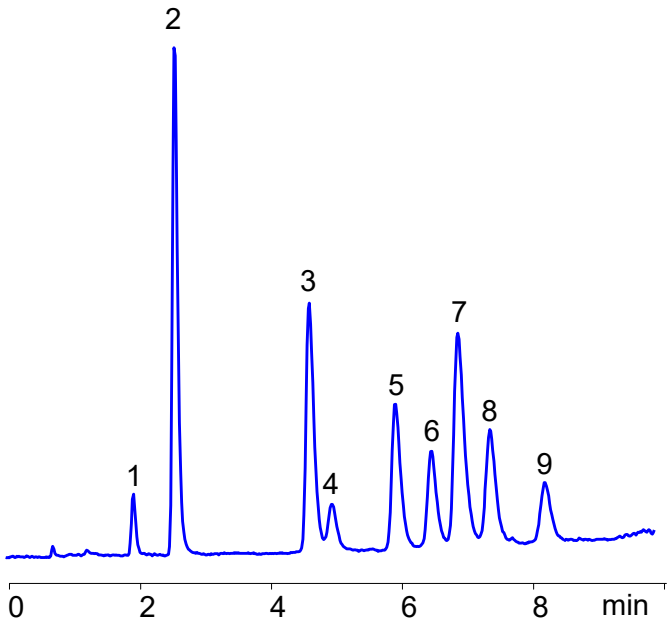
LC-MS Analysis of Isomers of Methylhistamine and Methylhistidine on Amaze MH Mixed-Mode Column



1. Bromide ion
2. Chloride ion
3. 1-Methylhistamine
4. α-Methylhistamine
5. Histamine
6. 1-Methylhistidine
7. 3-Methylhistamine
8. Histidine
9. 3-Methylhistidine

Column: Amaze MH
Dimensions: 3.0x100 mm, 3 um, 100A
Mobile Phase: ACN and AmFm gradient
Flow: 0.6 ml/min
Detection: MS/ELSD/CAD

Application Notes

A liquid chromatography-mass spectrometry (LC-MS) method was developed for the metabolomic profiling and separation of methylhistamine and methylhistidine isomers using the Amaze MH mixed-mode column. These compounds are biogenic amine metabolites of histamine and histidine, involved in neurological signaling, inflammation control, and nitrogen metabolism. Their close structural similarity and positional isomerism present significant chromatographic challenges.

The Amaze MH column is a tri-modal HILIC mixed-mode stationary phase exhibiting hydrophilic interaction (HILIC), cation-exchange, and anion-exchange properties. It contains an acidic group (pKa ≈ 4) and a basic group (pKa ≈ 11), allowing it to interact with both positively and negatively charged analytes. This design enables simultaneous retention of basic, zwitterionic, and amphoteric metabolites through a balance of electrostatic attraction, hydrogen bonding, and hydrophilic partitioning.

Separations were carried out using volatile ammonium formate buffers and acetonitrile gradients, optimized for MS detection and control of ionic strength. The method achieved baseline resolution of methylhistamine and methylhistidine isomers, with elution order governed by degree of methylation, polarity, and charge distribution.

This study demonstrates the utility of the Amaze MH mixed-mode column for metabolomic analysis of histamine-related pathways, providing high selectivity, reproducibility, and MS-compatible performance for the separation of closely related amine metabolites.