SIELC

Primesep™

Columns
Methods
Applications

“Creating New Dimensions in the World of Chromatography”

Content

Introduction

For decades liquid chromatography stationary phase design has been dominated by the elimination of the multiple or “unwanted” interactions that occur in mixed-mode separations. For instance, base-deactivated reversed-phases were developed to eliminate silanol interactions with amine-containing analytes. In size-exclusion chromatography it is assumed that only steric interactions and not adsorption interactions are both present and desired. In ion-exchange and ion-exclusion chromatography that are based on ionic interactions, nonionic interactions are generally viewed as complications to a separation and, thus, are undesirable. However, there is a way to benefit from multiple interactions on the stationary phase.

Primesep™ HPLC columns are designed for mixed-mode separations and capable of separating a tremendous range of compounds by different separation modes based only on the mobile phase selection. With an embedded ion-pairing group, the columns require no ion-pairing reagents in the mobile phase to retain and separate ionizable polar compounds.

These columns have shown to efficiently separate organic and inorganic ions in ion-exchange and ion-exclusion modes. The columns can be used to provide efficient separation in normal phase, reverse phase, and polar organic modes. Different modes of separation offer different column selectivity.

An organic pharmaceutical can be quantified with its inorganic counter ion on the same column at the same time. Also, inorganic cations and anions can be run together without an ion-chromatography system with a resin clean-up device. Unlike with reversed-phase columns, selectivity can be altered not only by varying organic modifier concentration, but also by changing acid modifier type and concentration. These tools open a new realm of choices to alter selectivity and elution order of analytes.

In most cases, separations require a simple mobile phase containing acetonitrile, water and TFA or formic acid. This simplifies the process of analytical method development and allows switching from one detection technique to another without changing a separation method. All common detection techniques such as MS, ELSD, UV, and RI are compatible with this volatile mobile phase.

The mobile phase allows simple scale up from analytical to preparative separations with no changes in the separation conditions.

The columns are resistant to dewetting in 100% aqueous mobile phase and are stable in pure organic and highly acidic conditions down to pH 1.0.

Any silanol or metal chelating interactions are completely eliminated and do not affect the efficiency of the separation.

The column chemistry is reproducible from lot to lot; absolute and relative retentions of neutral and charged compounds are maintained within close tolerances.

Novel Stationary Phase Properties

In ion-pairing chromatography, retention of ionizable species is controlled by concentration and type of ion-pairing reagents.

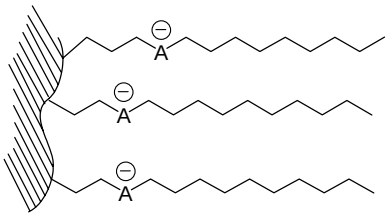
Pentanesulfonic acid, heptanesulfonic acid, sodium dodecylsulfate, tetrabutylammonium hydroxide are ion-pairing reagents that are typically used for retention of polar compounds in the reverse phase chromatography. By analogy, Primesep™ HPLC mixed-mode columns are offered in several modifications of the stationary phase with different strengths of ion-bearing groups for cation exchange mode (Primesep A, Primesep 100, Primesep 200, Primesep 300) and for anion-exchange mode (Primesep B and Primesep B2).

Primesep C column forms a strong complex with amines. The strength of the complex increases from tertiary to secondary, and primary amines. A pKa value for amines usually decreases in the same order. Contrary to ion-exchange separation, a reverse elution order is observed on Primesep C columns for the substituted amines

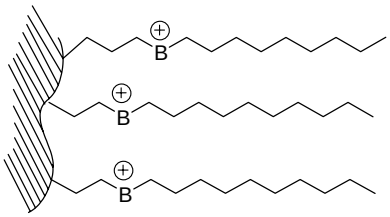
Mixed-Mode Primesep Columns

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.

Primesep A Primesep 200
Primesep 100 Primesep 300

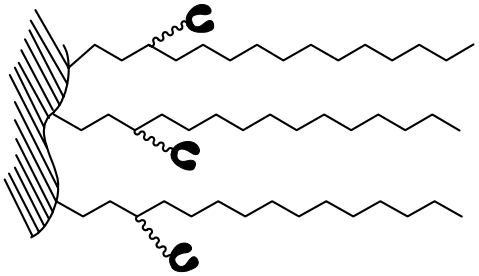


Primesep B
Primesep B2



Primesep C Column

A newly developed Primesep C column (C stands for “complex”) forms a weak complex with amino compounds and metal ions. With a reverse stationary phase as a basis for primary interaction, the column offers a typical RP retention profile for neutral compounds. In addition, embedded hosting groups interact with amines and other ions, and form a unique retention pattern. Amines with equal hydrophobicity retain on Primesep C in the following order: tertiary<secondary<primary. Alkali metals are retained in order $K^+<Na^+<Li^+$, which is a reverse order compared to the classical ion-exchange.

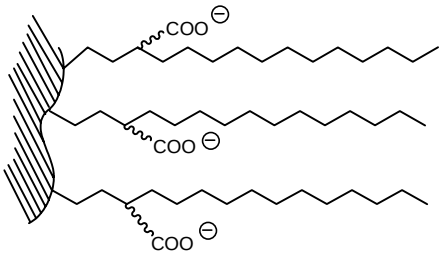


SWITCH Phase™ Technology

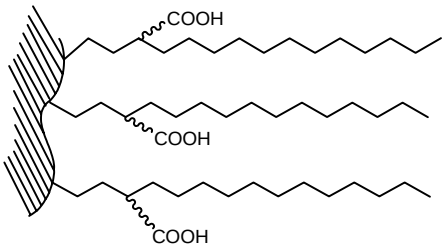
Columns based on SWITCH Phase™ technology change their properties depending on pH of the mobile phase. Embedded carboxylic acid is fully ionized at pH above transition point and loses charge when mobile phase pH goes below transition point. By controlling pH of the mobile phase, the polar properties of the stationary phase can be altered to tune your separation needs.

Primesep 300	Transition @ pH=3
Primesep 200	Transition @ pH=2
Primesep 100	Transition @ pH=1
Primesep A	Transition @ pH=0

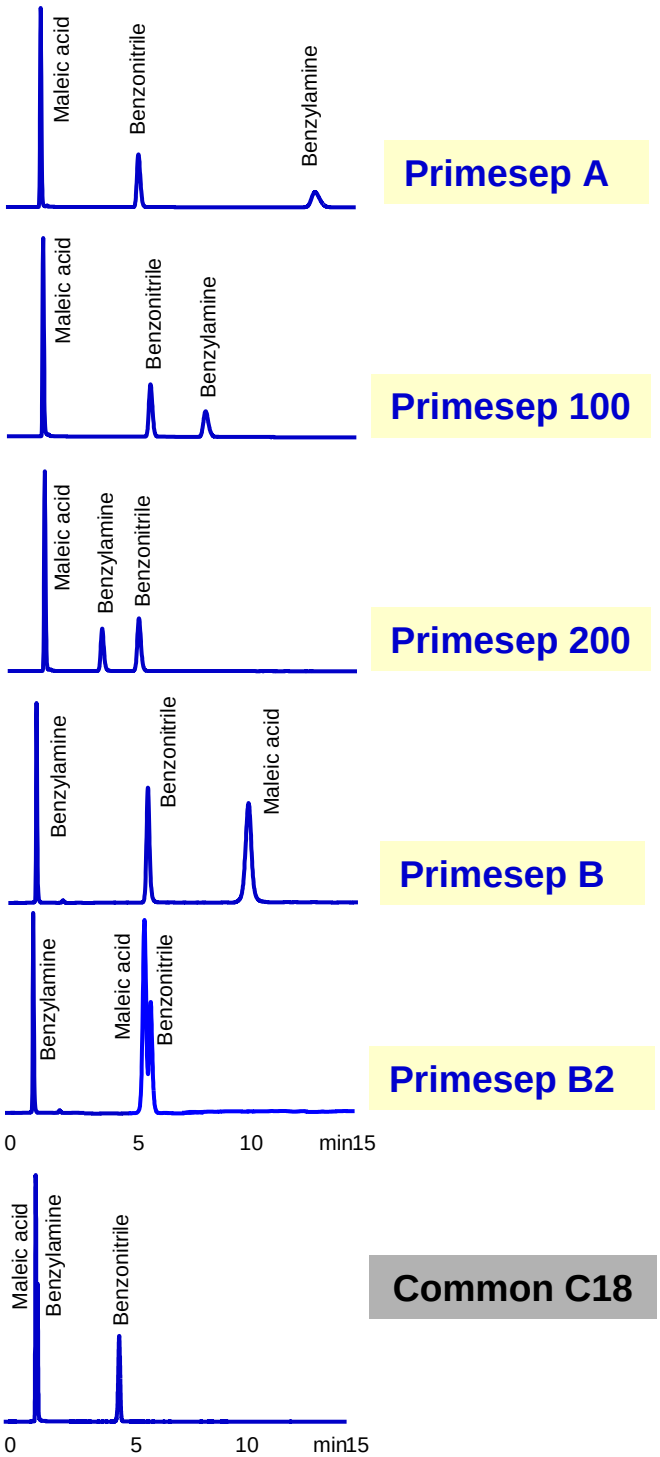
Primesep 300 at pH > 3.5



Primesep 300 at pH < 2.5



Primesep Columns Comparison



Primesep columns are different in the degree they retain ionic compounds.

Neutral compounds are retained on all columns similarly.

Primesep A, 100, 200 and 300 are cation exchange columns with different strengths of embedded functional groups. Primesep B and B2 are anion exchange columns.

Primesep is a silica based material which is stable in all organic solvents and water at pH range from 1.0 to 7.5

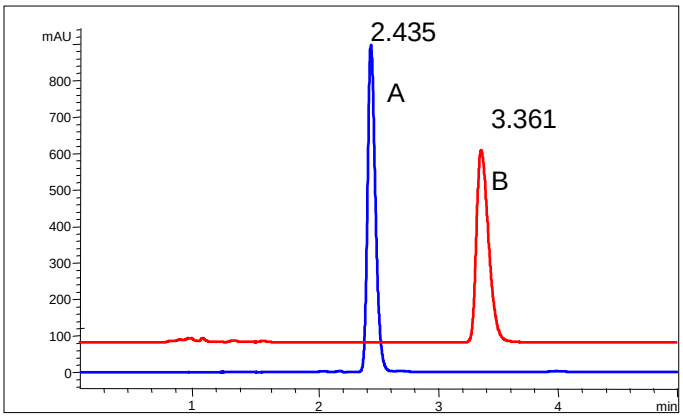
Columns: 150 x 4.6 mm x 5 um
Flow rate: 1.0 mL/min.
Detector: UV 210 nm
Mobile phase:
Water/MeCN/TFA-60/40/0.1

Primesep 100 Column Resists Loss of Retention in 100% Aqueous Mobile Phase

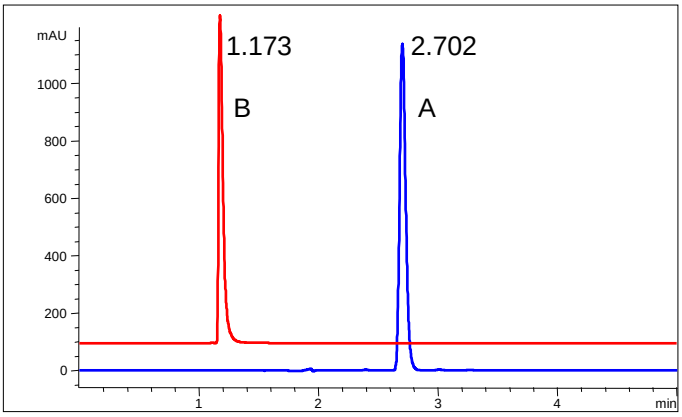
Reverse-phase columns normally do not perform well in 100% aqueous mobile phase. Dewetting of silica pores or collapse of the alkyl chains of the stationary phase causes a sudden loss of retention in this condition.

Primesep™ columns are designed with polar ionizable groups within a stationary phase layer, attracting enough water to keep the column in the wetted state with unfold alkyl chains. Our columns are comparable with YMC-AQ® and Waters Polarity®, but different in selectivity and ability to work in other modes of separation besides a reverse mode, such as normal separation, polar organic separation, ion-exchange, and ion-exclusion. These columns have no end capping chemistry.

Loss-of-end capping is a common cause of changing column properties and lost selectivity. Primesep column has only one type of ligand on silica surface. Loss of this ligand is due to aging or harsh use conditions does not affect relative contribution of each separation mode.



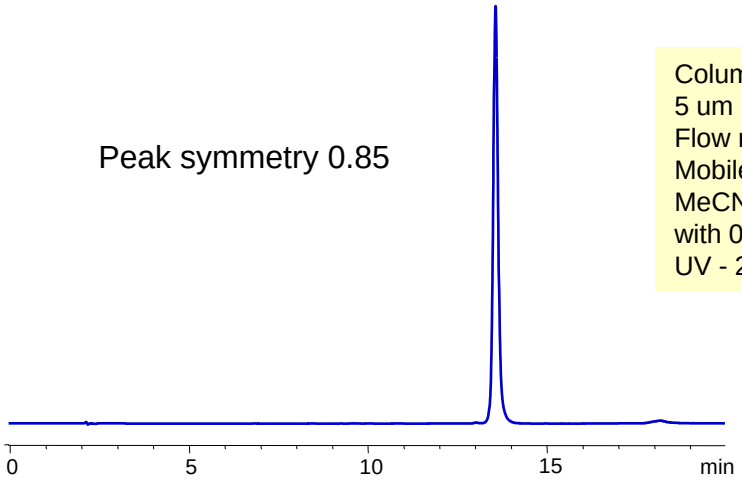
Column: Primesep 100
Mobile phase:
A Water/MeCN – 85/15%
B Water – 100%. The column was left with no flow at zero pressure for 24 hr
Sample: benzoquinone 0.1 mg/ml in water
Injection: 5 ul
Detector: UV 270 nm



Column: Conventional C8
Mobile phase: A Water/MeCN – 85/15%
B Water – 100%. The column was left with no flow at zero pressure for 1 hr
Sample: benzoquinone 0.1 mg/ml in water
Injection: 5 ul
Detector: UV 270 nm

Amitriptyline Test

The amitriptyline test shows residual silanol activity. Primesep columns demonstrate zero silanol interaction with any charged compounds. The strong cation or anion exchange groups completely mask any silanol effects.



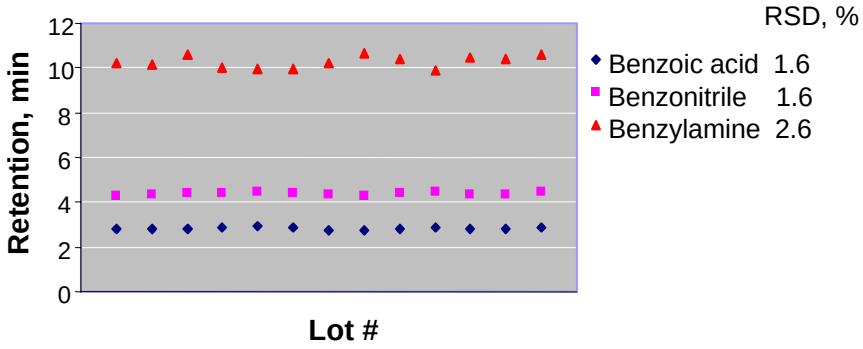
Column: Primesep B 150 x 4.6 mm x 5 um
Flow rate: 1.0 mL/min.
Mobile phase: Gradient MeCN/H_2O-10/90 to 30/70 in 15 min with 0.1 % TFA
UV - 250 nm

Lot to Lot Reproducibility of Primesep 100

Every Primesep™ column has a dual chemistry stationary phase with a hydrophobic long alkyl chain and an ionizable cationic or anionic embedded group. When the polar group bears a charge, it effectively shields any other less polar groups of the stationary phase. As a result, silanol groups, which cause unwanted interaction in many reverse-phase columns, are completely undetectable and do not affect the peak shape and selectivity.

