SIELC

# Primesep™

**Solving Problems of Pharmaceutical HPLC Analysis**


**“Creating New Dimensions in the World of Chromatography”**

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- Simultaneous analysis of inorganic components in the drugs and drug intermediates
- New approach to establish purity of reference standards with two alternative methods.

# Introduction

Reverse phase (RP) chromatography is a main technology for the separation of pharmaceuticals at all steps of the drug development and production. Problems that analytical chemists face when developing a new HPLC method are often associated with limitations of existing RP chromatography:

- Difficulties retention of polar compounds;
- Problems with separation of closely eluted or co-eluted peaks;
- Early eluted peak distortion;
- Inability to use isocratic method for compounds of very different polarity;
- Need for additional techniques for analysis of inorganic components of drugs and drug intermediates;
- Need for two alternative methods to establish purity of reference standards.

A recently introduced technology for HPLC separation based on mixed-mode stationary phases addresses most of these problems. The technology involves a new type of stationary phases chemically bonded to the silica support. The phases comprise of a brush type long alkyl chain with an embedded negatively or positively charged functional group in the middle of the alkyl chain. The evolved stationary phases offer several advantages compared to common reverse phases. This brochure will explain the benefits of using this new type of stationary phases for pharmaceutical applications and show examples of real life solutions achieved with the technology.

In most cases, separations require a simple mobile phase containing acetonitrile, water and TFA or formic acid. It 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, thus, 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.

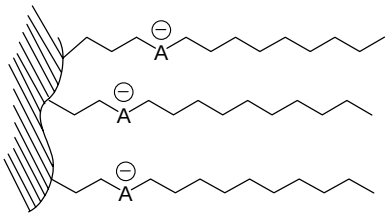
Pentanesulfonic acid, heptanesulfonic acid, sodium dodecylsulfate, tetrabutylammonium hydroxide are ion-pairing reagents that are typically used for retention of polar compounds in the reverse phase chromatography. By analogy, Primesep™ HPLC mixed-mode columns are offered in several modifications of 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 then to 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.

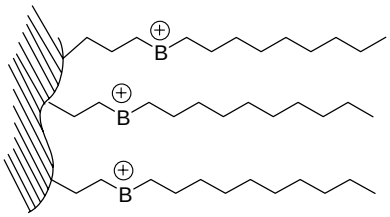
## Mixed-Mode Primesep Columns

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

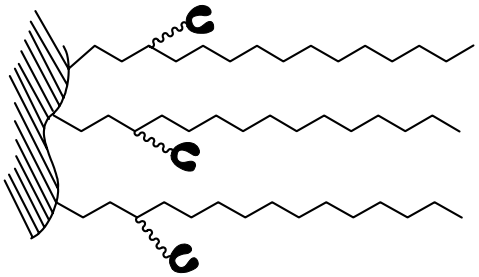



Primesep B
Primesep B2




## 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.

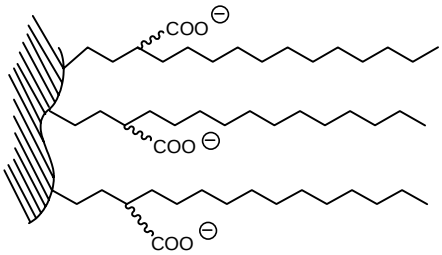


## SWITCH Phase™ Technology

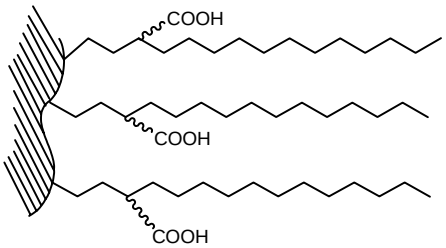
Columns based on SWITCH Phase™ technology change their properties depending on pH of the mobile phase. Embedded carboxylic acid is fully ionized at pH above transition point and loses charge when mobile phase pH goes below transition point. By controlling pH of the mobile phase, the polar properties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

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

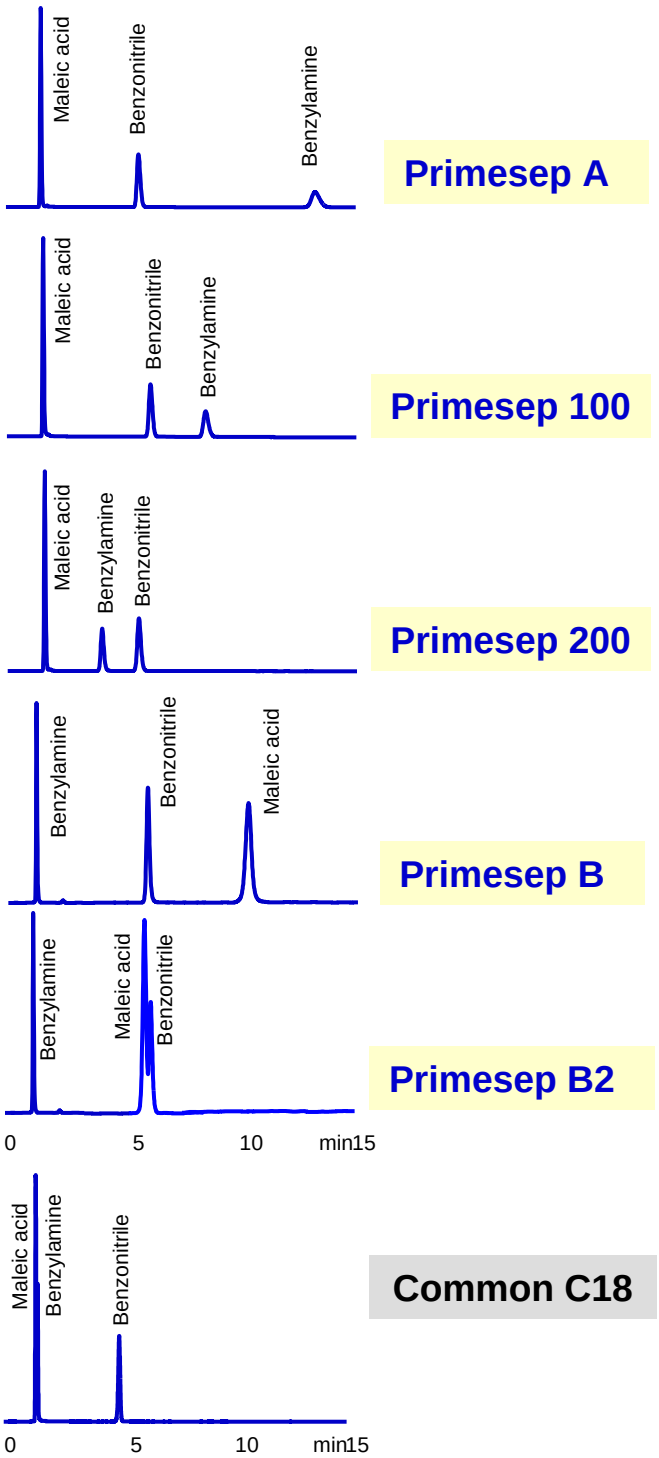
Primesep 300 at pH > 3.5




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

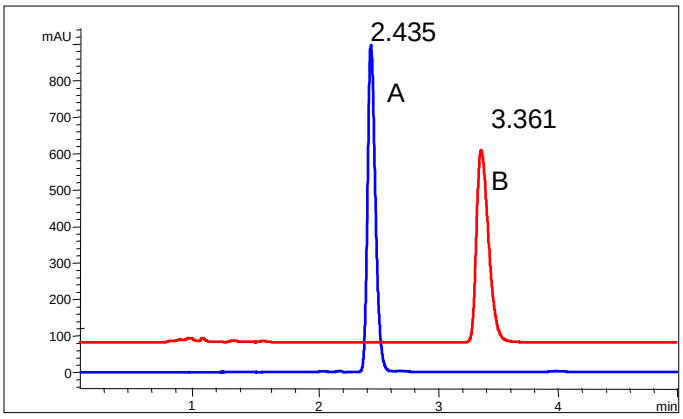
**Columns:** 150 x 4.6 mm x 5 um
**Flow rate:** 1.0 mL/min.
**Detector:** UV 210 nm
**Mobile phase:**
Water/MeCN/TFA-60/40/0.1

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

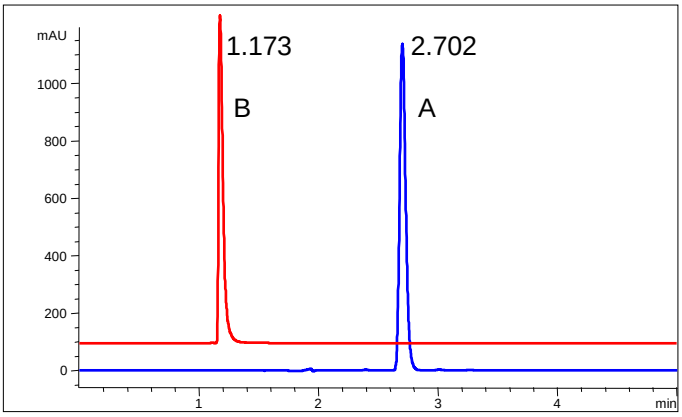
Usually reverse-phase columns do not perform well in 100% aqueous mobile phase. Dewetting of silica pores or collapse of the alkyl chains of the stationary phase causes a sudden loss of retention in this condition.

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 differ 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 and does not affect relative contribution of each separation mode.



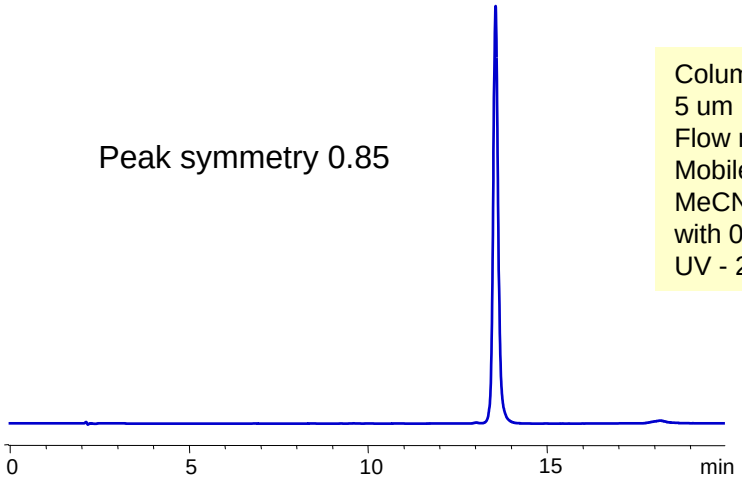

Column: Primesep 100
Mobile phase:
A Water/MeCN – 85/15%
B Water – 100%. The column was left with no flow at zero pressure for 24 hr
Sample: benzoquinone 0.1 mg/ml in water
Injection: 5 ul
Detector: UV 270 nm




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.



