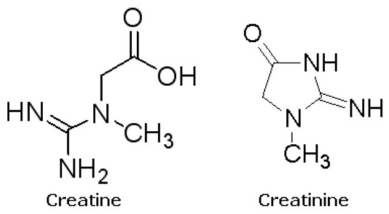
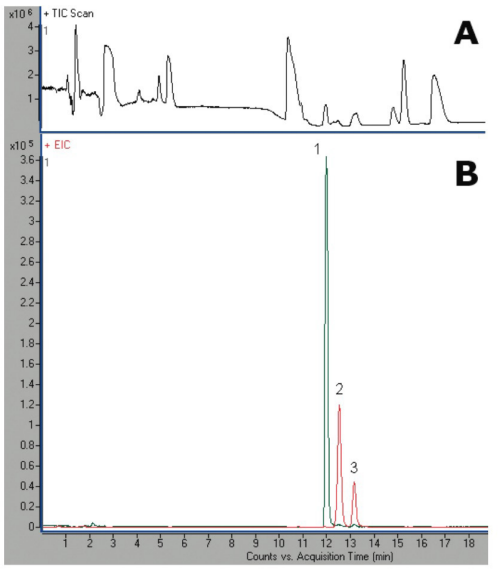
Metabolites in Urine

Isobaric compounds (creatine and 4-hydroxyproline) separated





A- TIC – Total ion chromatogram of synthetic urine sample
B- EIC – Extracted ion chromatogram of selected compounds (1,2,3)

Notes: Cr is a compound that is made primarily in the liver and then transported to muscles where it is used as an energy source. Once in the muscle, some of the Cr is spontaneously converted to Cm. It is important to monitor the Cr/Cm ratio. An increased ratio may be due to several diseases: congestive heart failure or dehydration. It may also be seen with gastrointestinal bleeding, or increased protein in the diet. The ratio may be decreased with liver disease (due to decrease in the formation of urea) and malnutrition. Cr and Cr analogs such as cyclocreatine were found to have antitumor, antiviral, and antidiabetic effects and to protect tissues from hypoxic, ischemic, neurodegenerative, or muscle damage.

Method Conditions

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

Catalog No.: 70000-7.5P

Dimensions: 4.6 x 75 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
40	95

Flow rate: 0.4 mL/min

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

Sample: Synthetic urine

Peaks: 1. Creatinine (Cr) 114.0662 $[M+H]^+$, RT = 11.98 min
2. Creatine (Cm) 132.0768 $[M+H]^+$, RT = 12.52 min
3. 4-Hydroxyproline 132.0655 $[M+H]^+$, RT = 13.16 min

Discussion

Creatine and creatinine are typically very difficult to separate from each other. A simple gradient with an MS friendly mobile phase was used to resolve these two compounds. In addition 4-hydroxyproline which has a very similar mass weight as creatine was also easily separated. A powerful combination of an Agilent MSD-TOF instrument (4 digit mass accuracy) and a Cogent Diamond Hydride LCMS column takes away guessing from the analysis of metabolites in a complex mixture as shown.

MANUFACTURED BY:
MICROSOLV TECHNOLOGY CORPORATION
9158 Industrial Blvd NE
Leland, NC 28451
p: 1.732.380.8900
f: 1.910.769.9435
customers@mtc-usa.com
www.Cogent-HPLC.com