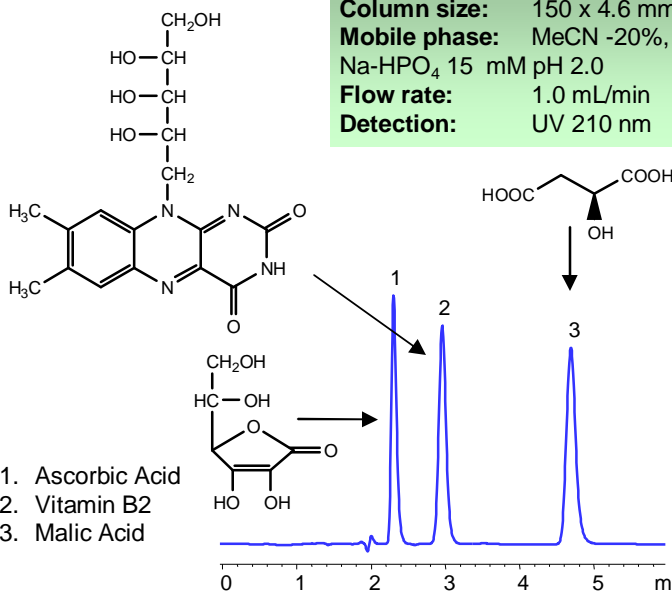


Figure 12. Analysis of cysteine-S-sulfate on Primesep SB column

Column: Primesep SB
Column size: 150 x 4.6 mm
Mobile phase: MeCN -20%,
 Na-HPO₄ 15 mM pH 2.0
Flow rate: 1.0 mL/min
Detection: UV 210 nm



1. Ascorbic Acid
2. Vitamin B2
3. Malic Acid

Figure 14. Retention and separation of vitamins and malic acid on Primesep SB column

Column: Primesep SB
Column size: 250 x 4.6 mm
Flow rate: 1.0 mL/min
Detection: UV 250 nm

1. 4-Aminoacetophenone
2. Phenol
3. Toluidine

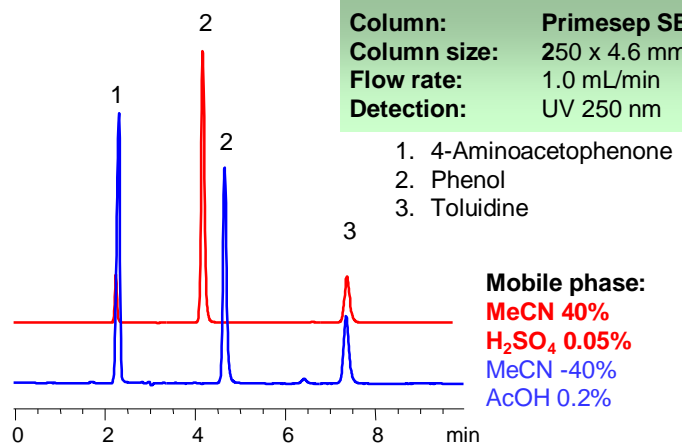


Figure 15. Separation and retention of weak basic and weak acidic compounds on Primesep SB column