Primesep™ multi-step manufacturing process guarantees good reproducibility of retention of neutral, acidic and basic compounds. The plot below shows the consistency of performance achieved on 13 lots of the stationary phases synthesized from 3 different lots of silica gel during one year.

Lot to Lot Reproducibility of Primesep 100 Silica



Column: Primesep 100
Mobile phase: Water/MeCN/TFA– 50/50/0.8
Sample Injection: 5 ul
Detector: UV 210 nm

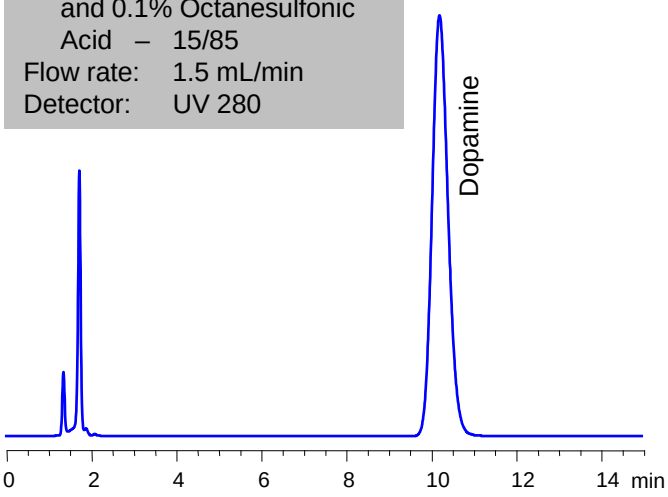
Retention of Polar Compounds without Ion-Pairing Reagents

In many fields, and in liquid chromatography particularly, the reverse-phase mode is a technique of choice to solve many separation problems. One of the limitations of reverse-phase columns is lack of retention of highly polar compounds on conventional stationary phases. Traditionally, mobile phase additives, such as ion-pairing reagents, have to be employed to achieve the separation of these compounds. In its turn, the use of ion-pairing reagents also has its limitations, i.e. artifacts when using gradient elution, incompatibility with Mass Spectrometry, Evaporating Light Scattering Detection, preparative chromatography, and more complex mobile phase preparations.

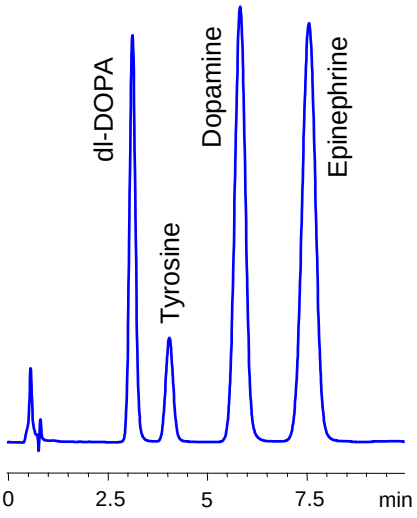
None of these limitations exist for Primesep™ mixed-mode stationary phases that are suitable for separations of polar and non-polar compounds at both analytical and preparative scales in isocratic and gradient modes. These stationary phases allow for a great degree of flexibility in the separation of a broad range of analytes on one stationary phase platform using simple mobile phases that are compatible with multiple detection modes.

Dopamine Separation

USP L1 type column,
300 x 4.6 mm
Mobile Phase:
$MeCN/H_2O$ with 1% AcOH and 0.1% Octanesulfonic Acid – 15/85
Flow rate: 1.5 mL/min
Detector: UV 280



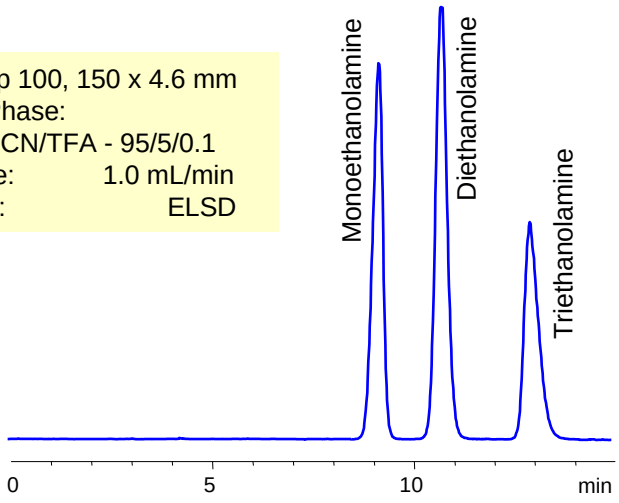
Primesep 200, 50 x 4.6 mm
Mobile Phase:
H_2O/MeCN/TFA - 90/10/0.02
Flow rate: 1.0 mL/min
Detector: UV 210



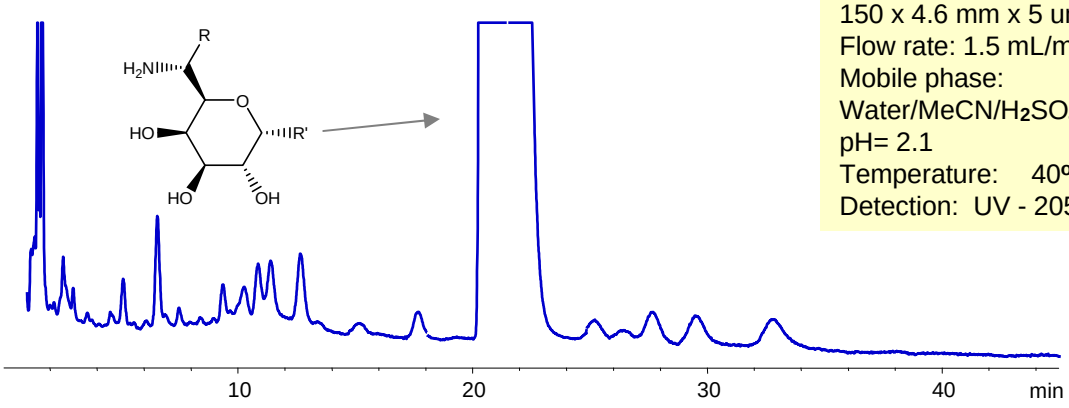
Ethanolamines Separation

Polar compounds are separated on Primesep columns by degree of polar interaction. Polar interaction includes electrostatic interaction which can be utilized by ion-exchange mechanism or hydrogen bonding which are adjustable by amount of water in the mobile phase.

Primesep 100, 150 x 4.6 mm
Mobile Phase:
H_2O/MeCN/TFA - 95/5/0.1
Flow rate: 1.0 mL/min
Detector: ELSD



Analysis of Amino Sugars

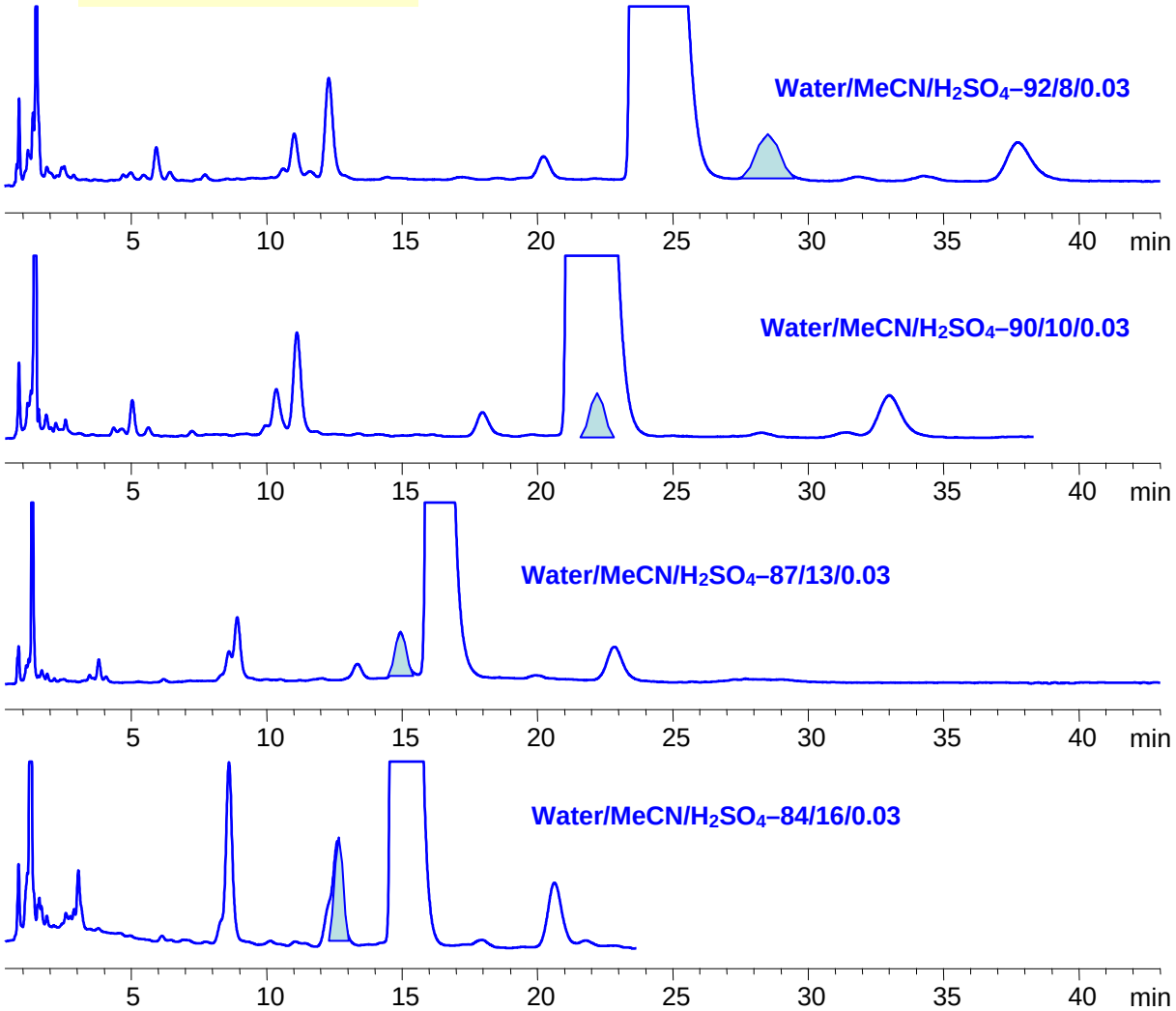


Column: Primesep 100
150 x 4.6 mm x 5 um
Flow rate: 1.5 mL/min.
Mobile phase:
Water/MeCN/H_2SO_4–8/92/0.03
pH= 2.1
Temperature: 40ºC
Detection: UV - 205 nm

Example of Method Development

Different compounds respond differently to changes in concentration of organic modifier. Neutral non-polar compounds are effected significantly, while polar compounds show a different response. This is a powerful tool for separation tuning on Primesep columns.

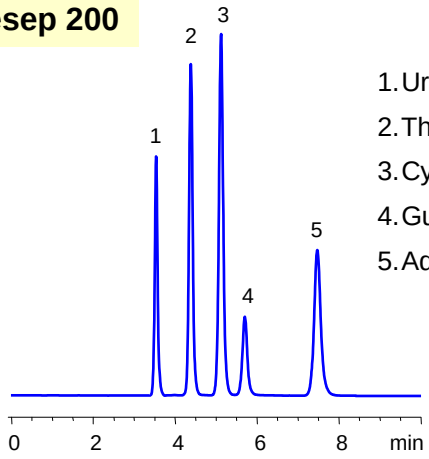
Column: Primesep 100
150 x 4.6 mm x 5 um
Flow rate: 1.5 mL/min.
Temperature: 40ºC
Detection: UV - 205 nm



Separation of Nucleobases

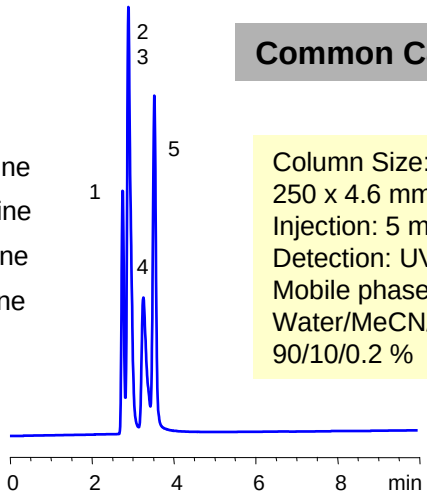
It's a known fact that polar organic compounds retain poorly on reverse-phase columns. These compounds often have an ionizable group in a molecule whose bearing charge makes the molecule even more polar and difficult to retain and separate. The Primesep 200 column is a unique solution for this situation.

Primesep 200



1. Uracil
2. Thymine
3. Cytosine
4. Guanine
5. Adenine

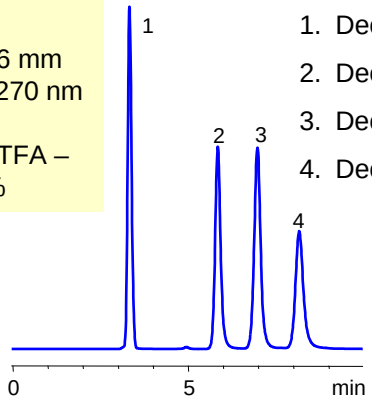
Common C8



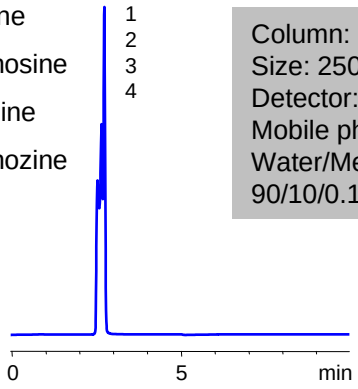
Column Size:
250 x 4.6 mm x 5 um
Injection: 5 mcl
Detection: UV 270 nm
Mobile phase:
Water/MeCN/TFA – 90/10/0.2 %

Separation of Nucleosides

Column:
Primesep 200
Size: 250 x 4.6 mm
Detector: UV 270 nm
Mobile phase:
Water/MeCN/TFA – 85/15/0.075 %



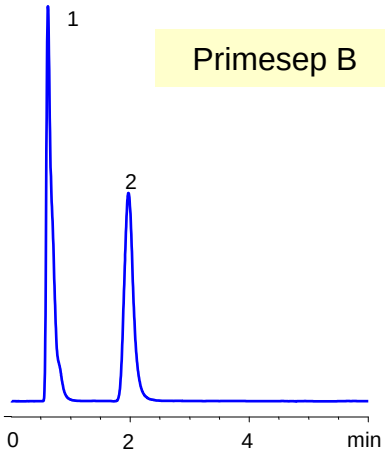
1. Deoxyuridine
2. Deoxyguanosine
3. Deoxycytidine
4. Deoxyadenozine



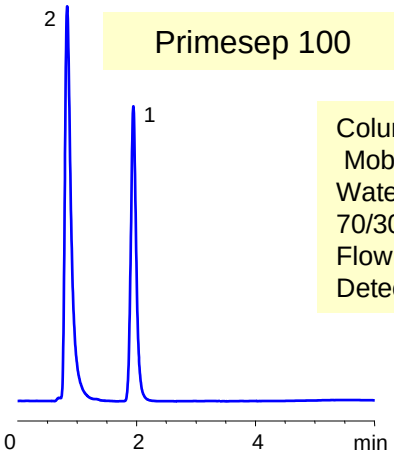
Column: Common C18
Size: 250 x 4.6 mm
Detector: UV 270 nm
Mobile phase:
Water/MeCN/TFA – 90/10/0.1 %

Ion-Exchange Mechanism in High Organic Mobile Phase. Analysis of Glyphosate.