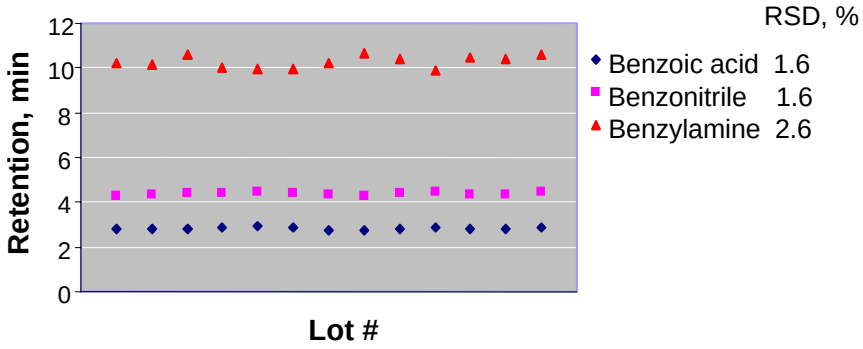

Column: Primesep B 150 x 4.6 mm x 5 um
Flow rate: 1.0 mL/min.
Mobile phase: Gradient MeCN/$H_2O$-10/90 to 30/70 in 15 min with 0.1 % TFA
UV - 250 nm

## 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**



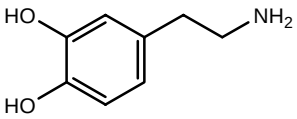

Column: Primesep 100
Mobile phase: Water/MeCN/TFA– 50/50/0.8
Sample Injection: 5 ul
Detector: UV 210 nm

# Retention of Polar Compounds in Reverse Phase System

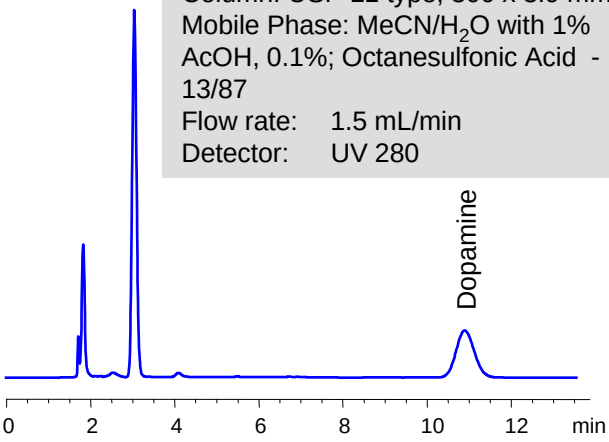
In many fields, and in liquid chromatography particularly, the reverse-phase mode is a technique of choice to solve many separation problems. One of the limitations of reverse-phase columns is lack of retention of highly polar compounds on conventional stationary phases. Traditionally, mobile phase additives, 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.

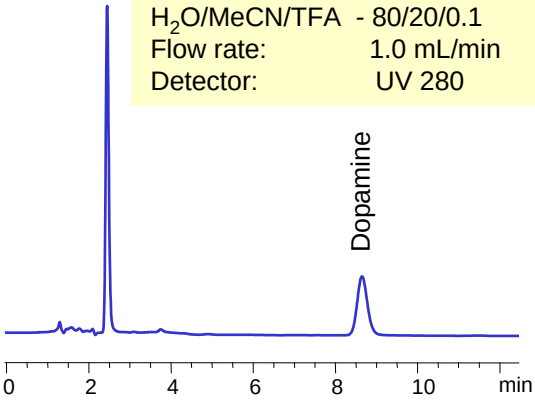
## Dopamine Degradation Method



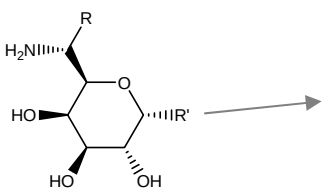

Column: USP L1 type, 300 x 3.9 mm
Mobile Phase: MeCN/$H_2O$ with 1% AcOH, 0.1%; Octanesulfonic Acid - 13/87
Flow rate: 1.5 mL/min
Detector: UV 280




Primesep 100, 150 x 4.6 mm
Mobile Phase:
$H_2O$/MeCN/TFA - 80/20/0.1
Flow rate: 1.0 mL/min
Detector: UV 280




## Analysis of Amino Sugars

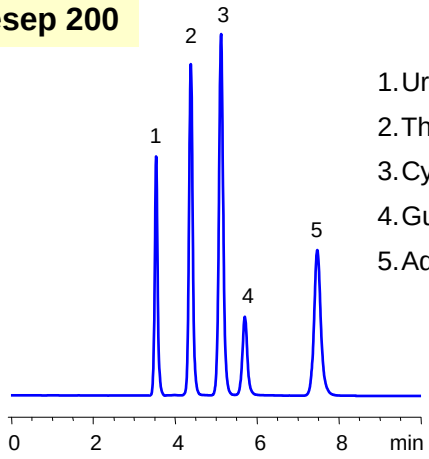



Column: Primesep 100
150 x 4.6 mm x 5 um
Flow rate: 1.5 mL/min.
Mobile phase:
Water/MeCN/$H_2SO_4$–8/92/0.03
pH= 2.1
Temperature: 40ºC
Detection: UV - 205 nm

## Separation of Nucleobases

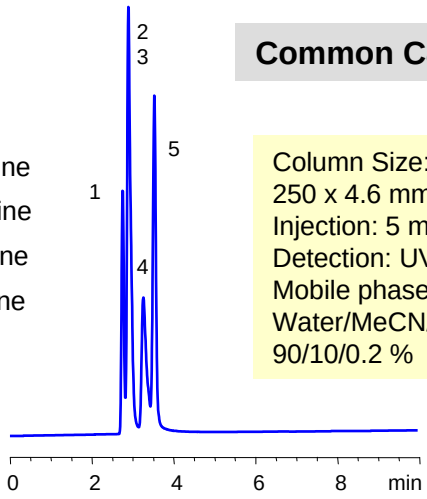
It's a known fact that polar organic compounds retain poorly on reverse-phase columns. These compounds often have an ionizable group in a molecule whose bearing charge makes the molecule even more polar and difficult to retain and separate. The Primesep 200 column is a unique solution for this situation.

**Primesep 200**




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

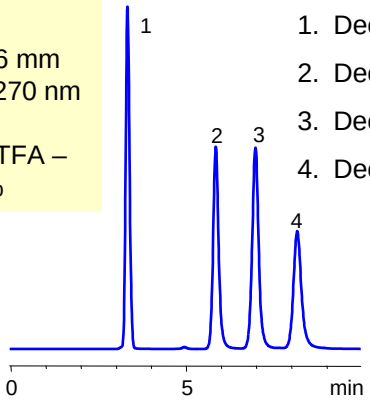
**Common C8**



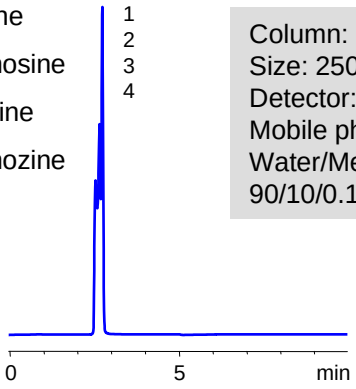

Column Size:
250 x 4.6 mm x 5 um
Injection: 5 mcl
Detection: UV 270 nm
Mobile phase:
Water/MeCN/TFA –
90/10/0.2 %

## 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

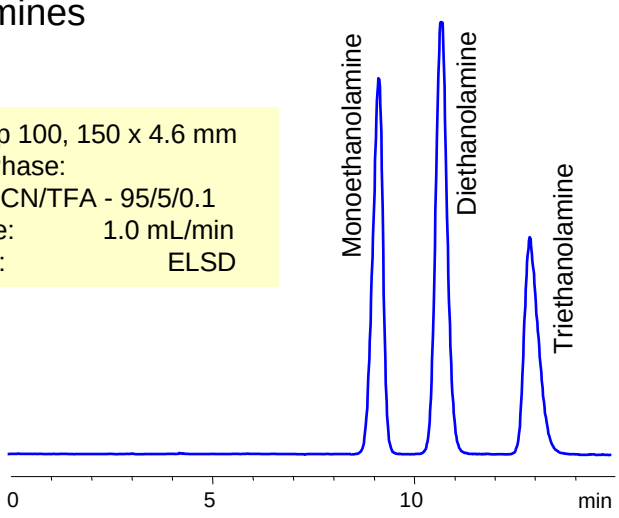



Column: Common C18
Size: 250 x 4.6 mm
Detector: UV 270 nm
Mobile phase:
Water/MeCN/TFA –
90/10/0.1 %

## Separation of Ethanolamines

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

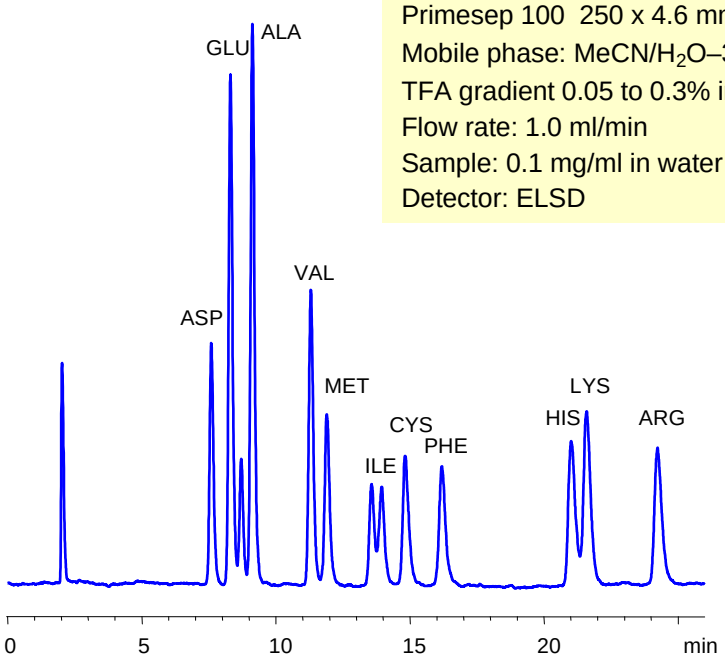
Primesep 100, 150 x 4.6 mm
Mobile Phase:
$H_2O$/MeCN/TFA - 95/5/0.1
Flow rate: 1.0 mL/min
Detector: ELSD



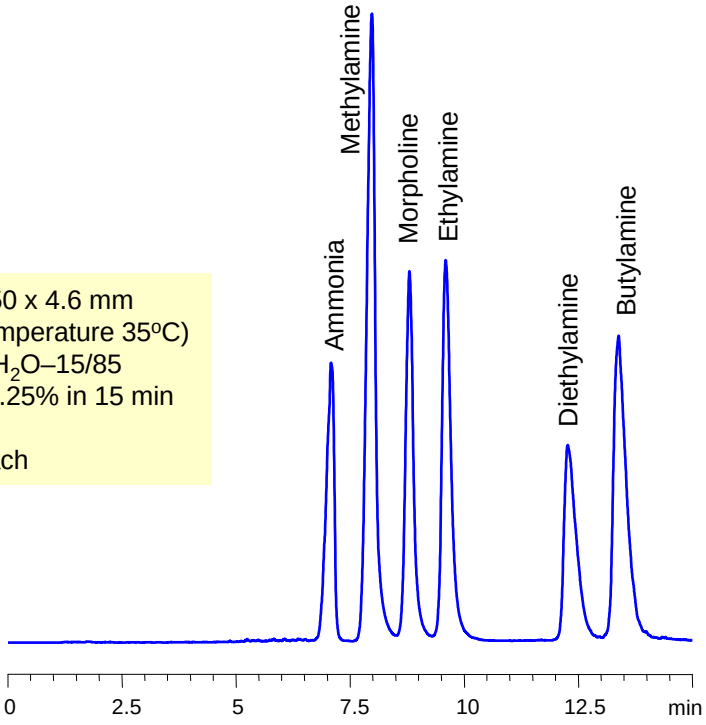
# Analysis of Amino Acids

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

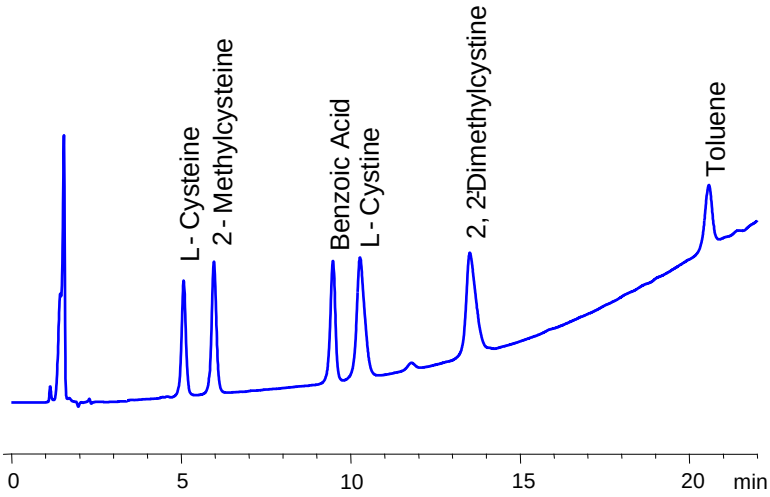



Primesep A column 150 x 4.6 mm
Detection: ELSD, (Temperature 35ºC)
Mobile phase: MeCN/$H_2O$–15/85
TFA gradient 0.05 to 0.25% in 15 min
Flow rate: 1.0 ml/min
Sample: 1.0 mg/ml each

