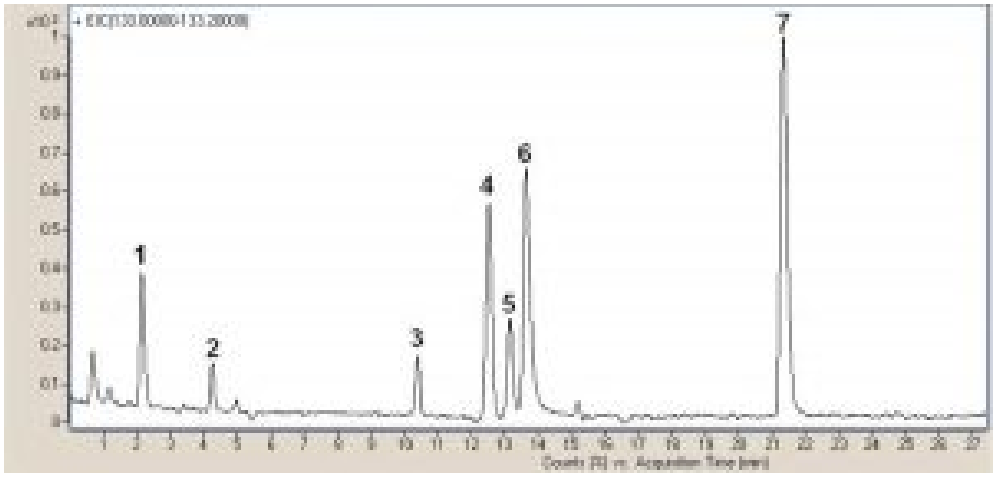
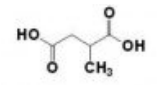
Isobaric Metabolites in Urine Analyzed with LCMS – AppNote

A Simple and Reproducible LCMS Method

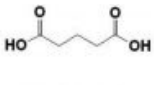
This Method demonstrates the ability to separate a wide variety of polar metabolites using Mass Spectrometry for detection. When monitoring the EIC at 133 m/z, seven Peaks were positively detected in a Urine sample by comparison with standards or by using accurate mass.

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.

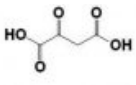




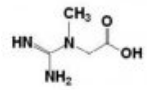
1. Methylsuccinic acid



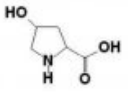
2. Glutaric acid



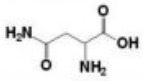
3. Oxaloacetic acid



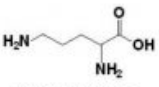
4. Creatine



5. 4 - Hydroxyproline



6. D,L - Asparagine



7. D,L - Ornithine

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Peak:

1. Methylsuccinic Acid, 133 m/z (M+H)+, RT = 2.14 minutes
2. Glutaric Acid, 133 m/z (M+H)+, RT = 4.25 minutes
3. Oxaloacetic Acid, 133 m/z (M+H)+, RT = 10.40 minutes
4. Creatine, 133 m/z (M+H)+, RT = 12.52 minutes
5. 4-Hydroxyproline, 133 m/z (M+H)+, RT = 13.15 minutes
6. D,L-Asparagine, 133 m/z (M+H)+, RT = 13.62 minutes
7. D,L-Ornithine, 133 m/z (M+H)+, RT = 21.31 minutes

Method Conditions:
Column: Cogent Diamond Hydride™, 4µm, 100Å
Catalog No.: 70000-15P-2
Dimensions: 2.1 x 150mm
Mobile Phase:
A: DI Water / 0.1% Formic Acid
B: Acetonitrile / 0.1% Formic Acid
Gradient:

Time *(minutes)*	%B
0	95
0.2	95
30	50
35	50
35.1	95

Post Time: 5 minutes
Flow Rate: 0.4mL / minute
Detection: ESI – POS – Agilent 6210 MSD TOF Mass Spectrometer
Sample Preparation: Synthetic Urine: 400µL of Acetonitrile was added to 100µL of Synthetic Urine and sample was centrifuged (3000g). Next 20µL of the supernatant was mixed with 10µL of 50:50 Acetonitrile / DI Water / 0.1% Formic Acid.

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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.

COGENT HPLC Columns™

Attachment

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