January 2007 Newsletter

Universal HPLC Column: Old Myth or Modern Reality?

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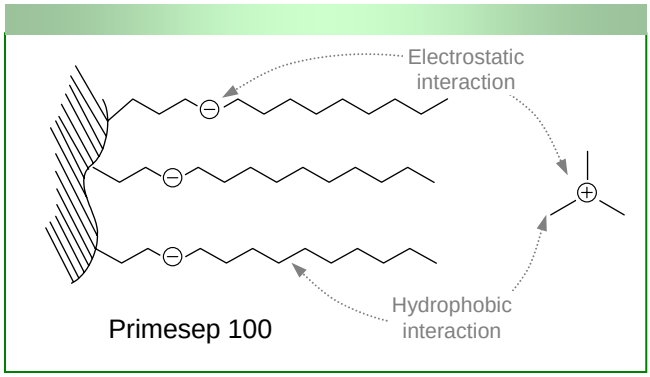

Figure 1. Simplified structure of Primesep 100 stationary phase with mixed-mode ligand attached to silica support and two main interactions occurred with charged analytes.

The liquid chromatography process involves the interaction of analytes with a stationary phase. Hydrophobic and electrostatic interactions are the two most commonly used forces in liquid chromatography. Traditionally these forces were used separately and stationary phases included only one. Therefore if analytes were similar and co-eluted, they typically could not be separated without changing columns.

Primesep 100 columns combine both hydrophobic and electrostatic forces in one mixed-mode stationary phase. Primesep 100 columns have a proprietary stationary phase with hydrophilic properties due to an embedded strong acid functionality and strong hydrophobic properties due to a long carbon chain (Fig. 1). With two functional groups of such different polarity, it is possible to tune a method to quickly separate similar analytes on the same column.

Primesep 100 columns provide good peak shape, retention and selectivity for acids, bases, amino acids and neutrals (Fig. 2). Mobile phases that are fully compatible with mass spectrometer detectors can be used (Fig. 2, 3, 4, 5, 6, 7, 8, 9, 13). Amino acids can be separated from parabens (Fig. 3) and fatty acids are baseline resolved (Fig. 5). A full range of amino acids can be separated (Fig. 4). Selectivity and peak order can be changed significantly by changing mobile phase composition (Fig. 2) or separation mode (Fig. 7). The polar-organic mode (HILIC) can be used to separate neutrals (Fig. 6). Pharmaceuticals can be separated by hydrophobic and ion-exchange interactions (Fig. 8, 10). Inorganic cations and amines can be separated by ion exchange (Fig 11, 12). Organic acids can be separating by combining hydrophobic and ion-exclusion interactions (Fig. 13). With an embedded ion-paring group, a Primesep 100 column requires no ion-pairing reagent in the mobile phase to retain and separate ionizable polar compounds. High loading is demonstrated for ionic analytes on a Primesep 100. Dopamine showed good peak shape on a 150 x 4.6 mm column at up to 200 ug on column (Fig. 9). McCalley *et al.*, Journal of Chromatography A, 2007, 1138 (1-2), 65-72, demonstrated up to 10 times higher loading capacity on Primesep 100 than on reversed-phase columns for other ionic bases.

Table 1. Type of molecules most suitable for separation by Primesep 100 column in RP conditions

	Hydrophobic molecules	**Hydrophilic** molecules
Strong acidic	Fig. 13	n/a
Weak acidic	Fig. 4	Fig. 2
Neutral	Fig. 2, 3, 6	Fig. 2, 7
Zwitterionic	Fig. 10	Fig. 2-4, 13
Weak basic	Fig. 2	Fig. 2
Strong basic	Fig. 9	Fig. 12

