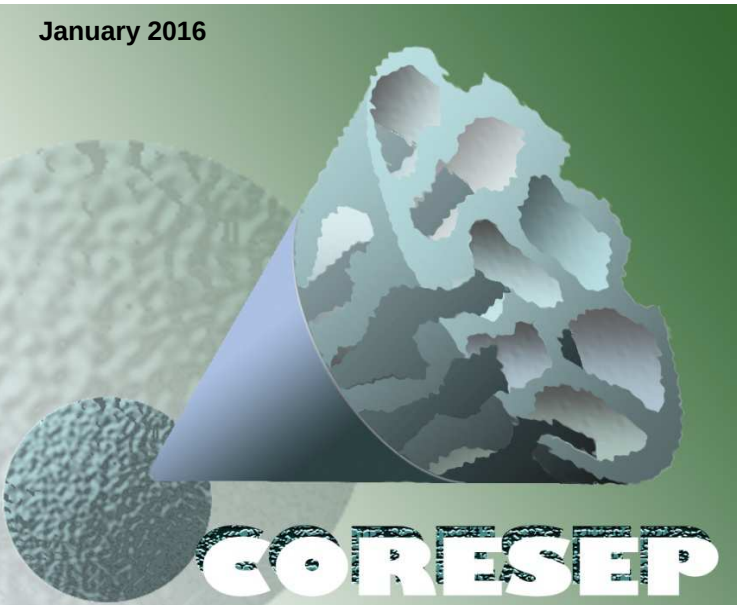


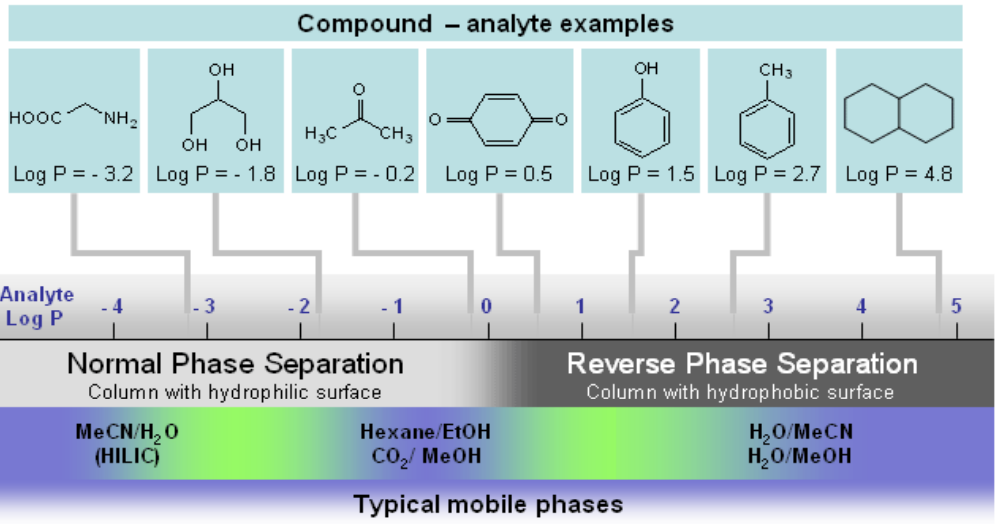
Mixed-Mode Core-Shell Columns

Introducing Coresep S HILIC/Ion-Exchange Column

SIELC Technologies, Inc., Wheeling, IL 60090

HILIC (Hydrophilic Interaction Liquid Chromatography) emerged as a powerful technique alternative to traditional reversed-phase chromatography. It is convenient for highly polar analytes when reverse-phase chromatography fails to produce significant retention. HILIC requires a very high concentration of acetonitrile (ACN, up to 99%) in the mobile phase [1].

SIELC Technologies has developed an HILIC/ion-exchange column based on core-shell particles. Core-shell silica enables the use of regular equipment for high-throughput, high-efficiency separation. In previous newsletters [1, 2] we described the benefits of using secondary interactions in reverse-phase separation.



SIELC Technologies continues to develop mixed-mode columns with core-shell particles with introduction of Coresep S mixed-mode HPLC column. Coresep S combines the efficiency of core-shell technology with the unique selectivity of HILIC/Ion-Exchange surface chemistry.

