

# APPLICATIONS

## Demonstrating Kinetex<sup>®</sup> PS C18 HPLC/UHPLC Column's Unique Reversed Phase Selectivity and Improved Chromatographic Performance through the Analysis of the Polar Base Berberine

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### Overview

The Kinetex PS C18 is a USP classified L1 column, that provides both a unique polar and hydrophobic selectivity and is 100 % aqueous stable. In addition, the solid support is a Kinetex core-shell (superficially porous) particle morphology that provides ultra-high column efficiency on any HPLC or UHPLC system.<sup>1</sup>

The gradient mobile phase system consisted of Water with 20mM Potassium Dihydrogen Phosphate buffer (pH 4.6) as the weak solvent and Acetonitrile as the strong organic solvent. A flow rate of 1.5 mL/min was used, and the column heater was set to ambient temperature (23 °C).

The berberine reference standard used to confirm peak identity was prepared by dissolving 1 mg (anhydrous) into 2 mL of Methanol within a Verex<sup>™</sup> HPLC injection vial. The Barberrry root sample was prepared by taking 500mg of homogenized root material and extracting in a 6mL mixture of 50:50 (Methanol/Water). The Barberrry root extraction was then vortexed/centrifuged, and the resulting supernatant was filtered through a Phenex<sup>™</sup> 0.45 µm Nylon syringe filter and transferred to a Verex HPLC injection vial. The goldenseal pill contents were extracted in 10mL mixture of 50:50 (Methanol/Water with 20mM phosphate buffer). The sample was then vortexed/centrifuged, and the supernatant was transferred to an HPLC injection vial. The goldenseal liquid sample was prepared by diluting in the mobile phase and transferred to an HPLC injection vial.

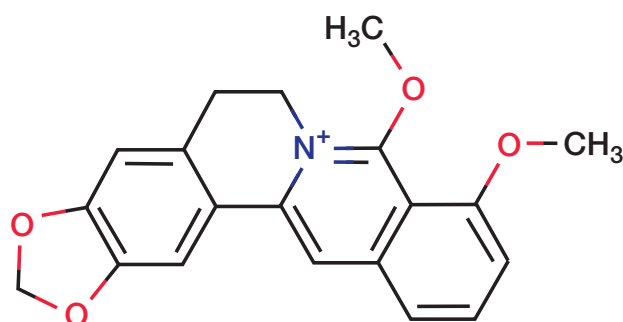
### Introduction

For this application study, the alkaloid compound berberine was selected to help demonstrate the Kinetex PS C18 HPLC/UHPLC column's unique multi-modal selectivity and improved chromatographic performance when applied to the analysis of polar basic compounds. Berberine specifically was selected as an example because it contains a quaternary ammonium cation group that typically presents challenges when analyzed under reversed phase conditions.

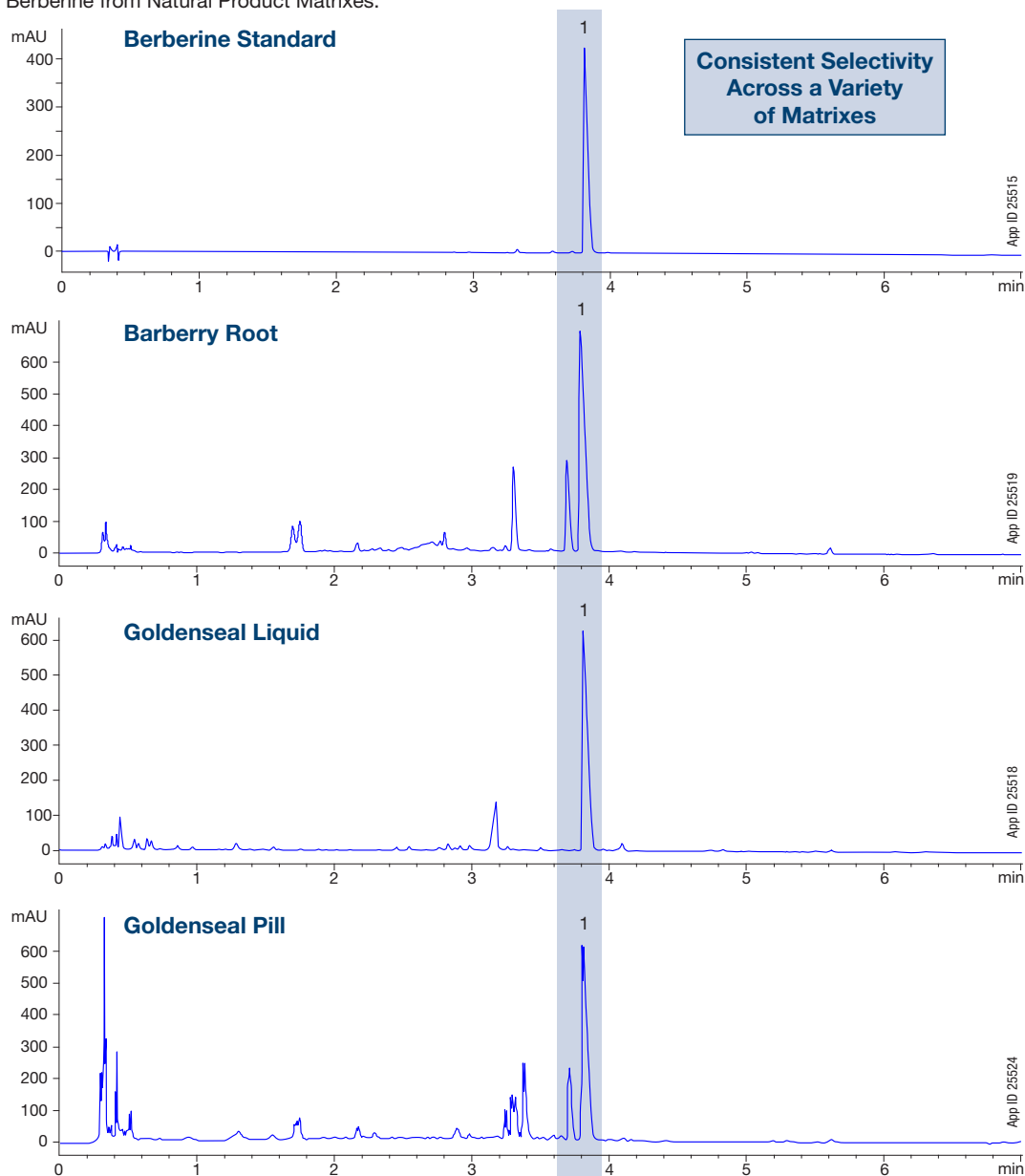
The unique selectivity and performance benefits of the Kinetex PS C18 were compared across a variety of natural product extracts to confirm consistent peak performance and selectivity. To further validate the Kinetex PS C18 phase's unique selectivity, when applied to polar basic compounds, a superficially porous (core-shell) C18 column was also run under identical conditions. The comparisons depicted, that when compared to a traditional C18 core-shell column the Kinetex PS C18 demonstrated a visually apparent increase in compound retention, improved peak shape, and greater peak sensitivity for polar basic compounds.