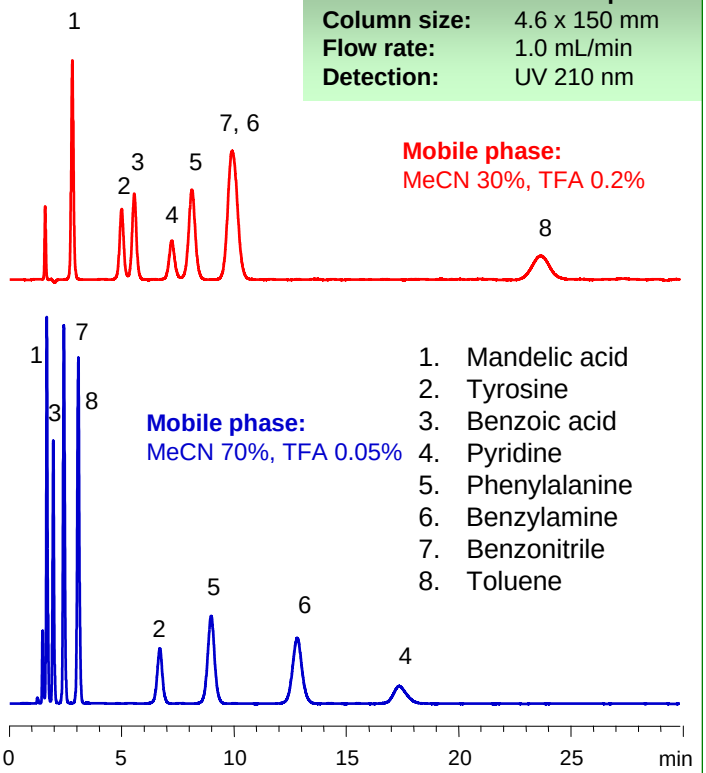

Figure 2. Ion-exchange and reverse phase mechanism in separation of acids, bases, amino acids, and neutral compounds.

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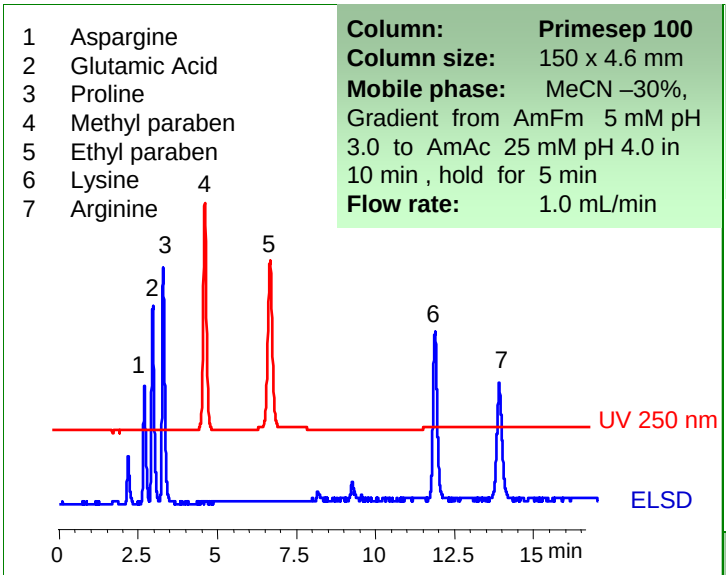
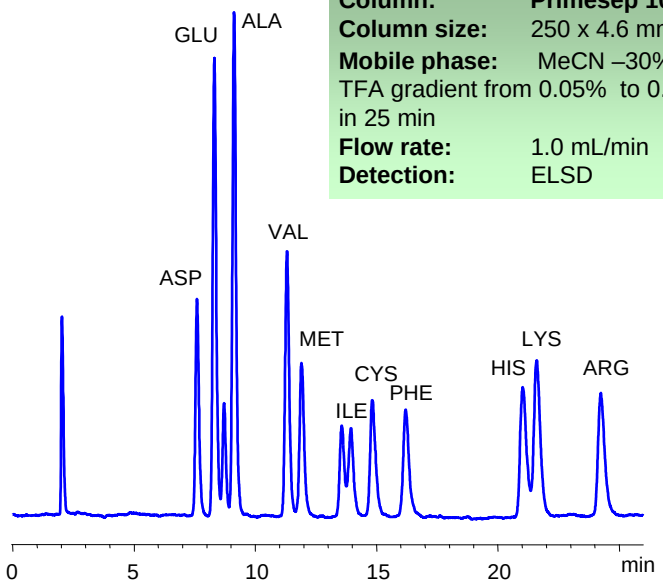


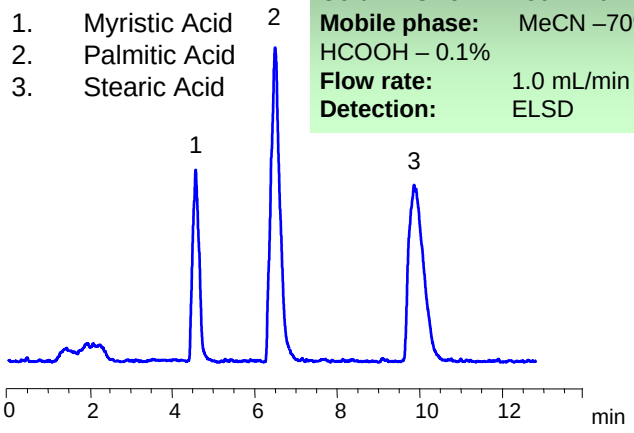
Column:	**Primesep 100**
Column size:	150 x 4.6 mm
Mobile phase:	MeCN –30%, Gradient from AmFm 5 mM pH 3.0 to AmAc 25 mM pH 4.0 in 10 min , hold for 5 min
Flow rate:	1.0 mL/min