## Retention of Polar and Hydrophobic Compounds

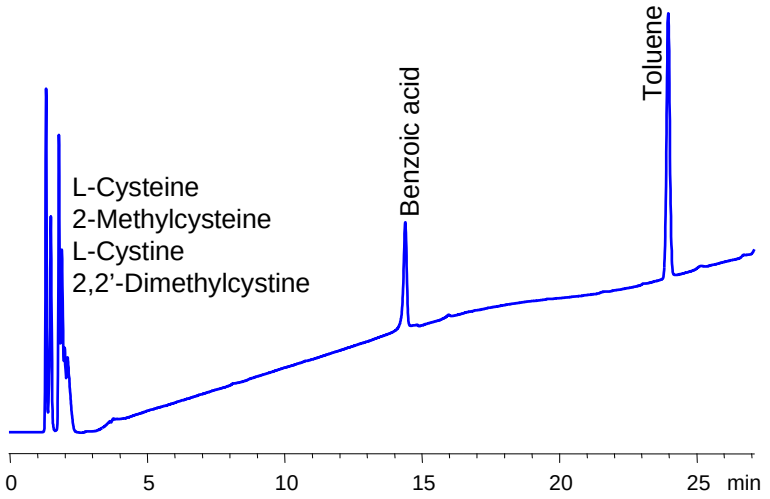
Complex mixtures with very polar and very hydrophobic compounds can be resolved with a gradient method.

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



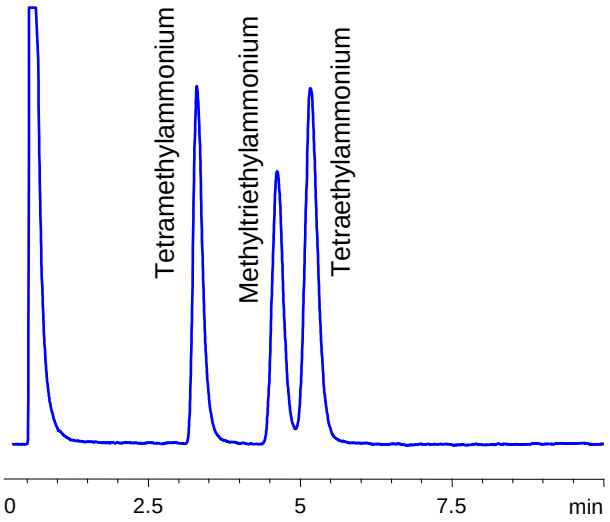

In similar conditions common reverse phase columns give no retention of polar compounds and require an ion-pairing reagent in the mobile phase.

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.



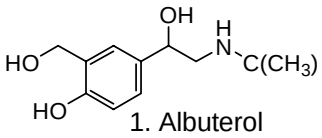

Primesep C 50 x 4.6 mm x 5 um
Mobile phase: MeCN/$H_2O$–15/85
TEA acetate 20 mM pH 5.0
Flow rate: 1.0 ml/min
Sample: 0.6 mg/ml each
Injection: 5 mc
Detector: ELSD, (Temperature 35°C)

# Resolution of Closely Eluted or Co-Eluted Peaks

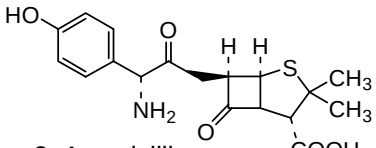
Primesep™ mixed-mode stationary phases provide multiple types of interactions with analytes. Ionizable compounds interact with the stationary phase by reverse-phase, ion-exchange or ion-exclusion mechanisms. The amount of the acid in the mobile phase influences the retention attributed to the ion-exchange 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 residues (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.
All these properties offer multiple ways to adjust selectivity of the Primesep column. If two or more peaks are not resolved with traditional reverse phase technology, the mixed-mode column can be a powerful tool to find optimum separation conditions.

## Albuterol and Amoxicillin

When two compounds are co-eluted on a reverse phase 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 the mobile phase properties. This often allows resolving co-eluted peaks without replacing the column.

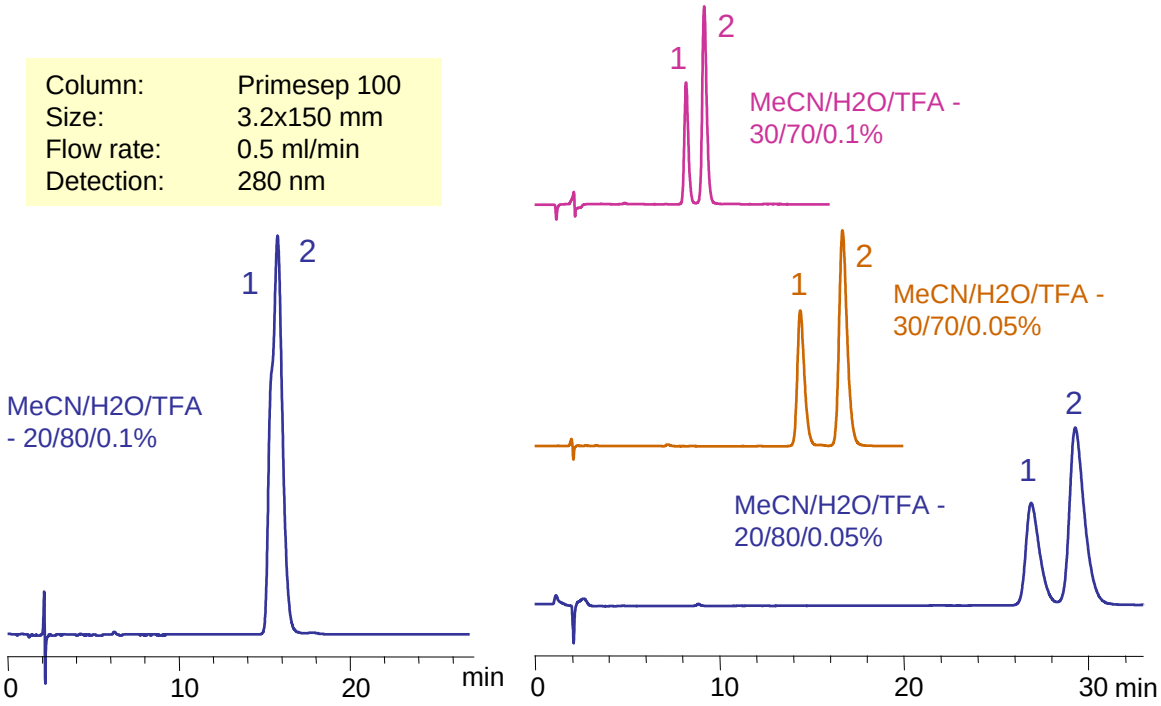


1. Albuterol



2. Amoxicillin

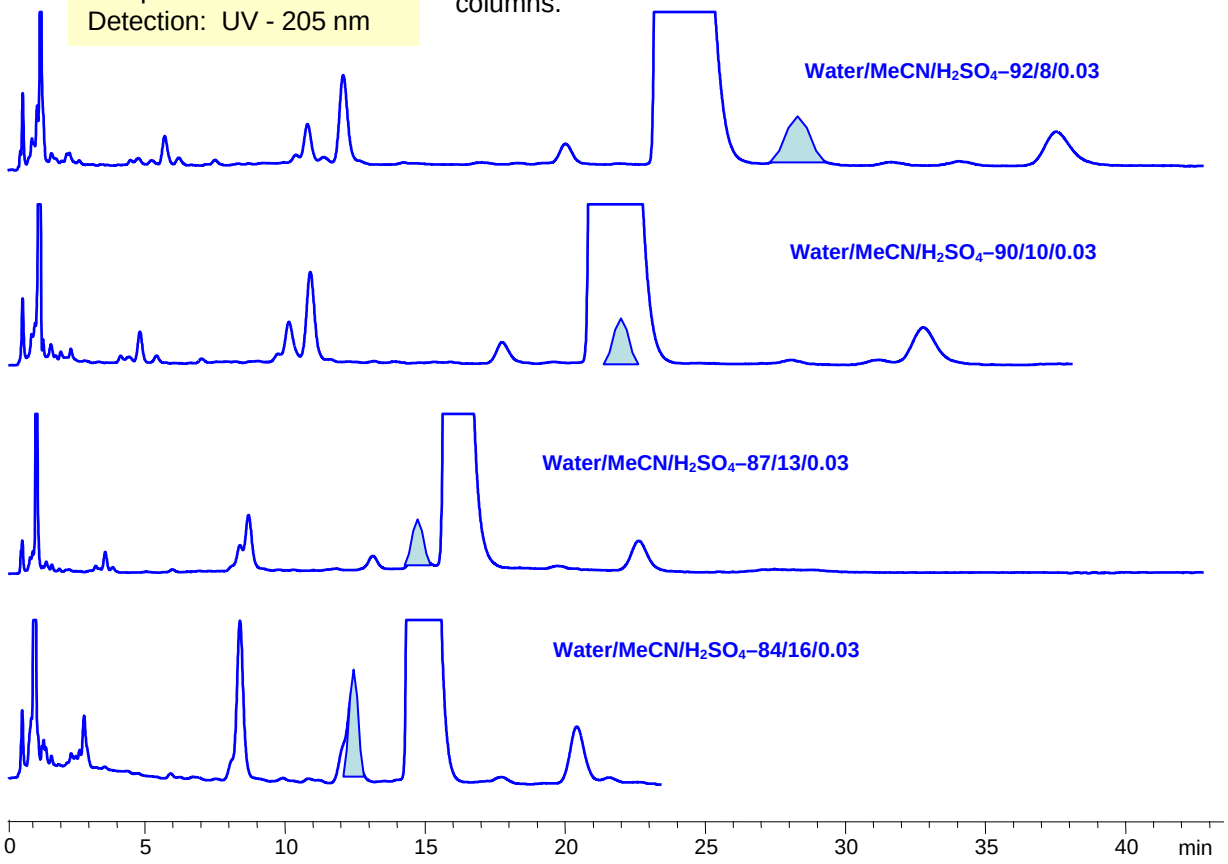
| | |
|---|---|
| Column: | Primesep 100 |
| Size: | 3.2x150 mm |
| Flow rate: | 0.5 ml/min |
| Detection: | 280 nm |



# Example of Method Development

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

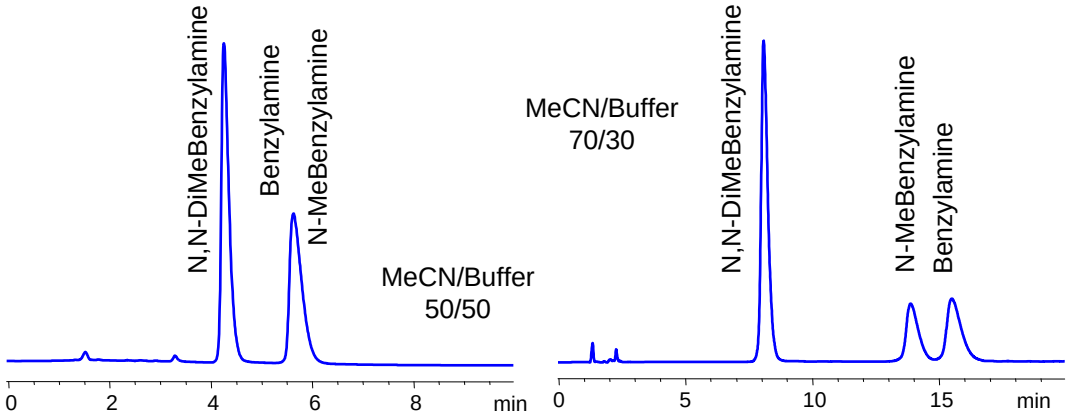
Various compounds respond differently to changes in the concentration of organic modifier. Neutral non-polar compounds are effected significantly, while polar compounds show a different response. This is a powerful tool for separation tuning on Primesep columns.




