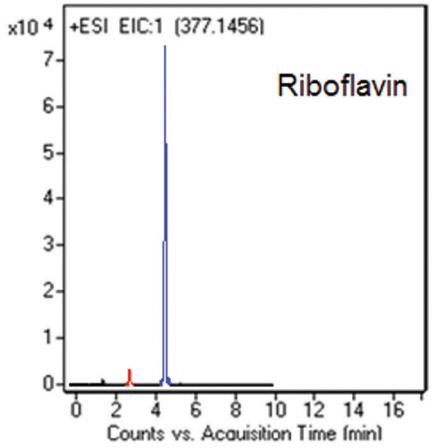
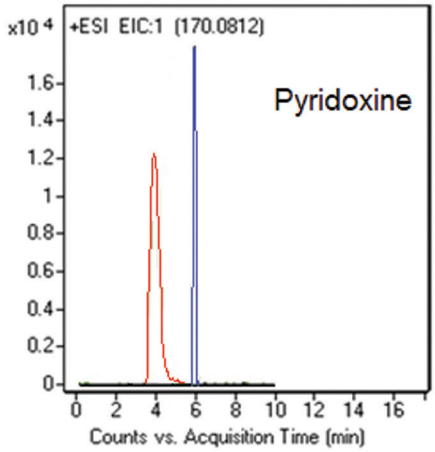
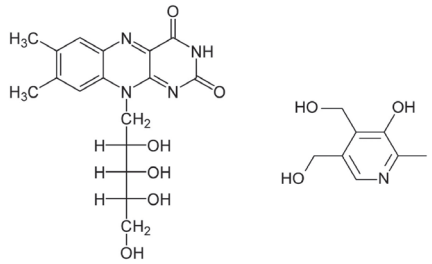
Vitamin Method Transfer to LC-MS

Different studied mobile phases for riboflavin and pyridoxine







Note: Riboflavin and pyridoxine are two types of B vitamins. These compounds are hydrophilic and hence may be difficult to retain by conventional reversed phase methods.

Method Conditions

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

Catalog No.: 70000-15P-2

Dimensions: 2.1 x 150 mm

Solvents: (Red Peaks)
A: DI H_2O / 10 mM ammonium formate / 0.05% formic acid (pH 3.5)
B: 95% ACN / 5% 10 mM ammonium formate (w/v) (pH 6.5)
(Blue Peaks)
A: 50% DI H_2O / 50% 2-propanol / 0.1% formic acid
B: ACN / 0.1% formic acid

Gradient:

time (min.)	%B
0	95
1.5	95
4	30
5	30
6	30
7	95

Post Time: 3 min

Injection vol.: 1µL

Flow rate: 0.4 mL/min

Detection: ESI – POS - Agilent 6210 MSD TOF mass spectrometer

Samples: **Stock Solution:** Riboflavin 5mg/L and pyridoxine 100mg/L in 50%A/50%B. The solution was filtered through a disposable 0.45µm filter (MicroSolv Tech Corp.). Sample for injection was diluted 1:100 with 50:50 solvent A:B mixture.

Peaks: Riboflavin 377.1456 m/z $[M+H]^+$
Pyridoxine 170.0812 m/z $[M+H]^+$

t_0: 0.9 min

Discussion

Hydrophilic vitamin analysis was developed using UV detection. The mobile phase was compatible with MS detection and the obtained separation was excellent. However, when the same mobile phase was used and the method transfer to MS detection was attempted the obtained peak intensities were very low and peak shape for pyridoxine was not acceptable (see red peaks in Figures). Since positive mode of ionization was used, the mobile phase was changed to formic acid-based solvents. The obtained peaks (see blue peaks in Figures), were much more symmetrical, well separated from each other and peak intensities were much higher.

MANUFACTURED BY:
MICROSOLV TECHNOLOGY CORPORATION
9158 Industrial Blvd NE
Leland, NC 28451
p: 1.732.380.8900
f: 1.910.769.9435
customers@mtc-usa.com
www.Cogent-HPLC.com