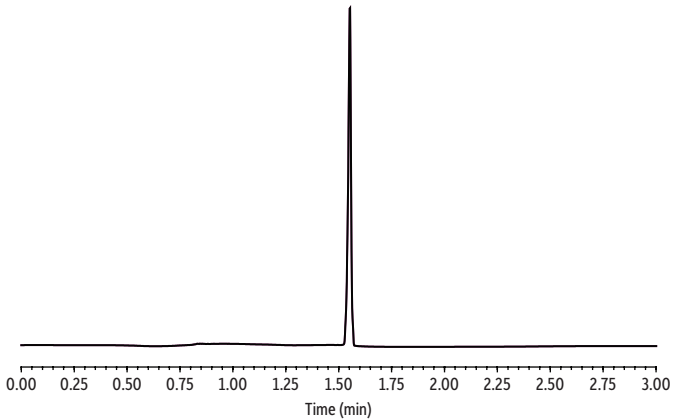


Methylmalonic Acid in Human Plasma (Trichloroacetic Acid Preparation)



LC\_CF0719

Peaks	tr (min)	Precursor Ion	Product Ion
1. Succinic acid	1.54	117.3	73.1

Column	Force C18 (cat.# 963431E)
Dimensions:	100 mm x 3.0 mm ID
Particle Size:	3 µm
Pore Size:	100 Å
Guard Column:	Force C18 EXP guard column cartridge 3 mm ID, (cat.# 963450253)
Temp.:	35 °C
Standard/Sample	
Conc.:	23 ng/mL Methylmalonic acid in double charcoal stripped human plasma
Inj. Vol.:	3 µL
Mobile Phase	
A:	0.5% Formic acid in water
B:	0.5% Formic acid in methanol

Time (min)	Flow (mL/min)	%A	%B
0.00	0.7	95	5
0.50	0.7	95	5
3.00	0.7	5	95
3.01	0.7	95	5
5.00	0.7	95	5

Detector	MS/MS
Ion Mode:	ESI-
Mode:	MRM
Instrument	HPLC
Sample Preparation	100 µL of sample (double charcoal stripped human plasma) was aliquoted for extraction. The sample contained a known endogenous level (13 ng/mL) of methylmalonic acid and was fortified with an additional 10 ng/mL for a total concentration of 23 ng/mL. The fortified sample was precipitated using 25 µL of 40% (w/v) trichloroacetic acid. 300 µL of 0.1% formic acid in water was added to the sample followed by a 10 second vortex at 3000 rpm. The sample was then centrifuged at 4000 rpm for 10 minutes at 10 °C. 250 µL of the supernatant was filtered using a Thomson SINGLE StEP standard filter vial (PVDF, 0.2 µm, Restek cat.# 25895) prior to analysis.