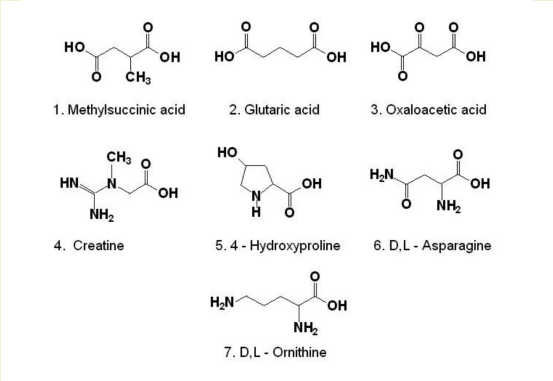
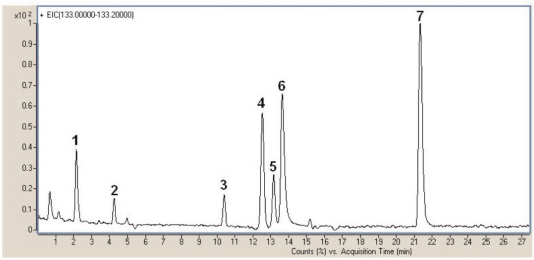
Determination of Important Isobaric Metabolites in Urine

A Simple & Reproducible MS Friendly Method





Notes: The determination of metabolites and amino acids in biological fluids is an important problem in clinical biochemistry and analytical chemistry. Changes in the concentrations of these compounds in urine, serum and other physiological fluids has proved to be correlated with several neurological disorders such as Alzheimer's disease, ischemic stroke as well as with a number of metabolic disorders such as phenylketonuria, argininemia, maple syrup urine disease and others.
Chromatogram presented is adapted from:
J.J.Pesek, M.T. Matyska, S. M. Fisher, T. R. Sana, Journal of Chromatography A, 1204 (2008) p55.

Method Conditions

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

Catalog No.: 70000-15P-2

Dimensions: 2.1 x 150 mm

Solvents: 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

Post Time: 5 min

Flow rate: 0.4 mL/min

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

Sample: Synthetic urine: 400µL of acetonitrile was added to 100 µL of synthetic urine and sample was centrifuged (3000 g). Next 20µL of the supernatant was mixed with 10µL of 50% acetonitrile/ 50% DI H_2O + 0.1% formic acid.

Peaks: 1. Methylsuccinic acid, 133 m/z $(M+H)^+$, RT = 2.14 min
2. Glutaric acid, 133 m/z $(M+H)^+$, RT = 4.25 min
3. Oxaloacetic acid, 133 m/z $(M+H)^+$, RT = 10.40 min
4. Creatine, 133 m/z $(M+H)^+$, RT = 12.52 min
5. 4-hydroxyproline, 133 m/z $(M+H)^+$, RT = 13.15 min
6. D,L - asparagine, 133 m/z $(M+H)^+$, RT = 13.62 min
7. D,L - ornithine, 133 m/z $(M+H)^+$, RT = 21.31 min

Discussion

This MS friendly method demonstrates the ability of Cogent Diamond Hydride column to separate a wide variety of polar metabolites using mass spectrometry for detection. When monitoring the EIC at 133m/z, seven peaks were positively detected in a urine sample by comparison with standards or by using accurate mass. The method described requires only a small sample volume and needs minimal manual sample preparation. Although primarily focused on the analysis of metabolites in urine, the method could also be applied to the determination of these compounds in other physiological or biological fluids.

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