# New Approach in Column Chemistry – Primesep C™.

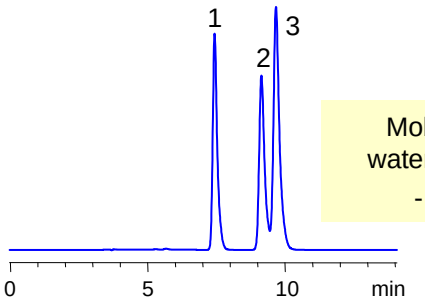
Primesep C column offers strong interaction with primary and secondary amines. This interaction increases with the decrease of water concentration in the mobile phase. By changing concentration of the organic modifier in the mobile phase, selectivity and elution order of the mixture of primary, secondary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

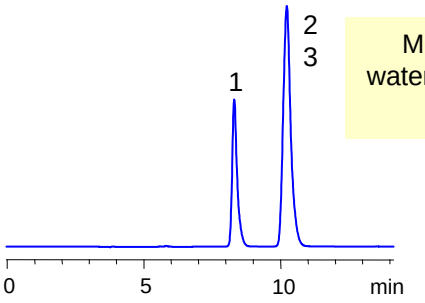


# Effect of Acidic Modifier on Selectivity

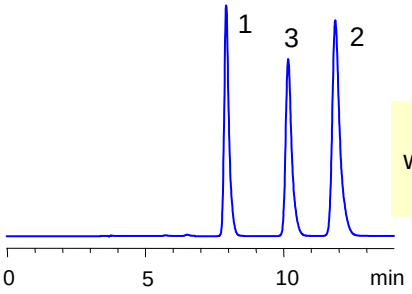
The Primesep B column has embedded amino-groups in the hydrophobic alkyl layer. When the acidic modifier in the mobile phase is changed, the hydrophobic amines form tight ion pairs with the modifier which can interact with neutral analytes by polar mechanism. Interaction with analytes is affected by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




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

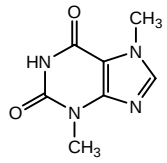



Mobile Phase: water/MeCN/ $HClO_4$ - 95/5/0.1

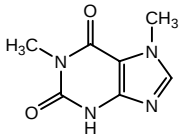



Mobile Phase: water/MeCN/ $H_2SO_4$ - 95/5/0.03

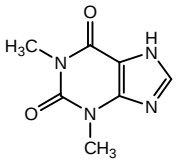
Primesep B 250 x 4.6 mm.
Flow rate:1.0 mL/min.
Sample: 0.3 mg/ml of each in MeCN/water
Injection: 5 µl
Temperature: 40ºC.
Detector: UV - 270 nm



Theobromine



1,7-Dimethylxanthine

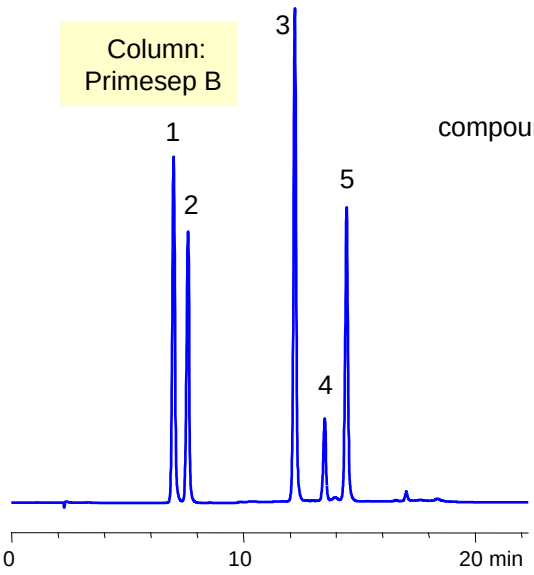


Theophylline

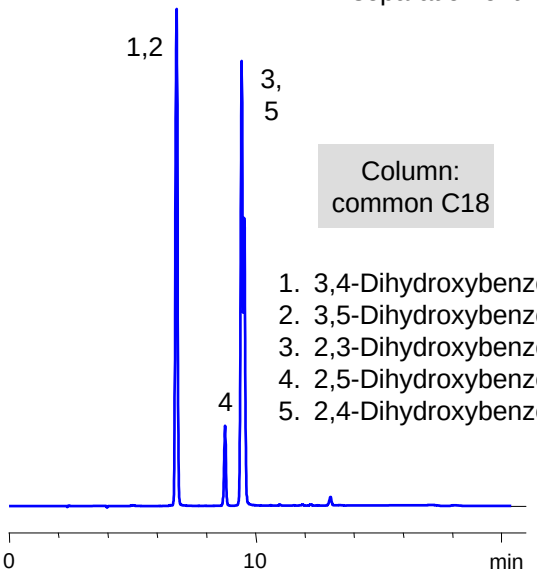
# Improvement of Selectivity by Anion-Exchange and Hydrophobic Interactions

On a Primesep B column, 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: Primesep B




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

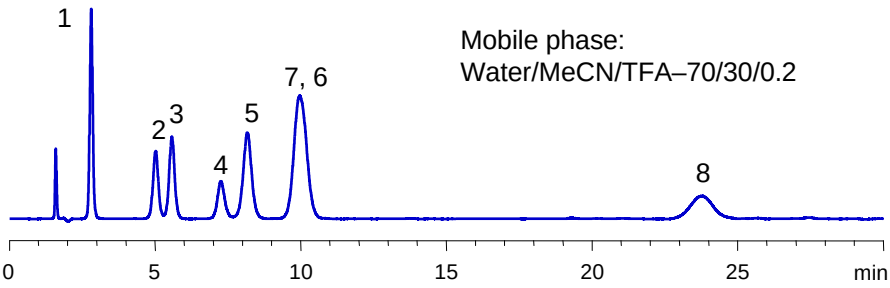



Column: common C18

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

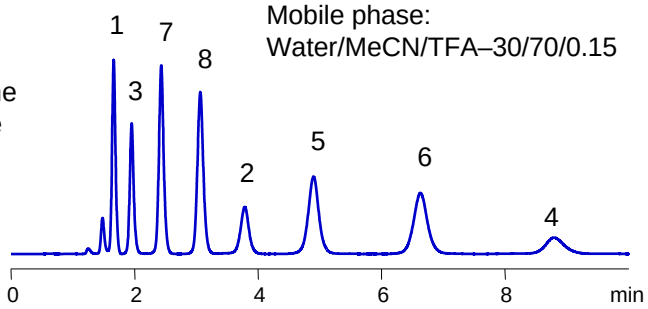
# Control of Retention and Resolution

Critical pairs were resolved in this mixture by changing the ion-exchange portion of interaction in this mixed-mode separation



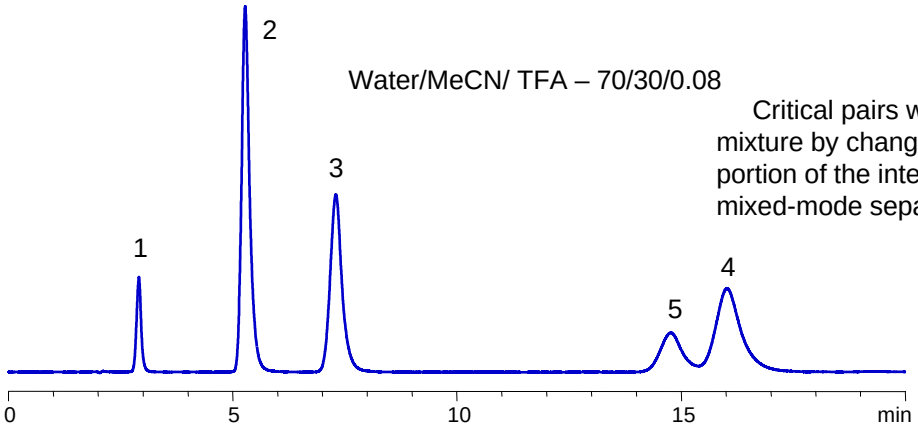

Mobile phase:
Water/MeCN/TFA–70/30/0.2

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



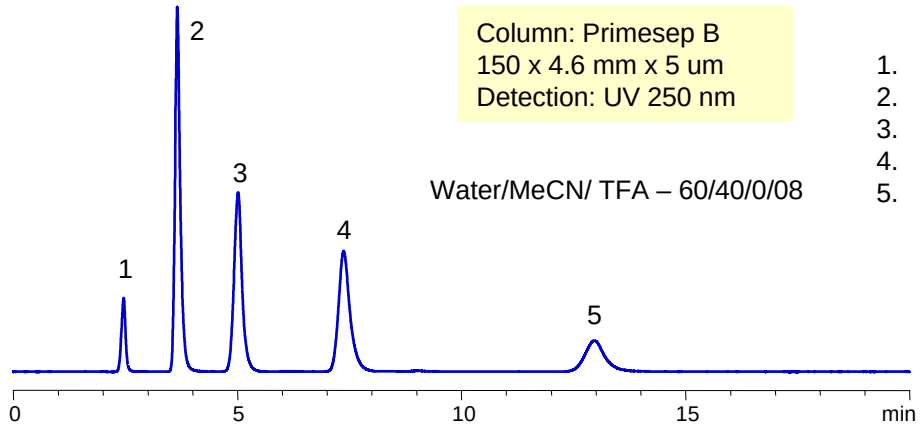

Mobile phase:
Water/MeCN/TFA–30/70/0.15

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




Water/MeCN/ TFA – 70/30/0.08

Critical pairs were resolved in this mixture by changing hydrophobic portion of the interaction in this mixed-mode separation




