Universal Stationary Phase for Reverse, Normal, Ion-Exchange and Ion-Exclusion Chromatography

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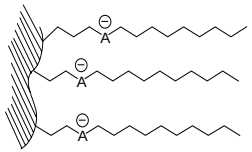
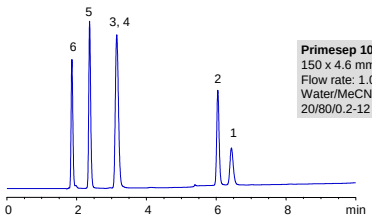
PrimesepTM mixed-mode stationary phases provide two functional groups for interaction with analytes. One is a very hydrophobic alkyl chain, another is a very hydrophilic acid residue (Primesep A, 100, 200), or a protonated base (Primesep B, Primesep B2, Primesep D).
With two functional groups of such difference in polarity, multiple separation modes can be performed on a single column.
Neutral compounds can be resolved in reverse, normal, or polar organic mode. Charged molecules can be resolved in reverse, normal, polar organic, ion-exchange, or ion-exclusion modes. Also, the combination of more than one mode is typical for ionizable molecules.
Selection of the mode of separation is governed by the type of the mobile phase selected for the particular separation. When a complex mixture is analyzed using Primesep columns, two or more interaction mechanisms help to tune the separation. Elution order and retention time can be adjusted in accordance with your analytical needs. The typical combinations of the mechanisms are: reverse phase – ion-exchange; reverse phase – ion exclusion; hydrophilic interaction – ion-exchange; chelating - reverse phase.

Schematic Structure of Primesep 100 and Primesep 200 Stationary Phases



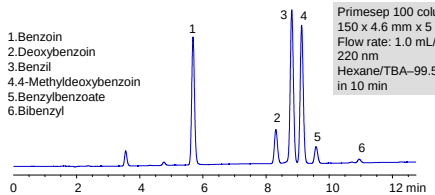
With an embedded ion-pairing group, a Primesep column requires no ion-pairing reagent in the mobile phase to retain and separate ionizable polar compounds.

Reverse - Phase Separation

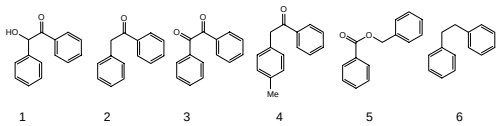


Primesep 100 column
150 x 4.6 mm x 5 um
Flow rate: 1.0 mL/min. UV - 250 nm
Water/MeCN/TFA–40/60/0.2-20/80/0.2-12 min

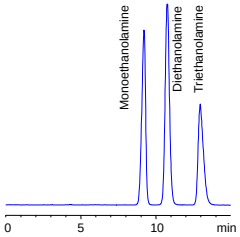
Normal - Phase Separation



Primesep 100 column
150 x 4.6 mm x 5 um
Flow rate: 1.0 mL/min. UV - 220 nm
Hexane/TBA–99.5/0.5 - 95/5 in 10 min



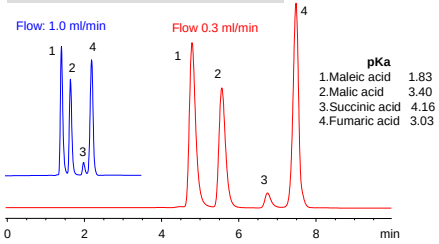
Ion-Exchange Mode Separation of Ethanolamines



Primesep 100, 150 x 4.6 mm
Mobile Phase:
H2O/MeCN/TFA - 95/5/0.1
Flow rate: 1.0 mL/min
Detector: ELSD

Ion-Exclusion Mechanism. Separation of Diacids.

Primesep 100 column 150 x 4.6 mm x 5 um
Mobile phase: Water/MeCN/H2SO4–75/25/0.03
Detection: UV 210 nm

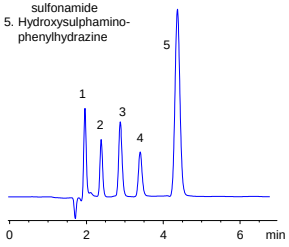


pKa	
1.Maleic acid	1.83
2.Malic acid	3.40
3.Succinic acid	4.16
4.Fumaric acid	3.03

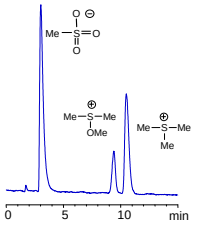
Polar-Organic (HILIC) Mode of separation.