Figure 3. Separation of amino acids and parabens with mass spec compatible mobile phase



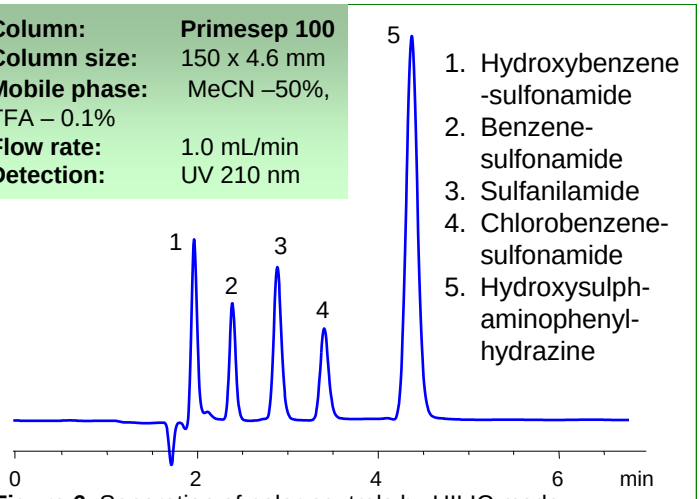
Column:	**Primesep 100**
Column size:	250 x 4.6 mm
Mobile phase:	MeCN –30%, TFA gradient from 0.05% to 0.3% in 25 min
Flow rate:	1.0 mL/min
Detection:	ELSD

Figure 4. Separation of 11 amino acids.



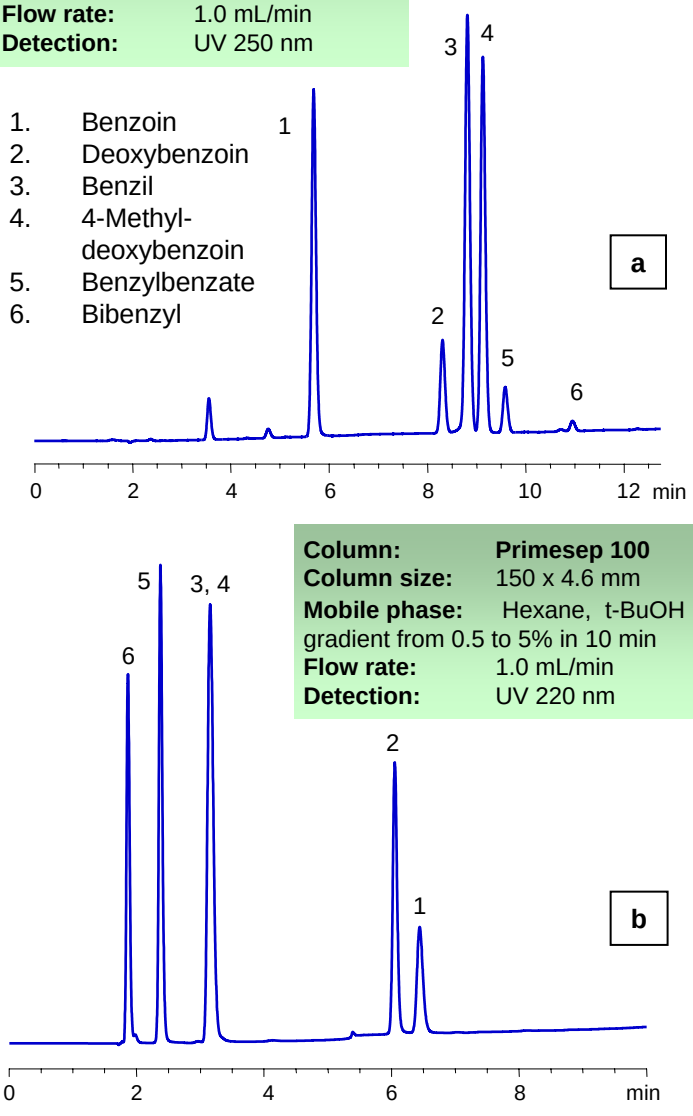
Column:	**Primesep 100**
Column size:	150 x 4.6 mm
Mobile phase:	MeCN –70%, HCOOH – 0.1%
Flow rate:	1.0 mL/min
Detection:	ELSD

Figure 5. Mass Spec compatible separation of fatty acids



Column:	**Primesep 100**
Column size:	150 x 4.6 mm
Mobile phase:	MeCN –50%, TFA – 0.1%
Flow rate:	1.0 mL/min
Detection:	UV 210 nm

Figure 6. Separation of polar neutrals by HILIC mode.



