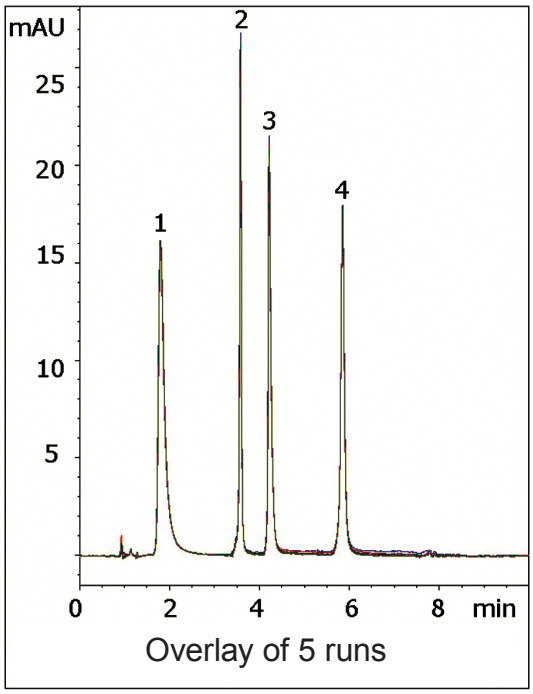
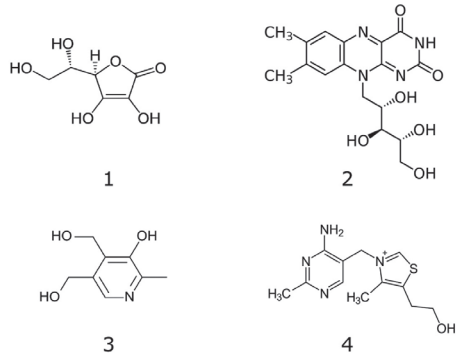
Hydrophilic Vitamin Analysis

Separation of ascorbic acid, riboflavin, pyridoxine, and thiamine





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.

Method Conditions

Column: **Cogent Diamond Hydride™**, 4µm, 100Å

Catalog No.: 70000-7.5P

Dimensions: 4.6 x 75 mm

Mobile Phase: A: DI H_2O / 10 mM ammonium formate / 0.05% formic acid (pH 3.5)
B: 95% acetonitrile / 5% 10 mM ammonium formate (pH 6.5)

Gradient:

time (min.)	%B
0	100
1.5	100
4	30
6	30
7	100

Post Time: 3 min

Injection vol.: 1µL

Flow rate: 1.0 mL/min

Detection: UV 266 nm

Samples: Mix of 300 mg/L ascorbic acid, 5 mg/L riboflavin, 100 mg/L pyridoxine, 20 mg/L thiamine in 50% 10 mM ammonium formate / 50% Acetonitrile diluent. Solution was filtered through 0.45µm nylon syringe filter (MicroSolv Tech Corp.). Peak identities were confirmed by individual standards.

Peaks: 1. Ascorbic Acid
2. Riboflavin
3. Pyridoxine
4. Thiamine

t_0: 0.9 min

Discussion

This LC-MS compatible method shows excellent separation and retention for all four analytes. If the analysis were done by reverse phase, LC-MS incompatible ion pair agents would likely be required to get this type of separation.

Ascorbic acid was found to have better retention near neutral pH but thiamine was retained too strongly under these conditions. Therefore a pH gradient was used in which the acidity of the mobile phase increases as well as the water content. The method is reliable and robust with respect to analyte retention and peak shape, as the overlay of five consecutive runs in the Figure demonstrates.

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