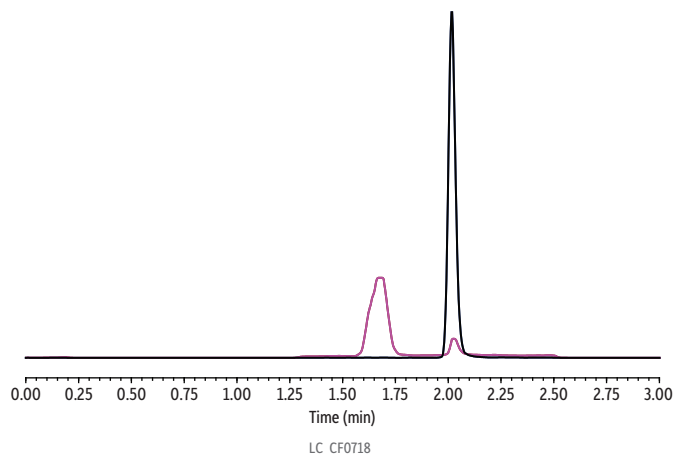


Methylmalonic Acid in Human Plasma on Force C18 by LC-MS/MS

- Easy sample prep (no derivatization).
- Compatible with legacy HPLC instruments.
- Fast 5-min total cycle time.
- Linear range = 10-500 ng/mL.



| Peaks | tr (min) | Precursor Ion | Product Ion |
|---------------------------|----------|---------------|-------------|
| 1. Succinic acid | 1.68 | 117.3 | 73.1 |
| 2. Methyl-D3-malonic acid | 2.02 | 120.2 | 76.2 |
| 3. Methylmalonic acid | 2.03 | 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.: 13 ng/mL Methylmalonic acid (endogenous) 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 containing 13 ng/mL of endogenous methylmalonic acid) was aliquoted for extraction. 5 µL of internal standard (2500 ng/mL MMA-D3 in water) was added to the sample. The 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.