APPLICATIONS

The Effectiveness of Polar Stationary Phase Modification on Peak Shape for Basic Compounds Under General Reversed Phase Conditions – A Comparison of Four Alkyl C18 Phases

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Introduction

Reversed phase chromatography is a useful mode for both the analysis of polar and non-polar compounds. However, basic compounds present a unique reversed phase method development challenges due to uncontrolled secondary interactions that can result in irreducibility and peak shape issues. These potential challenges can be mitigated by the incorporation of mobile phase additives such as buffers or ion-pairing reagents. But, the increased method complexity and related irreproducibility issues with the incorporation of ionic species limits the effectiveness and usability of such additives.

It has been observed that while typical alkyl C18 phases are prone to peak tailing for basic compounds, C18's that incorporate polar modification have the potential for differences in interaction mechanisms that relate to improvements in relative peak shape for basic compounds. In the case of the Kinetex PS C18, a covalently bonded polar functional group on the surface of the silica gel helps to gently repel basic compounds that would typically have the potential for unmitigated interactions with exposed silanol groups on the surface of standard C18 phase. In addition, the polar functional group on the surface of the silica gel has the additive effect of making the phase stable in 100 % aqueous mobile phase conditions.[1] This benefit of polar alkyl phases has been observed across different stationary phases with a variety of polar functional groups.[2]

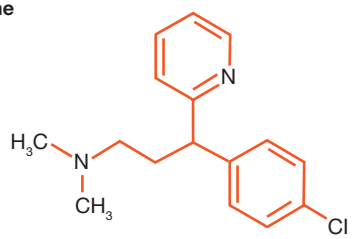
Described in this application note are the details for an investigation of basic compound peak shape across four different alkyl C18 stationary phases that are all based upon the same core-shell HPLC/UHPLC particle morphology. The goal of this application was to investigate the effect of a stationary phase modification and specifically the effect of a positive surface charge modification on peak shape for basic compounds under general reversed phase conditions.

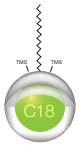
Experiment

Analytical reference standards for Chlorpheniramine were obtained through Sigma-Aldrich® (Saint Louis, MO). The reversed phase mobile phase was comprised of Water with 0.1 % Formic acid as the weak solvent A and Acetonitrile with 0.1 % Formic acid as the strong organic solvent B. Reference standards were prepared in water as the diluent to the concentrations of 0.1, 0.25, 0.5, 0.75, and 1.0 mg/mL, respectively. An Agilent® 1100 (Santa Clara, CA) HPLC system equipped with a UV-Vis detector set to a single wavelength of 254 nm and a reference wavelength of 360 nm was used for this experiment.

To investigate the impact of stationary phase modifications for the improvement of basic compound peak shape under general reversed phase conditions, increasing concentrations of the compound Chlorpheniramine were injected onto the four different alkyl C18 HPLC/UHPLC phases. All phases (C18, XB-C18, EVO C18, and PS C18) were run under identical conditions, on the same system, at the same time period, and are part of the same Kinetex core-shell particle family. All four Kinetex phases shared common material characteristics such as pore volume and surface area (**Table 1**) and only differed by secondary polar stationary phase modifications.

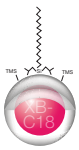
Chlorpheniramine



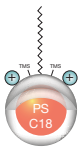


Kinetex 2.6µm C18 – A C18 that offers hydrophobic retention and methylene selectivity expect from a C18 column.

Kinetex 2.6µm EVO C18 – A C18 with an organo-silica grafting modification that provides pH stability 1 – 12.



Kinetex 2.6µm XB-C18 – A C18 that is modified with protective iso-butyl side chains for improved analysis of polar compounds.



Kinetex 2.6µm PS C18 – A C18 with a positive surface charge modification that provides both polar and hydrophobic selectivity, is stable in 100 % aqueous conditions, and demonstrates improved peak shape for basic compounds.

