Mixed-Mode Core-Shell Columns - Unique Selectivity, Efficiency and Speed. Coresep SB – A New Reversed-Phase Anion Exchange Column.

HELIX Chromatography, Inc., Prospect Heights, IL 60070

We would like to introduce a core-shell reversed-phase anion-exchange column Coresep SB. This column combines efficiency of core-shell technology with unique selectivity of reversed-phase anion-exchange stationary phase. Multiple interaction on a single column offer 2D type chromatography (Fig. 1) as a tool to separate complex mixtures with a variety of compounds possessing opposing properties: hydrophobic and hydrophilic, neutral and ionic, positively charged and negatively charged.

Coresep SB column is designed to retain and separate hydrophobic neutral, hydrophobic basic, hydrophobic acidic, and hydrophilic acidic compounds in one run.

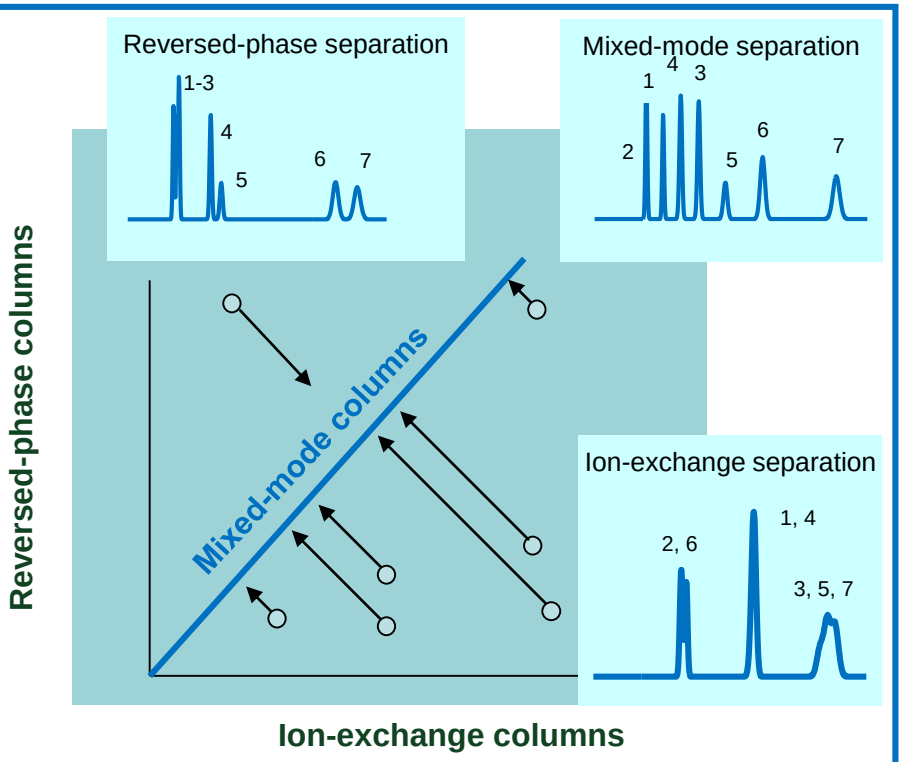

Fig. 1. 2D Property of mixed-mode separations compare to RP and IE modes

Based on the fact, that no two molecules have exactly the same hydrophobic and ionic properties, Coresep SB explores small differences in such properties for enhanced separation of various compounds

Core-shell mixed-mode columns achieve high efficiency while keeping back pressure below 5000 psi, which allows the use of a regular HPLC systems while achieving extremely sharp peaks and short run times.

Mixed-mode columns offer much higher capacity and retention than traditional reversed-phase columns.

Since the surface area of core-shell particles is reduced, mixed-mode phases address potential loadability issues whilst maintaining the selectivity advantages of multiple interactions. Newly optimized ligand density on Coresep SB column in combination of carefully selected ionic / hydrophobic ratios further increases the capacity of mixed-mode core-shell columns.

Coresep SB as well as other core-shell mixed-mode columns (Coresep 100 and Coresep S) are fully mass chromatography compatible. Mobile phase usually consist of various ratios of acetonitrile and water with buffer or acid. Presence of ions in the mobile phase facilitates ion-exchange mechanism and elution of ionized compounds.

Column: 3.2 x 100 mm, 2.7 um
Mobile phase: 50% ACN with 50 mmol NaH2PO4 buffer pH 3
Flow: 0.8 mL/min
Detection: UV 210 nm

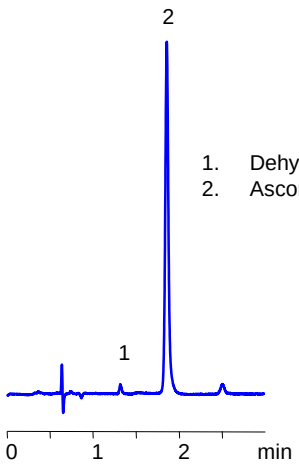

Fig . 2 Separation of dehydroxyascorbic and ascorbic acids

Copyright © 2017-2018 HELIX Chromatography, Inc., All rights reserved.