Column:	**Primesep 100**
Column size:	150 x 4.6 mm
Mobile phase:	MeCN gradient from 60 to 80% in 12 min, TFA 0.2%
Flow rate:	1.0 mL/min
Detection:	UV 250 nm

Column:	**Primesep 100**
Column size:	150 x 4.6 mm
Mobile phase:	Hexane, t-BuOH gradient from 0.5 to 5% in 10 min
Flow rate:	1.0 mL/min
Detection:	UV 220 nm

Figure 7. Reversal of peak order by changing from reversed-(a) to normal-mode (b) of separation.

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Column:	**Primesep 100**
Column size:	4.6 x 150 mm
Mobile phase:	MeCN –30%, TFA – 0.15%
Flow rate:	1.0 mL/min
Detection:	UV 210 nm

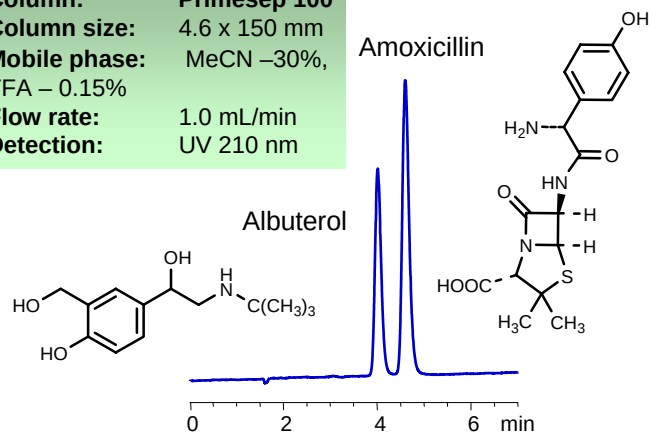

Figure 8. Pharmaceuticals separated by hydrophobic and ion-exchange interactions

Column:	**Primesep 100**
Column size:	4.6 x 150 mm
Mobile phase:	MeCN –10%, TFA – 0.1%
Flow rate:	1.0 mL/min
Detection:	UV 260 nm

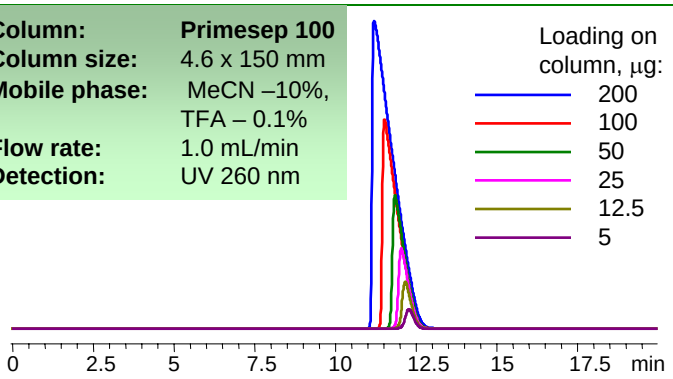

Figure 9. Dopamine loading study

Column:	**Primesep 100**
Column size:	4.6 x 150 mm
Flow rate:	1.0 mL/min
Mobile phase:	MeCN -30%, H_2SO_4-0.3%
Detection:	UV 210 nm

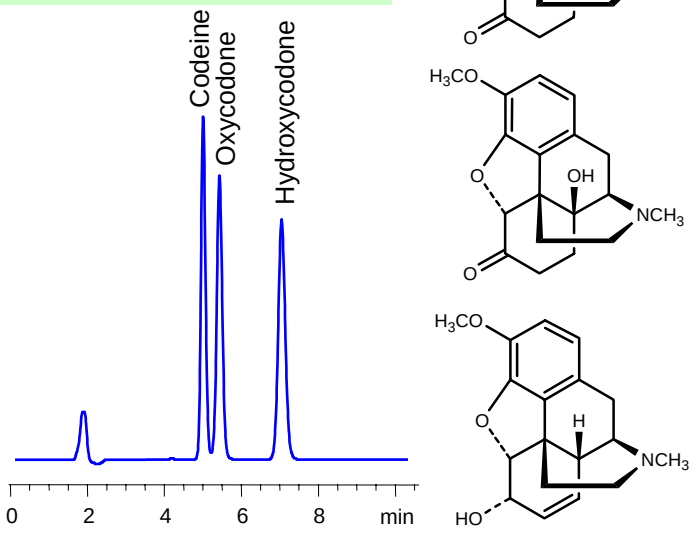

Figure 10. Codeine, oxycodone, and hydroxycodone (hydrocodone) separated by hydrophobic and ion-exchange interactions

