Primesep C Stationary Phase

SIELC Technologies, Inc. 15 E. Palatine Rd. Suite 107, Prospect Heights, IL 60070. Ph 847/229-2629, Fax 847/655-6079, mail@silec.com

Primesep columns are a new generation of HPLC stationary phases with a dual mechanism of interaction. These stationary phases combine reverse phase and ion-exchange properties in a single ligand attached to the silica surface. They are sometimes referred to as “mixed-mode” phases, as opposed to common reverse phase (RP) or ion-exchange (IE) separation media.

Primesep C is a special type of mixed-mode column. This column was developed for the analysis of hydrophobic and basic compounds. This column is complimentary to other dual mode columns such as Primesep 100 and Primesep 200. The important difference is the ion exchange embedded functional group, which can be switch **on** and **off** by the pH of the mobile phase. At a pH below 3 the ion-exchange properties of Primesep C column are significantly suppressed and RP is only mechanism of retention. At a pH over 4 both the IE and RP mechanism can be employed for separation. This unique property of the stationary phase becomes important when the analytes are basic compounds with very different pKa values. The pKa for basic compounds can vary significantly, as can the number of basic functional groups in the molecule. It is very difficult to separate compounds with vastly different basic properties using a single ion-exchange column. Primesep C with a pH gradient mobile phase allows one to retain the majority of basic compounds using MS friendly conditions (Fig. 1). Weak bases retain poorly by IE mechanism, so a column with strong IE properties is required. This can be achieved by starting the mobile phase at pH 5 or higher. Basic compounds with more than one amino group retain strongly and their elution is achieved by lowering pH of the mobile phase. If an organic gradient is applied at the same time, the compounds will also be separated based on their hydrophobic properties.

This generic dual gradient method can be used for many type of basic, neutral, and hydrophobic acidic compounds.

The mobile phase can be obtained with a dual solvent gradient from A to B prepared according to the following procedure: solvent A is prepared by dissolving 100 mM of ammonium acetate salt (AmAc) in water and adjusting the pH to 5.0 with acetic acid; 25% of this buffer is then mixed with 10% MeCN and 65% water. Similarly, ammonium formate buffer (AmFm) is prepared as a stock solution with 100 mM AmFm. The pH is then adjusted to 3.5 with formic acid; 25% of this stock solution is then diluted with 70% MeCN and 5% water to produce solvent B. If the pH of the mobile phase is kept constant with only an MeCN gradient, this complex mixture does not produce good separation. Foe example, at constant pH 5, poor peak shape for strong basses is usually observed. At constant pH 3.5, this column gives no retention of polar amines (Fig. 3).