### Berberine

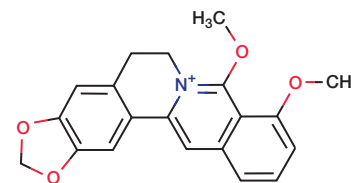
Molecular Formula: C<sub>20</sub>H<sub>18</sub>NO<sub>4</sub>  
LogP: -1.28



**Figure 1.**  
Berberine from Natural Product Matrixes.



**Berberine**  
Molecular Formula: C<sub>20</sub>H<sub>18</sub>NO<sub>4</sub>  
LogP: -1.28



**Conditions for all examples:**

**Column:** Kinetex<sup>®</sup> 2.6 μm PS C18  
Core-Shell 2.6 μm RP-MS C18  
**Dimensions:** 50 x 4.6 mm  
**Mobile Phase:** A: Water with 20 mM Potassium Dihydrogen Phosphate (pH 4.6)  
B: Acetonitrile  
**Gradient:**

Time (min)	% B
0	10
7	65
8	65
8.5	10
10	10

**Flow Rate:** 1.5 mL/min  
**Temperature:** 23 °C  
**Detector:** UV @ 235 nm  
**Sample:** 1. Berberine

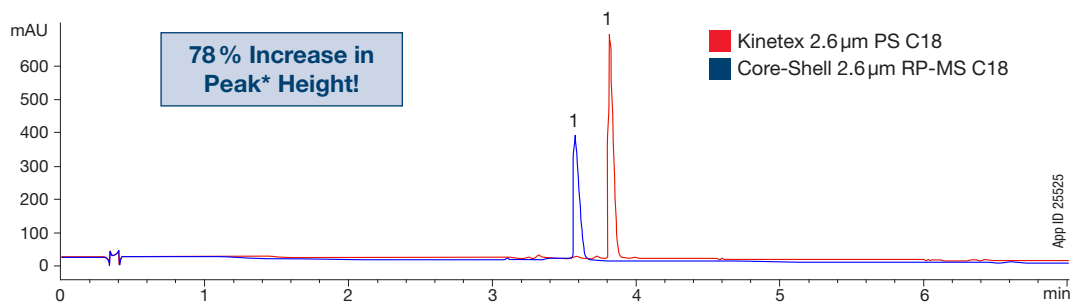
**Discussion**

**Figure 1** displays the Kinetex PS C18 multi-modal selectivity for berberine, a quaternary ammonium cation containing Alkaloid, in a variety of natural product matrixes. The selectivity of the Kinetex PS C18, which is attributed to its consistent positive surface charge and C18 ligand, demonstrated peak shape and sensitivity improvements for berberine across a variety of natural product matrixes and under typical reversed phase conditions. The berberine peaks were identified by both relative retention time and spiked injection. Peak performance and in-matrix separation were found acceptable.