Coresep S is designed to retain and separate hydrophilic neutral, hydrophilic acidic, hydrophobic and hydrophilic basic compounds. HILIC is a special case of normal phase chromatography that is designed for the retention of polar compounds.

Ion-exchange properties introduce another mechanism of retention, increasing column capacity and lowering the necessary concentration of ACN in comparison to traditional HILIC columns.

Coresep S exploits small differences in hydrophilic and ionic properties between molecules to provide enhanced separation relative to traditional methods. High efficiency, high ACN content, and low buffer concentration allow for increased sensitivity in MS detection. The Coresep S column is fully MS- and prep chromatography compatible.

Ion-exchange interactions in the Coresep S column allow for longer retention of basic compounds, relative to traditional HILIC columns, using the same concentration of ACN. Coresep S columns can be used in HILIC/cation-exchange mode, HILIC/anion-exclusion mode, or cation-exchange mode.

A mixture of amino acids was separated in under 2 minutes with an LC/MS-compatible mobile phase (Fig. 1). The retention time for amino acids depends on ACN concentration, buffer concentration, and buffer pH. Buffer pH affects the ionization state of the stationary phase and analytes, in the case of amino acids. Amino acids can exist in 3 different forms depending on the ionization state of the carboxylic acids. At a low pH, the carboxylic acids are protonated, and the amino acids behave like basic compounds; at pH 3-6, amino acids are zwitter-ionic and at their highest polarity for HILIC interactions.

Column: 3.2 x 100 mm, 2.7 um
Mobile phase: 85% MeCN with 15 mmol AmAc pH 5
Detection: UV 270 nm

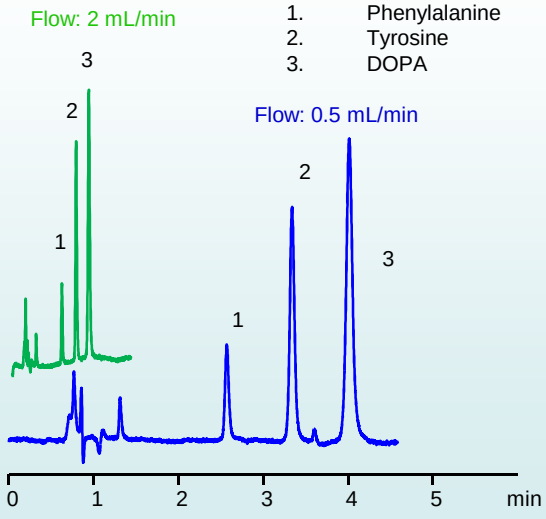


Fig 1. Fast and efficient separation of amino acids on Coresep S HILIC/mixed-mode column

Column: 4.6 x 150 mm, 2.7 um
Mobile phase: 85% MeCN with 15 mmol AmAc pH 5
Detection: UV 270 nm

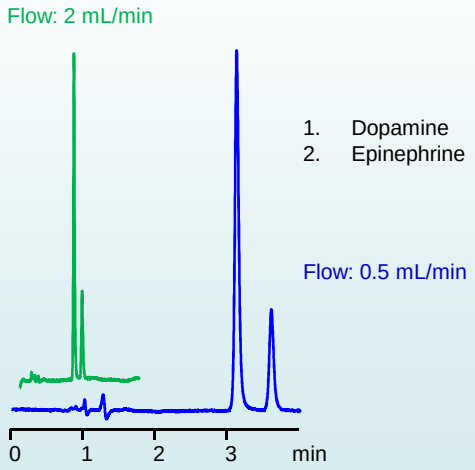


Fig.2 Fast analysis of hydrophilic basic neurotransmitters on Coresep S HILIC mixed-mode column

Column: 3.2 x 100 mm, 2.7 um
Mobile phase: 5% MeCN with 25 mmol AmAc pH 3
Detection: UV 250 nm