Discussion

Chlorpheniramine was selected as a selectivity probe for this investigation because of its known problematic peak shape under typical reversed phase conditions (**Figure 1**). The four phases selected for this investigation were all part of the same Kinetex core-shell particle family and chosen to compare the effect of a polar stationary phase modification on peak shape for basic compounds.

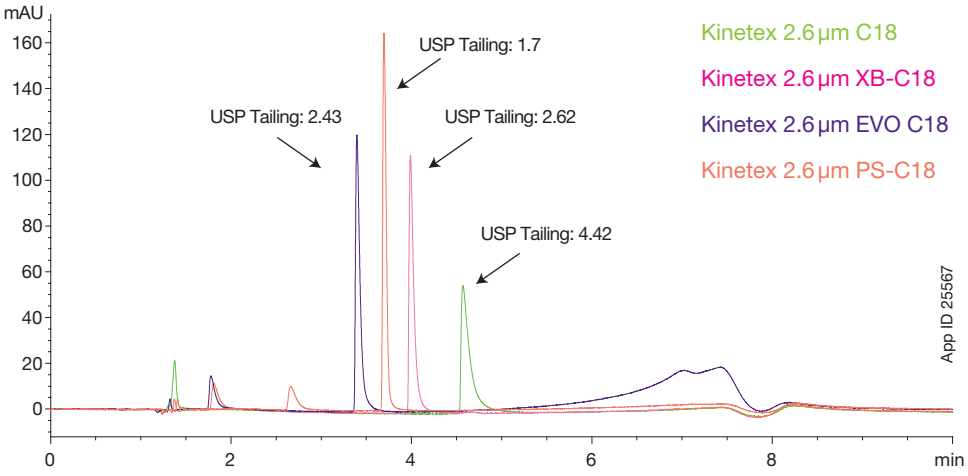
The Kinetex C18 represented an expected C18 phase selectivity and served as a comparative control for the other three modified C18 phases (**Figure 3**) and in particular the Kinetex PS C18 phase. Below are descriptions and phase depictions of the four different Kinetex phases used in this comparison.

Table 1.
Material Characteristics.

Packing Material	Available Particle Size (µm)	Pore Size (Å)	Effective Surface Area (m^2/g)	Effective Carbon Load (%)	pH Stability	Pressure Stability (bar)
PS C18	2.6	100	200	9	1.5 – 8.5*	1,000/600†
EVO C18	1.7, 2.6, 5	100	200	11	1.0 – 12	1,000/600†
C18	1.3, 1.7, 2.6, 5	100	200	12	1.5 – 8.5*	1,000/600†
XB-C18	1.7, 2.6, 5	100	200	10	1.5 – 8.5*	1,000/600†

* pH stability under gradient conditions. pH stability is 1.5 - 10 under isocratic conditions.
† 2.1 mm ID Kinetex columns are pressure stable up to 1000 bar.
When using Kinetex 1.3 µm or 1.7 µm, increased performance can be achieved, however high pressure-capable instrumentation is required.

Figure 1.
Overlay of Chlorpheniramine at 0.1 mg/mL Concentration.



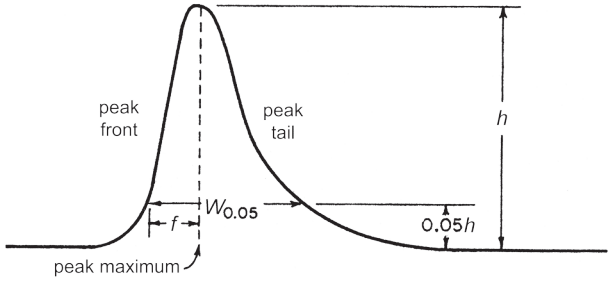
Conditions for all examples:

Column: Kinetex 2.6 µm C18
Kinetex 2.6 µm XB-C18
Kinetex 2.6 µm EVO C18
Kinetex 2.6 µm PS C18
Dimensions: 100 x 4.6 mm
Mobile Phase: A: Water with 0.1 % Formic Acid
B: Acetonitrile with 0.1 % Formic Acid
Gradient:

Time (min)	% B
0	10
6	50
6.01	10
10	10

Flow Rate: 1.0 mL/min
Temperature: 25 °C
Detector: UV-Vis @ 254 nm
Sample: 1. Chlorpheniramine

Figure 2.
USP Tailing Factor, <621> Chromatography**.



