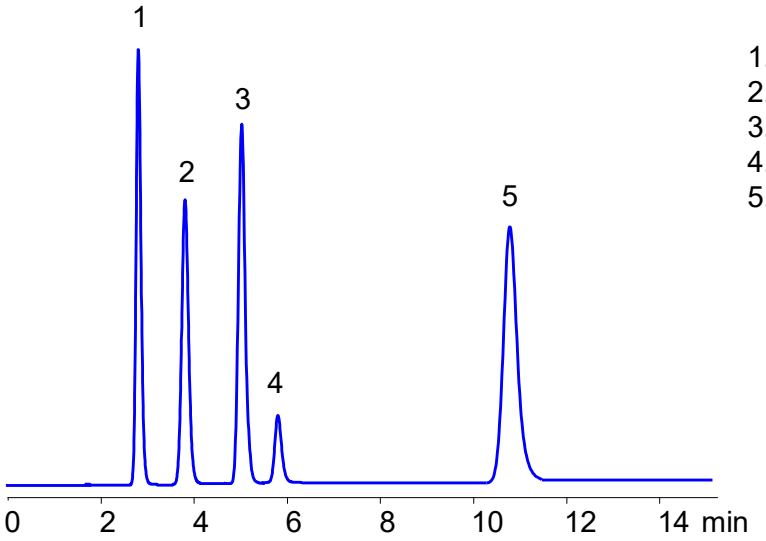
HPLC Analysis of Imidazole, Glutamic Acid, Alanine and Arginine on Amaze HD Mixed-Mode Column



1. Chloride ion
2. Imidazole
3. Glutamic acid
4. Alanine
5. Arginine

Column: Amaze HD
Dimensions: 3.0x100 mm,, 3 um, 100A
Mobile phase: ACN/Water/Ammonium formate pH 3 gradient
Flow rate: 0.6 ml/min
Detection: ELSD/CAD/MS

Application Notes

This HPLC method demonstrates the separation of biological metabolites and amino acids - Imidazole, Glutamic acid, Alanine, and Arginine - using the **Amaze HD mixed-mode column**. The **Amaze HD** stationary phase is a tri-modal HILIC mixed-mode phase combining hydrophilic interaction (HILIC), cation-exchange, and anion-exclusion properties. It features a short hydrophilic chain with an acidic ionizable group (pKa ≈ 3), providing strong and tunable retention for polar and ionic analytes. Retention is primarily governed by HILIC and cation-exchange mechanisms - HILIC increases with higher acetonitrile content, while cation-exchange is controlled by pH and buffer concentration.

Imidazole, a heterocyclic amine and key biological metabolite related to histidine, is retained through both HILIC and cation-exchange interactions. Glutamic acid, an acidic amino acid and neurotransmitter, exhibits strong HILIC and anion-exclusion retention due to its polarity and dual carboxyl groups. Alanine, a small neutral amino acid involved in metabolic energy cycles, interacts mainly through HILIC partitioning and elutes early in the chromatogram. Arginine, a strongly basic amino acid essential for protein synthesis and nitric oxide production, binds firmly to the negatively charged sites via cation-exchange, producing the longest retention.

The combined HILIC - cation-exchange selectivity of the Amaze HD column allows efficient separation of polar amino acids and small biological metabolites differing in polarity and charge. Excellent peak shape and reproducibility make this method ideal for profiling amino acids and related compounds in biochemical, nutritional, and pharmaceutical analyses.