By using mixed-mode columns you can avoid the use ion-pairing reagents for retaining highly hydrophilic acidic compounds. Coresep SB columns have an ion-pairing reagent chemically attached to the surface of the silica gel. There is no need to reserve a column to be used with ion-pairing reagent.

Hydrophilic acids like ascorbic, maleic, fumaric can be analyzed with regular RP columns, but require the use of ion-pairing reagent. Separation of dehydroxyascorbic and ascorbic acid (Fig. 2) is achieved within 2 minutes with ACN/water/phosphate buffer. Phosphate buffer can be replaced with ammonium formate for LC/MS applications. Benzoic, maleic and fumaric acid are retained and separated well on Coresep SB column, retention is based on the strength and ionization of acidic fragments of carboxylic acid. It can be controlled by amount of buffer and buffer pH.

Synergy effect of weak reversed phase and anion-exchange mechanisms allowed separation of homologous isomers of malonic acid with good resolution, peak shape and in short 2 minutes run (Fig. 3)

Column: 3.2 x 50 mm, 2.7 um
Mobile phase: 10% ACN with 0.15% sulfuric acid
Flow: 1 mL/min
Detection: 215 nm

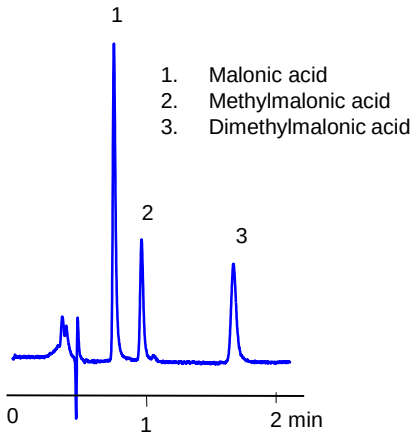

Fig. 3 Ultra-fast separation of malonic acids on mixed-mode reversed-phase anion-exchange column

Column: 3.2 x 100 mm, 2.7 um
Mobile phase: 25% ACN with 20 mmol AmFm pH 3
Flow: 1 mL/min
Detection: UV 210 nm

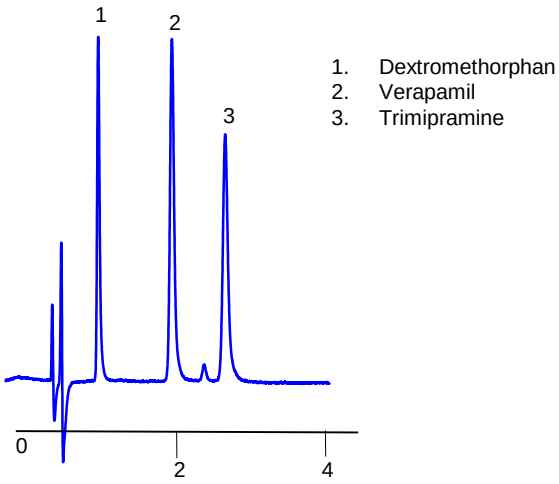

Fig. 4 Fast separation of hydrophobic basic drugs on core-shell mixed-mode column

Coresep SB has reverse-phase properties which allow to retain hydrophobic compounds, based on their hydrophobic properties. Presence of basic group close to the surface shields basic analytes from interaction with residual silanols, which contributes to good peak shape (Fig. 4). Presence of a basic group on the surface also allows to retain corresponding acidic counter-ion (Fig. 5) The method can be extended for analysis of other acidic counter-ions and corresponding hydrophobic drugs.

Column: 3.2 x 100 mm, 2.7 um
Mobile phase: 20% ACN with 0.2% TFA
Flow: 1 mL/min
Detection: UV 210 nm

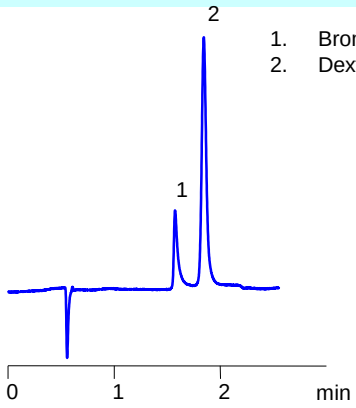

Fig. 5 Simultaneous analysis of hydrophobic basic drug and acidic counter-ion on core-shell mixed-mode column

Mixed-mode core-shell columns showed excellent selectivity towards separation of isomers. Two organic acids, 3-mitrophthalic and 4-nitrophtalic acids were separated on Coresep SB column (Fig. 6). Method can be extended to more complicated mixtures containing ionzable structural isomers. Column will not separate optical isomers. Another example shows separation of Maleic and fumaric acids are highly hydrophilic acidic compounds with no retention on traditional RP columns. The usual approach for analysis includes HILIC or ion-exchange chromatography. Since in most cases these acid are analyzed in aqueous solutions, HILIC approach might be problematic.

Copyright © 2017-2018 HELIX Chromatography, Inc., All rights reserved.

