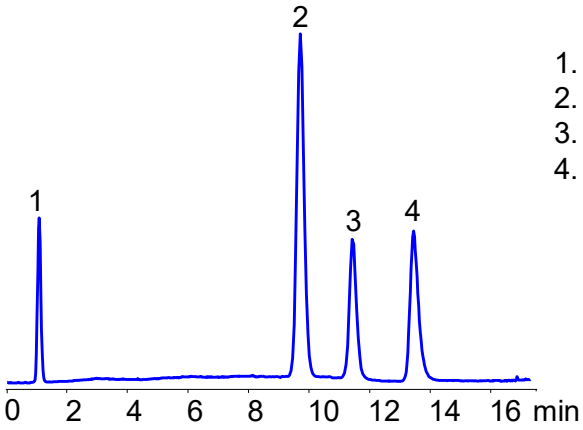
HPLC Analysis of Mannosamine, Galactosamine and Glucosamine on Amaze HD Mixed-Mode Column



1. Chloride ion
2. Mannosamine
3. Galactosamine
4. Glucosamine

Column:	Amaze HD
Dimensions:	3.0x150 mm,, 3 um, 100A
Mobile phase:	78% ACN with 50 mM AmFm pH 3
Flow rate:	0.6 ml/min
Detection:	ELSD/CAD/MS

Application Notes

We developed HPLC separation of mannosamine, galactosamine, and glucosamine using the **Amaze HD mixed-mode column**. The **Amaze HD** stationary phase is a tri-modal HILIC mixed-mode material combining hydrophilic interaction (HILIC), cation-exchange, and anion-exclusion mechanisms. The stationary phase features a short hydrophilic chain with an acidic ionizable group (pKa ≈ 3). This unique surface design provides strong and tunable retention for highly polar, weakly basic compounds such as amino sugars.

The method enables simultaneous control of retention and selectivity by adjusting both the organic content and ionic strength of the mobile phase. The HILIC mechanism dominates under high-acetonitrile conditions, where partitioning between the aqueous layer on the stationary phase and the mobile phase controls retention. The strength of HILIC interaction is controlled by amount of acetonitrile and buffer concentration. The cation-exchange mechanism, on the other hand, arises from electrostatic attraction between the protonated amino groups of the analytes and the negatively charged surface sites of the stationary phase. This interaction is strong but can be effectively modulated by pH and buffer concentration - lower pH increases provides a stronger retention, while higher ionic strength screens electrostatic forces and shortens retention times.

Mannosamine, galactosamine, and glucosamine are structural isomers differing only in the spatial orientation of hydroxyl groups, but their chromatographic behavior varies due to subtle differences in polarity and basicity. All are important biochemical building blocks involved in glycoprotein and polysaccharide biosynthesis. On the **Amaze HD** column, resolution of these isomers is achieved through a fine balance of HILIC and cation-exchange contributions, while anion-exclusion minimizes interference from acidic impurities and stabilizes the baseline.

The combination of strong, tunable HILIC and cation-exchange mechanisms makes the **Amaze HD** column particularly suitable for separating closely related polar amino sugars with excellent selectivity, reproducibility, and peak symmetry.