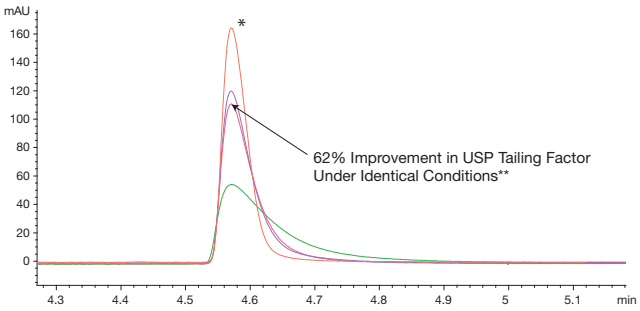
Symmetry factor (A^S): Also known as the "tailing factor", of a peak (see **Figure 4**) is calculated by:

$$A_S = W_{0.05}/2f$$

where $W_{0.05}$ is the width of the peak at 5 % height and *f* is the distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5 % of the peak height from the baseline.

** General Chapter <621> "Chromatography" in United States Pharmacopeia 40 National Formulary 35 (USP 40-NF 35, United States Pharmacopeial Convention, Rockville, Maryland, 2017), p. 6.

Figure 3.
Zoomed Overlay of Chlorpheniramine at 0.1 mg/mL - Adjusted for Overlay.



Kinetex 2.6 µm C18

Kinetex 2.6 µm XB-C18

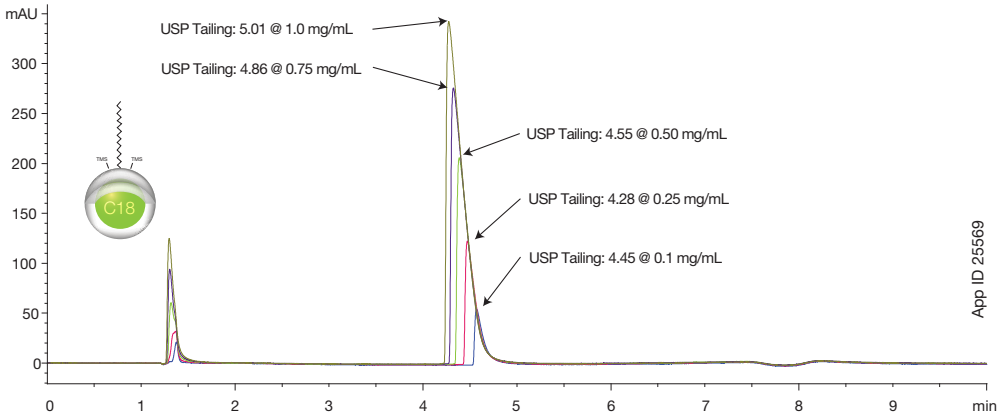
Kinetex 2.6 µm EVO C18

Kinetex 2.6 µm PS-C18

* Retention times for chlorpheniramine varied depending on the selectivity of the different phases. For comparison sake peaks were aligned in this overlay example.

** Improvement in USP Tailing Factor is based on comparison of the Kinetex C18 tailing vs. the Kinetex PS C18 tailing at referenced concentration.

