

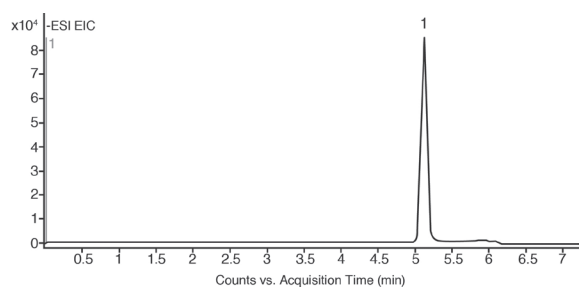
Applications of Cogent TYPE-C™ Columns

Clinical Applications continued

For many other Clinical applications go to www.mtc-usa.com and click on Knowledge Base.

3-Hydroxy-3-Methylglutaric Acid (HMG) in Urine by ANP LC-MS

Figure 61.

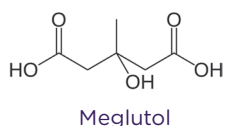


Method Conditions

Column: Cogent Diamond Hydride™, 4µm, 100Å
Catalog No.: 70000-15P-2
Dimensions: 2.1 x 150mm
Mobile Phase: A: DI H₂O / 10mm ammonium formate
 B: 95% acetonitrile/5% DI water/10mm ammonium formate (v/v)

Gradient:	time (min.)	%B
	0	95
	1	95
	5	30
	7	30
	8	95

Post Time: 3 min
Injection vol.: 1µL
Flow rate: 0.4mL/min
Detection: ESI - NEG - Agilent 6210 MSD TOF mass spectrometer
Peak: 1. 3-hydroxy-3-methylglutaric acid 161.0455m/z [M-H]⁻ in urine sample

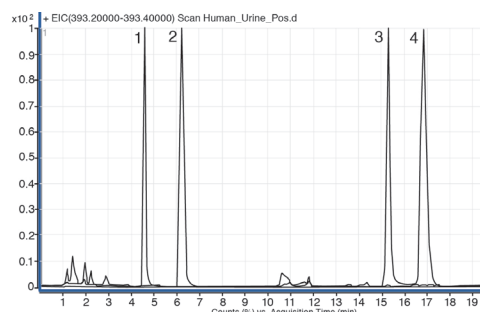


Discussion

A selective, specific, and sensitive method based on LC-MS analysis has been developed for the determination of 3-hydroxy-3-methylglutaric acid (a.k.a. meglutol) in urine samples. The method can be also used in the analysis of plasma samples after precipitation of plasma proteins with acetonitrile. The retention was achieved using a Cogent Diamond Hydride™ column. This method can be used for screening of large numbers of urine or plasma samples, due to simple sample preparation and rapid equilibration of the Cogent columns when gradient analysis is used.

Metabolites in Human Urine by ANP LC-MS

Figure 62.



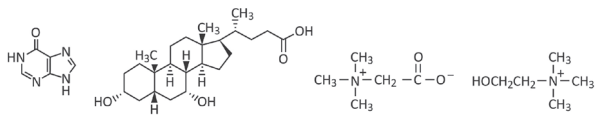
Method Conditions

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 (min.)	%B
	0.0	95
	0.2	95
	30.0	50
	35.0	50
	35.1	95
	40.0	95

Flow rate: 0.4mL/min
Detection: ESI - pos - Agilent 6210 MSD TOF mass spectrometer

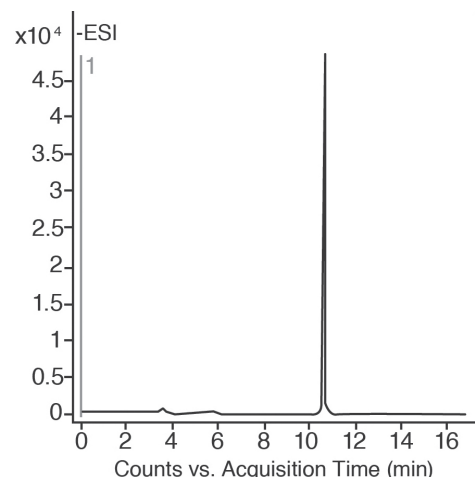
Sample: Human Urine - after simple extraction
Peaks: 1. Hypoxanthine; 137.04580m/z (M+H)⁺, RT = 4.98 min
 2. Chenodeoxycholic acid; 393.29990m/z (M+H)⁺, RT = 6.23 min
 3. Betaine; 118.08680m/z M⁺, RT = 15.27 min
 4. Choline; 104.10754m/z (M+H)⁺, RT = 16.82 min
Figure: EIC - extracted ion chromatogram of selected compounds (1,2,3,4)



1. Hypoxanthine 2. Chenodeoxycholic acid 3. Betaine 4. Choline

Galactose-1-Phosphate by ANP LC-MS

Figure 63.

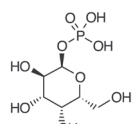


Method Conditions

Column: Cogent Diamond Hydride™, 4µm, 100Å
Catalog No.: 70000-15P-2
Dimensions: 2.1 x 150mm
Mobile Phase:
 A: DI H₂O / 0.1% formic acid (v/v)
 B: 90% acetonitrile/10% DI water/16.5mm ammonium acetate (v/v)

Gradient:	time (min.)	%B
	0	95
	1	95
	3	85
	6	85
	7	75
	9	75
	10	50
	12	50
	13	30
	15	30
	15.01	95

Post Time: 5 min
Injection vol.: 1µL
Flow rate: 0.4mL/min
Detection: ESI - NEG - Agilent 6210 MSD TOF mass spectrometer
Sample: Stock Standard: 1mg/mL galactose-1-phosphate in DI water, stored at -20°C
Peaks: Galactose-1-phosphate, 259.0224m/z (M-H)⁻



Galactose-1-phosphate

Discussion

This method is useful as a quantitative screening or routine clinical test to detect infants suspected of having a defect of galactose metabolism. It can also be used to monitor blood levels of galactose-1-phosphate in children with galactosemia who are on a lactose-free diet.