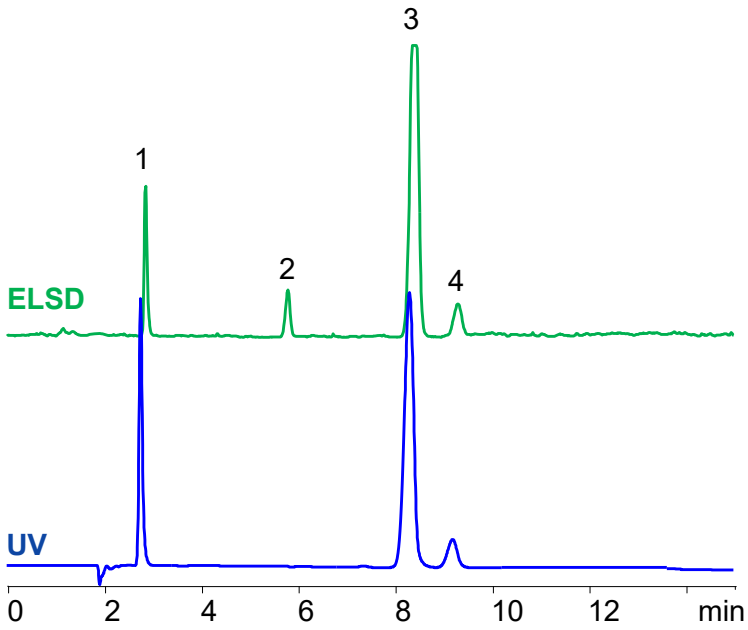
HPLC Separation of Polar Neuroactive Compounds Occurring in *Amanita muscaria* and Related Mushrooms on Amaze SC Mixed-Mode Column



1. Ibotenic acid
2. Muscarine
3. Muscimol
4. Thiomuscimol
5. p-TSA

Column:	Amaze SC
Dimensions:	4.6x150 mm, 3 um, 100A
Mobile phase:	10% ACN with 0.1% TFA
Flow rate:	1 ml/min
Detection:	222 nm, ELSD (45°C)

Application Notes

A group of neuroactive and toxic compounds characteristic of *Amanita muscaria*, *Amanita pantherina*, and related mushrooms - ibotenic acid, muscarine, muscimol, and thiomuscimol - were separated using the **Amaze SC mixed-mode column**. These compounds represent two structural classes: isoxazole amino acids (ibotenic acid and muscimol derivatives) and quaternary ammonium alkaloids (muscarine and thiomuscimol). Their high polarity and strong ionic character make them challenging to separate by conventional reversed-phase HPLC.

The **Amaze SC stationary phase** combines reversed-phase hydrophobic and strong cation-exchange functionalities within a single ligand, providing dual-mode selectivity. Retention and selectivity in this separation are governed by a synergistic combination of hydrophobic and electrostatic interactions. Muscarine, muscimol and thiomuscimol, being permanently charged cations at lower pH, are strongly retained through cation-exchange, while the zwitterionic and neutral forms of ibotenic acid exhibit balanced retention via both mechanisms.
An isocratic method with controlled ionic strength and pH provided baseline resolution of all four analytes within a short run time. The method demonstrates excellent reproducibility, stability, and compatibility with UV or ELSD detection, allowing for quantitative analysis of mushroom extracts and biological samples. Replacement of TFA with formic acid or ammonium formate will make the method compatible with mass spectrometry detection.

This example highlights the ability of the **Amaze SC mixed-mode column** to retain and resolve small polar neuroactive compounds that cannot be adequately separated on conventional reversed-phase or HILIC phases. The dual retention mechanism makes it a powerful tool for the analysis of complex biological or toxicological mixtures containing ionic amino acids and alkaloids, allowing a baseline separation of closely structurally related compounds and structural isomers.