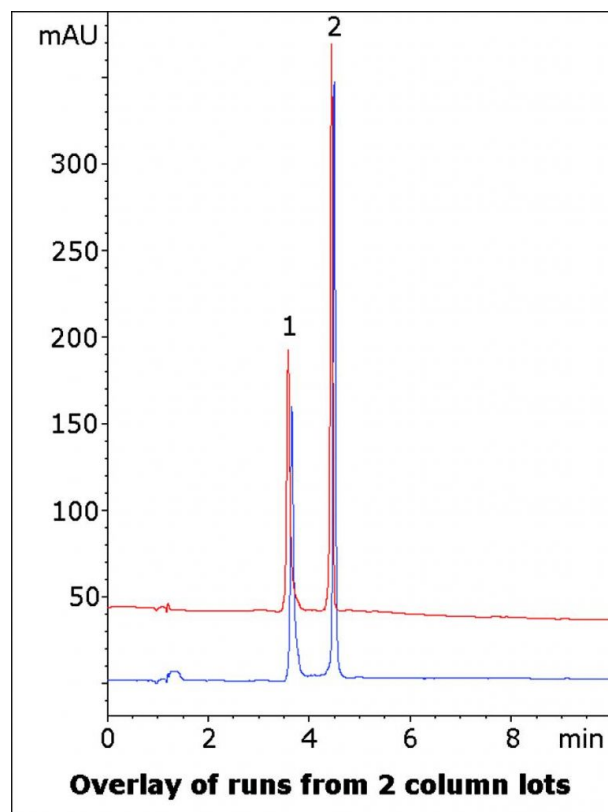


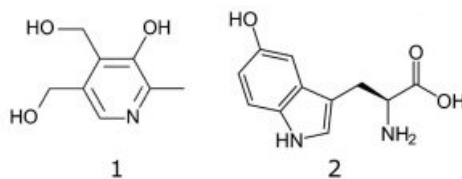
Hydroxytryptophan (5-HTP) Analyzed by HPLC- AppNote

Separation from Vitamin B6

Pyridoxine (Vitamin B6) is often added to formulations of 5-Hydroxytryptophan (5-HTP), as it assists in the conversion of 5- HTP to Serotonin. Therefore, it is of interest to separate these two compounds for an HPLC assay Method of this combination formulation.

The Method shown provides good resolution between the two peaks and both are well retained to allow for separation from matrix components that may be present. Runs from two Column lots are shown in the figure to illustrate lot-to-lot consistency. The Mobile Phase conditions are LCMS compatible as well, which expands the versatility of the Method.





Peaks:

1. Pyridoxine
2. 5-Hydroxytryptophan

Method Conditions

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

Catalog No.: 70000-7.5P

Dimensions: 4.6 x 75mm

Mobile Phase:

A: DI Water / 10mM Ammonium Acetate

B: 95% Acetonitrile / 5% Solvent A (v/v)

Time (minutes)	%B
0	100
1	100
6	50
7	100

Post Time: 3 minutes

Injection vol.: 2µL

Flow rate: 1.0mL / minute

Detection: UV @ 225nm

Sample Preparation: 0.1mg / mL 5-Hydroxytryptophan and 0.3mg / mL Pyridoxine USP reference standards in a diluent of 50:50 Solvent A / Solvent B. Peak identities were confirmed with individual standards.

t₀: 0.9 minutes

Note: 5-Hydroxytryptophan is a metabolic precursor to Serotonin. Since its use increases Serotonin production, it has a variety of applications involving inadequate Serotonin, including as an antidepressant, appetite suppressant,

and sleep aid. It is available as an Over-The-Counter dietary supplement.



Attachment

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