Primesep B



Primesep 100



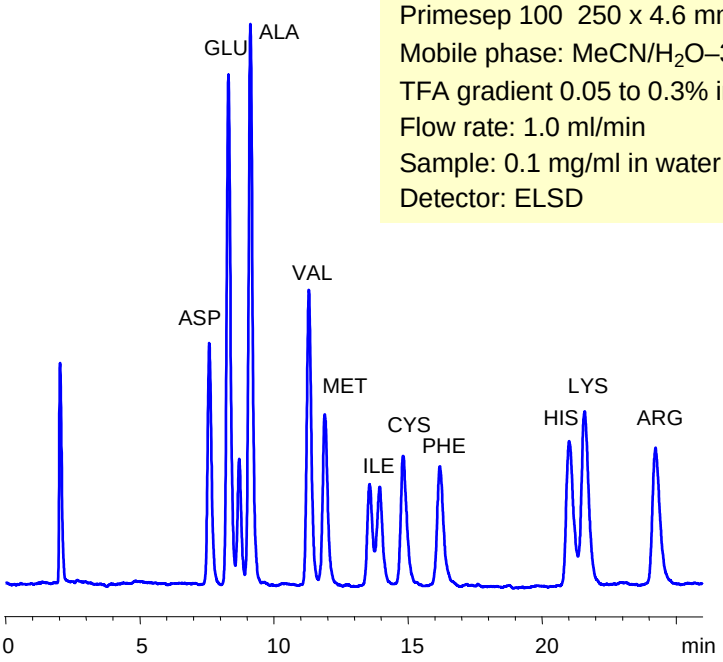
Column 50 x 4.6 mm
Mobile phase:
Water/MeCN/TFA– 70/30/0.1
Flow rate: 0.5 ml/min
Detection: ELSD

1. Isopropyl amine
2. Glyphosate

Analysis of Amino Acids

The presence of ion-exchange groups on a Primesep column makes it a perfect choice for separation of underivatized amino acids.

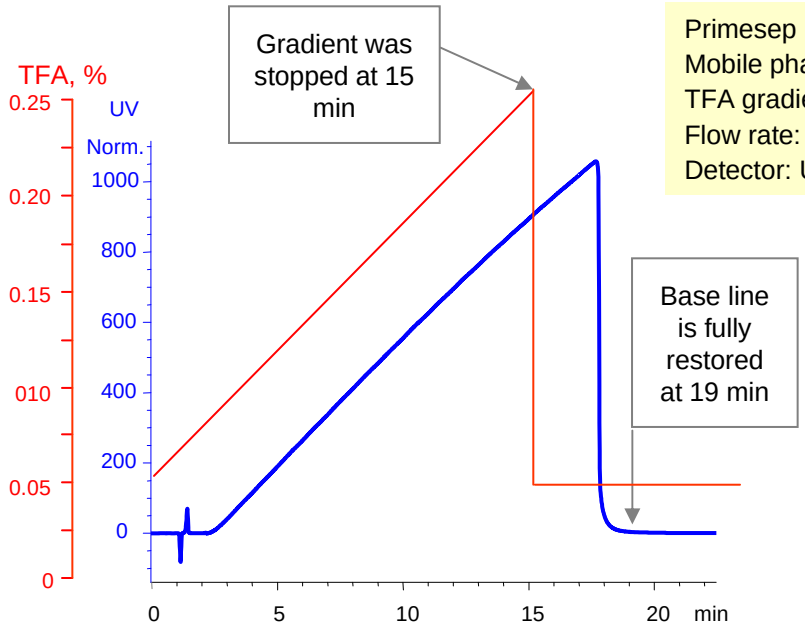
Acid gradient allows separation of compounds with significantly different pKa within a single chromatography run.



Primesep 100 250 x 4.6 mm
Mobile phase: MeCN/H_2O–30/70
TFA gradient 0.05 to 0.3% in 25 min
Flow rate: 1.0 ml/min
Sample: 0.1 mg/ml in water
Detector: ELSD

Extremely Fast Equilibration Time with Acid Gradient

The presence of the acidic groups on the Primesep column prevents retention of an acid on the stationary phase. It results in quick equilibration time equal to 2-3 column volumes. Thus, an acid gradient is a convenient option for separation of compounds with a drastically different pKa value.

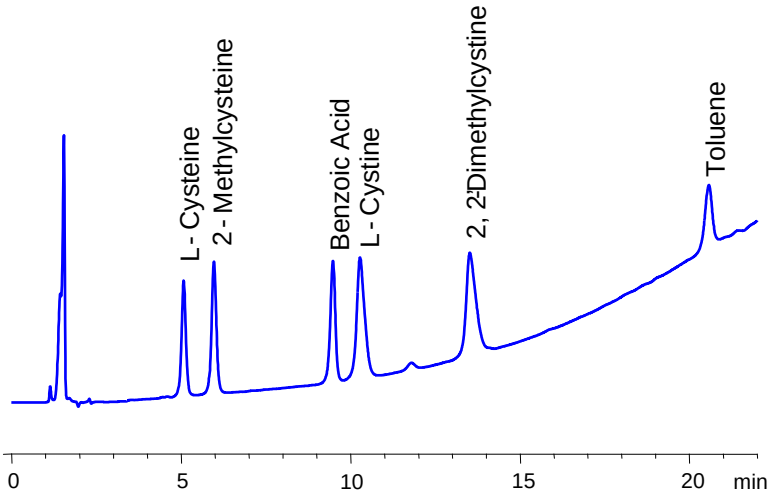


Primesep 100 150 x 4.6 mm
Mobile phase: MeCN/H_2O–15/85
TFA gradient 0.05 to 0.25% in 15 min
Flow rate: 1.0 ml/min
Detector: UV 210 nm

Retention of Polar Compounds

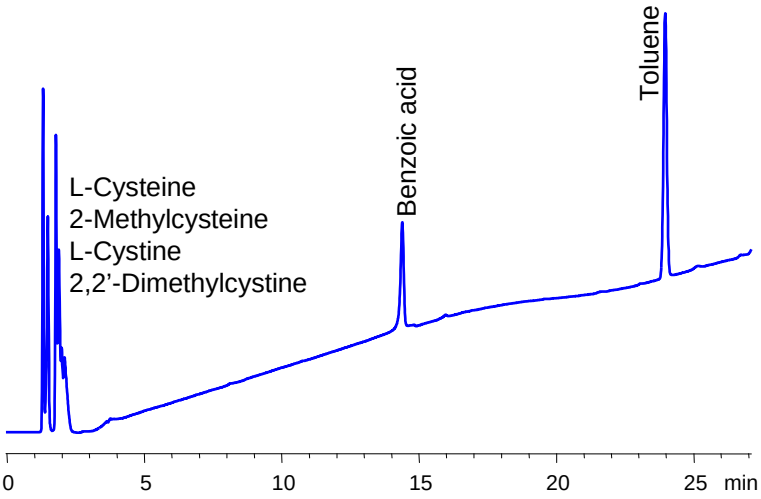
Complex mixtures with very polar and very hydrophobic compounds can be resolved with a gradient method.

Column: Primesep 100
150 x 4.6 mm x 5 um
Flow rate: 1.0 mL/min.
Mobile phase:
Water/MeCN/H_2SO_4-85/15/0.06 to 55/45/0.06 in 20 min + 5 min hold.
Detector: UV: 210 nm



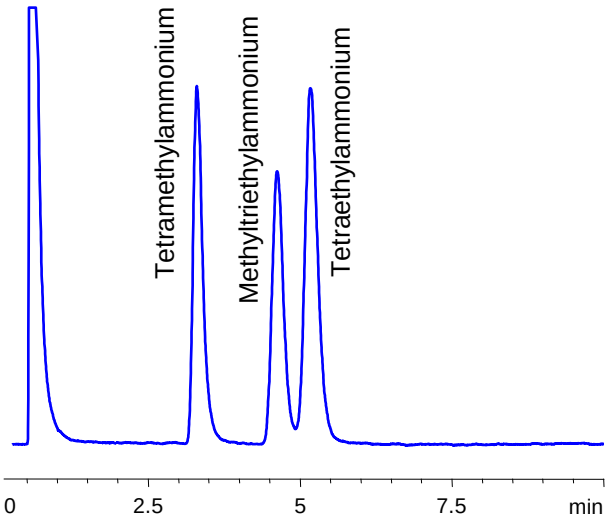
In similar conditions common reverse phase columns gave no retention of polar compounds and would require an ion-pairing reagent in the mobile phase.

Column: Common C8
150 x 4.6 mm x 5 um
Flow rate: 1.0 mL/min.
Mobile phase:
Water/MeCN/H_2SO_4-
100/0/0.06 to 40/60/0.06 in 25 min.
Detector: UV 210 nm



Separation of Quaternary Amines

Quaternary amines are strong bases. They are not volatile and can not be analyzed by GC. A typical HPLC separation will result in no or very little retention for these polar molecules. Primesep C column with volatile mobile phase allows to separate and quantitate quaternary amines with an ELSD or MS detection technique.

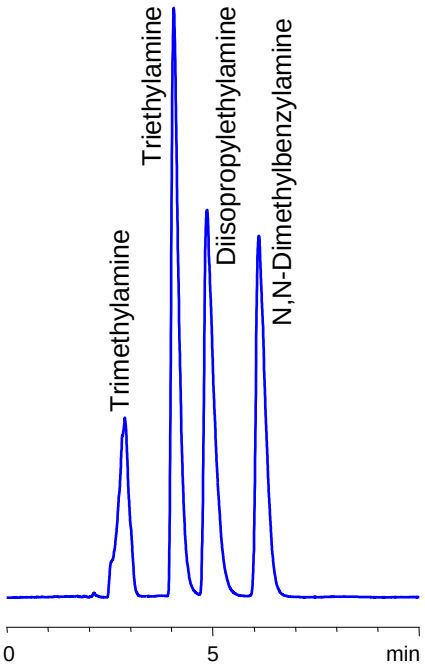


Primesep C 50 x 4.6 mm x 5 um
Mobile phase: MeCN/H_2O–15/85
TEA acetate 20 mM pH 5.0
Flow rate: 1.0 ml/min
Sample: 0.6 mg/ml each
Injection: 5 mc
Detector: ELSD, (Temperature 35°C)

Ion-Exchange and Hydrophobic Mechanism in Separation of Tertiary Amines.

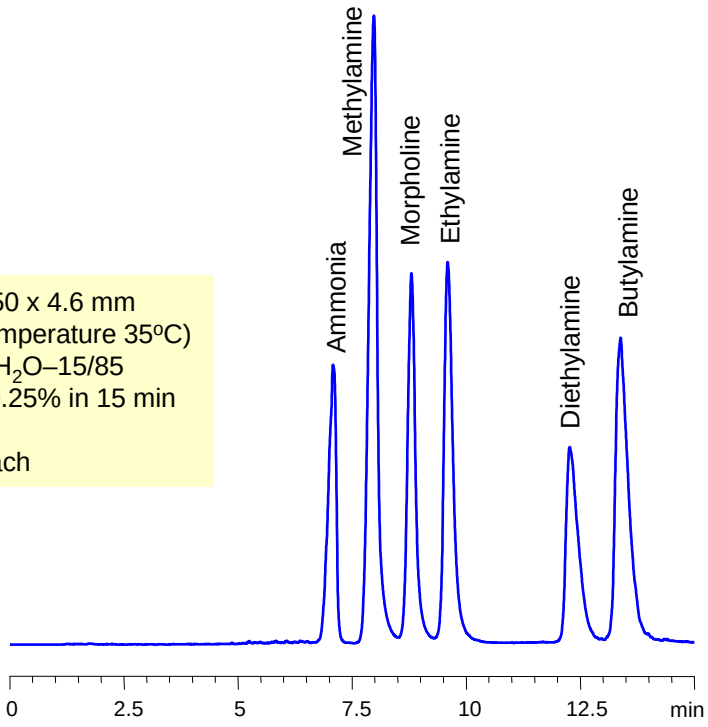
Strong bases, such as tertiary amines, retain too strongly on Primesep A or Primesep 100 columns. Primesep 200 is a weaker cation exchanger than Primesep 100 and Primesep A, and it separates strong bases in mild conditions.

Primesep 200 column 150 x 4.6 mm x 5 um
Mobile phase: MeCN/H_2O/TFA–20/80/0.15
Flow rate: 1.0 ml/min
Injection: 5 ul
Sample: 3.0 mg/ml each
Detector: ELSD, (Temperature 35°C)



Ion-Exchange and Hydrophobic Mechanism in Separation of Amines.

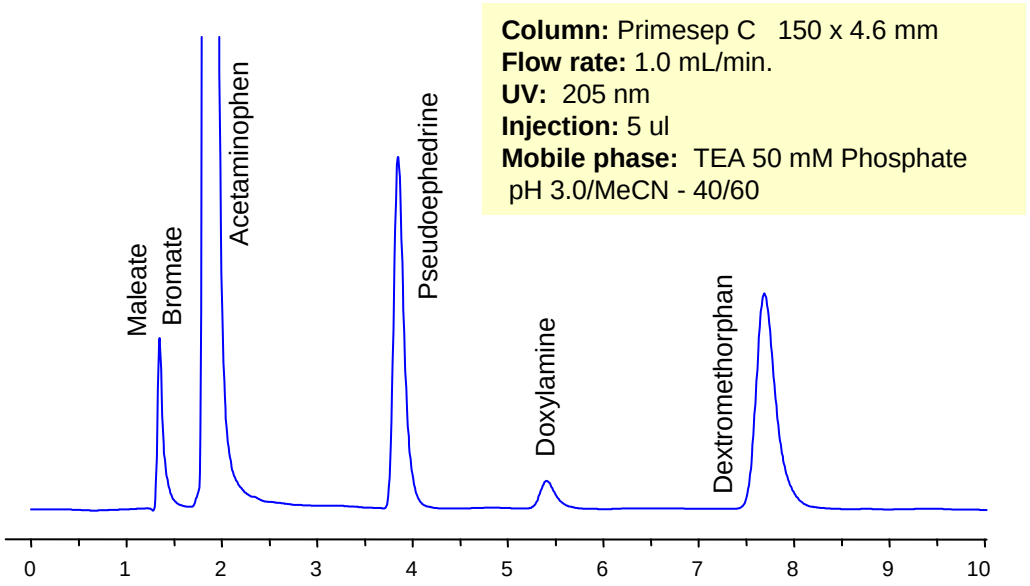
Primesep A column 150 x 4.6 mm
Detection: ELSD, (Temperature 35°C)
Mobile phase: MeCN/H_2O–15/85
TFA gradient 0.05 to 0.25% in 15 min
Flow rate: 1.0 ml/min
Sample: 1.0 mg/ml each

