Evolution of Mixed-mode HPLC: Are We There Yet?

Paraguat and Diguat separation on three

generations of mixed-mode columns

1. Paraguat

ί,

2. Diquat

Leading brand C18

150 x 4.6 mm

1.0 mL/min

Primesep D

150 x 4.6 mm

Mobile Phase: H2O/MeCN/TFA

Detection: UV 250 nm

Column

95/5/0 1

Column:

Flow: 1.0 ml /min

Mobile Phase

Detection: UV 250 nm

H2O/MeCN/TFA - 95/5/0.1

Size:

2 4 6 mir

G

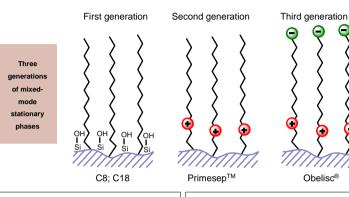
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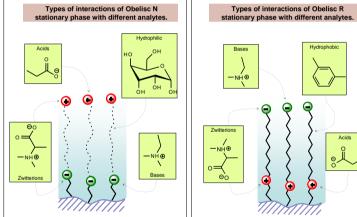
Abstract

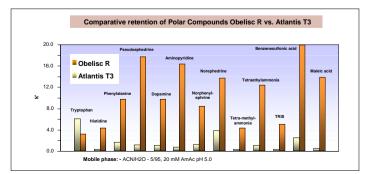
For decades, liquid chromatography stationary phase design has been dominated by the idea of eliminating multiple, or "unwanted" interactions that occur in mixed-mode separations. In reversed-phase chromatography base-deactivated phases were developed to eliminate silanol interactions with amine-containing analytes.

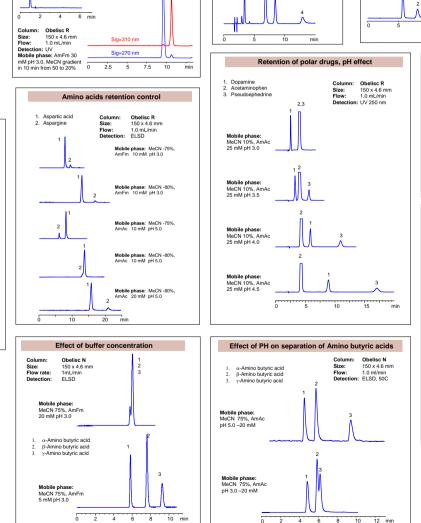
However, there are ways to benefit from multiple interactions on the stationary phase. SIELC Technologies was a first company that introduced Primesep - mixed-mode columns intentionally designed for multiple interactions. Now we offering a new generation of stationary phases, Obelisc®, an evolutional step in mixed-mode column technology. These columns are capable of separating a tremendous range of compounds by different separation modes based only upon mobile phase selection.

The ligand structure includes positive and negative charges separated by a long organic chain allows both positive and negative charges to simultaneously participate in electrostatic interaction. Two complimentary columns Obelisc R for reverse phase separation and Obelisc N for normal separation is differ in polarity of the long organic chain connected two opposite charges. Obelisc R has hydrophobic chain making the surface of the stationary phase hydrophobic in nature while Obelisc N has hydrophilic chain making this column suitable for HILIC. SFC and other normal phase applications.









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Column

Column size:

Mobile phase

MeCN 5%. Acetic acid -0.1%

Flow: Detection:

Hydrophilic molecules in MS

compatible mobile phase

Obaliec P

1.0 mL/min

UV 250 nm

Phenylalanine

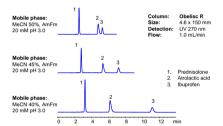
3-Aminobenzoic acid

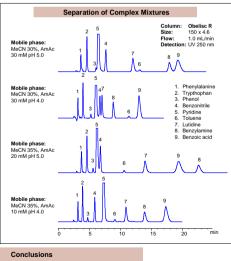
Tryptophan

Aspartame

150 x 4.6 mm

pH Effect on separation of neutral molecules . Indene Column Obelisc R Naphthalene Size: 150 x 4.6 Acenapthene Flow: 1.0 ml /min 4. Fluorene Detection: UV 250 nm Phenanthrene Mobile phase: MeCN / H2O 40/60, TFA 0.15% Mobile phase: MeCN / H2O 40/60, AmAc 10 mM pH 5.0 10 15 20 mir Selectivity adjusted by MeCN concentration





Mixed-mode columns that contain two types of interactions, ion-exchange and reversed-phase, or ion-exchange and normal phase with both positively charged and negative charged functional groups are first stationary phases of third generation of mixed-mode LC. Buffer concentration, buffer pH, and organic modifier concentration are three factors that allow to adjust selectivity and retention and obtain most convenient peaks spreading. Retention characteristics of polar compounds are significantly improved compared to traditional reversed-phase chromatography. Low buffer concentration is sufficient to control ion-exchange mode of interaction. High column capacity is typical with polar analytes. Mixed-mode type of stationary phases eliminates blind multiple-column search, and allows a systematic approach to method development.

SIELC Contact Information: 65 E. Palatine Rd. Suite 221, Prospect Heights, IL 60070 www.sielc.com 847-229-2629 Ph. 847-655-6079 Fax

pH Effect on separation of

zwitter-ionic molecules

Column:

Size: Flow:

Obelisc F

150 x 4.6 1.0 mL/mi

Detection: UV 250 nm

Mobile phase MeCN -30%, AmAc

20 mM pH 4.0

Mobile phase

20 mM pH 5.0

15

MeCN -30%, AmAc

1. 4-Amino benzoic acid

2 3-Amino benzoic acid

2-Amino benzoic acid