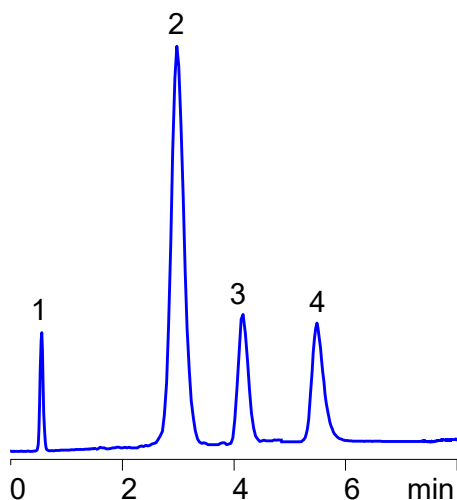


HPLC Separation of Alendronic, Phosphonic and Phosphoric Acids on Amaze HA Mixed-Mode Column



1. Sodium ion
2. Alendronic acid
3. Phosphonic acid
4. Phosphoric acid

Column: Amaze HA
Dimensions: 4.6x100 mm, 3 μ m, 100A
Mobile phase: ACN/Water/AmFm pH 2.5 gradient
Flow rate: 1 ml/min
Detection: ELSD/CAD/MS

Application Notes

The separation of structurally related phosphorus-containing acids - alendronic, phosphonic, and phosphoric acids - was achieved using the **Amaze HA mixed-mode column**, which combines anion-exchange, hydrophilic interaction (HILIC), and weak reversed-phase mechanisms. These highly polar, multi-acidic compounds are difficult to retain on conventional reversed-phase columns due to strong hydration and lack of hydrophobicity.

The **Amaze HA** stationary phase carries protonated amino ligands and polar hydrophilic groups, enabling electrostatic attraction, hydrogen bonding, dipole interactions, and mild hydrophobic effects. Under mildly acidic to neutral conditions, anion-exchange is the dominant retention mechanism, while HILIC and weak reversed-phase effects contribute to selectivity and peak shape. The degree of ionization and the ionic strength of the mobile phase strongly influence retention.

Separations were performed using ammonium formate or ammonium acetate buffer with a gradient of acetonitrile, providing control of ionic and hydrophilic interactions. Baseline resolution of all three acids was achieved, with elution order determined by acidity and anionic charge: alendronic acid eluted first, followed by phosphonic and phosphoric acids. The zwitterionic character of alendronic acid reduces its net negative charge, resulting in weaker electrostatic interaction and shorter retention.

This study demonstrates the versatility of the **Amaze HA mixed-mode column** for separating highly polar phosphorus-containing compounds, offering tunable selectivity through pH, buffer, and organic-modifier control for both analytical and preparative applications.