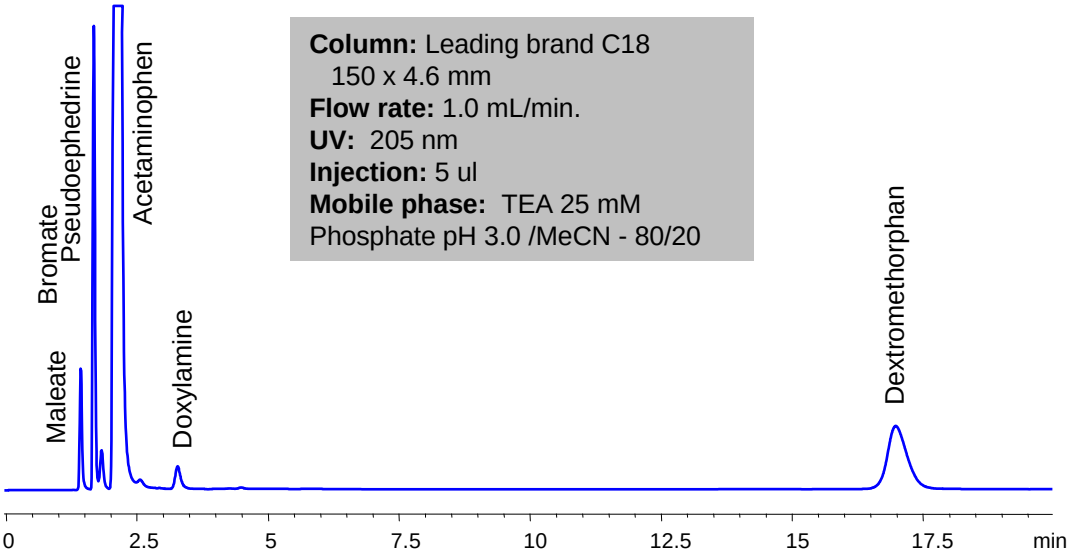


Retention of polar and hydrophobic compounds by isocratic method

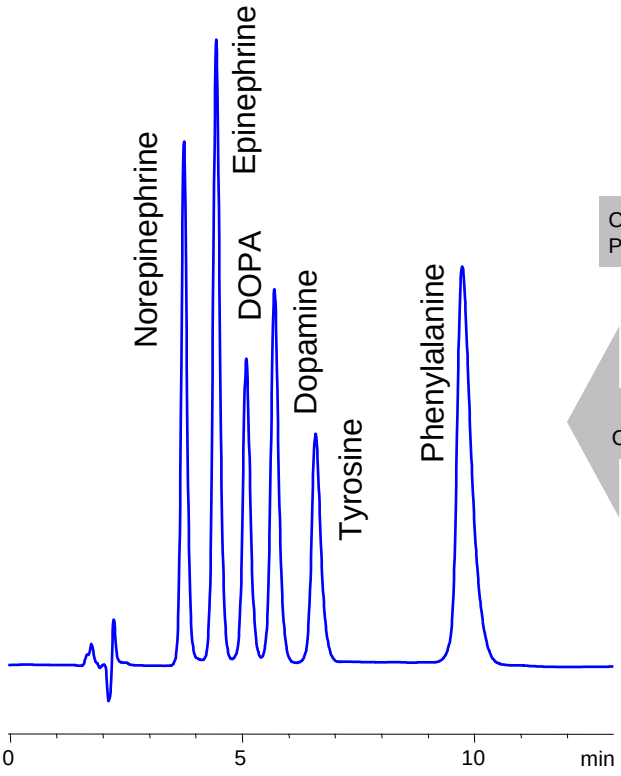
Primesep™ mixed-mode stationary phases provide multiple types of interactions with analytes. Ionizable compounds interact with the stationary phase by reverse-phase, ion-exchange or ion-exclusion mechanisms. The amount of the acid in the mobile phase influences the retention attributed to the ion-exchange interaction to the same degree as the organic modifier affects the retention in reverse-phase separation. Thus, the amounts of organic and acidic modifiers are both important for control of retention of ionizable analytes.

Separation of Active Ingredients in Cough and Cold Drugs





Ion-Exchange and Hydrophobic Mechanism In Separation of Neurotransmitters.

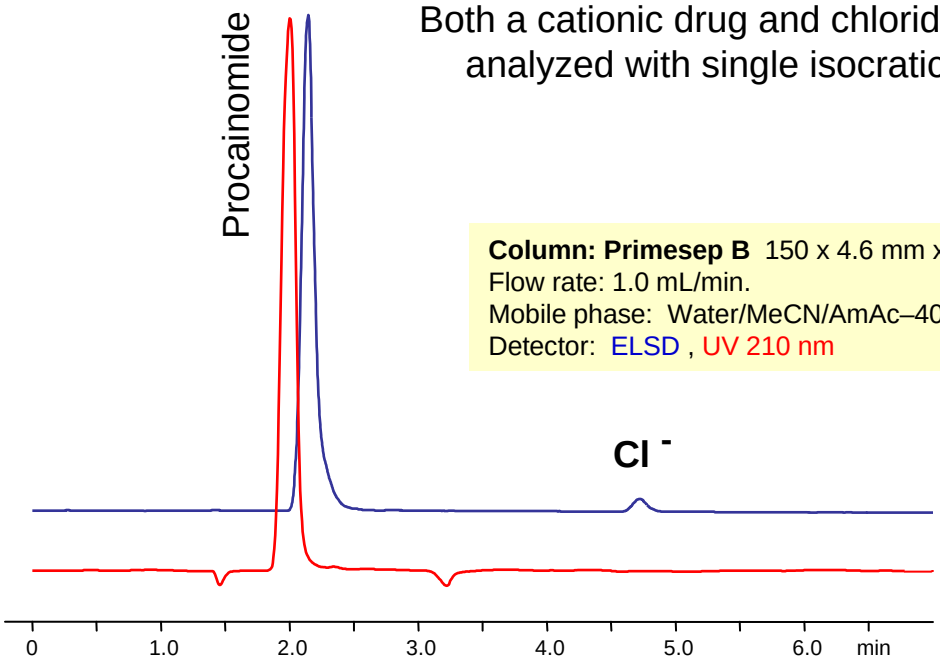


Catecholamines Pathway

Separation of Catecholamines

Phenylalanine
↓
Tyrosine
↓
DOPA
↓
Dopamine
↓
Norepinephrine
↓
Epinephrine

Primesep 200, 150 x 3.2 mm
Mobile Phase:
H_2O/MeCN/TFA - 90/10/0.1
Flow rate: 0.5 mL/min
Detector: UV 210 nm



Both a cationic drug and chloride anion are analyzed with single isocratic method

Column: Primesep B 150 x 4.6 mm x 5 um
Flow rate: 1.0 mL/min.
Mobile phase: Water/MeCN/AmAc–40/60/0.2
Detector: ELSD , UV 210 nm

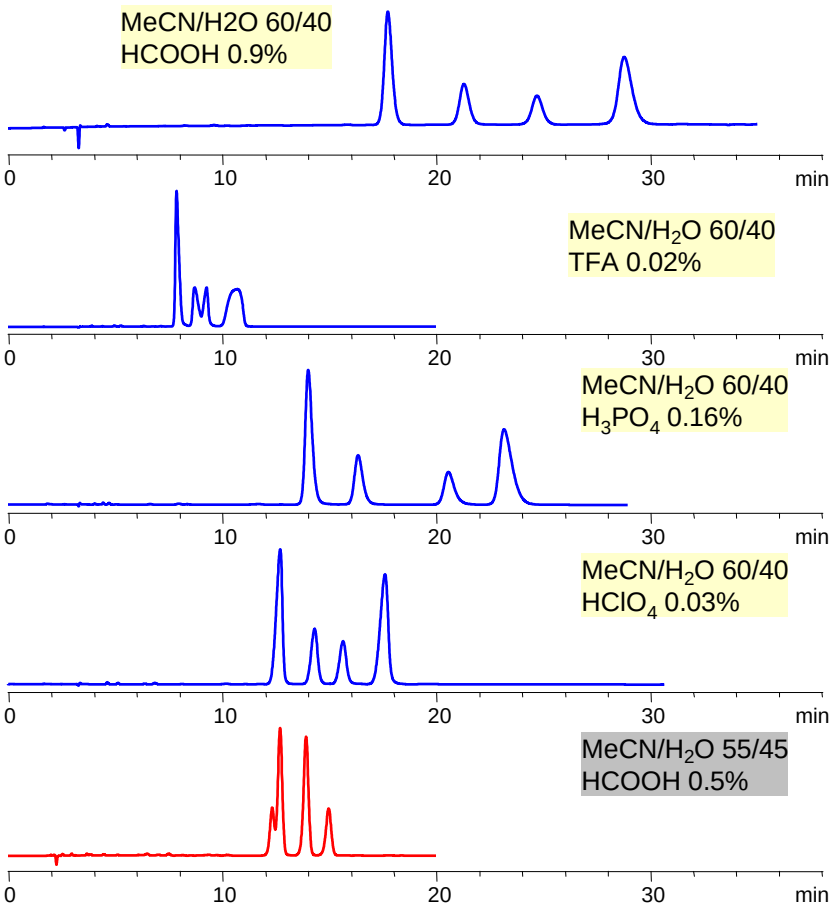
Unique Adjustable Selectivity

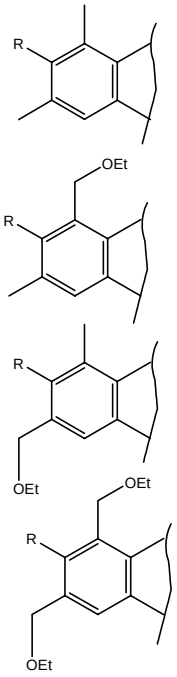
Primesep™ mixed-mode stationary phases provide multiple types of interactions with analytes. Ionizable compounds interact with the stationary phase by reverse-phase, ion-exchange or ion-exclusion mechanisms. The amount of the acid in the mobile phase influences the retention attributed to the ion-exchange interaction to the same degree as the organic modifier affects the retention in reverse-phase separation. Thus, the amounts of organic and acidic modifiers are both important for control of retention of ionizable analytes.

In addition to hydrophobic interactions, neutral compounds participate in different polar interactions with highly polar column functional groups. The behavior of polar groups can be modified by varying the mobile phase.

Basic functional groups on Primesep B column form salts with different acid residue (sulfate, perchlorate, trifluoroacetate, etc.), and each salt participates differently in polar interaction with neutral analytes. Analytes themselves can be ionized in many ways depending on the pH of the mobile phase, and retention time of your compounds can also be substantially altered by changing the pH of the mobile phase.

Effect of Acidic Modifier on Selectivity



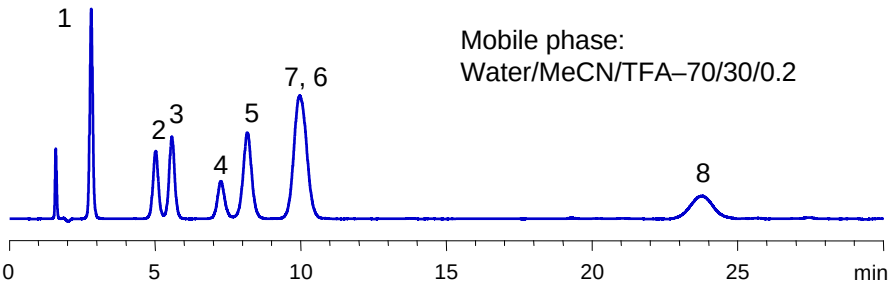


Primesep B (blue)
150 x 4.6 mm x 5 um
Common C18 (red)
250 x 4.6 x 5 um

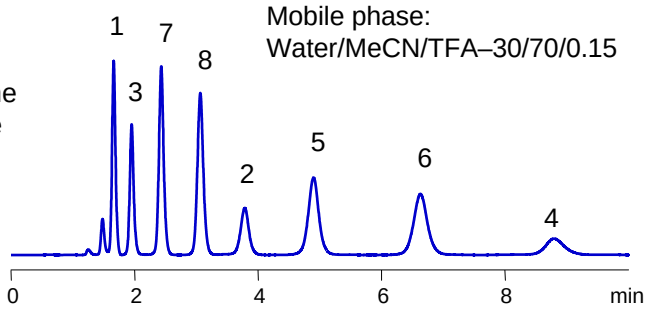
Flow rate: 1.0 mL/min.
UV - 250 nm

Control of Retention and Resolution

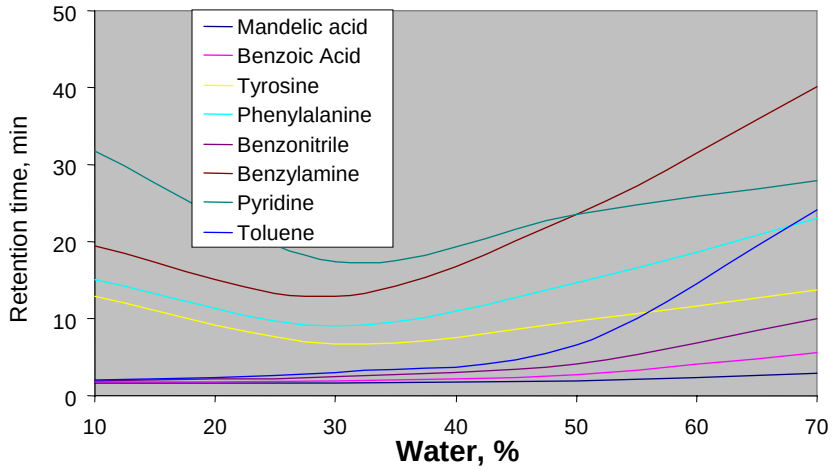
Critical pairs were resolved in this mixture by changing ion-exchange mechanism in mixed-mode interaction



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

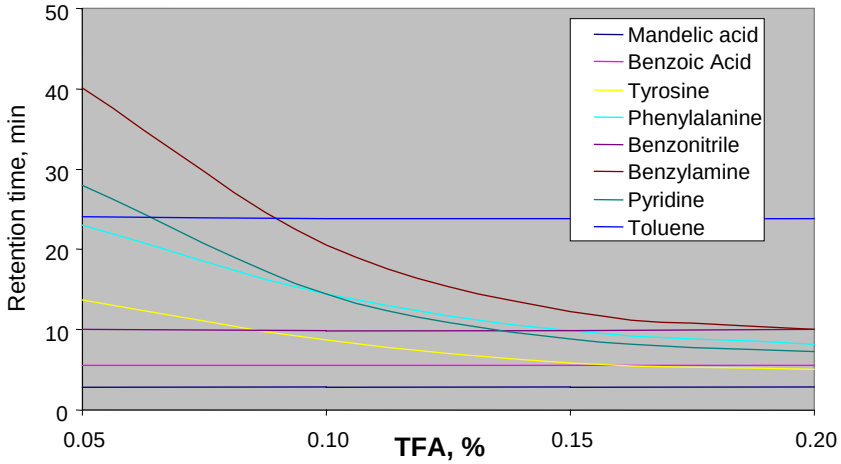


Primesep 100
150 x 4.6 mm x 10 um
Flow rate: 1.0 mL/min.
Detector UV - 210 nm



Effect of Water Concentration

Primesep 100 column
150 x 4.6 mm x 10 um
Flow rate: 1.0 mL/min.
Mobile phase:
Water/MeCN with
TFA-0.05%
Detector: UV - 210 nm



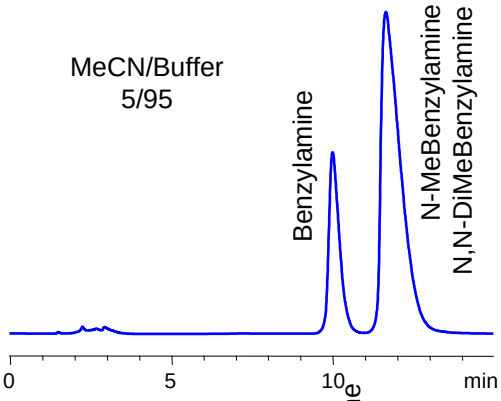
Effect of TFA Concentration

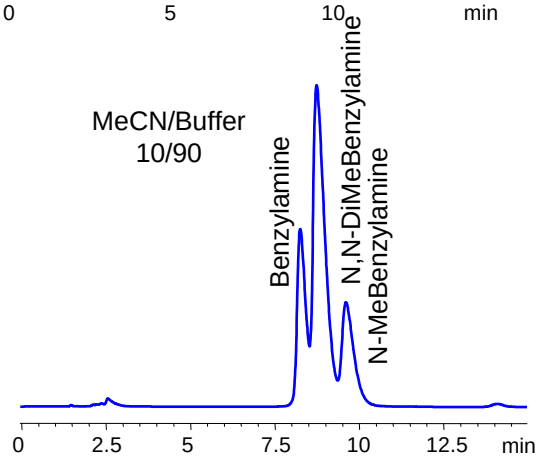
Primesep 100 column
150 x 4.6 mm x 10 um
Flow rate: 1.0 mL/min.
Mobile phase:
Water/MeCN – 70/30
Detector: UV - 210 nm

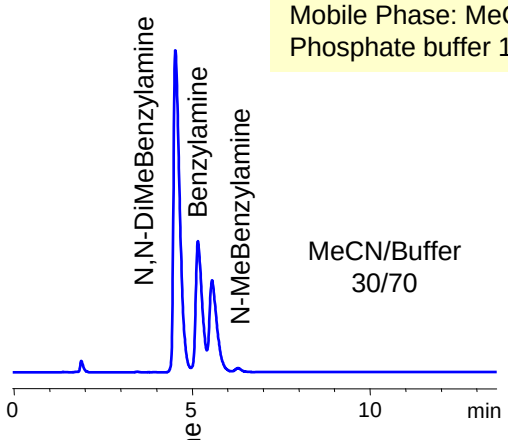
New Approach in Column Chemistry – Primesep C™.