Column: Primesep B
150 x 4.6 mm x 5 um
Detection: UV 250 nm

Water/MeCN/ TFA – 60/40/0/08

1. Fumaric acid
2. Benzoic acid
3. Phthalic acid
4. Naphthoic acid
5. Maleic acid

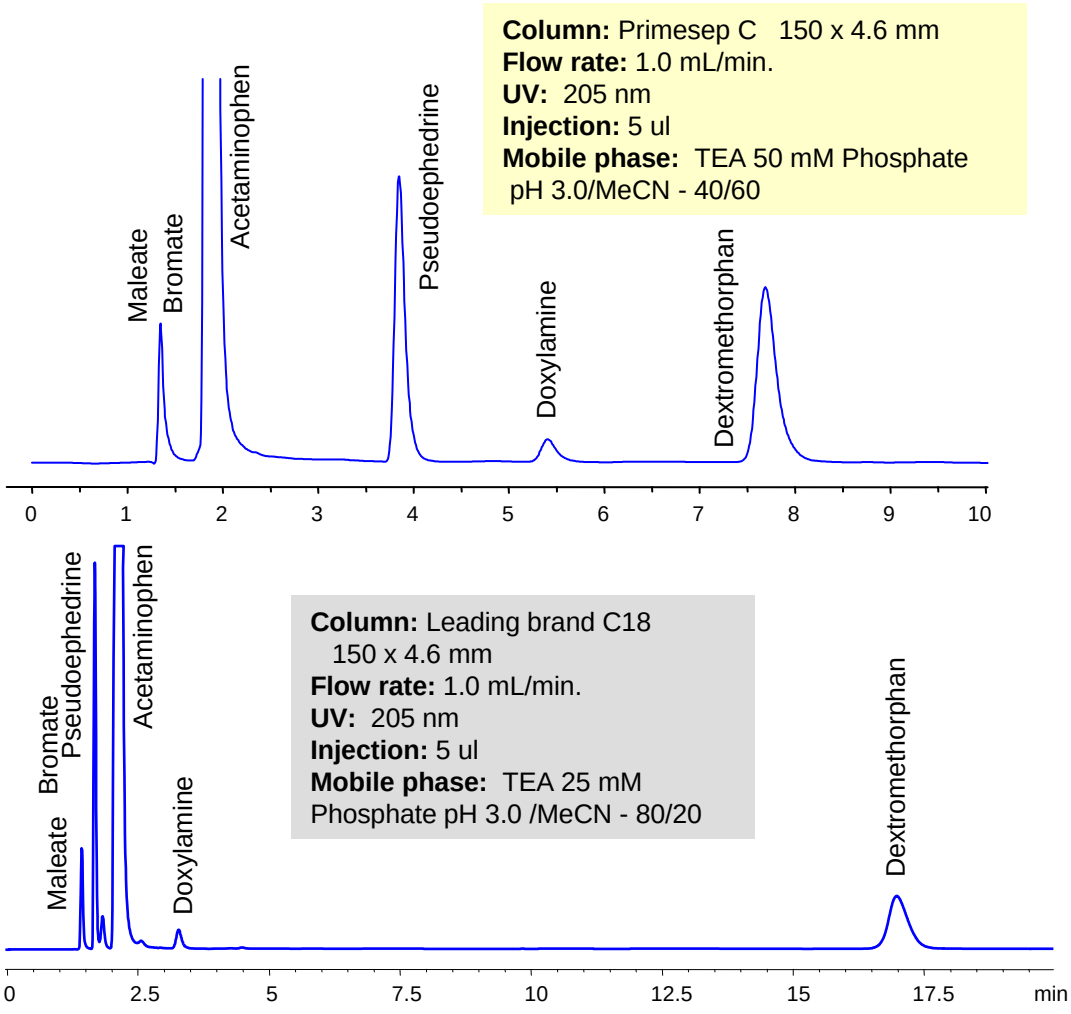
# Replacement of Gradient Method for Compounds of Different Polarity with Isocratic Method

Primesep™ mixed-mode stationary phases provide multiple types of interactions with analytes. Ionizable compounds interact with the stationary phase by reverse-phase and ion-exchange mechanisms.

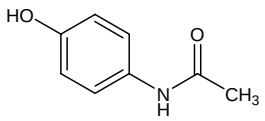
On these dual columns, the compounds of different polarity can be analyzed in a single isocratic mode by employing different separation mechanisms for compounds of different nature. Polar ionizable compounds can be retained to the same degree or even stronger than hydrophobic compounds do.

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.

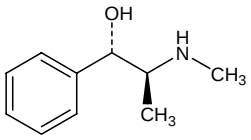
## Separation of Active Ingredients in Cough and Cold Drugs



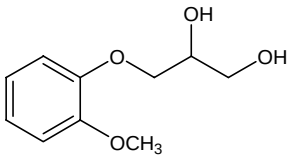
# Separation of Tylenol® Sinus Active Ingredients



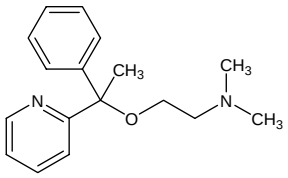

Acetaminophen




Pseudoephedrine

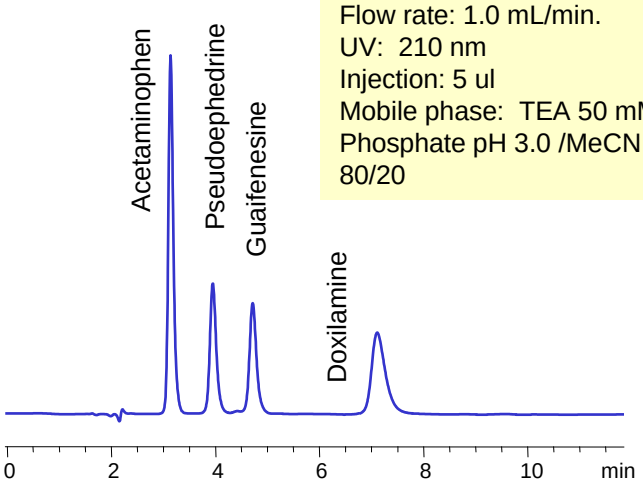



Guaifenesine

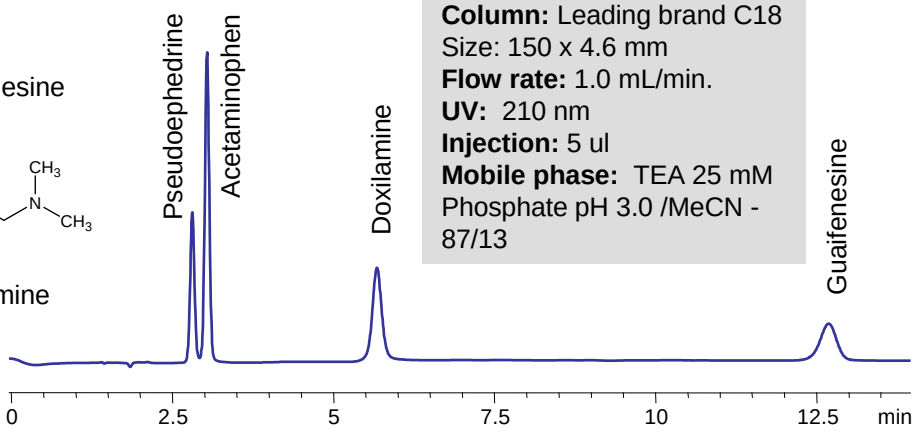



Doxylamine

Column: Primesep C
Size: 150 x 4.6 mm
Flow rate: 1.0 mL/min.
UV: 210 nm
Injection: 5 ul
Mobile phase: TEA 50 mM Phosphate pH 3.0 /MeCN - 80/20



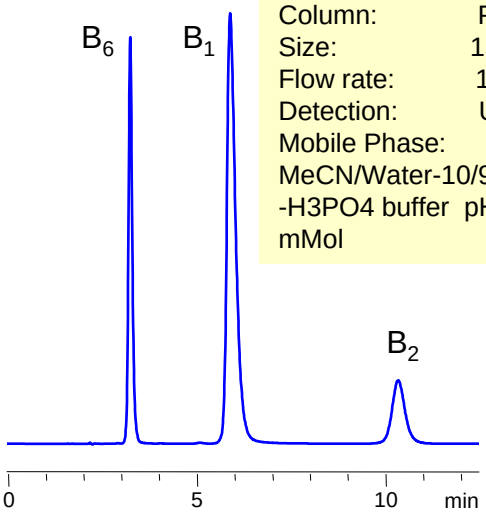

**Column:** Leading brand C18
Size: 150 x 4.6 mm
**Flow rate:** 1.0 mL/min.
**UV:** 210 nm
**Injection:** 5 ul
**Mobile phase:** TEA 25 mM Phosphate pH 3.0 /MeCN - 87/13



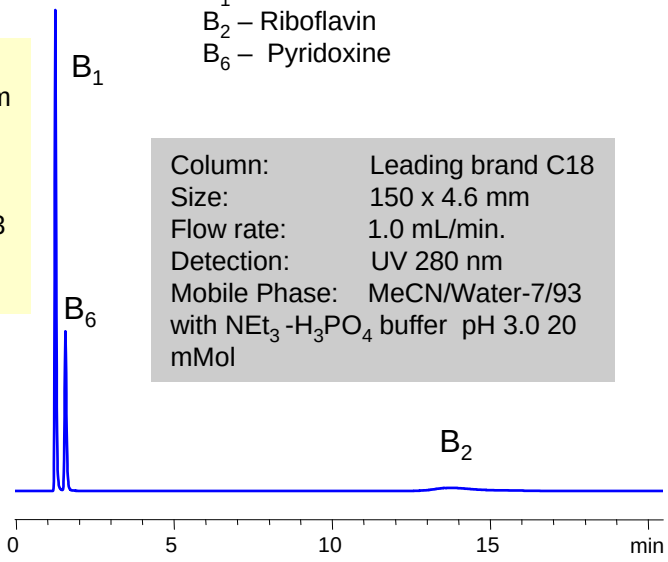

## Separation of Vitamins B mixture

$B_1$ – Thiamine
$B_2$ – Riboflavin
$B_6$ – Pyridoxine

Column: Primesep C
Size: 150 x 4.6 mm
Flow rate: 1.0 mL/min.
Detection: UV 280 nm
Mobile Phase: MeCN/Water-10/90 with Net3 -H3PO4 buffer pH 3.0 20 mMol




Column: Leading brand C18
Size: 150 x 4.6 mm
Flow rate: 1.0 mL/min.
Detection: UV 280 nm
Mobile Phase: MeCN/Water-7/93 with $NEt_3$ -$H_3PO_4$ buffer pH 3.0 20 mMol



# Improving of Shape of Early Eluted Peaks and Strong Bases

Polar compounds are eluted rapidly from RP columns. To achieve some retention of polar compounds, a low organic or zero organic mobile phase is required. In this situation the properties of the sample diluents become very important to obtain an undisturbed peak. A strong organic diluent can cause splitting of the early eluted peaks making the identification and quantitation impossible.

Another problem is often observed with amines that are introduced in a column as a salt of acid different from the mobile phase's acidic component. This also can cause peak distortion, as there is not enough time, and/or the excess of the buffer in the mobile phase is not sufficient to quickly exchange all the contra-ions of the amines in the sample.

It is not always possible to use a low organic diluent, since other components of the sample may not be soluble in it.

Desalting prior to injection or replacing contra ions of the sample is also a difficult operation. Even if it is possible, it requires an additional step in the sample preparation.

A similar technique applies to preparative chromatography when a high concentration of the sample is desirable, and organic diluent is capable of dissolving more of the sample than water alone is.

Primesep columns often retain polar compounds by ion-exchange mechanism in addition to RP, so a higher concentration of organic component in the mobile phase can be used. As a result, the concentration of organics in the sample diluent becomes not so important for obtaining undisturbed peaks.

