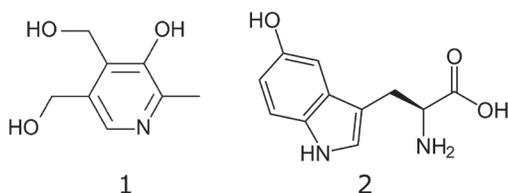
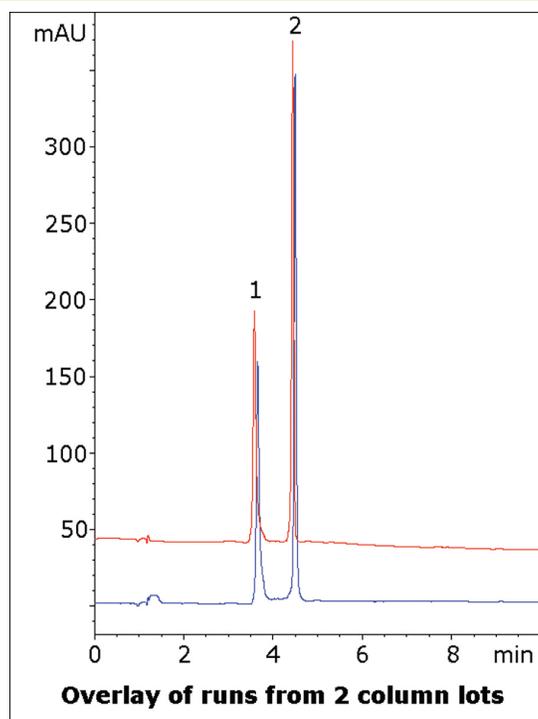


# Hydroxytryptophan (5-HTP)

## Separation from vitamin B6



**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 over-the-counter as a dietary supplement.

### Method Conditions

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

**Catalog No.:** 70000-7.5P

**Dimensions:** 4.6 x 75 mm

**Solvents:** A: DI H<sub>2</sub>O / 10 mM ammonium acetate  
B: 95% acetonitrile / 5% solvent A (v/v)

Gradient:	time (min.)	%B
	0	100
	1	100
	6	50
	7	100

**Post Time:** 3 min

**Injection vol.:** 2µL

**Flow rate:** 1.0 mL/min

**Detection:** UV 225 nm

**Sample:** 0.1 mg/mL 5-hydroxytryptophan and 0.3 mg/mL pyridoxine USP reference standards in a diluent of 50/50 solvent A / solvent B. Peak identities were confirmed with individual standards.

**Peaks:** 1. Pyridoxine  
2. 5-Hydroxytryptophan

**t<sub>0</sub>:** 0.9 min

### Discussion

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 here provides excellent 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 LC-MS compatible as well, which expands the versatility of the method.