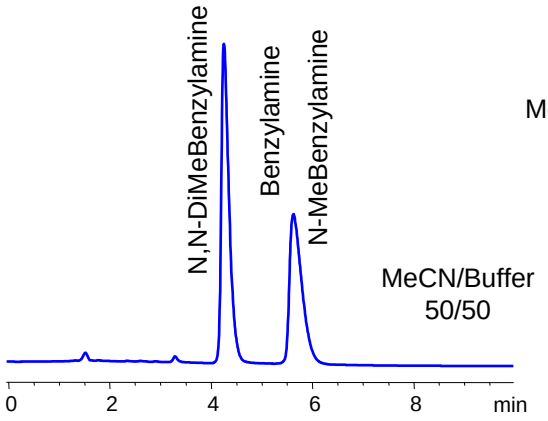
Primesep C column offers strong interaction with primary and secondary amines. This interaction increases with the decrease of water concentration in the mobile phase. By changing concentration of the organic modifier in the mobile phase, selectivity and elution order of the mixture of primary, secondary and tertiary amines can be tuned.

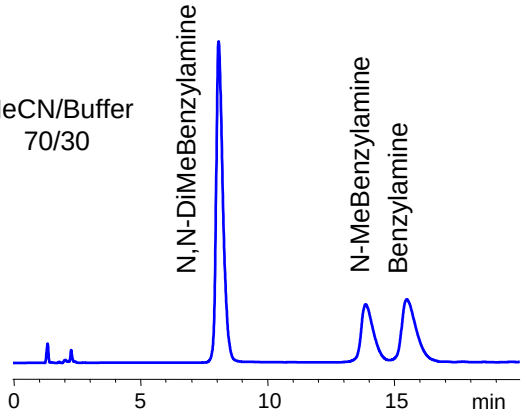
Column: Primesep C, 150x4.6 mm
Flow rate: 1.0 mL/min.
Detection: UV 210
Mobile Phase: MeCN/TEA-Phosphate buffer 10 mM pH 3.0

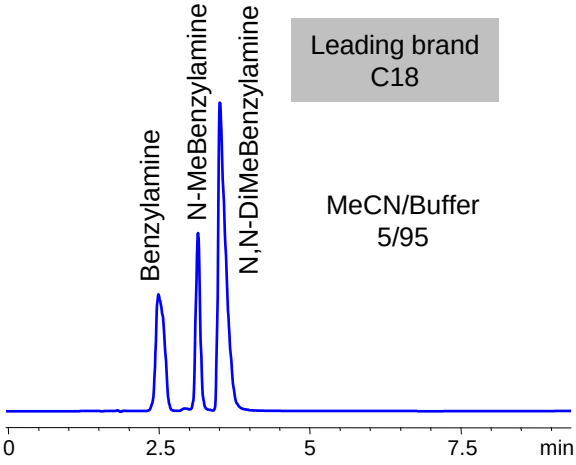


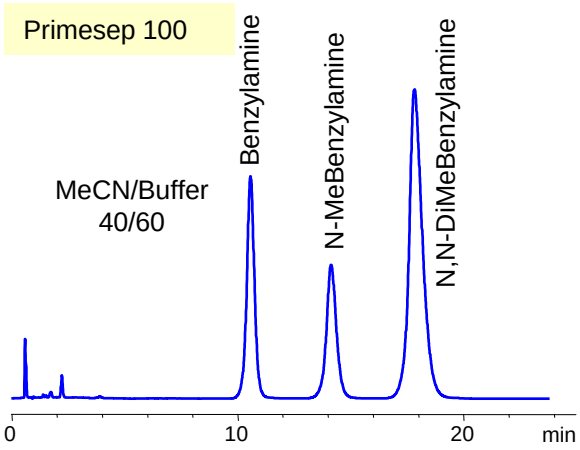




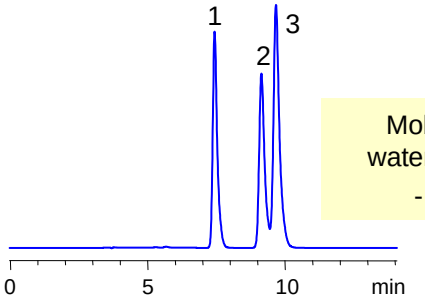






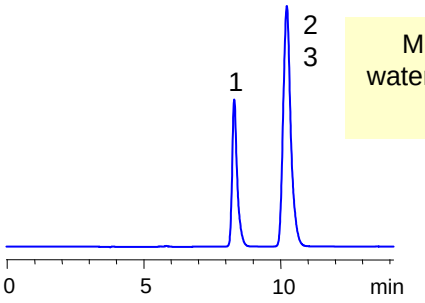


Effect of Acidic Modifier on Selectivity

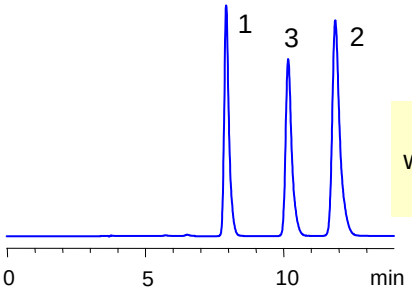


Mobile Phase: water/MeCN/TFA - 95/5/0.1

The Primesep B column has embedded amino-groups in the hydrophobic alkyl layer. When the acidic modifier in the mobile phase is changed, the hydrophobic amines form tight ion pairs with the modifier which can interact with neutral analytes by polar mechanism. Interaction with analytes is affected by the type of acid modifier.

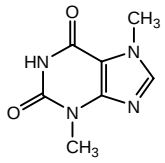


Mobile Phase: water/MeCN/ $HClO_4$ - 95/5/0.1

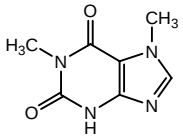


Mobile Phase: water/MeCN/ H_2SO_4 - 95/5/0.03

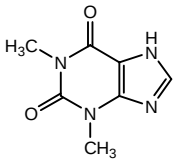
Primesep B 250 x 4.6 mm.
Flow rate:1.0 mL/min.
Sample: 0.3 mg/ml of each in MeCN/water
Injection: 5 µl
Temperature: 40ºC.
Detector: UV - 270 nm



Theobromine



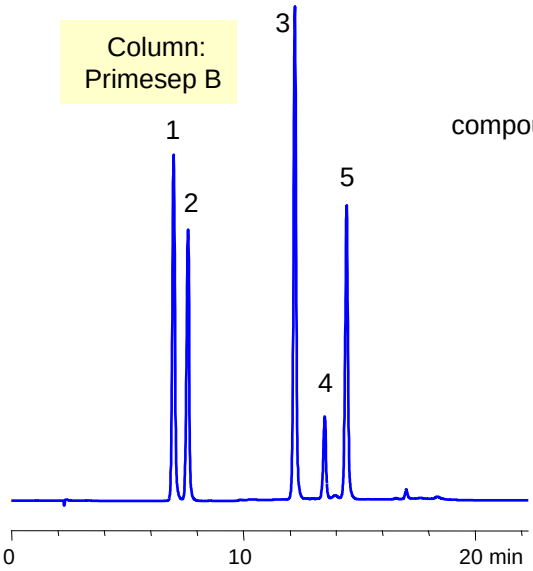
1,7-Dimethylxanthine



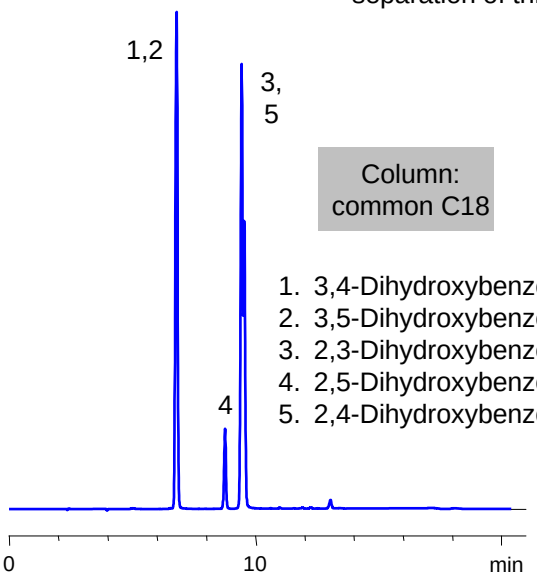
Theophylline

Improvement of Selectivity by Anion-Exchange and Hydrophobic Interactions

Column: Primesep B



On a Primesep B column, a separation of structurally similar compounds can be achieved by using their differences in hydrophobic properties, ion exchange properties, or combination of both. Columns with single type interactions do not produce efficient separation of this mixture.

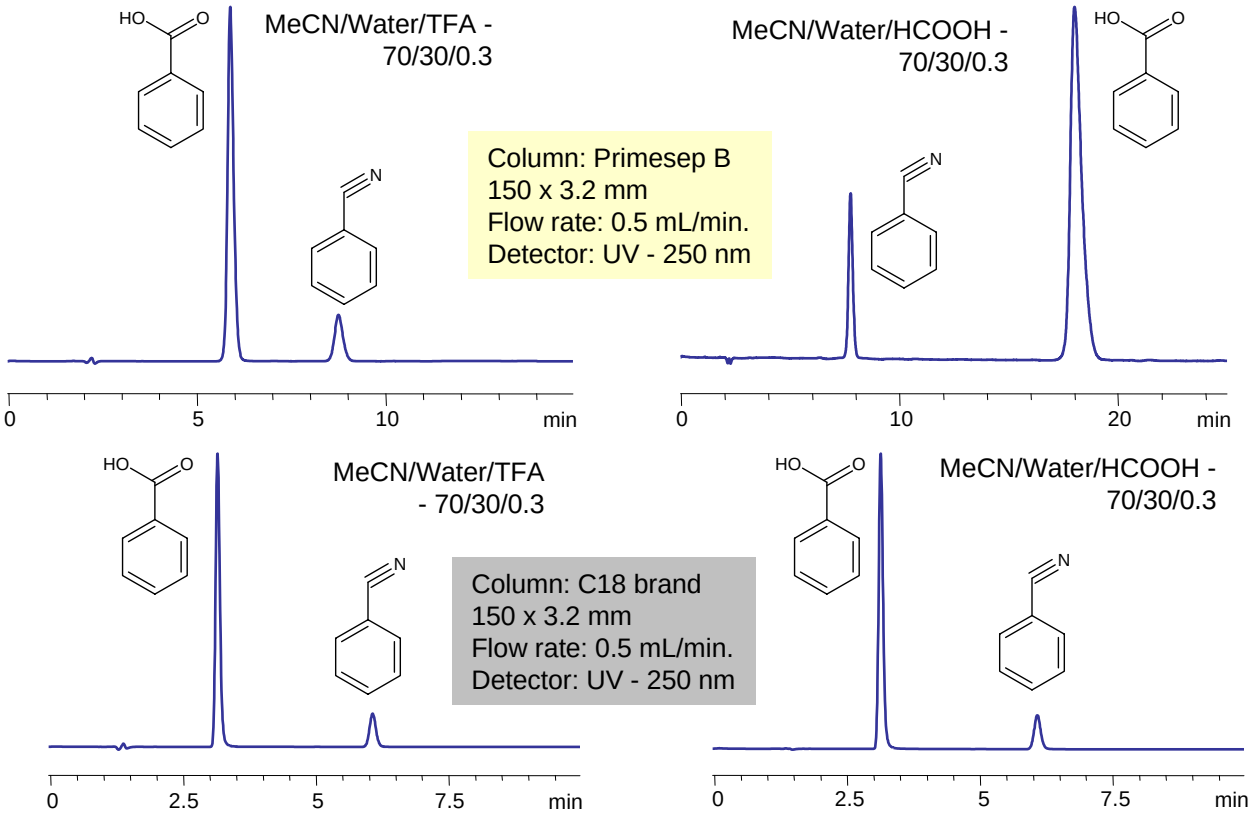


Column: common C18

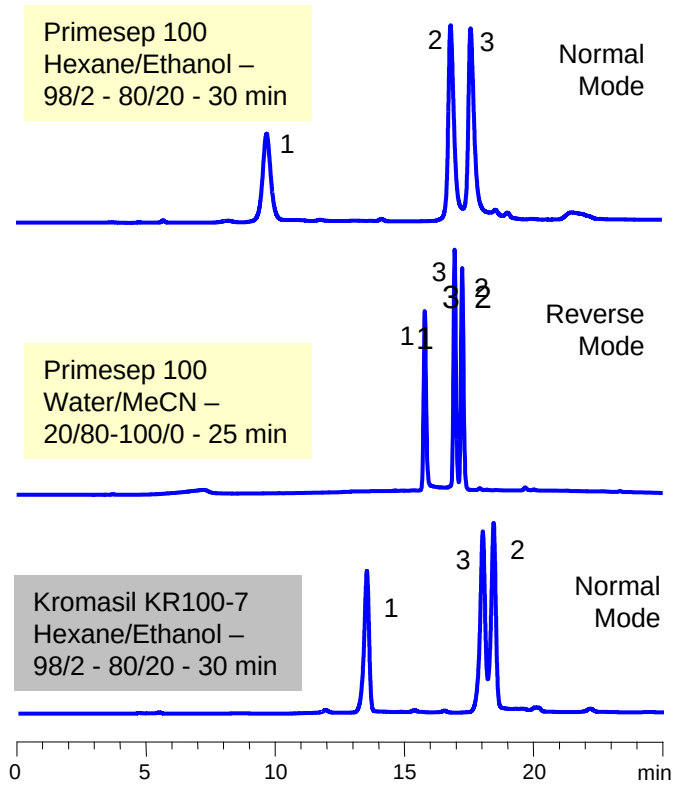
1. 3,4-Dihydroxybenzoic Acid
2. 3,5-Dihydroxybenzoic Acid
3. 2,3-Dihydroxybenzoic Acid
4. 2,5-Dihydroxybenzoic Acid
5. 2,4-Dihydroxybenzoic Acid

Column: 150 x 4.6 mm Flow rate: 1.0 mL/min.
Mobile phase: H_2O/MeCN/TFA -100-50/0-50/0.03-0.1 in 15 min
Sample: 0.2 mg/ml of each in MeCN/water
Injection: 5 µl
Temperature: 40ºC. Detector: UV - 270 nm

Control of Elution Order



Primesep B has an embedded anion exchange functional group, allowing a reversal retention order by using HCOOH instead of TFA in the mobile phase. Separation on typical reverse phase columns are not effected by type of acidic modifier.