Column: 3.2 x 100 mm, 2.7 um
Mobile phase: 50% ACN with 0.1% sulfuric acid
Flow: 1 mL/min
Detection: UV 250 nm

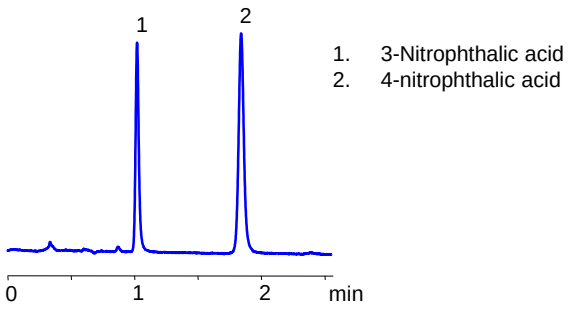

Fig. 6 Separation of isomers of nitrophthalic acid on core-shell mixed-mode column

If a mixture contains a hydrophobic acid it is usually not retained on HILIC column. Coresep SB column was used for separation and analysis of benzoic, maleic and fumaric acids. Retention for benzoic acid (pKa 5) comes from RP interaction and stronger acids, like maleic (pKa 1.9 and 6.1) and fumaric (pKa 3.03 and 4.44) are retained mostly by ion-exchange interaction (Fig .7)

Unique chemistry of mixed-mode core-shell, also allows one to retain and separate mono-, di- and tri-phosphates of oligonucleosides. These compounds are highly hydrophilic due to the presence of sugar fragments and presence of phosphates. Mono-, di- and triphosphates can be separated in one run or isolated in groups depending on the number of phosphate residues. (Fig. 8) A much higher concentration of the buffer is required to elute triphosphate from the column. The method remains LC/MS compatible and prep chromatography compatible.

Column: 3.2 x 100 mm, 2.7 um
Mobile phase: 50% ACN with 50 mmol NaH2PO4 buffer pH 3
Flow: 0.8 mL/min
Detection: UV 210 nm

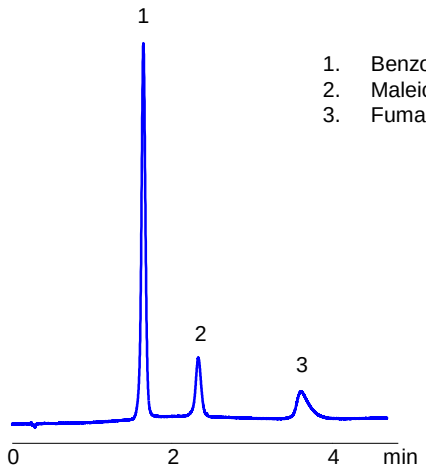

Fig. 7 Retention and separation of organic acids on core-shell reversed-phase anion-exchange column

Column: 4.6 x 150 mm, 2.7 um
Mobile phase: 5% ACN, AmAc (pH 4.5) from 20 mmol to 300 mmol in 45 minutes
Flow: 1.5 mL/min
Detection: UV 210 nm

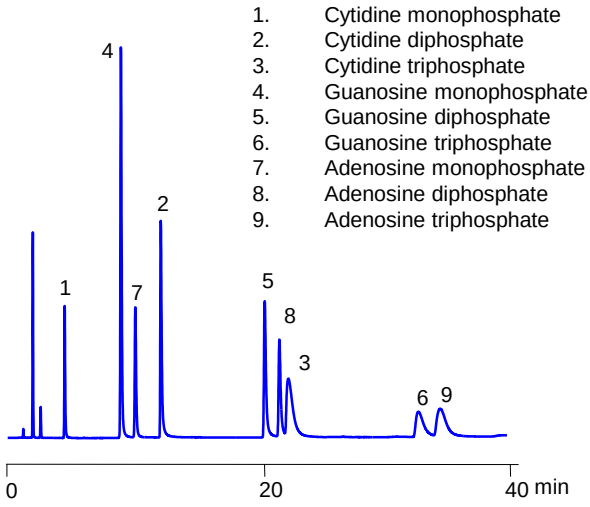

Fig. 8 Separation of 9 oligonucleotides on Coresep SB column in reversed-phase anion-exchange mode

Coresep™ part number generator

CSB - 4 15 2 9

Column id

4.6 mm	**4**
3.0 mm	3
2.0 mm	2
1.0 mm	1

Column length

150 mm	**15**
100 mm	10
50 mm	5
25 mm	2
10 mm	1
Guard	G

Packing size

2.7 um	**2**

Pores size

90 A	**9**

Contacts:
HELIX Chromatography, Inc.
15 E. Palatine Rd., Suite 118
Prospect Heights, IL 60070
Ph. 847-777-1335
Fax 847-594-0801
mail@helixchrom.com
www.helixchrom.com

Promotional code: 2ND40
Valid until July 15, 2018

Buy one Coresep column and get the second one 40% off

Copyright © 2017-2018 HELIX Chromatography, Inc., All rights reserved.