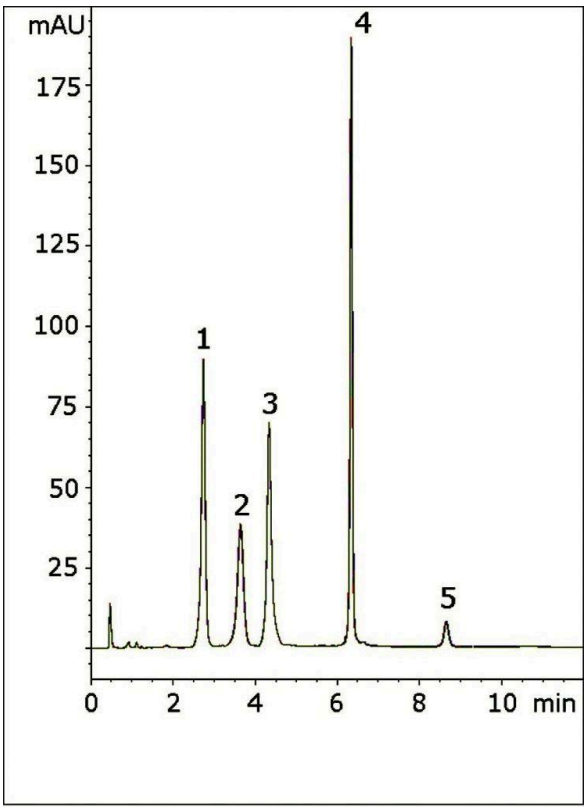
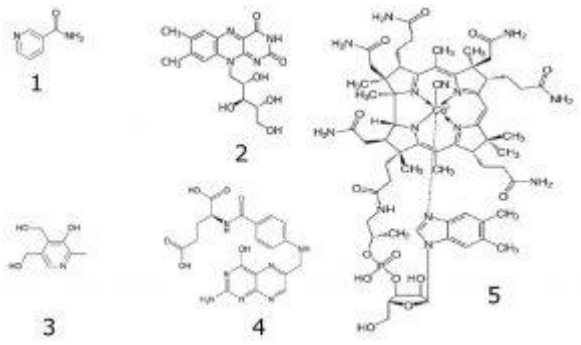
Niacinamide, Riboflavin, Pyridoxine, Folic Acid and Cyanocobalamin Vitamins Analysis with HPLC - AppNote

Separation of Five Highly Polar Vitamins

The B Vitamins are all highly polar and often ion pair agents are needed in Reverse Phase in order to retain them. However, these agents are incompatible with LCMS and therefore limit the applications of these methods. In this method using the Diamond Hydride Column, good separation is obtained with MS-compatible mobile phase solvents.





Peaks:

1. Niacinamide
2. Riboflavin

3. Pyridoxine

4. Folic Acid

5. Cyanocobalamin

Method Conditions

Column: Cogent Diamond Hydride™, 4µm, 100Å
Catalog No.: 70000-7.5P
Dimensions: 4.6 x 75mm

Mobile Phase:

A: DI Water / 10 mM Ammonium Formate

B: 95% Acetonitrile / 5% Solvent A

Gradient:

Time *(minutes)*	%B
0	100
2	100
9	50
10	100

Post Time: 2 minutes
Flow rate: 1.0 mL/minute
Detection: UV @ 266 nm
Injection vol.: 2µL

Sample Preparation: Mix of 0.1 mg/L Niacinamide, 0.01 mg /mL Riboflavin, 0.3 mg / mL Pyridoxine, 0.05 mg / mL Folic Acid, 1.0 mg / mL Cyanocobalamin in 50% Solvent A/ 50% Solvent B diluent. Peak identities were confirmed by individual standards.
t_0: 0.9 minutes

Note: *The word "Vitamin" was originally spelled "vitamine" when it was first coined by biochemist Casimir Funk. It was derived from the words "vital" and "amine" because it was believed at the time that all Vitamins were chemical Amines. The "e" was dropped from the word when it was discovered that this is not the case.*

COGENT HPLC Columns™

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