Separation of Steroids. Reversed vs. Normal Mode

Neutral steroids are resolved in reverse and normal mode. The separation pattern vary in different separations modes and allows to select appropriate condition for a specific detection technique or a preparative separation. Also, a single column allows to have two alternative separation techniques to isolate impurity that may co-elute in a different separation mode.

Primesep 100 offers functional groups for interaction with the analyte that are different from silanol groups which are responsible for normal phase interaction on bare silica.

Column: 250 x 4.6 mm
Flow rate: 1.0 mL/min. UV - 240 nm

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

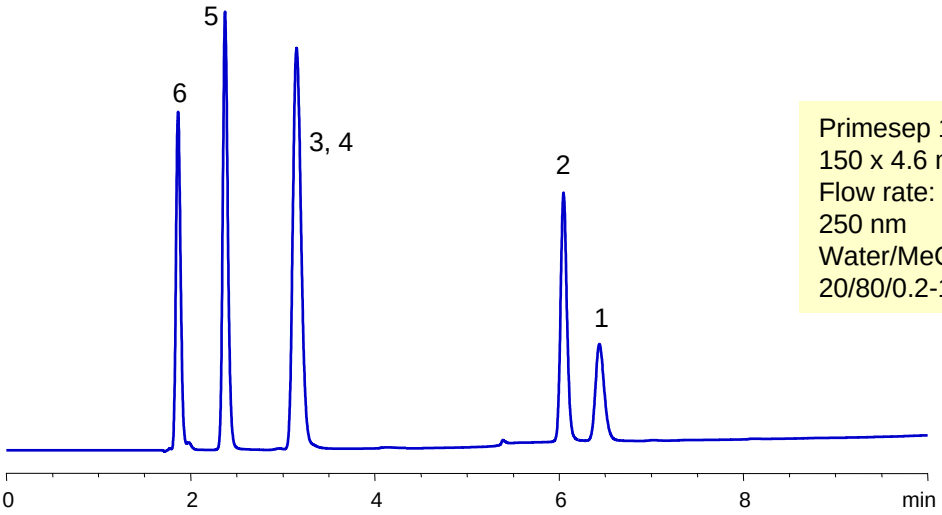
Primesep™ mixed-mode stationary phases provide two functional groups for interaction with analytes. One is a very hydrophobic alkyl chain, another is a very hydrophilic acid residue (Primesep A, 100, 200), or a protonated base (Primesep B).

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.

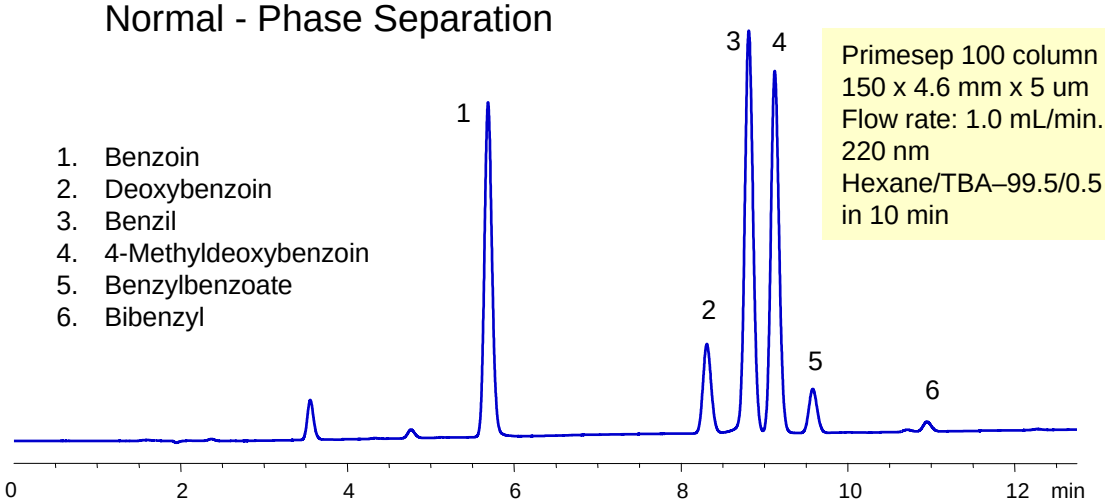
Reverse - Phase Separation



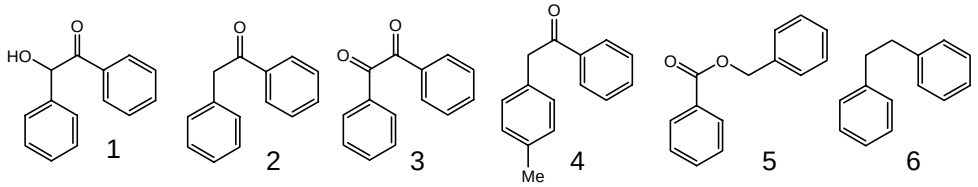
Primesep 100 column
150 x 4.6 mm x 5 um
Flow rate: 1.0 mL/min. UV - 250 nm
Water/MeCN/TFA–40/60/0.2-20/80/0.2-12 min

Normal - Phase Separation

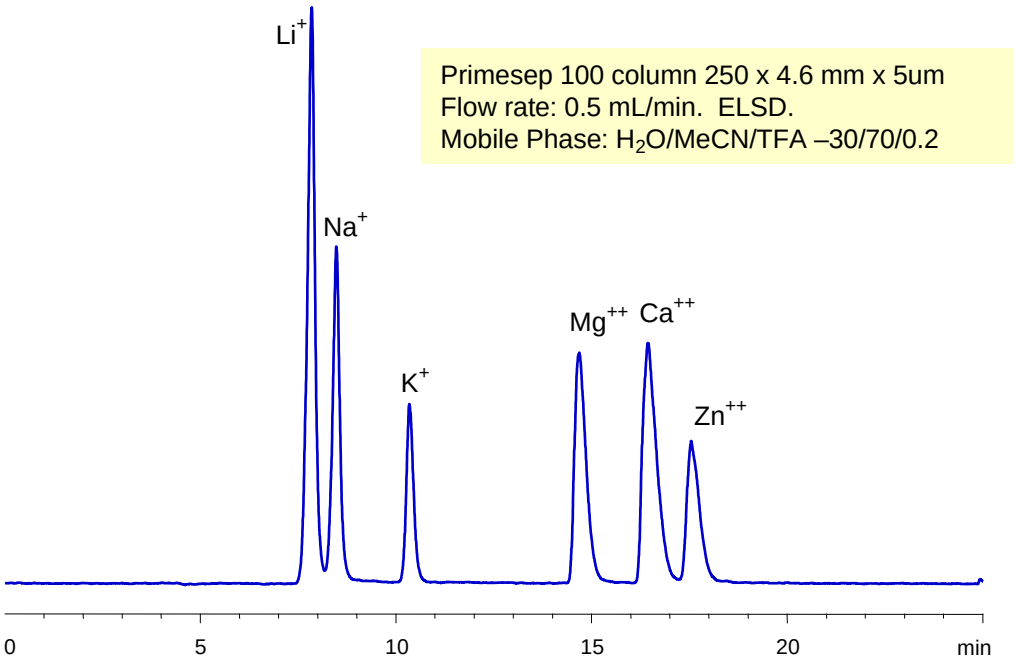
1. Benzoin
2. Deoxybenzoin
3. Benzil
4. 4-Methyldeoxybenzoin
5. Benzylbenzoate
6. Bibenzyl



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



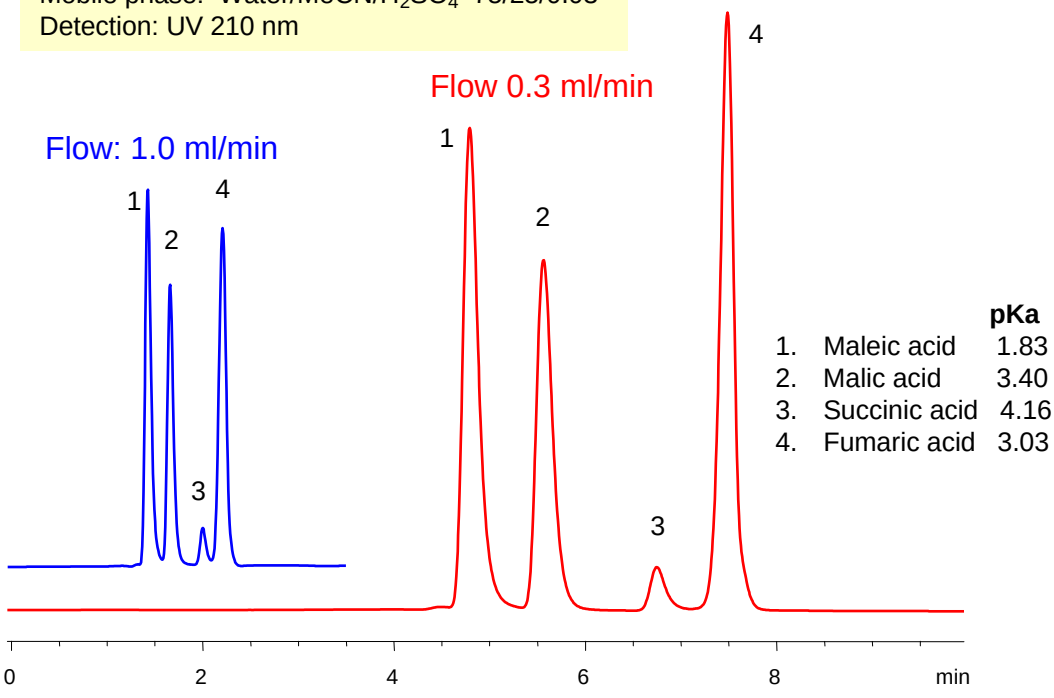
Ion-Exchange Mode. Separation of Inorganic Cations



Primesep 100 column 250 x 4.6 mm x 5um
Flow rate: 0.5 mL/min. ELSD.
Mobile Phase: H_2O/MeCN/TFA –30/70/0.2

Ion-Exclusion Mechanism. Separation of Diacids.

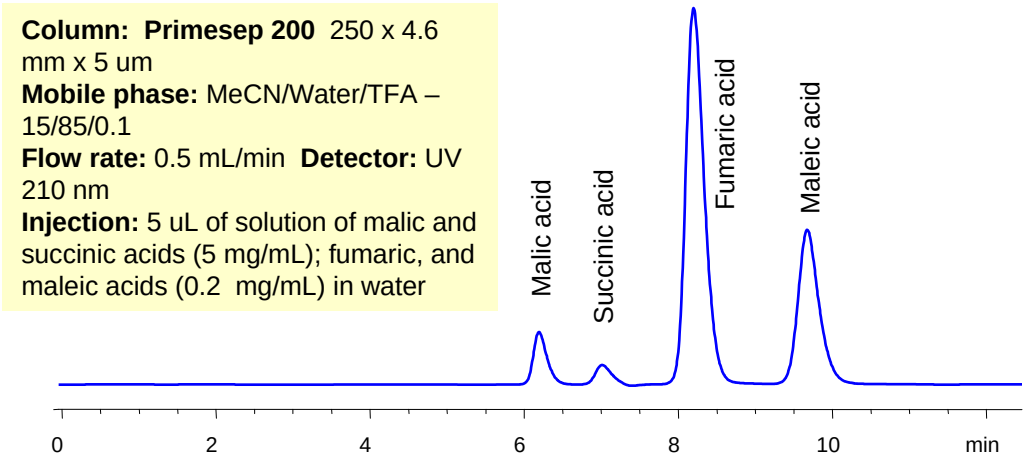
Primesep 100 column 150 x 4.6 mm x 5 um
Mobile phase: Water/MeCN/H_2SO_4–75/25/0.03
Detection: UV 210 nm



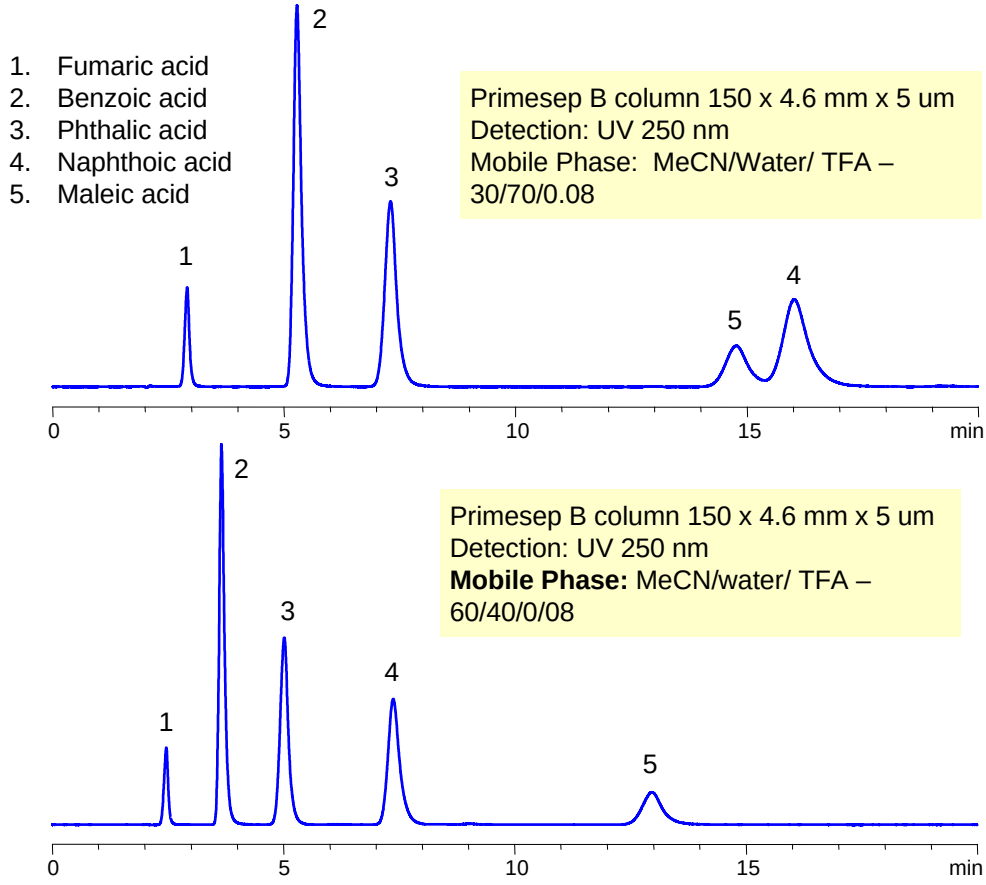
		pKa
1.	Maleic acid	1.83
2.	Malic acid	3.40
3.	Succinic acid	4.16
4.	Fumaric acid	3.03

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-Exclusion and Reverse Phase Mechanism in Separation of Diacids.



