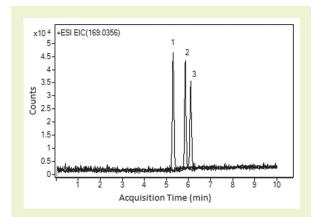
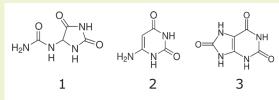


Uric Acid and Metabolites

LC-MS method for allantoin, 6-aminouracil, and uric acid (UA)





Note: Humans lack the enzyme (uricase) which converts UA to allantoin. However the compound still can be detected in human urine samples. UA can react with an oxidant in a step reaction and the end product is allantoin. When UA is degraded it can't be renewed. The presence of allantoin in urine is an indicator of a disease state (oxidative stress, renal failure, diabetes, etc.). The allantoin/UA ratio is also elevated in Down syndrome and chronic lung disease. 6-aminouracil is a direct product of reaction of UA with nitric oxide, which has a role in cardiovascular disease.

Method Conditions

Column: Cogent Diol™, 4µm, 100Å

Catalog No.: 40060-15P-3 **Dimensions:** 3.0 x 150 mm

Mobile Phase: A: DI $H_2O / 0.1\%$ formic acid (v/v)

95

B: Acetonitrile / 0.1% formic acid (v/v)

 Gradient:
 time (min.)
 %B

 0
 95

 6
 30

 7
 30

8

Injection vol.: 1 µL

Flow rate: 0.4mL/min

Detection: ESI - POS - Agilent 6210 MSD TOF mass spectrometer

Sample: Standards of uric acid and its main metabolites were prepared in DI H_2O at concentrations of 20 microg/mL each. The sample for injection (a mixture of the three compounds)

was diluted by a factor of 3.

Peaks: 1. Allantoin 159.0513 m/z [M+H]+

2. 6-aminouracil 128.0455 m/z [M+H]+

3. Uric acid 169.0356 m/z [M+H]+

to: 0.9 min

Discussion

The presented method for analysis of UA and its highly polar metabolites is simple and doesn't require mobile phase additives or pre-column derivatization. The peaks are symmetrical and the MS signal is not diminished, as is the case when ammonium formate or acetate is used as a mobile phase additive. The analyzed metabolites are signature end products for UA degradation in the presence of oxidants and can therefore be used as biomarkers for different disease states.

Cogent columns can have very fast equilibration between gradient runs and can be successfully used in studies of pathways in human pathology.