The dual selectivity and performance benefits of the Kinetex PS C18, compared across a variety of natural product extracts, confirm consistent column performance.

**Figure 2** is further validation of the phase's applicability for the analysis of polar bases. In **Figure 2** are representative chromatographic overlays comparing a conventional core-shell C18 column to the Kinetex PS C18, under identical method conditions. The Kinetex PS C18 demonstrated a visually apparent increase in compound retention, improved peak shape, and greater peak sensitivity for polar basic compounds compared to a conventional C18.

**Figure 2.**  
Berberine Comparisons of Kinetex<sup>®</sup> PS C18 vs. Core-Shell RP-MS C18.



**Conditions for all examples:**

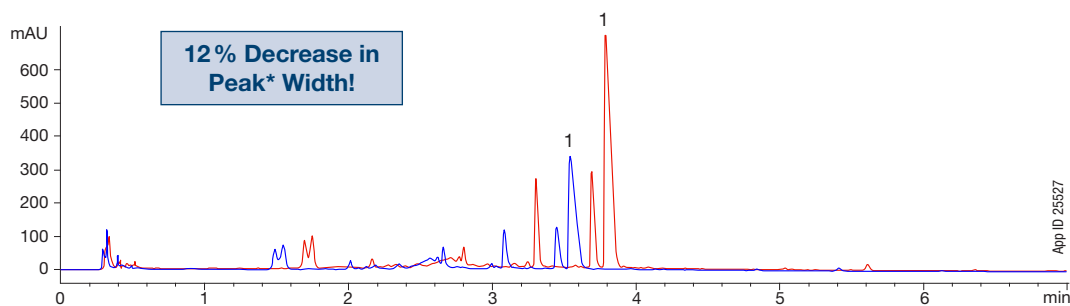
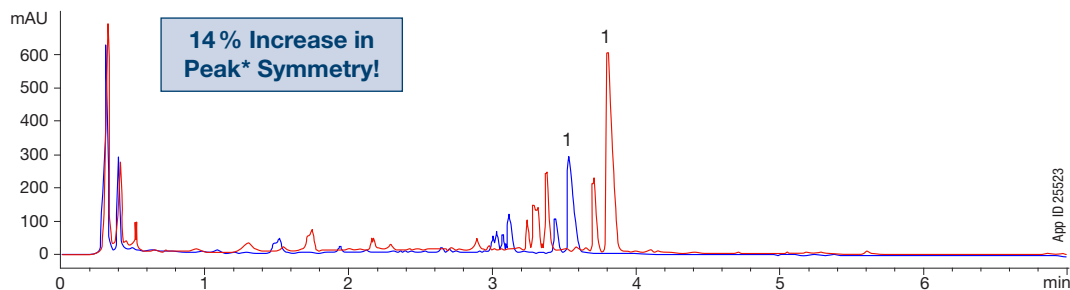
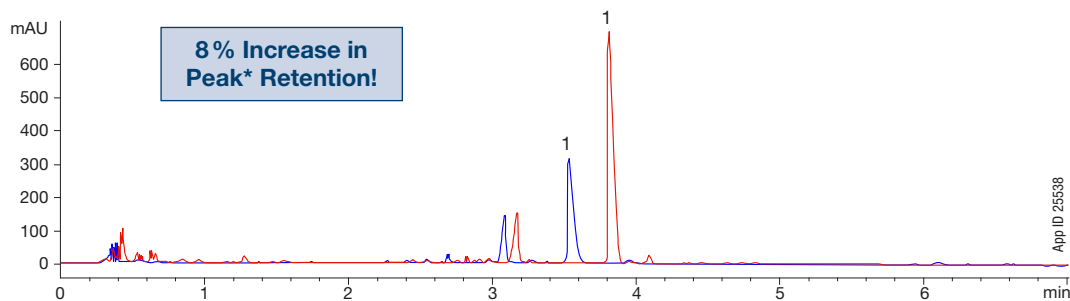
**Column:** Kinetex 2.6 μm PS C18  
Core-Shell 2.6 μm RP-MS C18

**Dimensions:** 50 x 4.6 mm

**Mobile Phase:** A: Water with 20 mM Potassium Dihydrogen Phosphate (pH 4.6)  
B: Acetonitrile

Gradient	Time (min)	% B
	0	10
	7	65
	8	65
	8.5	10
	10	10

**Flow Rate:** 1.5 mL/min  
**Temperature:** 23 °C  
**Detector:** UV @ 235 nm  
**Sample:** 1. Berberine



\* Peak improvement percentage were based off of berberine peak values and peak identity was confirmed by spiked injection.

## 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	AJO-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-YO	00D-4780-YO	00F-4780-YO	AJO-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	AJO-8949 for 4.6 mm ID

† SecurityGuard ULTRA Cartridges require holder, Part No.: AJO-9000.

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## References

1. Fabrice Gritti, Georges Guiochon. Facts and Legends About Columns Packed with sub-3-µm Core-Shell Particles. *LC-GC North America*. 2012, 30(7), 586-595.



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Comparative separations may not be representative of all applications.

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