

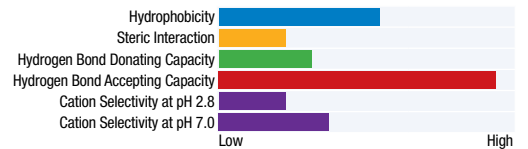
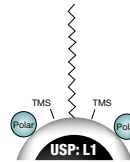
APPLICATIONS

Reference Analysis of B Vitamin Complex (Hydroxocobalamin, Thiamine, and Pyridoxine) from an Injectable Solution using a Kinetex[®] 2.6 μm Polar C18 HPLC/UHPLC Column

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Overview

B complex vitamins are a class of essential water-soluble vitamins that can be administered via an injectable solution. Because of the relative hydrophilic nature of these compounds, they present a reversed phase analytical challenge. Several reversed phase methods have been proposed that use an ion-pairing agent to increase observed retention. In this application, we investigated a non-ion-pairing reversed phase method that leverages the unique dual selectivity of the Kinetex Polar C18. This column combines a polar modified surface and the hydrophobic selectivity of a C18 column. The result is a USP classified L1 column, that provides both increased polar retention and is 100 % aqueous stable. In addition, the Kinetex solid support is a superficially porous (core-shell) particle morphology that provides ultra-high column efficiency on any HPLC/UHPLC system.¹



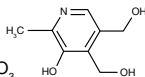
Column: Kinetex 2.6 μm Polar C18
Dimensions: 150 x 4.6 mm
Part No.: 00F-4759-E0
Mobile Phase: A: Water with 30 mM Potassium Phosphate (pH 3.0)
 B: Acetonitrile
Gradient:

Time (min)	% B
0	0
8	80

Flow Rate: 1.0 mL/min
Injection Volume: 10 μL
Temperature: 40 °C
Detection: UV @ 254 nm
Sample: 1. Pyridoxine (2.1 min)
 2. Hydroxocobalamin (3.6 min)
 3. Thiamine (8.9 min)
 4. Proprietary Excipient (9.7 min)

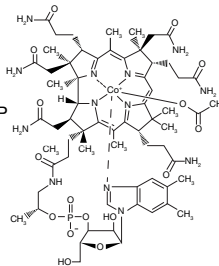
Pyridoxine

Molecular Formula: C₈H₁₁NO₃
 Basic pKa: 5.58
 LogD: -2.4 (@ pH 3.0)



Hydroxocobalamin

Molecular Formula: C₆₂H₈₉CoN₁₃O₁₅P
 Acidic pKa: 1.84
 LogD: 2.53 (@ pH 3.0)



Thiamine

Molecular Formula: C₁₂H₁₇N₄OS⁺
 Basic pKa: 5.54
 LogD: -4.8 (@ 3.0)

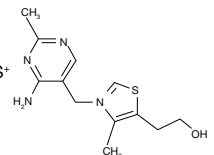
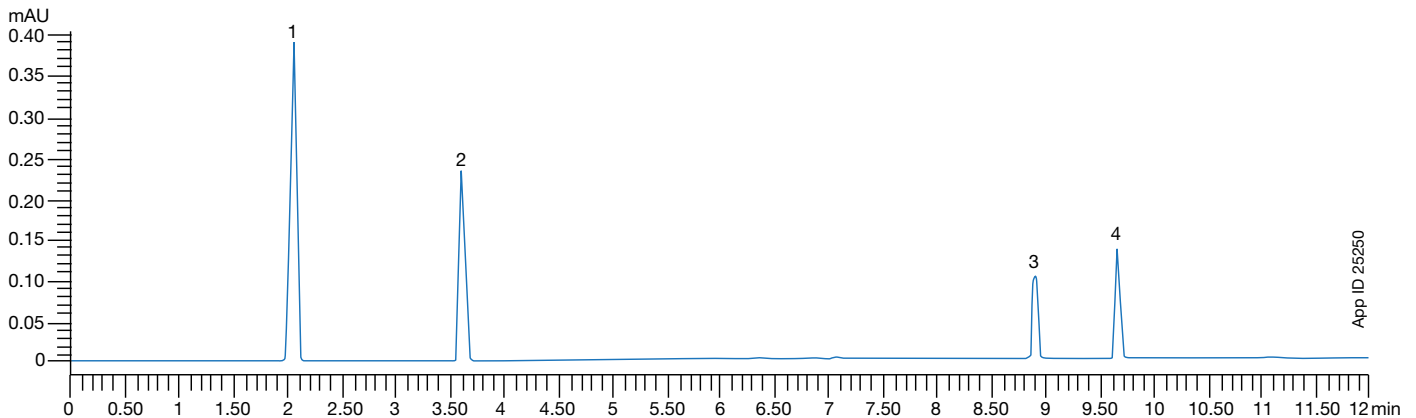


Figure 1.
Representative Separation of Pyridoxine, Hydroxocobalamin, and Thiamine



App ID 25250

APPLICATIONS

Kinetex® 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
Polar C18	00A-4759-AN	00B-4759-AN	00D-4759-AN	00F-4759-AN	AJ0-9532 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
Polar C18	00B-4759-Y0	00D-4759-Y0	00F-4759-Y0	AJ0-9531 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	3/pk
Polar C18	00B-4759-E0	00D-4759-E0	00F-4759-E0	AJ0-9530 for 4.6 mm ID

[†] SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000.

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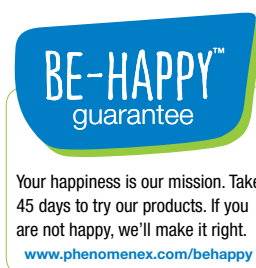
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References

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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