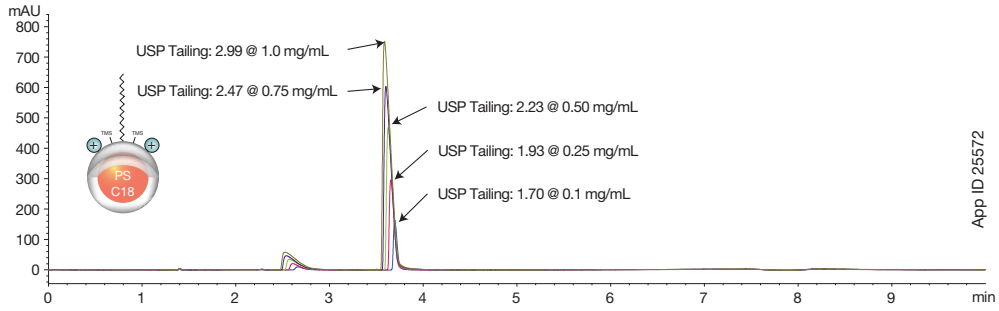
Figure 4.
Overlay of Chlorpheniramine (0.1, 0.25, 0.50, 0.75, and 1.0 mg/mL) on the Kinetex 2.6 µm C18.



Conditions for all examples:

Column:	Kinetex 2.6 µm C18 Kinetex 2.6 µm XB-C18 Kinetex 2.6 µm EVO C18 Kinetex 2.6 µm PS C18
Dimensions:	100 x 4.6 mm
Mobile Phase:	A: Water with 0.1 % Formic Acid B: Acetonitrile with 0.1 % Formic Acid
Gradient:	**Time (min) % B** 0 10 6 50 6.01 10 10 10
Flow Rate:	1.0 mL/min
Temperature:	25 °C
Detector:	UV-Vis @ 254 nm
Sample:	1. Chlorpheniramine

Figure 5.
Zoomed Overlay of Chlorpheniramine (0.1, 0.25, 0.50, 0.75, and 1.0 mg/mL) on the Kinetex 2.6 µm PS C18.



Conclusions

In order to investigate the effect of a positive surface charged modification on basic compound peak shape, four different alkyl C18 stationary phases were selected. All phases were based upon the same particle morphology and only differed by a secondary stationary phase modification. By simply utilizing the Kinetex PS C18 under identical running conditions a 62 % improvement in USP tailing factor was observed at concentration 0.1 mg/mL (**Figure 2**). This improvement in peak shape was observed over a dynamic concentration range of the polar basic probe chlorpheniramine (**Figures 3 & 4**).

The Kinetex PS C18's controlled incorporation of a bonded positive surface charge provided improved peak shape for the polar basic compound chlorpheniramine, in comparison to other alkyl C18 phases of equal particle morphology and under identical running conditions.

Therefore, the Kinetex PS C18 is a USP classified L1 column that provides a unique combination of both polar and hydrophobic selectivity, is stable in 100 % aqueous conditions, and demonstrates improved peak shape for basic compounds.

Kinetex® Core-Shell LC Column Ordering Information

2.6 µm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
PS C18	00A-4780-AN	00B-4780-AN	00D-4780-AN	00F-4780-AN	AJ0-8951
					for 2.1 mm ID

2.6 µm MidBore™ Columns (mm)				SecurityGuard ULTRA Cartridges‡
Phases	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
PS C18	00B-4780-Y0	00D-4780-Y0	00F-4780-Y0	AJ0-8950
				for 3.0 mm ID

2.6 µm Analytical Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
PS C18	00B-4780-E0	00D-4780-E0	00F-4780-E0	00G-4780-E0	AJ0-8949
					for 4.6 mm ID

‡ SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000.

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2. Thomas H. Walter, Pamela Iraneta, Mark Capparella. Mechanism of Retention Loss When C8 and C18 HPLC Columns Are Used with Highly Aqueous Mobile Phases. *J. of Chromatogr.A 2005, 1075, 177-183.*
3. General Chapter <621> "Chromatography" in United States Pharmacopeia 40 National Formulary 35 (USP 40-NF 35, United States Pharmacopeial Convention, Rockville, Maryland, 2017), p. 8.

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