Improving of a Peak Shape of the Charged Compounds

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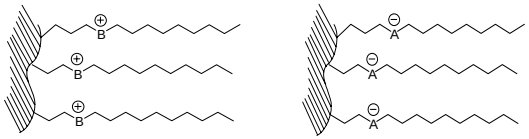
Charged basic analytes often produce poor peak shape on silica based columns due to residual silanol interaction of the charged molecules with oppositely charged silica surface. Strong buffer usually requires to improve the peak shape. A well endcapped column will produce symmetrical peaks with weak basses, but strong bases will still produce unsymmetrical peaks and low efficiency as result.
Another new solution for this type situation was obtained by using mixed-mode columns with the same surface charged as of analyte. Recently several new silica based reverse phase stationary phases with both negatively charged and positively charged surfaces became commercially available. If analyte charge and surface charge are the same then no secondary ion-exchange interaction is possible. The pure reverse phase interaction then offers a symmetrical peak shape and high efficiency for compounds with broad pKa value including quaternary amines.
Separation of basic proteins such as histones significantly improves when surface of reverse phase stationary phase bears positive charge.
The difference in selectivity also observed in systems with the same charge of analytes and stationary phase surface compare with that of the regular reverse phase columns.

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.

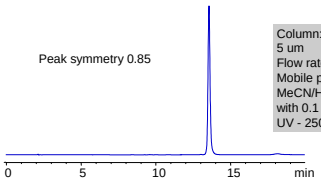
Schematic Structure of Primesep Basic and Acidic Stationary Phases



Primesep B
Primesep B2
Primesep D

Primesep A
Primesep 100
Primesep 200

Amitriptyline Test

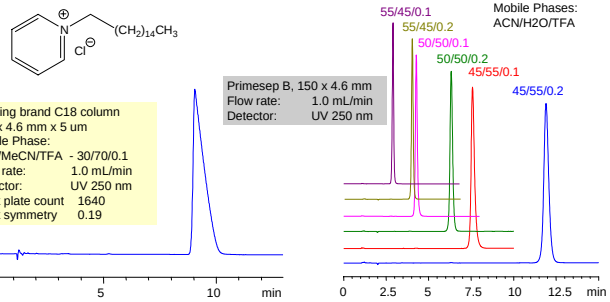


Column: Primesep B 150 x 4.6 mm x 5 um
Flow rate: 1.0 mL/min.
Mobile phase: Gradient
MeCN/H2O-10/90 to 30/70 in 15 min with 0.1 % TFA
UV - 250 nm

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.

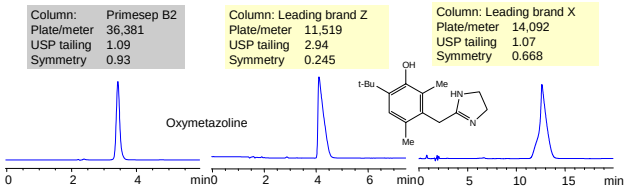
Column surface and analyte have the same charge

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.



Leading brand C18 column
150 x 4.6 mm x 5 um
Mobile Phase:
H2O/MeCN/TFA - 30/70/0.1
Flow rate: 1.0 mL/min
Detector: UV 250 nm
Peak plate count 1640
Peak symmetry 0.19

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



Column: Primesep B2
Plate/meter 36,381
USP tailing 1.09
Symmetry 0.93

Column: 150 x 4.6 mm
Flow rate: 1.0 mL/min.
Detection: UV 250 nm
Mobile phase: MeCN/H2O/TFA – 20/80/0.1%

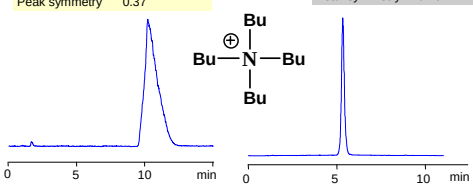
Column: Leading brand Z
Plate/meter 11,519
USP tailing 2.94
Symmetry 0.245

Column: 150 x 4.6 mm
Flow rate: 1.0 mL/min.
Detection: UV 250 nm
Mobile phase: MeCN/H2O/TFA – 35/80/0.1%

Column: Leading brand X
Plate/meter 14,092
USP tailing 1.07
Symmetry 0.668

Column: 150 x 4.6 mm
Flow rate: 1.0 mL/min.
Detection: UV 250 nm
Mobile phase: MeCN/H2O/TFA – 20/80/0.1%

Tetrabutylammonium Hydroxide



Leading brand C18 column
150 x 4.6 mm x 3.5 um
Mobile Phase:
H2O/MeCN/TFA - 70/30/0.1
Flow rate: 1.0 mL/min
Detector: ELSD
Peak plate count 640
Peak symmetry 0.37

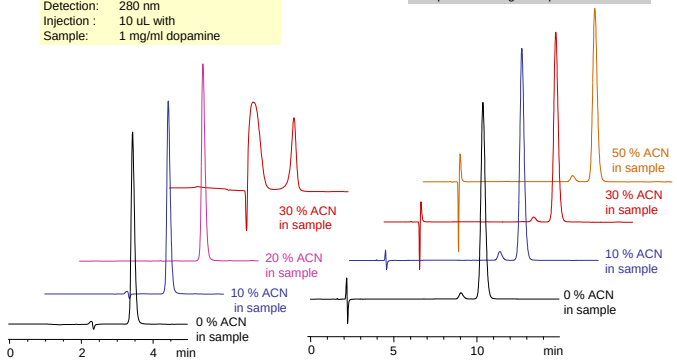
Primesep B, 150 x 4.6 mm
Mobile Phase:
H2O/MeCN/TFA - 70/30/0.15
Flow rate: 1.0 mL/min
Detector: ELSD
Peak plate count 5200
Peak symmetry 0.70

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 mixed-mode 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



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.

1. Albuterol
2. Amoxicillin

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

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

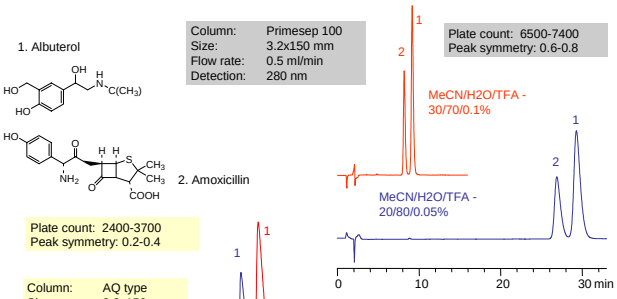
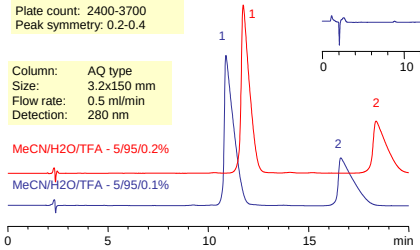


Plate count: 2400-3700
Peak symmetry: 0.2-0.4

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



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