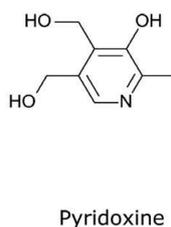
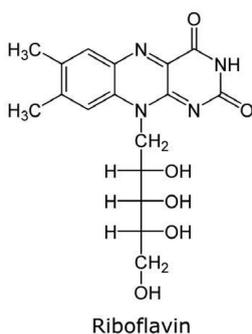
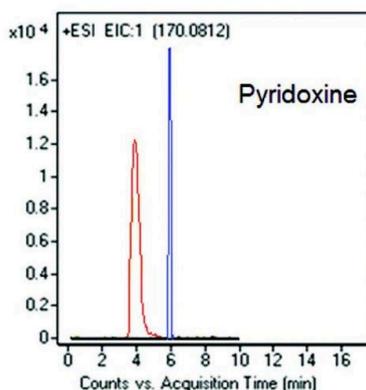
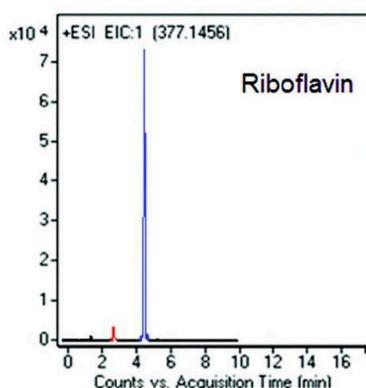


## Riboflavin Pyridoxine Hydrophilic Vitamins Analyzed with LC-MS - AppNote

### Different Mobile Phase for LCMS from a UV Method was Needed

Hydrophilic Vitamin analyses were developed using UV detection. However, when the same Method and Mobile Phase was tried with MS Detection, the Peak intensities were very low and Peak Shape for Pyridoxine was not acceptable (see *Red Peaks in Figures below*).

The Mobile Phase was changed and did not include Ammonium Formate since Positive Mode of Ionization was used with the MS. The Peaks (see *Blue Peaks in Figures*), were much more Symmetrical, well Separated from each other and Peak intensities were much higher. pH should always be considered when transferring methods from UV to LCMS.



## Peaks:

Riboflavin 377.1456 m/z [M+H]<sup>+</sup>

Pyridoxine 170.0812 m/z [M+H]<sup>+</sup>

## Method Conditions

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

**Catalog No.:** [70000-15P-2](#)

**Dimensions:** 2.1 x 150 mm

**Mobile Phases:**

### *For Red Peaks*

- A: DI Water with 10 mM Ammonium Formate / 0.05% Formic Acid (pH 3.5)
- B: 95:5 Acetonitrile / 10 mM Ammonium Formate (w/v pH 6.5)

### *For Blue Peaks*

- A: 50:50 DI Water / 2-Propanol with 0.1% Formic Acid
- B: Acetonitrile with 0.1% Formic Acid

**Gradient:**

| Time (minutes) | %B |
|----------------|----|
| 0              | 95 |
| 1.5            | 95 |
| 4              | 30 |
| 5              | 30 |
| 6              | 30 |
| 7              | 95 |

**Post Time:** 3 minutes

**Injection vol.:** 1 µL

**Flow rate:** 0.4 mL / minute

**Detection:** ESI – POS - Agilent 6210 MSD TOF Mass Spectrometer

**Sample Preparation:**

- Stock Solution: Riboflavin 5mg / L and Pyridoxine 100mg / L in 50:50 Solvent A / Solvent B. The solution was filtered through a 0.45µm Syringe Filter (MICROSOLV Tech Corp.).
- Samples for Injection were diluted 1:100 with 50:50 Solvent A:B mixture.

**t<sub>o</sub> :** 0.9 minutes

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**Note:** Riboflavin and Pyridoxine are two types of B Vitamins and are shown in two different Chromatograms above. These compounds are hydrophilic and hence may be difficult to retain by conventional Reversed Phase Methods.

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**Attachment No 244 Hydrophilic Vitamins Analyzed with LCMS pdf** 0.4 Mb [Download File](#)

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