Strong bases like quaternary amines typically demonstrate poor chromatography performance due to substantial silanol interaction even with the best deactivated silica based columns. A strong ionic mobile phase is usually employed to improve peak shapes and efficiency of separation. 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 stationary phase with positively charged analytes, thus offering efficient separation and symmetrical peak shape. Retention is still controllable by varying the amount of organic modifier in the mobile phase and separating compounds by their hydrophobic properties. The hydrophobic interaction is reduced due to the repulsion effect of the ion-exclusion process.

## AQ Type Columns vs. Mixed-Mode

Poor peak shapes of basic compounds at low organic are typical for AQ type columns. High ion-strength of the mobile phase is required to improve the peak shapes. Primesep 100 column provides symmetrical peaks for compounds of basic nature with low ion-strength mobile phase and allows to use a higher organic concentration in the mobile phase.

| | |
|---|---|
| Column: | Primesep 100 |
| Size: | 3.2x150 mm |
| Flow rate: | 0.5 ml/min |
| Detection: | 280 nm |

Plate count: 2400-3700
Peak symmetry: 0.2-0.4

Column: AQ type
Size: 3.2x150 mm
Flow rate: 0.5 ml/min
Detection:280 nm

1. Amoxicillin
2. Albuterol

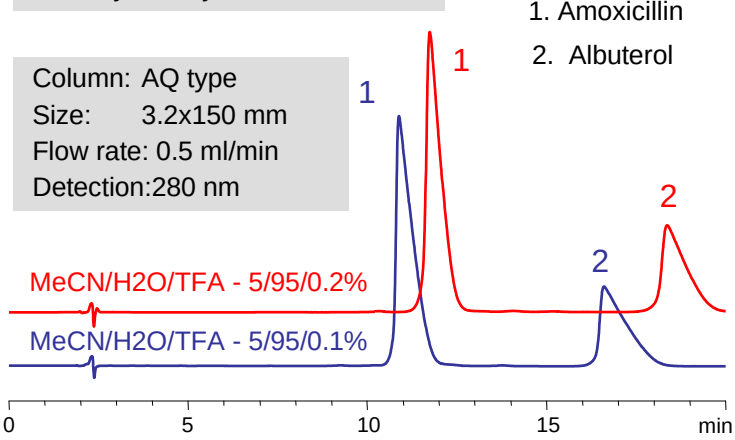
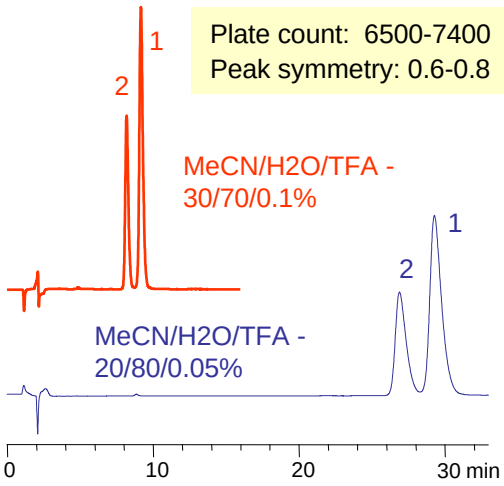


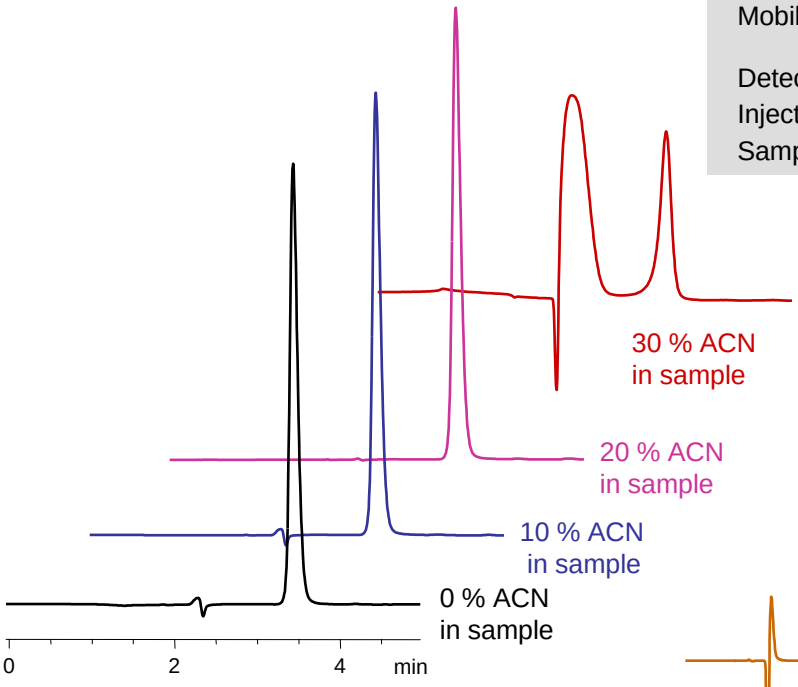

Plate count: 6500-7400
Peak symmetry: 0.6-0.8

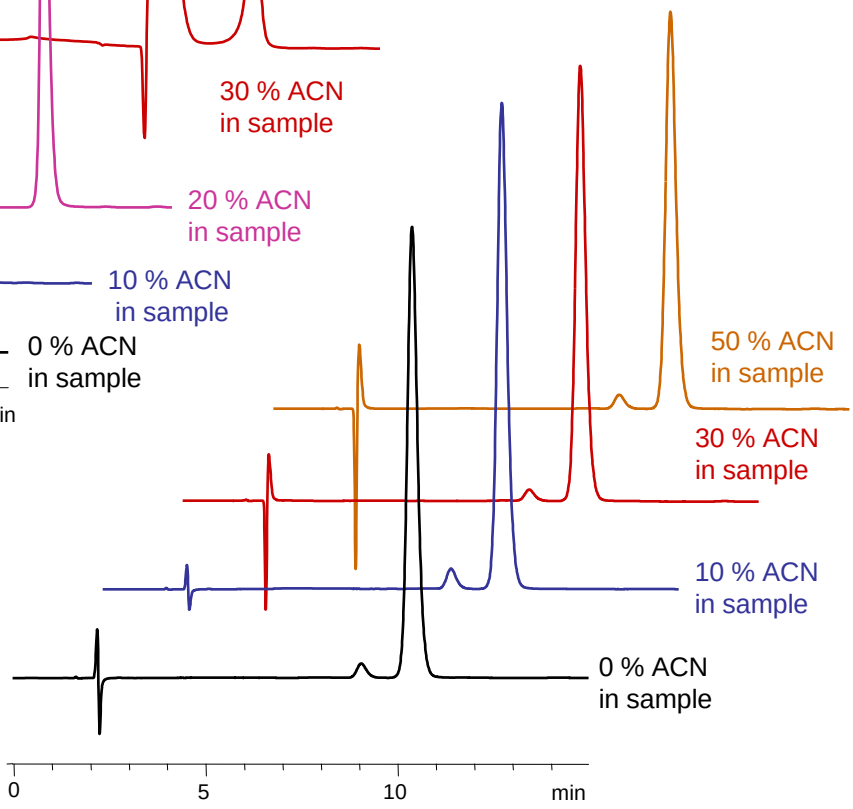


# Study of Dopamine Sample Diluents

This example shows the effect of a sample diluent on a peak shape of dopamine. When the concentration of the organics reaches 30%, dopamine peak shape and the retention get inconsistent which makes the identification and quantitation impossible.
When Primesep 100 column is used for the same application, there is no peak distortion observed even at 50% organic concentration in the sample diluent. This is a convenient feature of this technology which allows to use a wide spectrum of the solvents as a sample diluent.

| | |
|---|---|
| **Column:** | **AQ Type C18** |
| **Size:** | 3.2 x 150 mm |
| Flow rate: | 0.5 ml/min |
| Mobile phase: | 5/95/0.1 MeCN/H2O/TFA |
| Detection: | 280 nm |
| Injection : | 10 uL with |
| Sample: | 1 mg/ml dopamine |



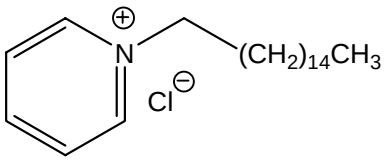




| | |
|---|---|
| Column: | Primesep 100 |
| Size: | 3.2x150 mm |
| Flow rate: | 0.5 ml/min |
| Mobile phase: | MeCN/H2O/TFA-10/90/0.2 |
| Detection: | 280 nm |
| Injection : | 10 uL |
| Sample: | 1 mg/ml dopamine |

# 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

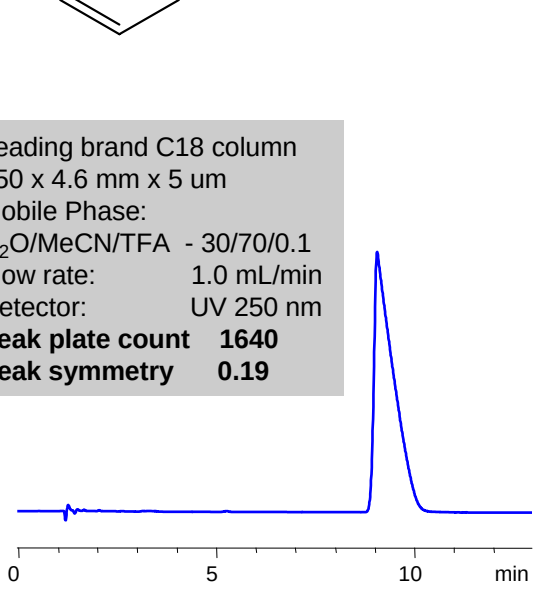
MeCN/water/TFA – 50/50/0.1

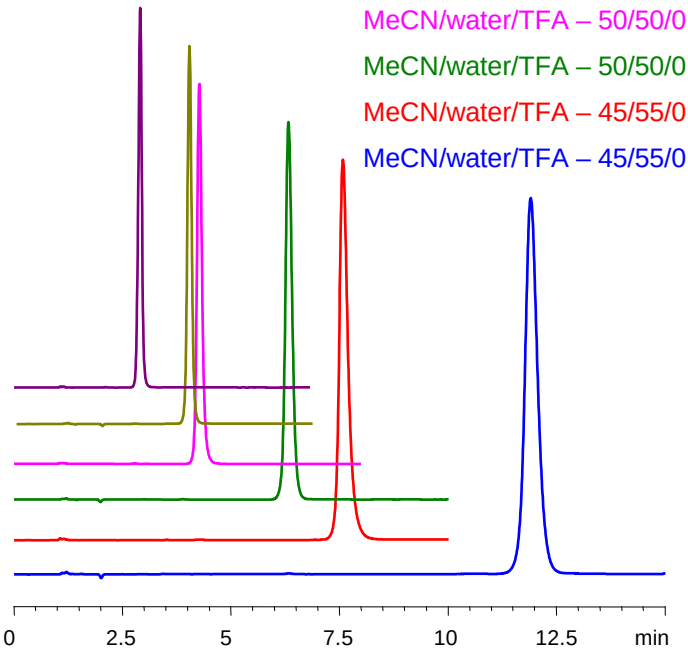
MeCN/water/TFA – 50/50/0.2

MeCN/water/TFA – 45/55/0.1

MeCN/water/TFA – 45/55/0.2

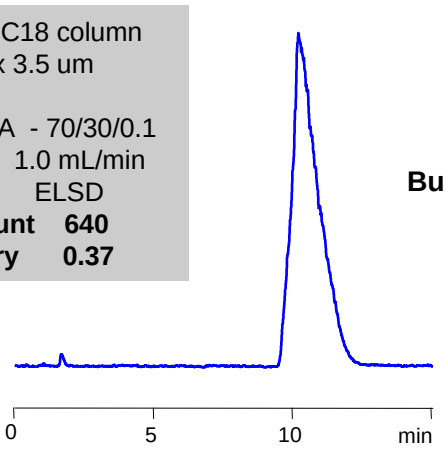
Leading brand C18 column
150 x 4.6 mm x 5 um
Mobile Phase:
$H_2O$/MeCN/TFA - 30/70/0.1
Flow rate: 1.0 mL/min
Detector: UV 250 nm
**Peak plate count 1640**
**Peak symmetry 0.19**