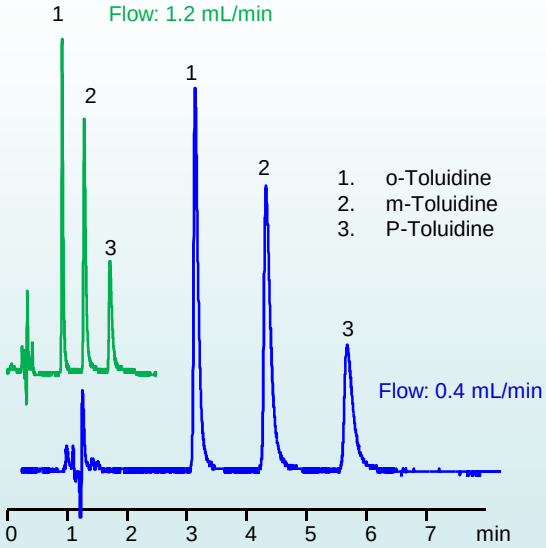


Fig. 3 Separation of toluidine isomers in IE mode on Coresep S HILIC/mixed-mode column

HILIC mode is required for the retention of zwitterionic compounds because of the difficulty in to have stationary phase be ionized enough to retain weak basic compounds such as amino acids.

Basic drugs can be retained and separated by the HILIC/cation-exchange mechanism on Coresep S columns. Dopamine and epinephrine can be separated within 1 minute with high efficiency and a perfect peak shape (Fig. 2). In the case of hydrophobic basic molecules, ion-exchange mode can be employed without HILIC being present. At low concentrations of ACN, only cation-exchange mode is present and the retention time is dependent on pH and buffer concentration. A mixture of isomers of toluidine was separated within 3 minutes (Fig. 3).

In general, Coresep S columns offer alternative selectivity not only to reverse-phase columns, but also to traditional HILIC columns (cyano, diol, bare silica, zwitter-ionic). Retention time and elution depends on the concentration of ACN , buffer pH, buffer concentration, and the nature of the buffer.

The retention time of compounds can be adjusted independently. Various mobile phases can be employed to suit the particular detection technique that is used (Fig. 4).

Column: 3.2 x 100 mm, 2.7 um
Flow: 1 mL/min
Detection: UV 250 nm

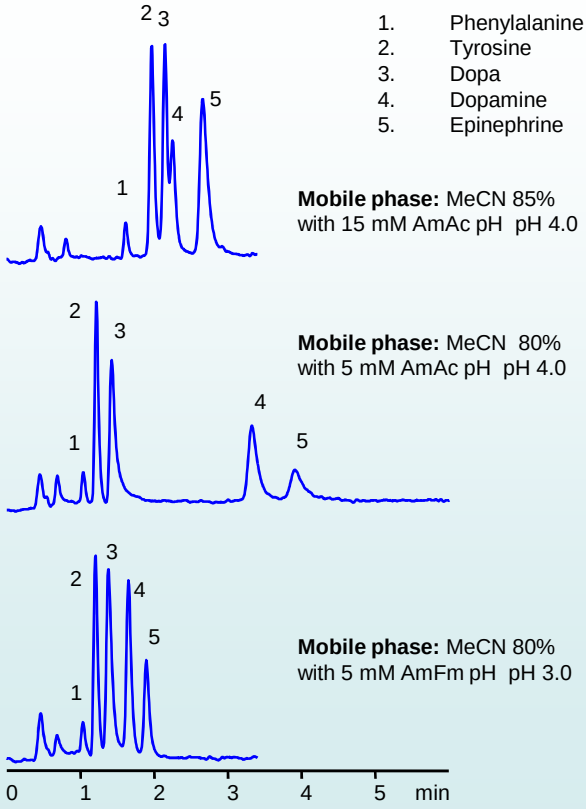


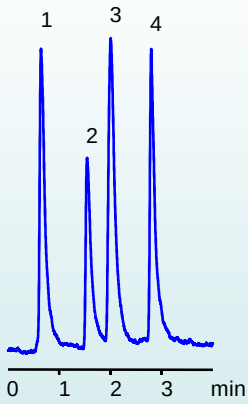
Fig. 4 Effect of mobile phase composition on separation of neurotransmitters

As a traditional HILIC column, Coresep S can be used to separate hydrophilic sugars and sugar substitutes. Separation of 4 of these compounds was achieved in under 4 minutes (Fig. 5).

HILIC mode alone was used for the separation of uracil and uridine in under 1 minute with excellent peak shape (Fig. 6). Other nucleobases and nucleotides can be separated by this approach.

