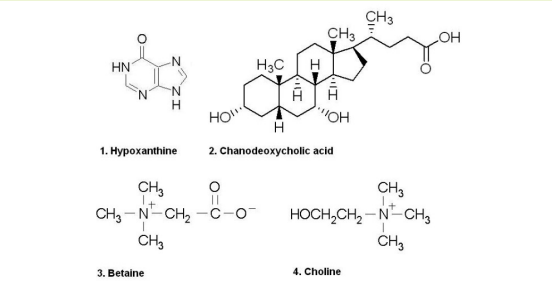
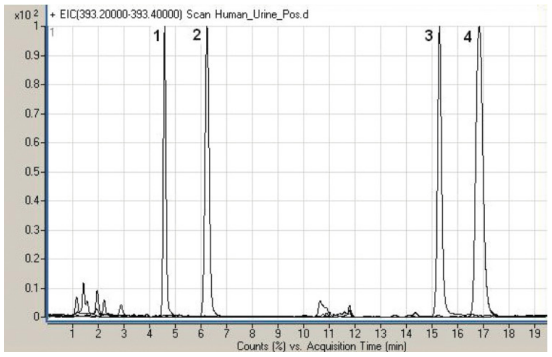
Metabolites in Human Urine

Simple LCMS friendly method reproducible using (ANP) Aqueous Normal Phase LCMS





Notes: For the screening of "enzyme defects" in humans, measuring the excretion of purines in urine is considered a good diagnostic tool. High or low excretion levels of hypoxanthine was seen in cases of different types of leukemia. Betaine is an important nutrient and due to its many metabolic roles there is an increasing demand for its measurement. Choline appears to be a good tracer for the detection of a prostate lesion, however, further well-organized studies are needed before it can be used clinically.
The use of Cogent Diamond Hydride columns in ANP combined with the powerful MS Agilent system prove useful for carrying out these studies as well as for the routine screening and monitoring of metabolites in patients.

Method Conditions

Column: **Cogent Diamond Hydride™**, 4µm, 100Å

Catalog No.: 70000-15P-2

Dimensions: 2.1 x 150 mm

Mobile Phase: A: DI H_2O/ 0.1% formic acid
B: Acetonitrile/ 0.1% formic acid

Gradient:

time (min.)	%B
0	95
0.2	95
30	50
35	50
35.1	95
40	95

Flow rate: 0.4 mL/min

Detection: ESI – pos - Agilent 6210 MSD TOF mass spectrometer

Sample: Human Urine – after simple extraction

Peaks: 1. Hypoxanthine 137.04580 m/z $(M+H)^+$, RT = 4.98 min
2. Chenodeoxycholic acid 393.29990 m/z $(M+H)^+$, RT = 6.23 min
3. Betaine 118.08680 m/z $(M+H)^+$, RT = 15.27 min
4. Choline 104.10754 m/z $(M+H)^+$, RT = 16.82 min
Figure: EIC – extracted ion chromatogram of selected compounds (1,2,3,4)

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Discussion

This method can be used for routine assays of urinary purines (hypoxanthine), bile acids (chenodeoxycholic acid) and nutrients (betaine, choline) in biological fluids. The method is very sensitive (due to the high content of organic component (acetonitrile) in the MP and the use of "MS friendly" (formic acid), reproducible (% RSD for gradient analysis is below 0.5%) and accurate (MW to 3-4 decimal points).

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