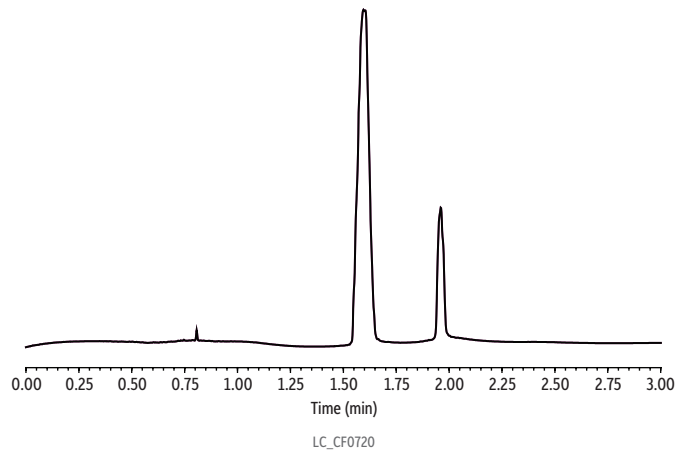


Methylmalonic Acid in Human Plasma (Formic Acid Preparation)



Peaks	tr (min)	Precursor Ion	Product Ion
1. Succinic acid	1.60	117.3	73.1
2. Methylmalonic acid	1.96	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 300 µL of 0.5% formic acid in methanol 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.