Demonstrating the Kinetex® PS C18 HPLC/UHPLC Column's Resistance to Dewetting and 100 % Aqueous Stability

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Introduction

The Kinetex PS C18 is the latest addition to the Kinetex core-shell (superficially porous) HPLC/UHPLC column family. The phase's selectivity is well suited for the analysis of different classes of acids, bases, and neutral compounds under typical reversed phase conditions. However, the Kinetex PS C18 selectivity is particularly applicable for the analysis of polar compounds that contain one or more basic functional groups and that typically exhibit poor peak performance on traditional C18 phases. The PS C18 is a USP classified L1 column that provides a unique combination of both polar and hydrophobic selectivity. In addition, the Kinetex core-shell (superficially porous) particle morphology provides ultra-high column efficiency on any HPLC or UHPLC system.[1]

Traditional C18 HPLC/UHPLC columns are prone to retention time inconsistencies when subjected to 100 % aqueous running conditions. This inconsistency in retention time is attributed to variations in the amount of high aqueous mobile phases that gets forced out of the column's hydrophobic pore structure from run to run when the flow is stopped and the back-pressure is released.[2] The Kinetex PS C18 phase incorporates a polar functional group that is covalently bonded on the surface of the silica gel. The location and polarity of this group promotes aqueous solvation of the stationary phase's pores, even at lower percentages of organic mobile phase modifiers. Therefore, avoiding pore exclusion and negative retention time variations. This benefit of polar-embedded alkyl phases has been observed across different stationary phase with a variety of polar functional groups.[3] In addition to the compatibility with 100 % aqueous conditions, polar-embedded C18 phases have reversed phase selectivity that is both unique and distinct from traditional C18 phases.

Described in the experimental details is a procedure for investigating potential retention time variation under high aqueous (>99 % water) mobile phases conditions. The procedure involves stopping and restarting flow rate cycles and monitoring the retention of two polar basic probes (Dopamine and Epinephrine).

Experiment

Analytical reference standards for the Dopamine and Epinephrine were obtained through Sigma-Aldrich® (Saint Louis, MO). The 100 % aqueous mobile phase was comprised of a 20 mM Potassium Phosphate Monobasic buffer that was adjusted to the pH of 2.5 with phosphoric acid. A reference standard mixture of Epinephrine and Dopamine was prepared in mobile phase diluent to the concentration of 0.5 mg/mL, each. An Agilent® 1100 HPLC system equipped with UV-Vis detection set to 270 nm was used for this experiment.

To investigate the stability of the Kinetex 2.6 µm PS C18 when exposed to 100 % aqueous conditions, the column was exposed to one-hour cycles of alternating active and halted flow, in which the column was first subjected to active pump operation and then pump halt. At the start of each active pumping cycle the same sequence, as follows, and was repeated for a total of 4-cycles (**Figure 1**).

Sequence:

Column Equilibration: 100 % aqueous mobile phase for 10 minutes at 1.5 mL/min prior to sample injection.

Sample Injection: 60 injections (1-minute run time for each injection).

Halt pump and allow back-pressure to reach zero bar for 60 minutes.

Figure 1.
Retention Time vs. Number of Injection

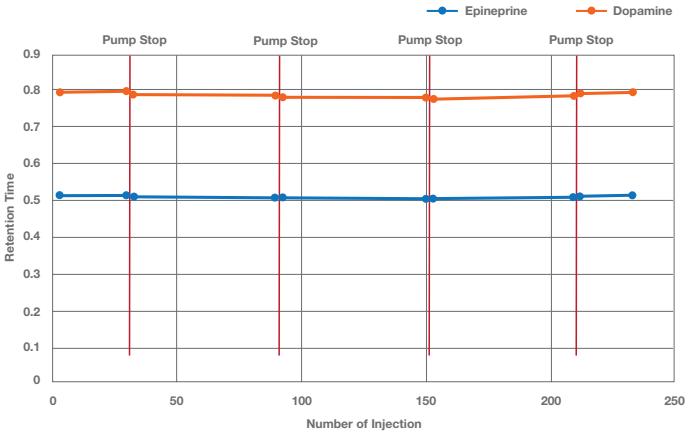
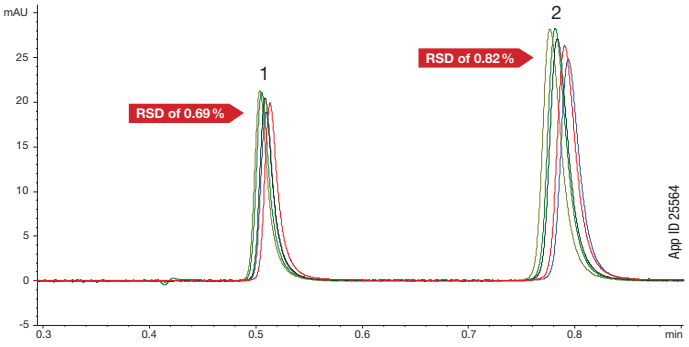


Figure 2.
Chromatographic Overlay of Sequence Injections of Dopamine and Epinephrine



Column: Kinetex 2.6 µm PS C18
Dimensions: 50 x 4.6 mm
Mobile Phase: 100 % Water with 20 mM Potassium Phosphate Monobasic (pH 2.5)
Flow Rate: 1.5 mL/min
Temperature: 25 °C
Detector: UV-Vis (@ 270 nm)
Sample: 1. Dopamine
2. Epinephrine

Conclusion

During this investigation, we found that the retention times of Epinephrine and Dopamine were consistent and stable for more than 200 injections over a total of 4-cycles of pump operation and halts.

There was no significant change in either the retention time of Dopamine with an average percent change of 0.62 % ± 0.41 % or Epinephrine with an average percent change of 1.10 % ± 0.78 % and these results indicated that the Kinetex 2.6 µm PS C18 is suitable for use in 100 % aqueous condition.

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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3. LCGC Editors (2014), Chromatography Technical Tips – Polar Analytes Retention and Phase Collapse. [Blog] The LCGC Blog. Available at: http://www.chromatographyonline.com/lcgc-blog-chromatography-technical-tips-polar-analyte-retention-and-phase-collapse [Accessed 30 May. 2019].

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