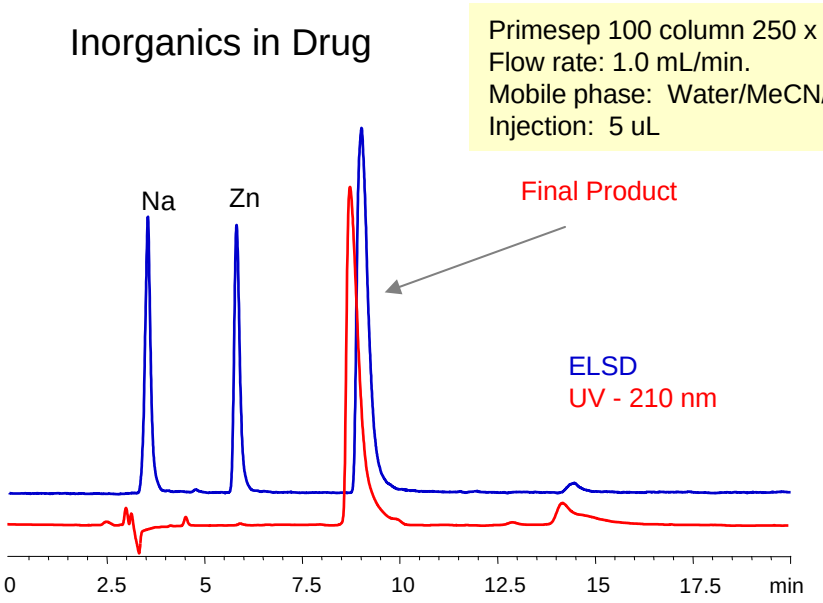
Ion-Exchange and Reverse Phase Mechanism in Separation of Acids.



Simultaneous Separation of Inorganic Ions and Organic Compounds in Single HPLC Method

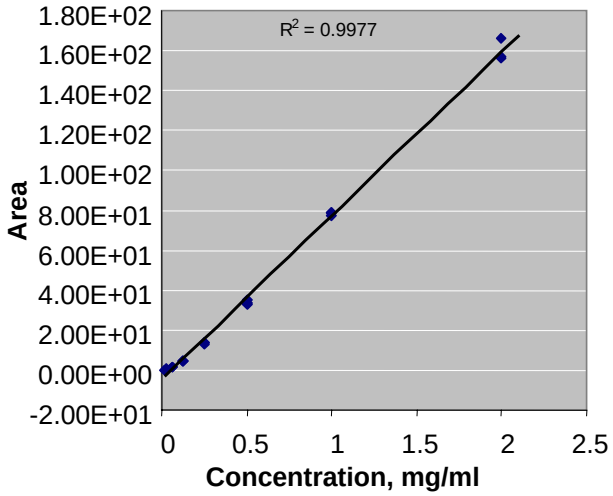
In many instances, ionizable compounds exist as salts of organic molecules with inorganic counter ions. This is common for drugs, surface active compounds, biological molecules, and many other industrial and research substances. Typically, two independent analytical methods are created for analysis of such salts – reverse phase for organic part and ion chromatography method, or titration, for inorganic part.

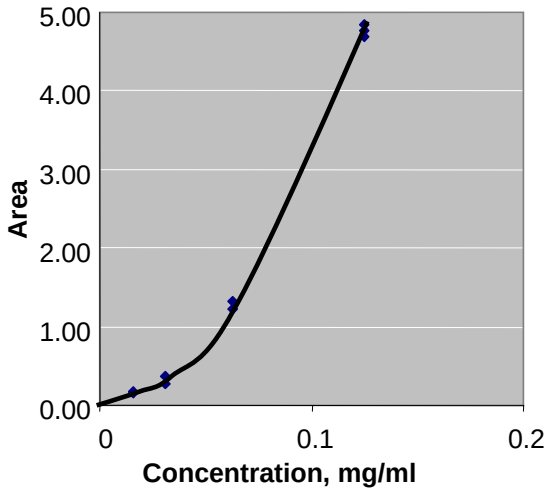
Primesep™ columns offer a unique ability to analyze both parts of such salts at the same time. ELSD in combination with standard UV detector is a convenient tool for this purpose.



Quantitation of inorganic components of the mixture can be performed using an ELSD signal. This detector is inherently not linear, but if the concentration of inorganic ion is significant, which is usually the case in pharmaceuticals, then significant linear region can be exploited with ELSD technology.

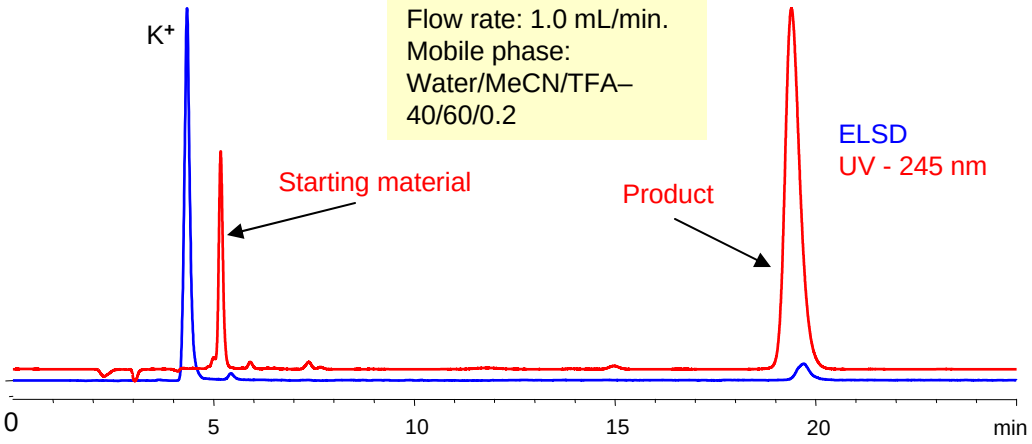
At low concentration, a non-linear treatment of calibration curve should be used to get accurate results.



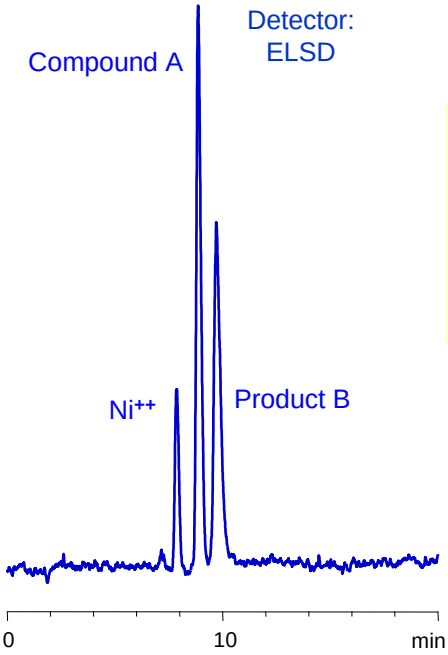


Quantitation of Potassium

Primesep 100 column
250 x 4.6 mm x 5 um
Flow rate: 1.0 mL/min.
Mobile phase:
Water/MeCN/TFA–
40/60/0.2

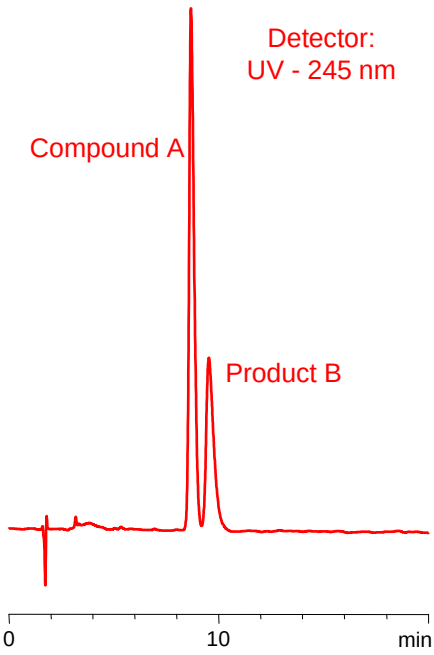


Analysis of Nickel in Hydrogenation Reaction

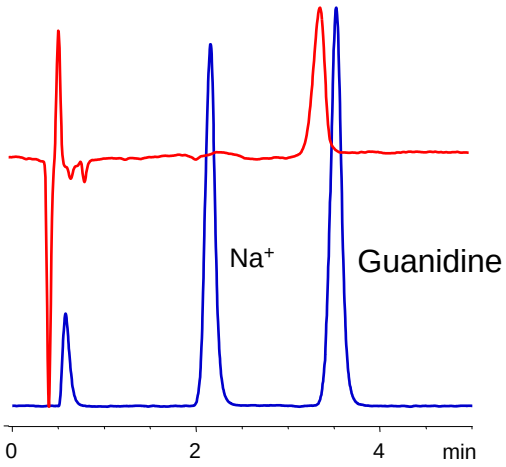


Primesep 100 column
150 x 4.6 mm x 5 um
Flow rate: 1.0 mL/min.
Temperature 45°C
Mobile phase:
Water/MeCN/TFA–
60/40/0.1

$$A \xrightarrow[[Ni]]{H_2} B$$



Analysis of Guanidine

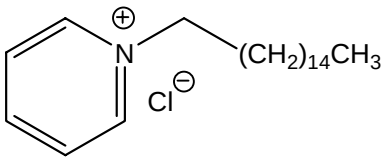


Column: Primesep 100 50 x 4.6 mm 5 um
Mobile phase: MeCN/H_2O/TFA – 25/75/0.05
Flow rate: 1.0 ml/min
Detector: ELSD , UV 200 nm

Improving Peak Shape of Strong Bases by Ion-Exclusion Mechanism

Strong bases like quaternary amines do not perform well chromatographically due to the strong silanol interaction even with the best deactivated silica based columns. A strong ionic mobile phase is often employed to improve the peak shape and the separation efficiency. Another approach can be used with mix-mode stationary phases. Primesep B column with a positively charged surface completely eliminates any ion-exchange interaction of the stationary phase with positively charged analytes and, thus, offers efficient separation and a symmetrical peak shape. Retention is still controllable by varying the amount of organic modifier in the mobile phase that provides separation of the compounds according to their hydrophobic properties. Hydrophobic interaction is reduced due to the repulsion effect of the ion-exclusion process.

Case Study for Cetylpyridinium Ions

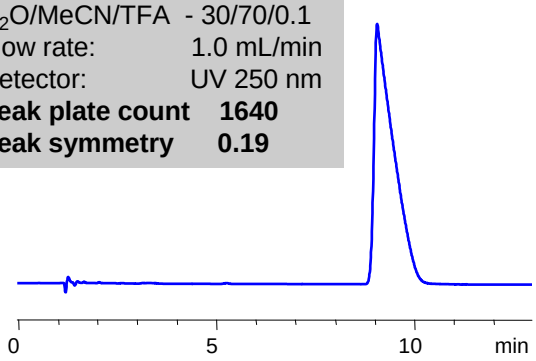


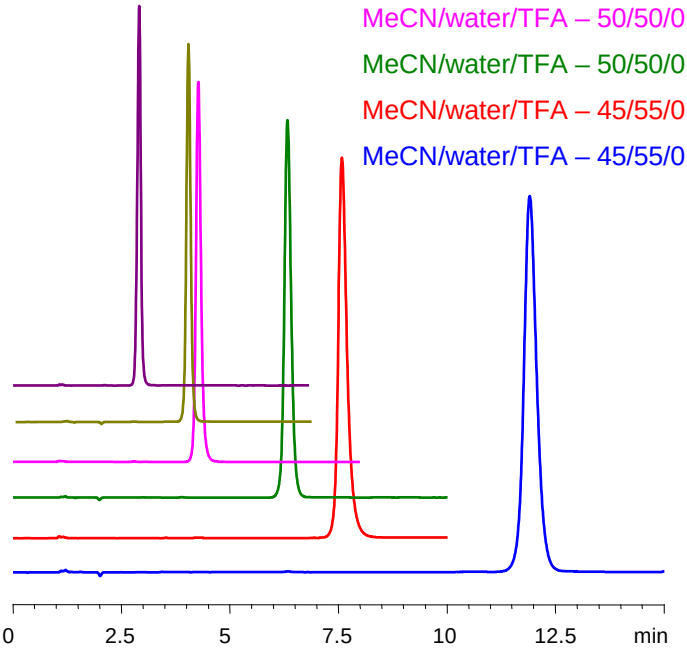
Primesep B, 150 x 4.6 mm
Flow rate: 1.0 mL/min
Detector: UV 250 nm

Mobile Phases:

MeCN/water/TFA – 55/45/0.1
MeCN/water/TFA – 55/45/0.2
MeCN/water/TFA – 50/50/0.1
MeCN/water/TFA – 50/50/0.2
MeCN/water/TFA – 45/55/0.1
MeCN/water/TFA – 45/55/0.2

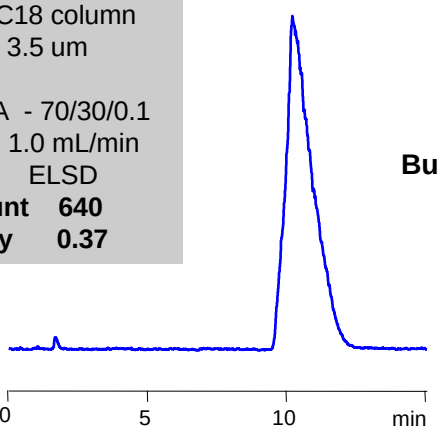
Leading brand C18 column
150 x 4.6 mm x 5 um
Mobile Phase:
H_2O/MeCN/TFA - 30/70/0.1
Flow rate: 1.0 mL/min
Detector: UV 250 nm
Peak plate count 1640
Peak symmetry 0.19

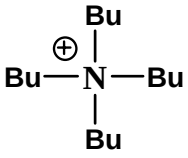




Tetrabutylammonium hydroxide

Leading brand C18 column
150 x 4.6 mm x 3.5 um
Mobile Phase:
H_2O/MeCN/TFA - 70/30/0.1
Flow rate: 1.0 mL/min
Detector: ELSD
Peak plate count 640
Peak symmetry 0.37





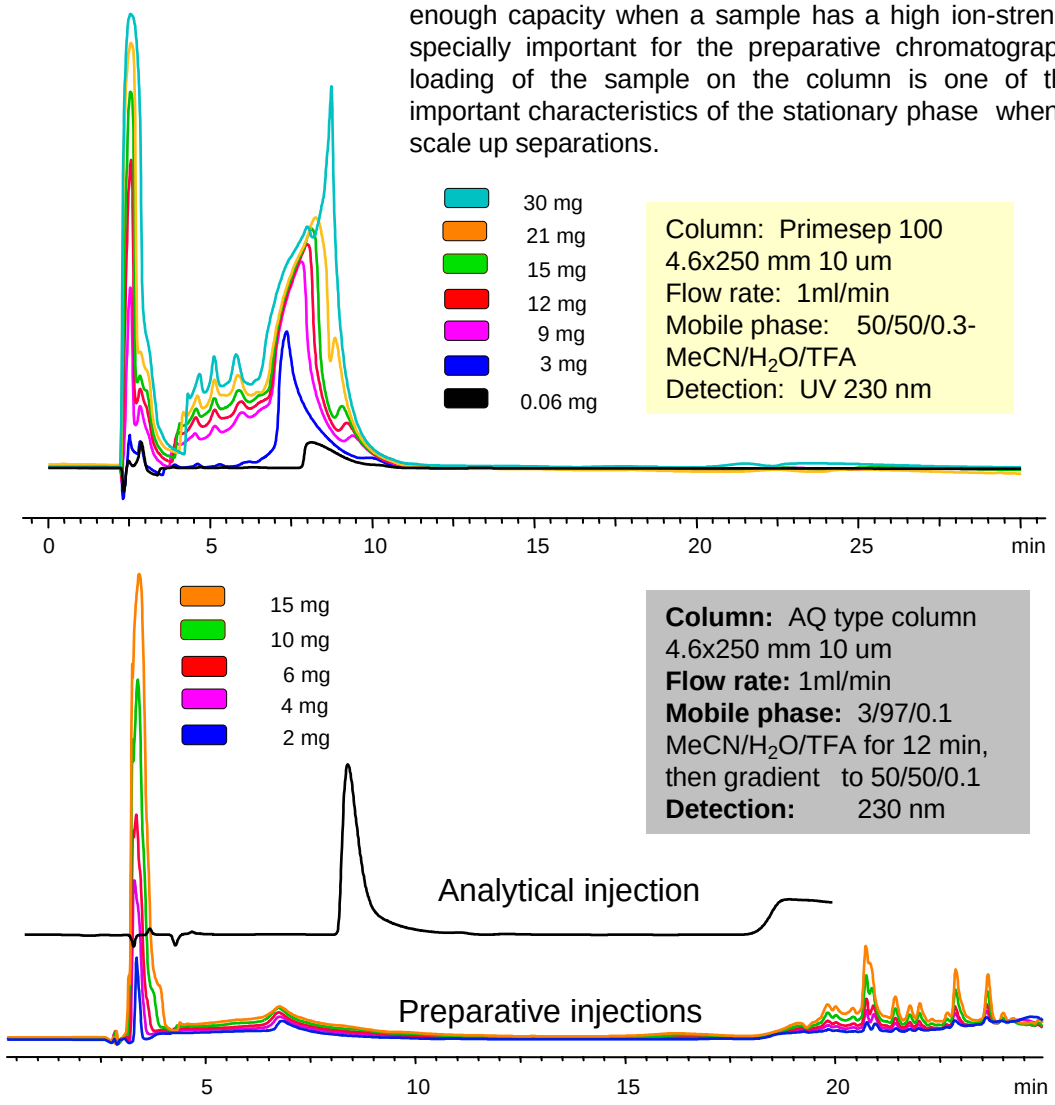
Primesep B, 150 x 4.6 mm
Mobile Phase:
H_2O/MeCN/TFA - 70/30/0.15
Flow rate: 1.0 mL/min
Detector: ELSD
Peak plate count 5200
Peak symmetry 0.70

ELSD, MS, and Preparative Chromatography Compatible Technology for Analysis and Separation of Complex Mixtures

Ion-pairing reagents are not compatible with preparative chromatography, LC-MS, and ELS detection. Primesep columns offer an alternative way of retention of polar compounds through ion-exchange mechanism with embedded ion-bearing groups.

