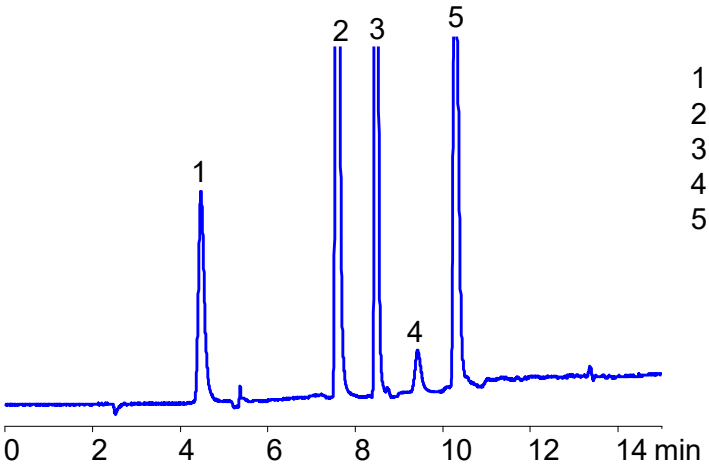
HPLC UV Analysis of Animal Supplements Composition on Amaze SC Column



1. Pantothenic acid
2. Nicotinic acid
3. Cyanocobalamin
4. Carnitine
5. Benzoic acid

Column:	Amaze SC
Dimensions:	4.6x150 mm, 3 um, 100A
Mobile phase:	ACN gradient from 0 to 60% in 12 minutes, 20 mM phosphate buffer pH 3.5
Flow rate:	1 ml/min
Detection:	210 nm

Application Notes

A mixed-mode HPLC method was developed for the simultaneous determination of five biologically active components commonly found in animal nutritional supplements - pantothenic acid, nicotinic acid, cyanocobalamin (vitamin B_{12}), carnitine, and benzoic acid - using the **Amaze SC mixed-mode** column. These compounds include vitamins, metabolic cofactors, and acids, representing a wide range of polarity, charge, and functional diversity that makes their separation challenging on conventional reversed-phase columns.

The **Amaze SC** stationary phase combines strong acidic ion-exchange sites with hydrophobic ligands, enabling a dual-mode retention mechanism based on reversed-phase partitioning and cation-exchange interactions. Under mildly acidic to neutral mobile-phase conditions, basic and zwitterionic analytes such as carnitine and cyanocobalamin are retained primarily through electrostatic attraction, while neutral and weakly acidic molecules - pantothenic, nicotinic, and benzoic acid - are governed by hydrophobic interactions.

Gradient of acetonitrile with isocratic ammonium phosphate buffer concentration was used to achieve baseline separation of these five compounds. UV detection provided reliable quantitation, taking advantage of the strong absorbance of aromatic analytes and the moderate UV response of the remaining compounds.

The retention order reflecting molecular charge, polarity, and aromaticity. The **Amaze SC mixed-mode column**, featuring strong acidic cation-exchange sites, delivers predictable and tunable selectivity for structurally diverse bioactive ingredients in complex nutritional formulations.