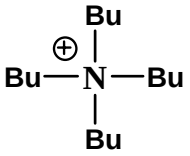



## Tetrabutylammonium Hydroxide

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**







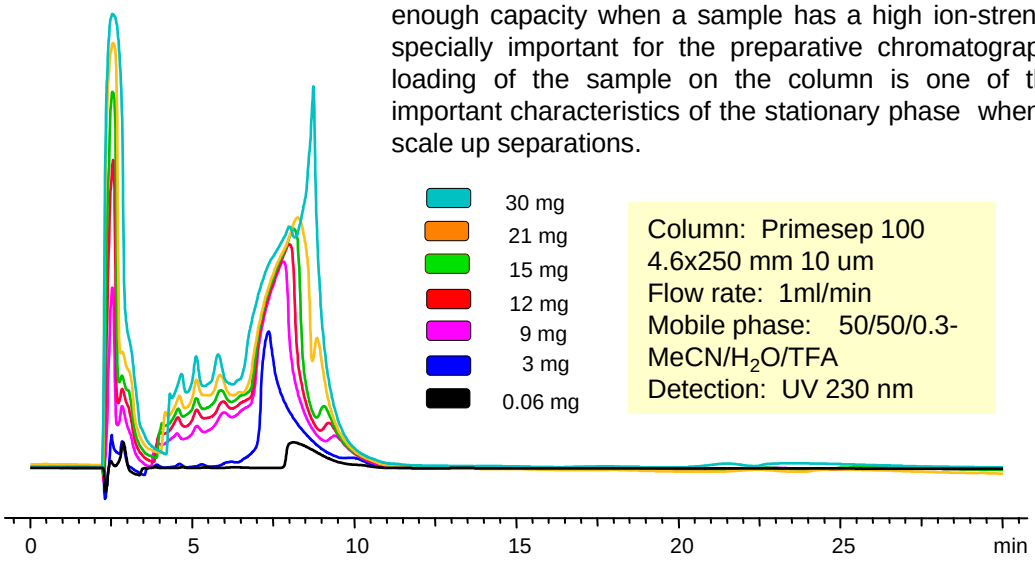
**Primesep B**, 150 x 4.6 mm
Mobile Phase:
$H_2O$/MeCN/TFA - 70/30/0.15
Flow rate: 1.0 mL/min
Detector: ELSD
**Peak plate count 5200**
**Peak symmetry 0.70**

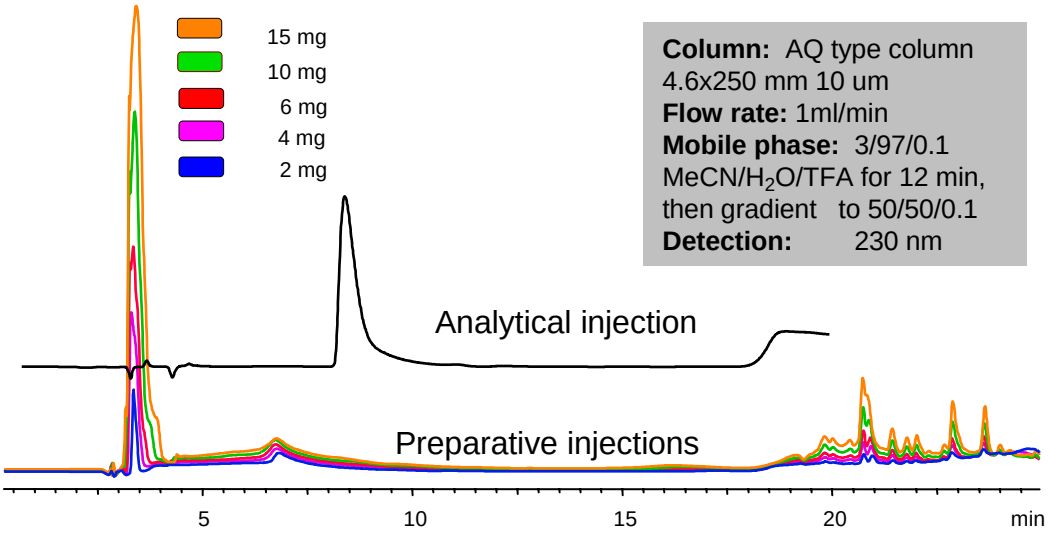
# High Loadability of Primesep Phases toward Polar Ionizable Compounds and High Ion Strength Samples.

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 a 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.

# New Approach to Establish Purity of Reference Standards with Two Alternative Methods

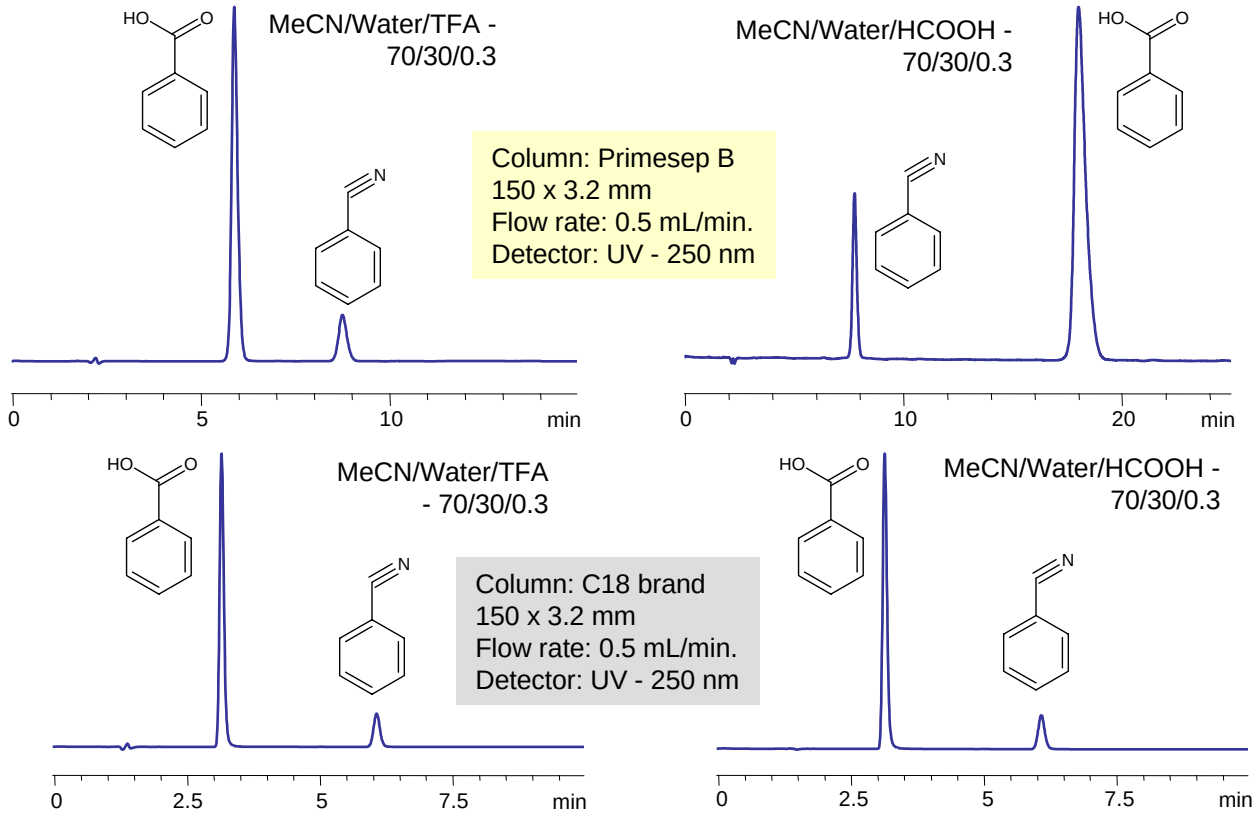
FDA requires establishing purity of a reference standard with at least two alternative methods. Typically, the reverse phase chromatography and the normal phase TLC method are used to identify possible impurities which are not identifiable by either method alone. This approach is labor intensive, as TLC is not a well-automated technique, requires specialized equipment, alternative data handling process, occupies a significant bench and hood space, and uses toxic reagents and flammable solvents in open containers.

The mixed mode chromatography can be used alone or in combination with a regular reverse phase technique to create a universal approach for establishing purity of the reference standards. There are three ways to do this:

1. Using two columns - reverse phase and mixed-mode to provide two different separation media for an orthogonal separation.
2. Using two mixed-mode columns – reverse phase anion-exchange and reverse phase cation-exchange to produce an orthogonal platform for the reference standards
3. Using one mixed mode column in two different modes of separation (normal and reverse, reverse and ion-exchange, polar organic mode and ion-exchange etc.) to produce an orthogonal platform for the reference standards

## Control of Elution Order

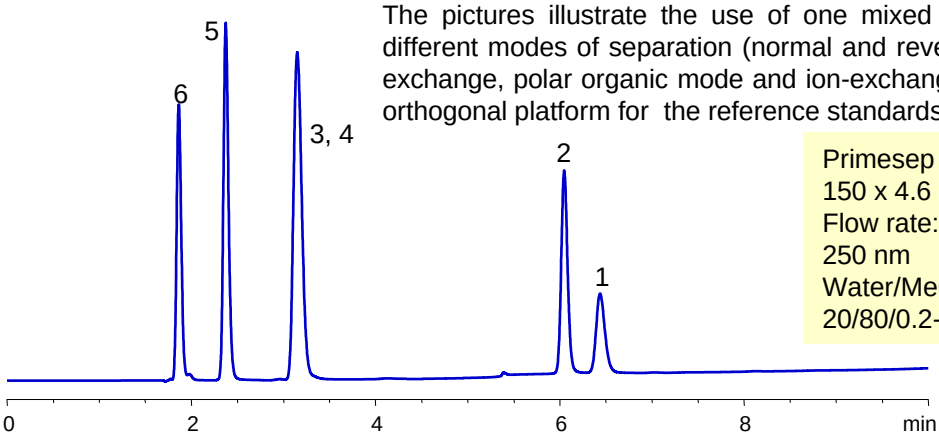
Two columns – a regular reverse phase column and a mixed-mode column - provide two different separation media for an orthogonal separation.