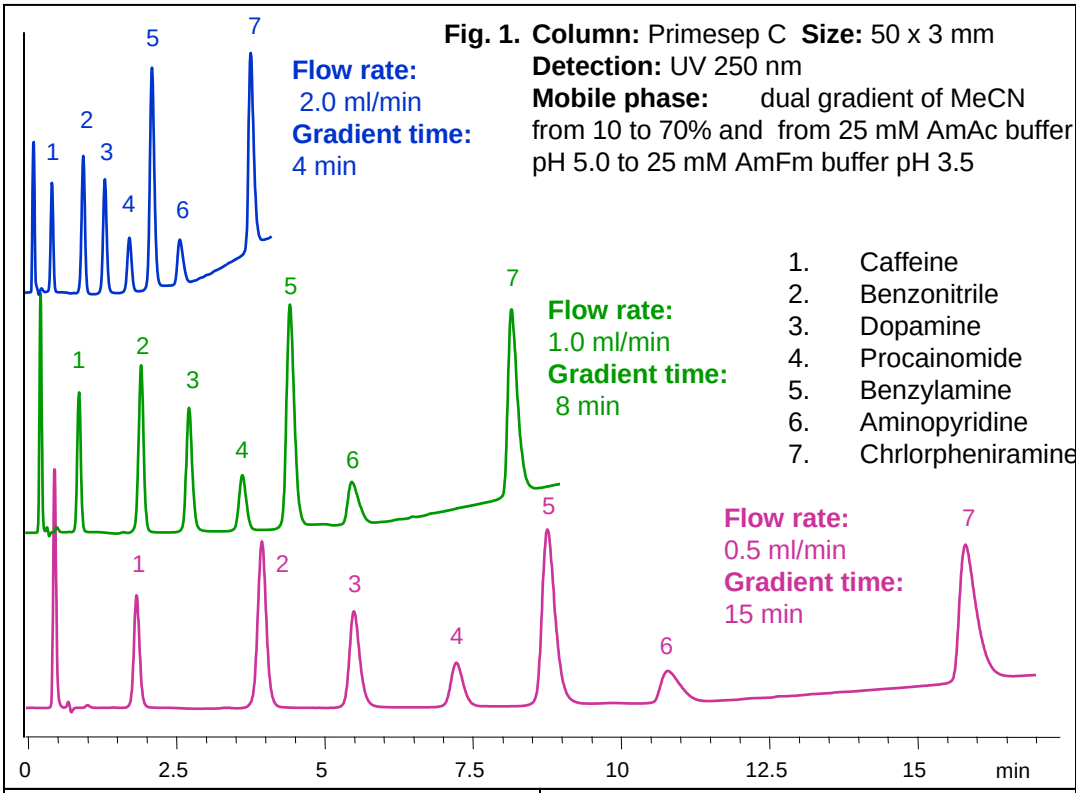


Fig. 1. **Column:** Primesep C **Size:** 50 x 3 mm
Detection: UV 250 nm
Mobile phase: dual gradient of MeCN from 10 to 70% and from 25 mM AmAc buffer pH 5.0 to 25 mM AmFm buffer pH 3.5

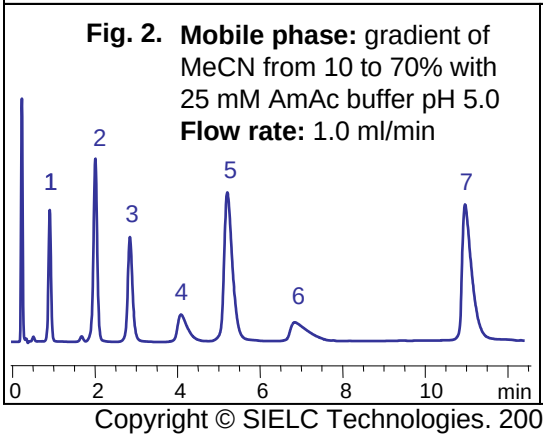


Fig. 2. **Mobile phase:** gradient of MeCN from 10 to 70% with 25 mM AmAc buffer pH 5.0
Flow rate: 1.0 ml/min

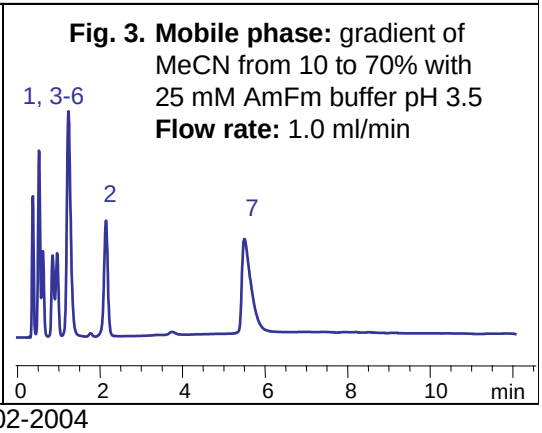


Fig. 3. **Mobile phase:** gradient of MeCN from 10 to 70% with 25 mM AmFm buffer pH 3.5
Flow rate: 1.0 ml/min

Table. 1. pH effect on surface charge of Primesep C column and charge of different compounds

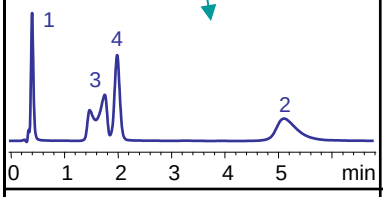
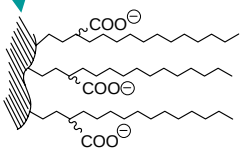
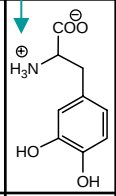
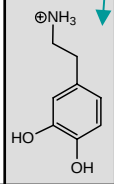
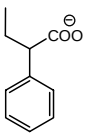
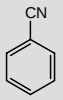
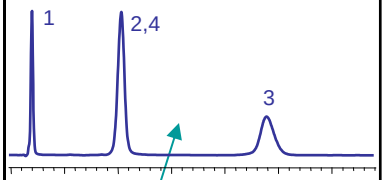
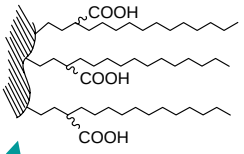
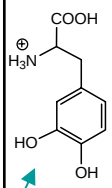
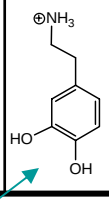
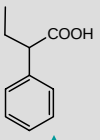
Column: Primesep C
Size: 50x3 mm
Mobile phase: 25 mM AmAcFm pH 4.0 with 10% MeCN
Detection: 250 nm

