Featured Application: *Methylmalonic Acid (MMA) on Force C18*

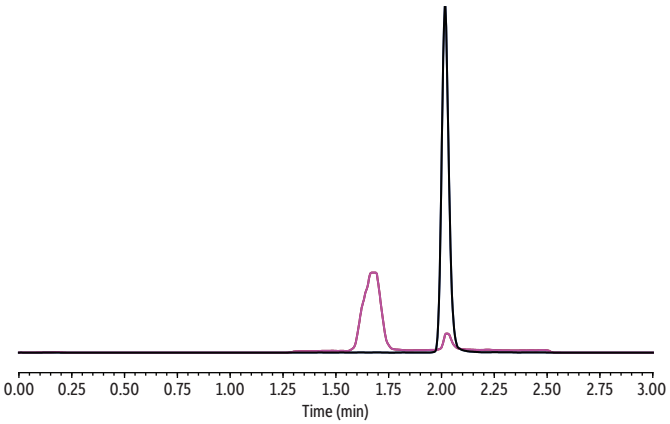
Simplified Methylmalonic Acid Analysis without Derivatization in Human Plasma by LC-MS/MS

- Baseline separation of isomers ensures accurate reporting.
- Simple protein crash sample preparation, no derivatization required.
- Fast 5-minute analysis is suitable for high-throughput labs.

Vitamin B12 plays an essential role in metabolic energy production, and deficiency can be difficult to diagnose without testing as it can manifest through a wide variety of symptoms. In clinical testing of plasma samples, elevated levels of methylmalonic acid (MMA) can be used to diagnose functional vitamin B12 deficiency as well as methylmalonic acidemia, an inherited metabolic disorder. Methylmalonic acid determination is a very sensitive test and is more specific than a homocysteine test, but it typically requires extensive sample pre-treatment using liquid-liquid extraction, derivatization, solvent evaporation, and/or SPE. Additionally, chromatographic resolution can be difficult to achieve between methylmalonic acid and its naturally occurring isomer, succinic acid.

To overcome these obstacles, we developed a simplified method for methylmalonic acid analysis. The approach shown here uses a protein crash sample preparation and does not require derivatization. By eliminating derivatization, considerable time and resource savings are realized and a significant source of variation is removed. For analysis, a direct injection of the supernatant is made onto a Force C18 column. LC-MS/MS results show that methylmalonic acid is clearly resolved from succinic acid, which eliminates potential isobaric interference and makes peak identification and quantitation straightforward. In addition, the 5-minute total chromatographic analysis time makes this method suitable as a high-throughput assay for the clinical diagnosis of vitamin B12 deficiency and methylmalonic acidemia.

Peaks	t_R (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



LC_CF0718

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
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
Notes 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 (2,500 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 3,000 rpm. The sample was then centrifuged at 4,000 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.

Notes Want even better performance when analyzing metal-sensitive compounds? Check out Inert LC columns at www.restek.com/inert.

Force C18 LC Columns

- A traditional end-capped C18 ideal for general-purpose use in reversed-phase chromatography.
- Wide pH range (2–8) provides excellent data quality for many applications, matrices, and compounds.
- High carbon load (20%) offers high hydrophobic retention.

Catalog No.	Product Name	Units
9634232	Force C18, 1.8 µm, 30 x 2.1 mm LC Column	ea.
9634252	Force C18, 1.8 µm, 50 x 2.1 mm LC Column	ea.
9634212	Force C18, 1.8 µm, 100 x 2.1 mm LC Column	ea.
963425E	Force C18, 1.8 µm, 50 x 3.0 mm LC Column	ea.
963421E	Force C18, 1.8 µm, 100 x 3.0 mm LC Column	ea.
9634332	Force C18, 3 µm, 30 x 2.1 mm LC Column	ea.
9634352	Force C18, 3 µm, 50 x 2.1 mm LC Column	ea.
9634312	Force C18, 3 µm, 100 x 2.1 mm LC Column	ea.
9634362	Force C18, 3 µm, 150 x 2.1 mm LC Column	ea.
963435E	Force C18, 3 µm, 50 x 3.0 mm LC Column	ea.
963431E	Force C18, 3 µm, 100 x 3.0 mm LC Column	ea.
963436E	Force C18, 3 µm, 150 x 3.0 mm LC Column	ea.
9634315	Force C18, 3 µm, 100 x 4.6 mm LC Column	ea.
9634365	Force C18, 3 µm, 150 x 4.6 mm LC Column	ea.
9634552	Force C18, 5 µm, 50 x 2.1 mm LC Column	ea.
9634512	Force C18, 5 µm, 100 x 2.1 mm LC Column	ea.
9634562	Force C18, 5 µm, 150 x 2.1 mm LC Column	ea.
963455E	Force C18, 5 µm, 50 x 3.0 mm LC Column	ea.
963451E	Force C18, 5 µm, 100 x 3.0 mm LC Column	ea.
963456E	Force C18, 5 µm, 150 x 3.0 mm LC Column	ea.
9634515	Force C18, 5 µm, 100 x 4.6 mm LC Column	ea.
9634565	Force C18, 5 µm, 150 x 4.6 mm LC Column	ea.
9634575	Force C18, 5 µm, 250 x 4.6 mm LC Column	ea.

