

APPLICATION

Rapid and Accurate Vitamin B12 Extraction and Analysis using Kinetex[®] 5 µm EVO C18 Core-Shell Column and HPLC/UV

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Overview

Nutraceutical label claim verification is a continual concern for the dietary supplements industry and its consumers. Manufacturers and various contract labs are pressed to use and discover accurate, reproducible and efficient methods that are able to confirm the quality and quantity of the target within the sample. In addition, selectivity from other added components is also a concern. Vitamin B12, also called cobalamin, is a water-soluble vitamin with a key role in the normal functioning of the brain and nervous system, and for the formation of blood.

The label claim of the sample taken indicated a 1 mg/pill concentration of vitamin B12. After using the equation ($y = 0.3265x - 0.0115$) and the area count (17.9185) from the sample injection; we can calculate the concentration of the final product to be 1.0983 mg/pill, in the pill tested. Demonstrating the sensitivity, reproducibility, and stability of the Kinetex EVO C18 column during the analysis.

Sample Preparation

- 1) 1 pill dissolved in 20 mL of 50:50 Methanol:Water
- 2) Sonicated at 45 °C for 20 minutes
- 3) Centrifuged at 4700 rpm for 5 minutes
- 4) Decanted and filtered liquid portion through 0.45 µm Phenex[™] PTFE filter (Part No. AF0-1102-12)
- 5) Injected 2 µL of sample onto HPLC system

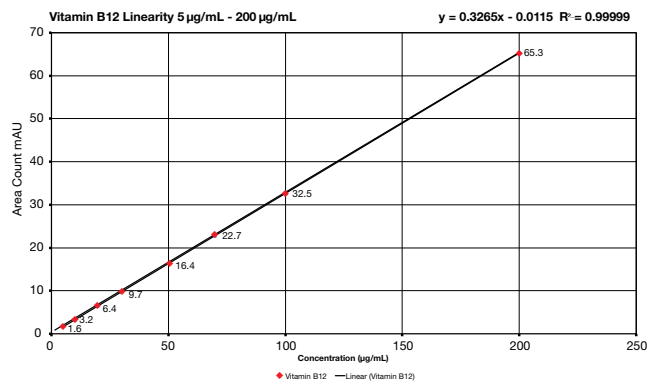
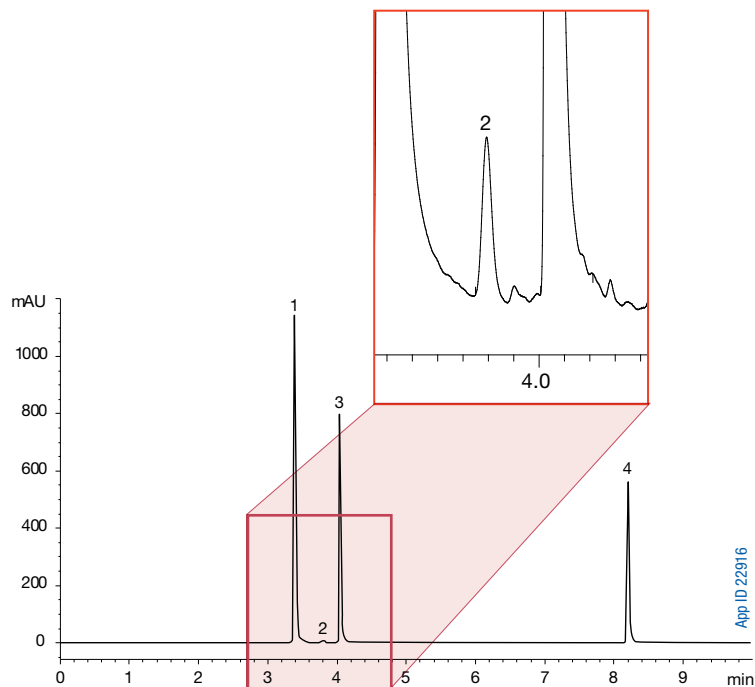
HPLC Conditions

Column: Kinetex 5 µm EVO C18
Dimensions: 150 x 4.6 mm
Part No.: 00F-4633-E0
Mobile Phase: A: 3 g of Sodium 1-Hexanesulfonate monohydrate with 0.4 mL Triethylamine with 15 mL of Acetic acid into 985 mL of Milli-Q[®] Water
 B: Acetonitrile

Gradient:	Time (min)	% B
	0	5
	10	98
	12	98
	12.1	5
	15	5

Flow Rate: 1 mL/min
Injection Volume: 2 µL
Temperature: Ambient
Detection: UV @ 278 nm

Sample: 1. Vitamin B6
 2. Vitamin B12
 3. Vitamin B1
 4. Diclofenac



APPLICATION

Kinetex Ordering Information

5 µm Minibore Columns (mm)

Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	SecurityGuard™ ULTRA Cartridges†
EVO C18	00A-4633-AN	00B-4633-AN	00D-4633-AN	00F-4633-AN	3/pk AJ0-9298 for 2.1 mm ID

5 µm MidBore™ Columns (mm)

Phases	50 x 3.0	100 x 3.0	150 x 3.0	SecurityGuard™ ULTRA Cartridges†
EVO C18	00B-4633-YO	00D-4633-YO	00F-4633-YO	3/pk AJ0-9297 for 3.0 mm ID

5 µm Analytical Columns (mm)

Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	SecurityGuard™ ULTRA Cartridges†
EVO C18	00B-4633-E0	00D-4633-E0	00F-4633-E0	00G-4633-E0	3/pk AJ0-9296 for 4.6 mm ID

5 µm Axia™ Packed Preparative Columns (mm)

Phases	50 x 21.2	100 x 21.2	150 x 21.2	250 x 21.2	SecurityGuard™ PREP Cartridges*
EVO C18	00B-4633-P0-AX	00D-4633-P0-AX	00F-4633-P0-AX	00G-4633-P0-AX	15 x 21.2 /ea AJ0-9304 for 21.2 mm ID

5 µm Axia Packed Preparative Columns (mm)

Phases	100 x 30	150 x 30	250 x 30	SecurityGuard™ PREP Cartridges**
EVO C18	00D-4633-U0-AX	00F-4633-U0-AX	00G-4633-U0-AX	15 x 30 /ea AJ0-9305 for 30 mm ID

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

* PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8223

** PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8277

Find more information on Kinetex core-shell HPLC and UHPLC columns at:

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Axia column and packing technology is patented by Phenomenex. U.S. Patent No. 7,674,383.

Kinetex EVO is patented by Phenomenex. U.S. Patent Nos. 7,563,367 and 8,658,038 and foreign counterparts.

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