Traditionally, neutral surfactants have been analyzed by reverse-phase chromatography. We achieved highly efficient separations of Triton X-100 surfactant on Coresep S columns in HILIC mode (Fig. 7). Such complex mixtures can be analyzed within 3 minutes with a simple ACN gradient. These methods can be applied to other surfactants that possess a basic group of neutral hydrophilic entities at the end of the molecule.

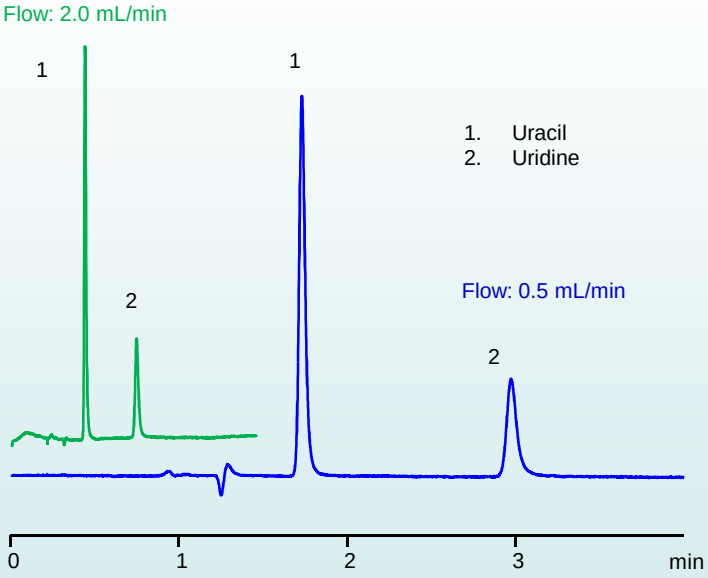
Column: 3.2 x 100 mm, 2.7 um
Mobile phase: MeCN gradient from 90% to 30% in 5 min with 10 mM AmFm pH 3.0
Flow: 0.8 mL/min
Detection: UV 210 nm



1. Saccharin
2. Xylitol
3. Sorbitol
4. Sucrose

Fig. 5 Analysis of sugars and sweeteners in HILIC mode on core-shell/mixed-mode column

Column: 3.2 x 100 mm, 2.7 um
Mobile phase: MeCN/water-98/2
Flow: 1 mL/min
Detection: 250 nm



1. Uracil
2. Uridine

Fig. 6 Retention and separation of uridine and uracil on Coresep S HILIC/mixed-mode column

Column: 3.2 x 100 mm, 2.7 um
Detection: UV 210 nm

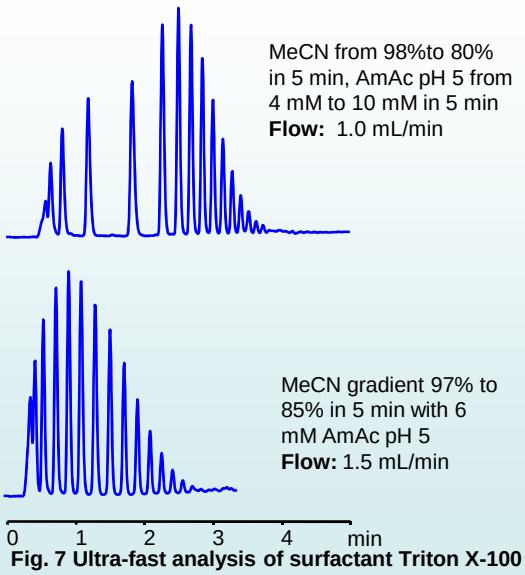


Fig. 7 Ultra-fast analysis of surfactant Triton X-100 in HILIC mode on Coresep S column

Coresep™ part number generator

CSS – 46 . 150 . 0209

Column id	
4.6 mm	**46**
3.0 mm	32
2.0 mm	20
1.0 mm	10
0.5 mm	05

Column length	
150 mm	**150**
100 mm	100
50 mm	50
25 mm	25
10 mm	10
Guard	G

Packing size	
5 um	05
2.7 um	**02**

Pores size	
90 A	**09**

All data were obtained in SIELC Technologies labs.

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