



Simultaneous Quantitation of Vitamins B9 and B12 in Milk Powder Extracted with Strata™-X PRO and Using a Kinetex™ 2.6 µm F5 Column by LC-MS/MS

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Overview

Vitamin B9 (Folic Acid) is crucial for proper brain function and plays an important role in mental and emotional health. Folic acid also works closely with vitamin B12 to help make red blood cells and help iron function properly in the body. Two of the most important vitamins in supplemental milk powder given to aid the health of children are vitamins B9 and B12. The determination of vitamins B9 and B12 is a challenge due to their stability under light and matrix complexity. The phospholipid from the milk powder must be removed to reduce any ion suppression that could occur during LC-MS/MS analysis.

A quantitative method for the analysis of Vitamin B9 and B12 in milk powder samples was developed using a Kinetex 2.6 µm F5 column and Strata-X PRO Solid Phase Extraction (SPE). In this application note we illustrate the use of the versatile Kinetex F5 column for the separation of Vitamin B9 and B12 vitamins. The core-shell Kinetex 2.6 µm F5 particle provided high HPLC efficiency at very low HPLC pressures, while delivering effective baseline separation of the two vitamins.

Strata-X PRO SPE was used to remove phospholipids prior to MS analysis. The successful removal of phospholipid interferences from the milk powder resulted in high % recoveries. Strata-X PRO SPE eliminates conditioning and equilibration steps compared to traditional protein precipitation methods and other types of SPE, thereby saving analysis time and resources.

Sample Preparation

Step	Description
Pretreatment:	To 1 g of milk powder (sourced from local market), add 10 mL of 5 % Phosphoric Acid in Water and vortex at 1000 rpm for 10 min. Centrifuge the sample for 10 min at 10,000 rpm and collect the supernatant.
Load:	5 mL of the pre-treated sample through a Strata-X PRO, 500 mg / 6 mL SPE cartridge (8B-S536-HCH).
Dry:	Extract to dryness under a gentle stream of Nitrogen at room temperature.
Wash:	With 5 mL of Water / Methanol (95:5, v/v).
Elute:	With 5 mL of Methanol.
Reconstitute:	Evaporate and reconstitute with 1 mL Water.

LC Conditions

Column: Kinetex 2.6 µm F5

Dimensions: 100 x 3.0 mm

Part No.: [00D-4723-Y0](#)

Mobile Phase: A: 5 mM Ammonium Formate + 0.1 % Formic Acid in Water

B: 5 mM Ammonium Formate + 0.1 % Formic Acid in Methanol

Gradient:	Time (min)	%B
	0	5
	1	5
	7	95
	9	95
	9.1	5
	11	5

Flow Rate: 0.5 mL/min

Injection Volume: 20 µL

Temperature: 40 °C

LC System: SCIEX® ExionLC™

Detection: MS/MS

Detector: SCIEX QTRAP® 4500

MS/MS Conditions

Source Temperature: 550 °C

GS1: 55 psi

GS2: 55 psi

CUR: 30 psi

CAD: 8 psi

IS: 5500 V

Table 1. MRM Transitions

Analyte Name	Q1 (m/z)	Q3 (m/z)
Vitamin B9 – 1	442.1	295.0
Vitamin B9 – 2	442.1	176.1
Vitamin B12 – 1	678.5	147.1
Vitamin B12 – 2	678.5	359.1



Figure 1. Representative Chromatogram of 1 ng/mL Standard on a Kinetex™ 2.6 μm F5 Column.