1. Hydroxybenzenesulfonamide
2. Benzenesulfonamide
3. Sulfanilamide
4. Chlorobenzene-sulfonamide
5. Hydroxysulphamino-phenylhydrazine

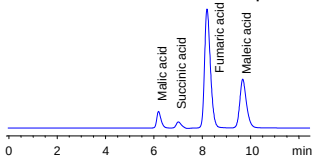
Primesep 100 150 x 4.6 mm
Flow rate 1.0 mL/min. UV 210 nm Mobile phase: Water/MeCN/TFA–50/50/0.1



Primesep 100 150 x 4.6 mm
Flow rate: 1.5 mL/min. Detection: ELSD
Mobile phase: Water/MeCN/TFA – 10/90/0.1



Ion-Exclusion and Reverse Phase Mechanism. Diacids Separation.

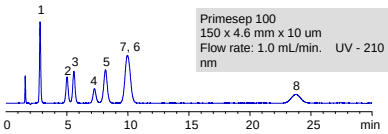


Column: Primesep 200 250 x 4.6 mm x 5 um
Mobile phase: MeCN/Water/TFA – 15/85/0.1
Flow rate: 0.5 mL/min Detector: UV 210 nm
Injection: 5 uL of solution of malic and succinic acids (5 mg/mL); fumaric, and maleic acids (0.2 mg/mL) in water

When a complex mixture is analyzed using Primesep columns, two or more interaction mechanisms help to tune the separation. Elution order and retention time can be adjusted in accordance with your analytical needs. The typical combinations of the mechanisms are: reverse phase – ion-exchange; reverse phase – ion exclusion; hydrophilic interaction – ion-exchange; chelating - reverse phase.

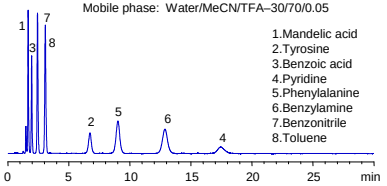
Ion-Exchange and Reverse Phase Mechanism in Separation of Acids, Bases, Amino Acids, and Neutral compounds.

Mobile phase: Water/MeCN/TFA–70/30/0.2



Primesep 100
150 x 4.6 mm x 10 um
Flow rate: 1.0 mL/min. UV - 210 nm

Mobile phase: Water/MeCN/TFA–30/70/0.05

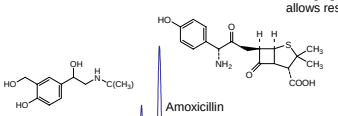


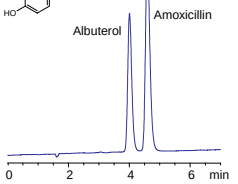
1.Mandelic acid
2.Tyrosine
3.Benzoic acid
4.Pyridine
5.Phenylalanine
6.Benzylamine
7.Benzonitrile
8.Toluene

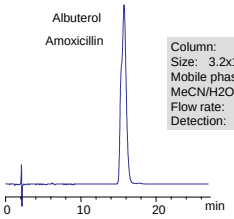
Ion-Exchange and Reverse Phase Mechanism Can Be Controlled Independently.

Column: Primesep 100
150 x 4.6 mm
Mobile phase: MeCN/H2O/TFA–30/70/0.15
Flow rate: 1.0 ml/min
Detection: UV 210 nm

When two compounds are co-eluted on RP column, there are a few options available to improve the separation of these compounds: temperature variation, replacement of an organic component of mobile phase (ACN-MeOH), and pH adjustment. When both compounds are of the same nature, such changes will have little or no effect on the resolution. The most significant effect can be usually obtained by changing the column. This is why it takes a range of the columns for successful method development. Primesep columns, in opposite, are changing themselves by changing mobile phase properties. This often allows resolving co-eluted peaks without replacing the column.







Column: Primesep 100
Size: 3.2x150 mm
Mobile phase: MeCN/H2O/TFA–20/80/0.1
Flow rate: 0.5 ml/min
Detection: 280 nm

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