At pH > 3.5 the Primesep C column surface becomes negatively charged and it interacts with charged analytes

Analyte has two opposite charges. Hydrophilic analyte shows no retention even with charged surface.

Column surface and analyte have opposite charges. Retention observed for polar compounds

Column surface and analyte have the same charge. Hydrophobic acidic compound shows little retention.

Chromatogram example	Mobile phase pH	Primesep C column's surface charge	1 DOPA	2 Dop-amine	3 2-Phenyl-butiric acid	4 Benzo-nitrile
	> 5					
	< 4					

Analyte has no charge, but surface is negatively charged. Reduced hydrophobic interaction is usually observed.

Analyte and column surface have no charge. Retention observed is due to hydrophobic interaction

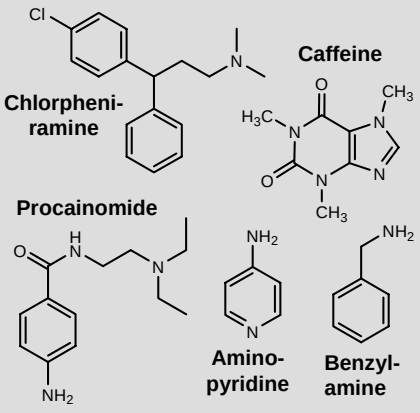
Column: Primesep C
Size: 50x3 mm
Mobile phase: 25 mM AmAcFm pH 4.0 with 10% MeCN
Detection: 250 nm

At pH 3, Primesep C loose its charge and is similar to a RP column with embedded polar group.

Column surface has no charge. Polar positively charged analytes are not retained.

Both analyte and column surface have no charge. Strong hydrophobic retention is usually observed.

Other compounds used in this study

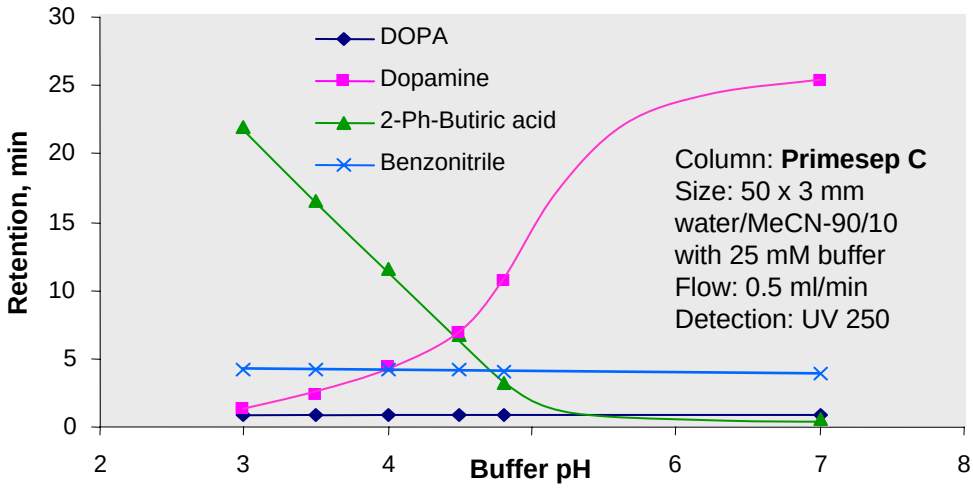


Primesep C part number:

C – 046 . 150 . 0510

Column id		
Column length	150 mm	
Particle and pore size		5 uM 100 A

Fig 4. pH effect on retention of acidic, basic, neutral, polar and hydrophobic compounds



Buffer in pH range from 3-5 is ammonium acetate-formate (AmAcFm), made by adjusting the pH of 100 mM ammonium acetate with formic acid, and then diluting with MeCN and water to 25 mM. The mobile phase at pH 7 is obtained by dissolving ammonium acetate in water and MeCN without pH adjustment.

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031 336 90 00 • www.scantecnordic.se