Load Study of HCl Salt of Polar Compound

The amounts of ion-bearing groups on Primesep columns are comparable to the amount of ion-bearing groups on regular ion-exchange columns. A Primesep column offers a high capacity ion-exchange mechanism. In many instances, RP columns designed for retention of polar compounds do not offer enough capacity when a sample has a high ion-strength. It is specially important for the preparative chromatography. The loading of the sample on the column is one of the most important characteristics of the stationary phase when used in scale up separations.



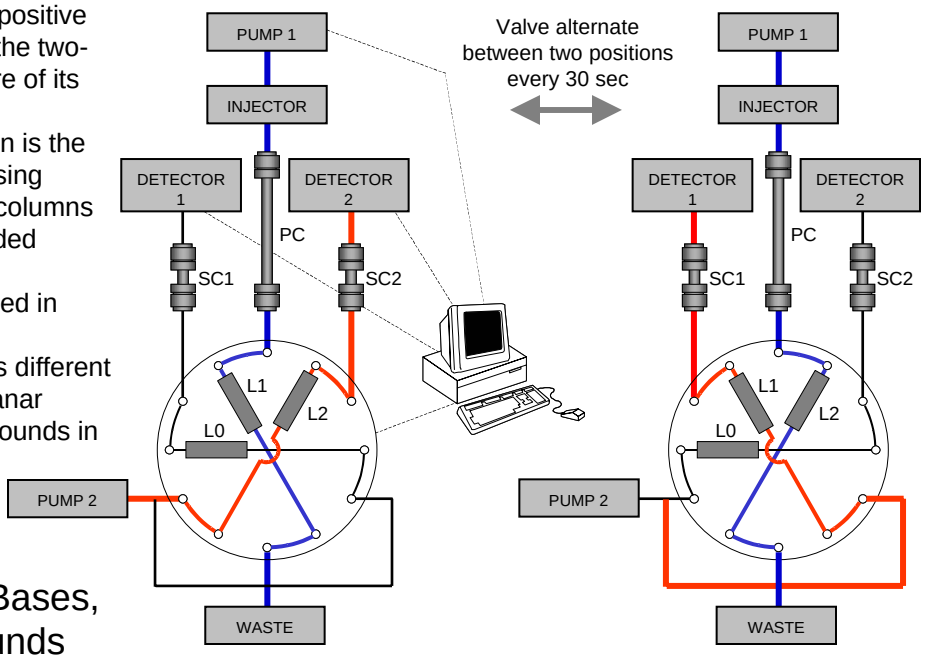
The combination of ion-exchange and reverse separation modes in a single column offers wide selection of conditions to tailor your separation in the way that is most convenient and economical. The last is very important when the preparative separation is required. If ionizable compounds are separated, a chromatographer can choose the conditions where a high concentration of an organic modifier is present in the mobile phase. Thus, the cost of solvent removal can be significantly reduced, and the organic solvent can be recycled. Another benefit of using Primesep preparative columns is an ability to reverse the elution order of differently charged components of the mixture that helps isolate a particular component in the mixture.

2-D HPLC Separation with Mixed-Mode Primesep Columns

A multidimensional technique involving coupled columns is more powerful compared to its one-dimensional counterpart, provided the retention mechanisms in the two dimensions are orthogonal. Mixed mode columns offer unique selectivity and hence are ideal for multi-dimensional separation. The stationary phase consisting of embedded ion-pairing reagent (acidic or basic) and hydrophobic functional group offers mixed mode retention for the charged species. The retention of charged species is effected by the concentration of organic content and ionogenic modifier level in the mobile phase.

With this technique, the primary column effluent is sampled alternatively into dual secondary column using an electronically controlled valve for further separation. Since columns differ in their electivity, chemical components co-eluting on the primary column are likely to resolve in the secondary column. The primary column separation is comparable with a conventional HPLC whereas the secondary column separation is faster. The high-speed separation in the secondary column enables a partial or complete transfer of primary column effluent to the secondary column, which results in comprehensive two-dimensional liquid separations.

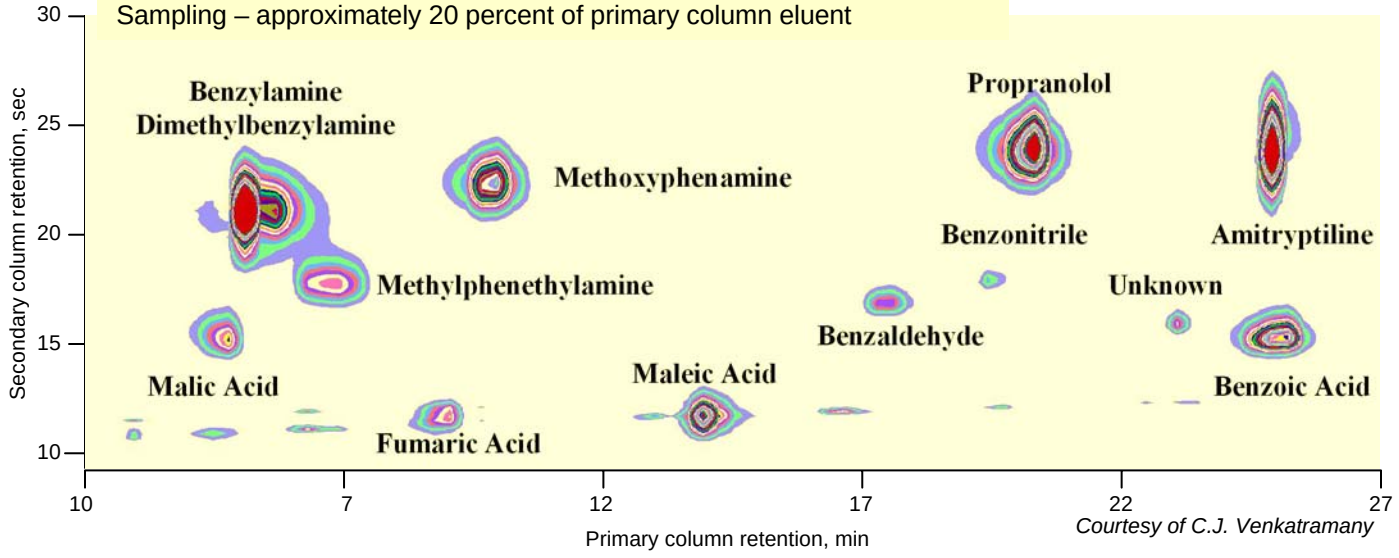
Each chemical component has a unique pair of retention times enabling positive identification and its location in the two-dimensional plane as a measure of its physical property. The key to a successful orthogonal separation is the right column selection. By choosing Primesep B and Primesep 100 columns with oppositely charged embedded functional groups the significant difference in selectivity is obtained in each dimension. The two-D chromatographic map separates different classes of compounds in the planar space, helping to identify compounds in a complex mixture.



2-D Mapping of a Mixture of Acids, Bases, and Neutral Compounds

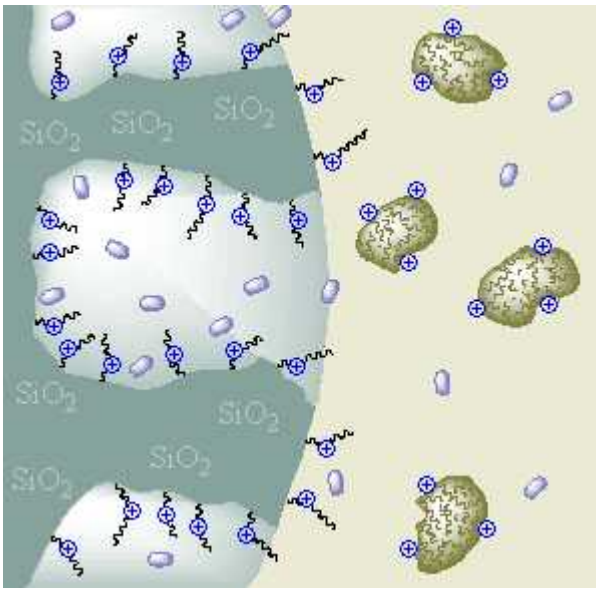
Primary column **Primesep B**, 150 x 4.6 mm 5 um; flow rate 0.5 mL/min
Secondary column **Primesep 100**, 20 x 4.6 mm 5 um; flow rate 2.5 mL/min
Detection UV 215 nm
Sampling – approximately 20 percent of primary column eluent

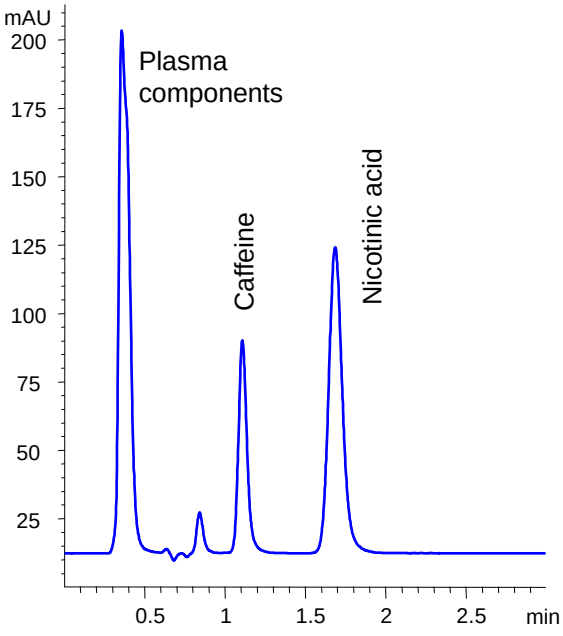
PC - primary column
SC – secondary columns
L – intermediate loops



Courtesy of C.J. Venkatramany

Direct Plasma Analysis



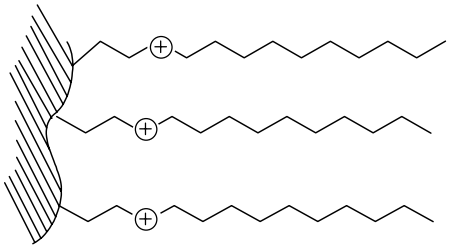


Column:	Primesep D
Dimensions:	50 x 4.6 mm x 5 um
Flow rate:	1.0 mL/min
Mobile phase:	MeCN/H_2O/HCOOH-33/67/0.2
Detection:	UV - 250 nm
Sample:	Caffeine and nicotinic acid 0.2 mg/mL each dissolved in plasma EDTA diluted with H2O 1:1
Injection #200:	5 uL

Analysis of plasma and other biological fluids by HPLC usually requires several steps of sample preparation. Such tasks as solid-phase extraction, liquid-liquid extraction, centrifugation, and filtration usually are necessary steps prior to actual HPLC analysis in order to remove proteins and peptides from plasma to protect HPLC column. When plasma is analyzed directly, the efficiency of the column degrades quickly due to irreversible adsorption of some components of the plasma. Multi-step process increases the cost and compromises accuracy of determination.

SIELC Technologies now offers a simple solution which allows to analyze a broad range of small molecules in plasma or other biofluids via a single-column system without any sample preparation or common mobile phase.

This approach is based on unique stationary phase of Primesep™ D column that is comprised of two types of functional groups -- an anion exchange group and long alkyl chain -- chemically bonded to silica support.



This combination of very polar positively-charged group and very hydrophobic alkyl group in a single ligand on a surface of the stationary support allows direct injection of plasma and other biofluids. At pH around 3.0, which can be obtained by adding of formic acid to the mobile phase of MeCN-H2O, most proteins became positively charged and are excluded from interaction with the surface of the stationary phase. Small negatively charged molecules can be separated and retained on Primesep D column by anion-exchange mode or reverse phase mode chromatography. Small hydrophobic molecules are retained and can be separated by regular RP mode on Primesep™ D column.

Primesep™ D column demonstrates high analyte recovery and high selectivity. Proteins and peptides elute as a sharp peak in void or pre-void time and they are not interfered with analytes peaks. Simple bypass valve can be used to remove protein peak from reaching the detector by diverting flow to waste in first 40 seconds (for 50 mm long column -- time may need to be increased if longer columns are used).

SIELC

formerly Allsep Technologies

For decades liquid chromatography stationary phase design has been dominated by the goal to eliminate multiple, or “unwanted”, interactions and to obtain a simple and predictable retention mechanism. Unfortunately, the simplification of the retention process limits the ability to control elution order of the analytes and the scope of available applications this system can be used for. As a response to this limitation, hundreds of different reverse-phase columns were introduced in the last years to cover a variety of analytical situations.

In contrast, PrimesepTM stationary phases were intentionally designed with two major interactions offered on the same column. Both interactions are independently adjustable with mobile-phase composition producing unlimited states of the stationary phase. The hydrophobic interaction is controlled by the amount of organic modifier in the mobile phase (as in any reverse-phase column), while the ion-exchange interaction is controlled by the ion-strength and pH of the mobile phase (as in other ion-exchange columns). This unique property allows using one stationary phase for numerous applications, including analyses of polar and non-polar, ionizable and neutral, organic and inorganic compounds. The behavior of PrimesepTM columns is predictable and reproducible. The method development process is simple and versatile.

Create a column part number by using chart below

A - 46 . 150 . 0510

Type of packing

Primesep A	**A**
Primesep B	B
Primesep B2	B2
Primesep C	C
Primesep P	P
Primesep 100	100
Primesep 200	200
Primesep 300	300

Column id

22 mm	220
4.6 mm	**46**
3.2 mm	32
2.1 mm	21
1.0 mm	10

Column length

250 mm	250
150 mm	**150**
100 mm	100
50 mm	050

Packing size

5 mc m	**5**
10 mc m	10
20 mc m	20

Pores size

100 A	**10**
200 A	20
300 A	30

BGB GC|LC MS|CE

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