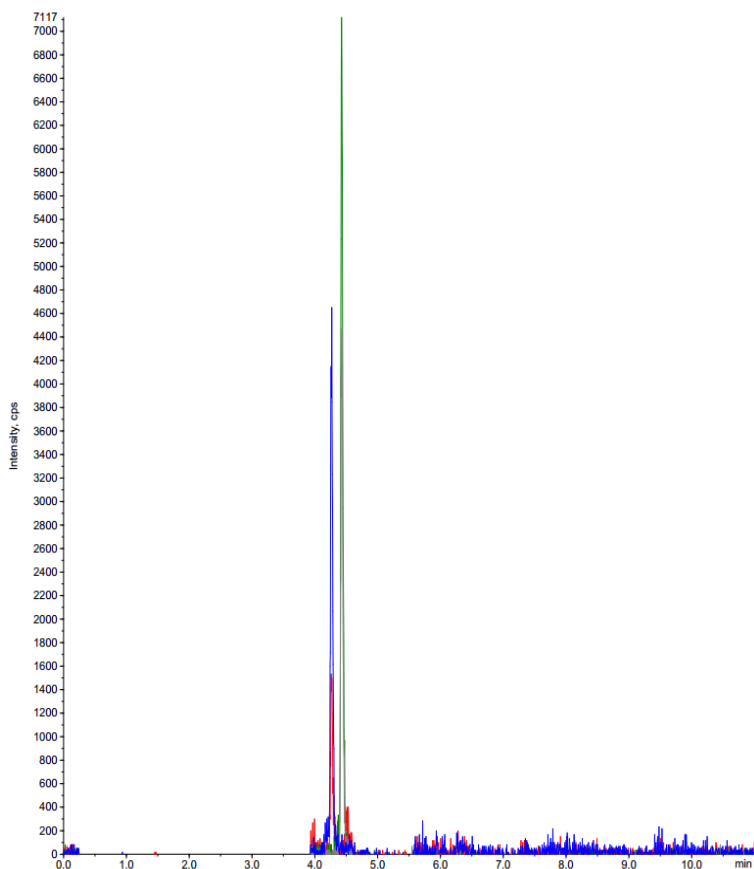


Figure 2. Linearity Curve of Vitamin B9.

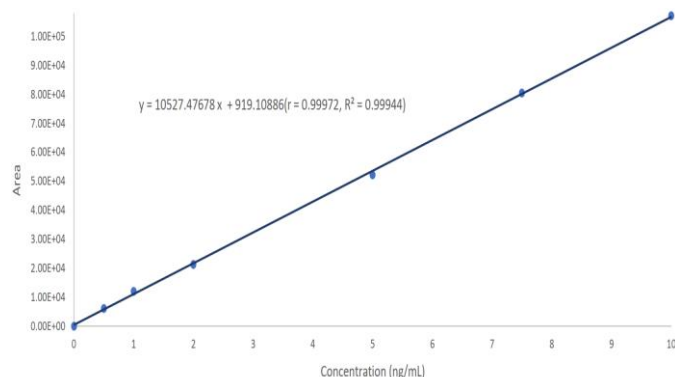


Figure 3. Linearity Curve of Vitamin B12.

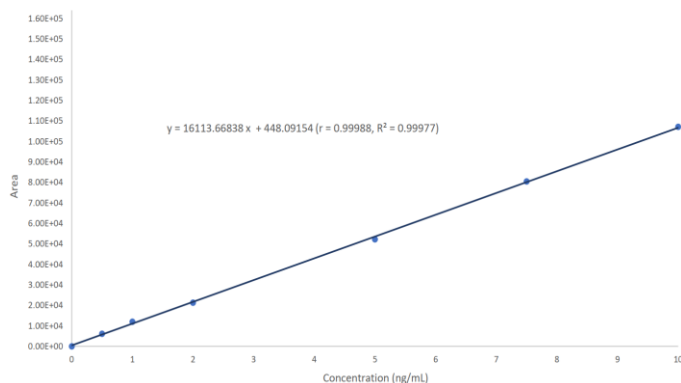


Table 2. % Recovery of Vitamins B9 and B12 in Milk Powder Extracted with Strata™-X PRO.

Nominal Concentration (ng/mL)	% Recovery	
	Vitamin B9	Vitamin B12
0.50	98.76	101.42
1.00	105.38	101.23
2.00	96.77	96.60
5.00	97.47	100.95
7.50	100.72	99.18
10.0	100.90	100.63



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