Column:	**Primesep 100**
Column size:	4.6 x 250 mm
Flow rate:	0.5 mL/min
Mobile phase:	MeCN -30%, TFA - 0.2%
Detection:	ELSD

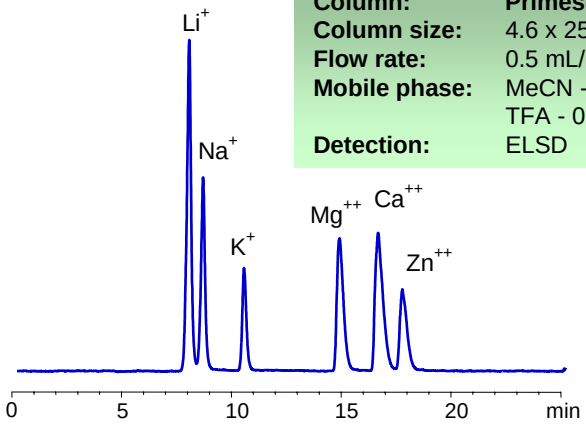

Figure 11. Inorganic cations separated by ion exchange

Column:	**Primesep 100**
Column size:	4.6 x 150 mm
Mobile phase:	MeCN –95%, TFA – 0.1%
Flow rate:	1.0 mL/min
Detection:	ELSD

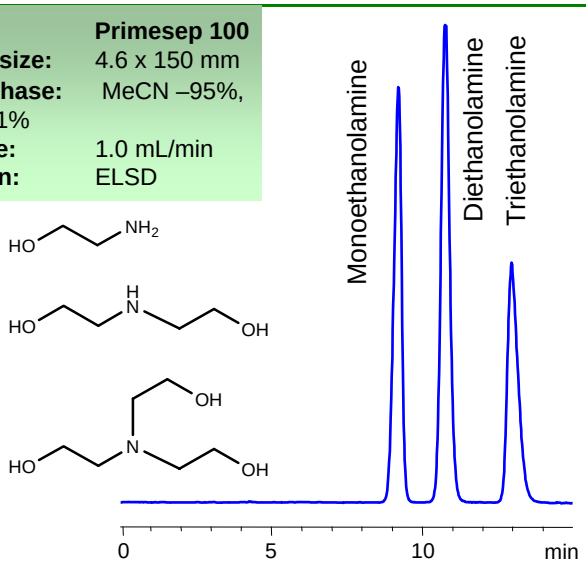

Figure 12. Ethanolamines separated by hydrophobic and ion-exchange interactions

Column:	**Primesep 100**
Column size:	4.6 x 150 mm
Mobile phase:	MeCN –95%, TFA – 0.1%
Flow rate:	1.0 mL/min
Detection:	ELSD

1. Pyridine-dicarboxylic acid
2. Picolinic acid
3. Isonicotinic acid
4. Nicotinic Acid

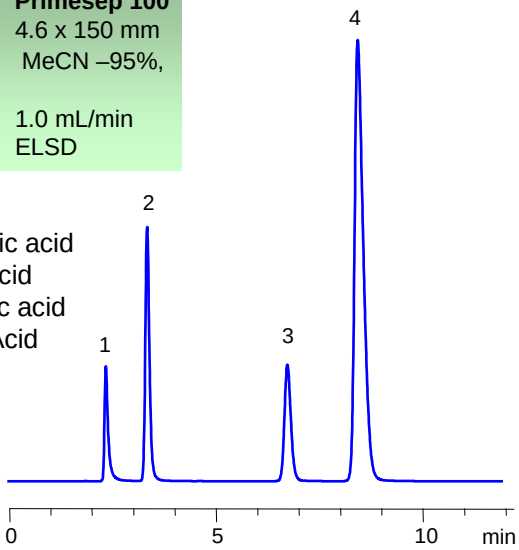

Figure 13. Separation of organic acids by combining hydrophobic and ion-exclusion interactions.

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