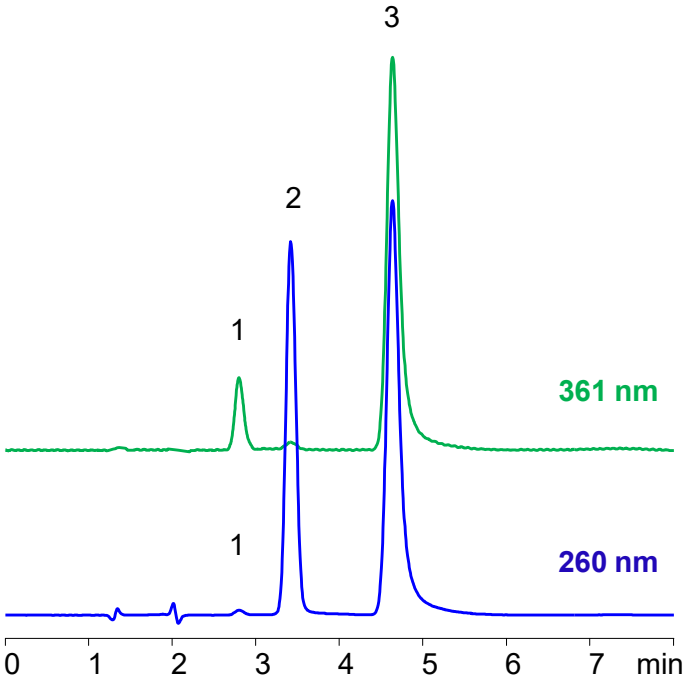
UV HPLC Analysis of Iron in Nutraceutical Formulation Containing Vitamin C and Vitamin B12 on Amaze HA Mixed-Mode Column



1. Cyanocobalamin
2. Ascorbic Acid
3. Iron ion

Column:	Amaze HA
Dimensions:	2.1x150 mm, 3 um, 100A
Mobile phase:	ACN/Water/EDTA/Ammonium formate
Flow rate:	0.2 ml/min
Detection:	260, 360 and 550 nm
Injection:	1 uL

Application Notes

This method describes the UV HPLC analysis of iron in a complex nutraceutical formulation containing Vitamin C (ascorbic acid) and Vitamin B12 (cyanocobalamin) using the **Amaze HA mixed-mode column**. The **Amaze HA** stationary phase combines reversed-phase and anion-exchange retention mechanisms, providing high selectivity for both ionic and hydrophobic components present in vitamin–mineral formulations. The stationary phase features a hydrophobic alkyl chain and a strong anion-exchange group, enabling simultaneous retention of inorganic, polar, and hydrophobic compounds in one analytical run.

Iron ions are inherently non-UV active; therefore, EDTA (ethylenediaminetetraacetic acid) is added to the mobile phase to form UV-absorbing iron-EDTA complexes. This approach allows indirect detection and quantification of iron species under standard UV conditions. The resulting complexes are well retained through anion-exchange interactions with the stationary phase due to their negatively charged carboxylate groups, while vitamins and other neutral species are separated primarily through reversed-phase and hydrophilic mechanisms.

Vitamin C, a strong antioxidant, interacts weakly with the anion-exchange sites and elutes early due to its high polarity. Vitamin B12, a large, moderately hydrophobic cobalamin derivative, exhibits substantial reversed-phase retention and distinct UV absorbance, enabling clear separation from iron-EDTA peaks.

The **Amaze HA mixed-mode column** provides superior resolution and peak shape for this multi-component system. The method enables precise quantification of iron in fortified formulations while simultaneously monitoring vitamin integrity, making it ideal for nutraceutical quality control and stability studies.