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

## Reverse - Phase Separation

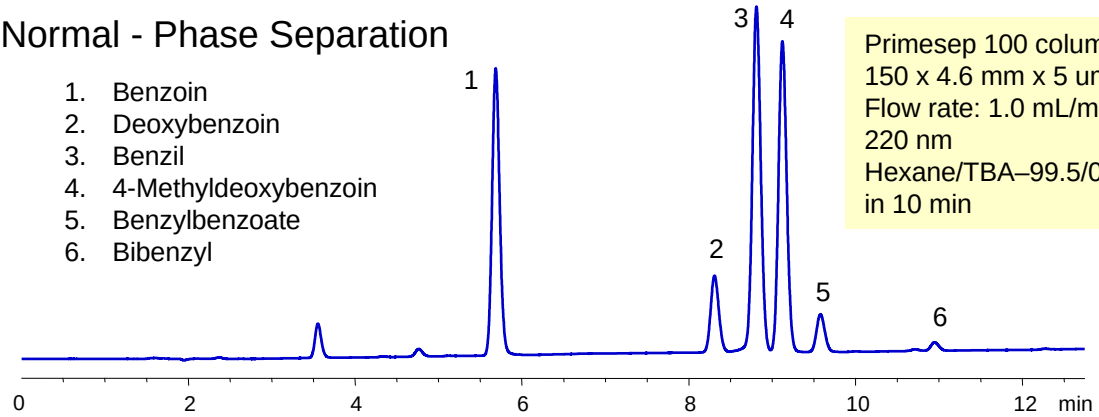
The pictures illustrate the use of one mixed mode column in two different modes of separation (normal and reverse, reverse and ion-exchange, polar organic mode and ion-exchange etc.) to produce an orthogonal platform for the reference standards



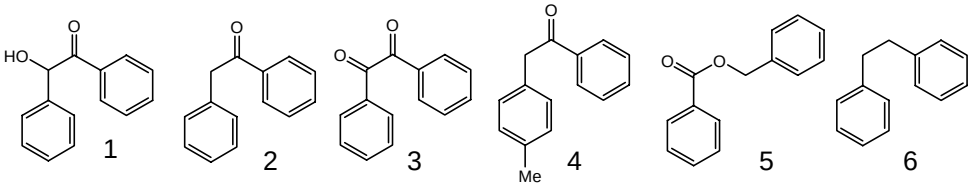

Primesep 100 column
150 x 4.6 mm x 5 um
Flow rate: 1.0 mL/min. UV - 250 nm
Water/MeCN/TFA–40/60/0.2-20/80/0.2-12 min

## Normal - Phase Separation

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

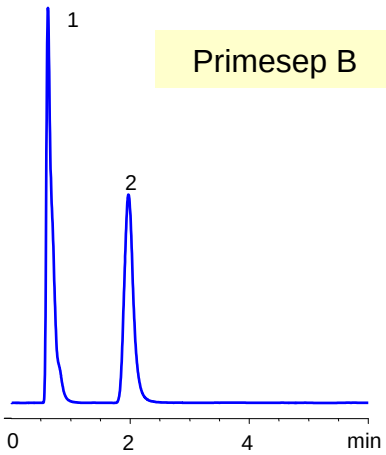



Primesep 100 column
150 x 4.6 mm x 5 um
Flow rate: 1.0 mL/min. UV - 220 nm
Hexane/TBA–99.5/0.5 - 95/5 in 10 min

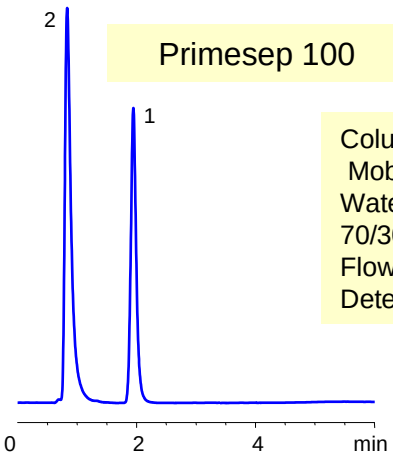



## Analysis of Glyphosate.

Two mixed-mode columns – a reverse phase anion-exchange column and a reverse phase cation exchange column - produce an orthogonal platform for the reference standards. Retention and elution order is orthogonal for charged molecules.




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

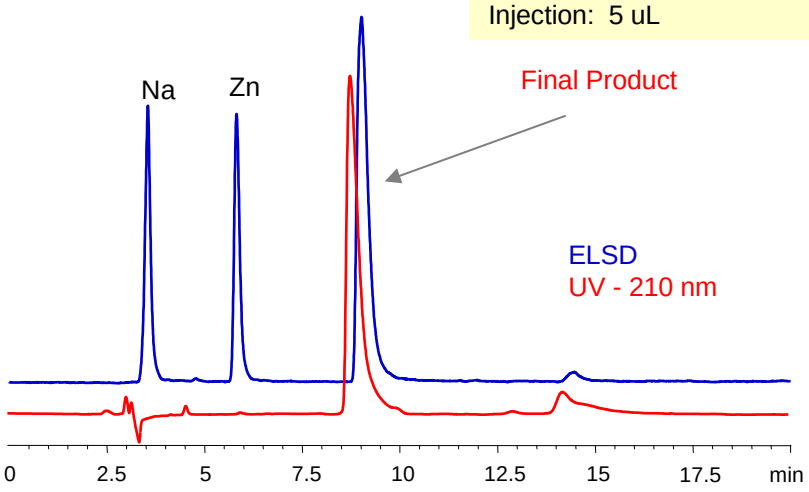
# Simultaneous Separation of Inorganic Ions and Organic Compounds in Single HPLC Method

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.

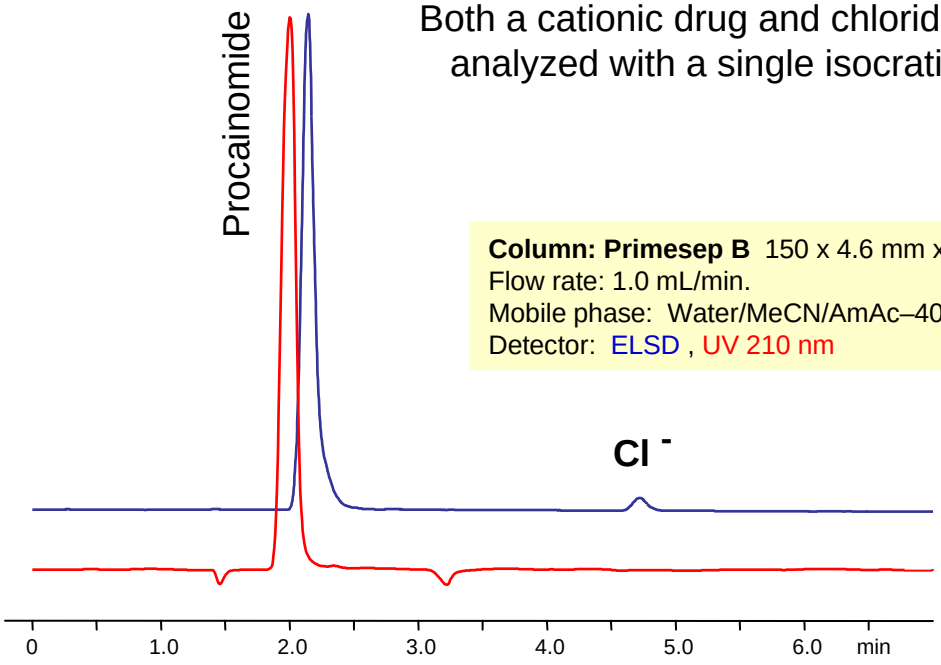
Primesep™ columns offer a unique ability to analyze both parts of such salts at the same time. ELSD in combination with standard UV detector is a convenient tool for this purpose.

## Inorganics in Drug

Primesep 100 column 250 x 4.6 mm x 10 um
Flow rate: 1.0 mL/min.
Mobile phase: Water/MeCN/TFA–50/50/0.2
Injection: 5 uL



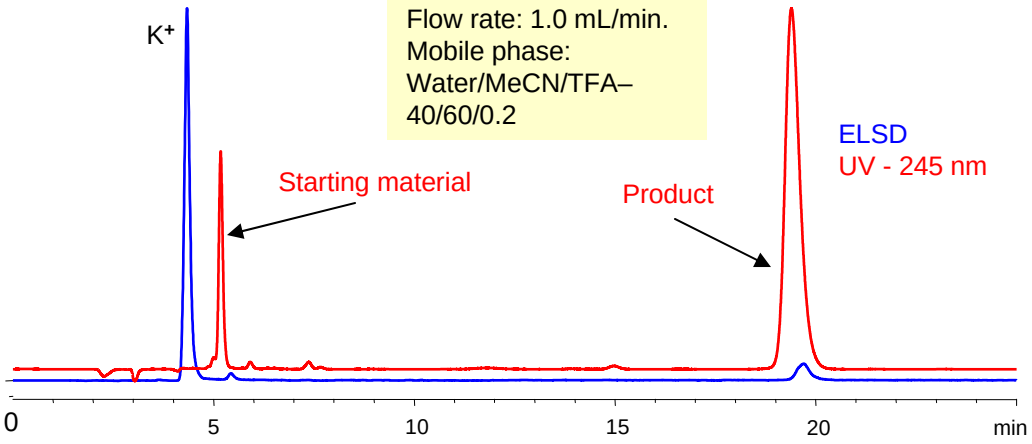

## Both a cationic drug and chloride anion are analyzed with a single isocratic method



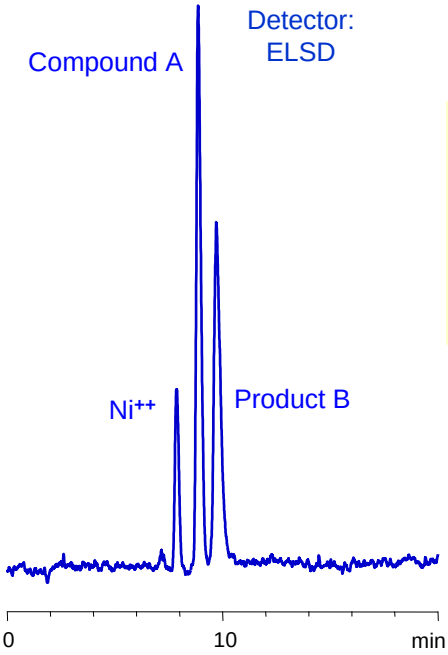

**Column: Primesep B** 150 x 4.6 mm x 5 um
Flow rate: 1.0 mL/min.
Mobile phase: Water/MeCN/AmAc–40/60/0.2
Detector: ELSD , UV 210 nm

## 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

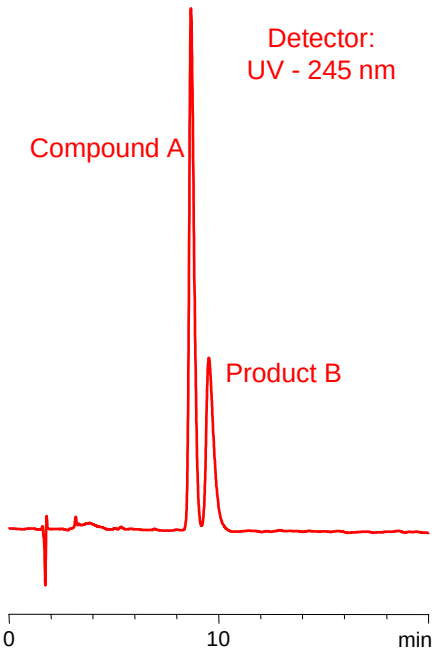


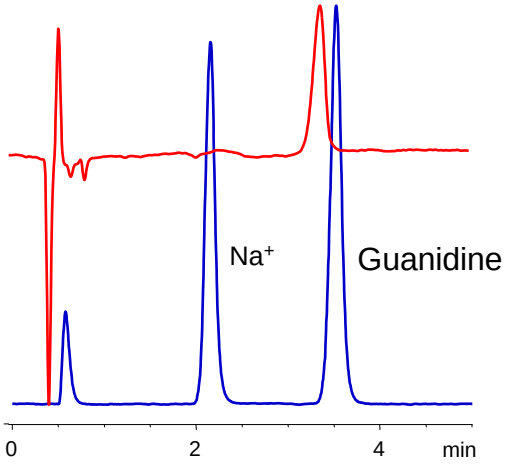

## Analysis of Nickel in Hydrogenation Reaction




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