In mammalian brain, high enzymatic levels are in telencephalic brain regions; very low levels in plasma/serum, notably altered by liver dysfunction. An actual role of guanine deaminase in specialized organ metabolism and synaptic physiology is uncertain, and relatively little is known about the enzyme characteristics outside of rabbit, with likely lower Km values in plasma/serum. 3

Efficient Separation of Highly Polar Purine Metabolites using Superficially Porous Particle HPLC Columns

Standard Microplate Enzyme Assay and LC/MS Conditions

Analysis of Enzyme Inhibitors

Conclusive remarks on potential inhibitors of guanine deaminase have been investigated using the LC/MS assay described, with bovine liver enzyme. As shown for the example of the known competitive inhibitor, 5-azacytosine monophosphate (AMP), a series C18 columns stable in organic solvents and multiple washes, efficient separation of the enzyme reaction product, xanthine and Uric acid, is obtained. Each assay was performed a minimum of three times with highly selective interference due to column conditioning performance.

Conclusions

A highly selective and sensitive LC/MS method is demonstrated for the analysis of the enzyme GDA. The HPLC, AQ C18, 2.7 µm column was chosen for its ability to separate the very polar guanine metabolites and potential inhibitors of interest. The column's robust stability in 100% methanol conditions allowed for full throughput and the assessment of hundreds of different measurements. Studying enzyme kinetics with highly selective LC/MS methods avoid many of the limitations of previous methods including lack of selectivity in complex samples. Selection of the enzyme reaction product through SIM in the ion trap allows specific detection of the product without interference due to similarly potential inhibitors.