Stationary Phase Category: C18, octadecylsilane (L1)
Ligand Type: End-capped C18
Particle: 1.8 µm, 3 µm, or 5 µm fully porous silica
Pore Size: 100 Å
Carbon Load: 20%
End-Cap: yes
Surface Area: 300 m^2/g

Recommended Usage:
pH Range: 2.0–8.0
Maximum Temperature: 80 °C
Maximum Pressure: 1034 bar/15,000 psi* (1.8 µm), 600 bar/8700 psi (3 µm); 400 bar/5800 psi (5 µm)

* For maximum lifetime, recommended maximum pressure for 1.8 µm particles is 830 bar/12,000 psi.

Properties:
- Compatible with moderately acidic to neutral mobile phases (pH 2–8).
- Excellent data quality in food, environmental, bioanalytical, and other applications.

Switch to a C18 when:
- You need a general-purpose column for reversed-phase chromatography.
- You need to increase retention of hydrophobic compounds.

Want even better performance when analyzing metal-sensitive compounds? Check out Inert LC columns at **www.restek.com/inert**

Force Inert C18 LC Columns

- Inert LC column technology reduces nonspecific binding of chelating analytes, enabling sensitive analysis and smooth integration of peaks.
- Ideal for the analysis of metal-sensitive compounds.
- Increased response and analyte recovery, allowing lower detection limits.
- Improved peak shape without passivation or mobile phase additives.
- Part of Restek's Force Inert C18 column line featuring 1.8 and 3 µm fully porous silica.

Catalog No.	Product Name	Units
9634252-T	Force Inert C18, 1.8 µm, 50 x 2.1 mm LC Column	ea.
9634212-T	Force Inert C18, 1.8 µm, 100 x 2.1 mm LC Column	ea.
9634352-T	Force Inert C18, 3 µm, 50 x 2.1 mm LC Column	ea.
9634312-T	Force Inert C18, 3 µm, 100 x 2.1 mm LC Column	ea.
963435E-T	Force Inert C18, 3 µm, 50 x 3.0 mm LC Column	ea.
963431E-T	Force Inert C18, 3 µm, 100 x 3.0 mm LC Column	ea.

Stationary Phase Category: C18, octadecylsilane (L1)
Ligand Type: End-capped C18
Particle: 1.8 µm or 3 µm fully porous silica
Pore Size: 100 Å
Carbon Load: 20%
End-Cap: yes
Surface Area: 300 m^2/g

Recommended Usage:
pH Range: 2.0–8.0
Maximum Temperature: 80 °C
Maximum Pressure: 1034 bar/15,000 psi* (1.8 µm); 600 bar/8700 psi (3 µm)

*For maximum lifetime, recommended maximum pressure for 1.8 µm particles is 830 bar/12,000 psi.

Properties:
- Compatible with moderately acidic to neutral mobile phases (pH 2–8).
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Switch to a C18 when:
- You need a general-purpose column for reversed-phase chromatography.
- You need to increase retention of hydrophobic compounds.

Force C18 Guard Cartridge, EXP

- Free-Turn architecture lets you change cartridges by hand without breaking inlet/outlet fluid connections—no tools needed.
- Patented titanium hybrid ferrules can be installed repeatedly without compromising high-pressure seal.
- Auto-adjusting design provides ZDV (zero dead volume) connection to any 10-32 female port.
- Guard column cartridges require EXP direct connect holder (cat.# 25808).
- Pair with EXP hand-tight fitting (cat.# 25937–25938) for tool-free installation.
- For use with 3 or 5 µm Force LC columns. For 1.8 µm Force columns, use a 0.2 µm UltraShield filter.

Catalog No.	Product Name	Units
963450252	Force C18 Guard Cartridge, 5 x 2.1 mm EXP, 3-pk.	3-pk.
963450253	Force C18 Guard Cartridge, 5 x 3.0 mm EXP, 3-pk.	3-pk.
963450250	Force C18 Guard Cartridge, 5 x 4.6 mm EXP, 3-pk.	3-pk.

Force Inert C18 Guard Cartridge, EXP

- Premium inert coating reduces nonspecific binding of chelating analytes.
- Free-Turn architecture lets you change cartridges by hand without breaking inlet/outlet fluid connections—no tools needed.
- Patented titanium hybrid ferrules can be installed repeatedly without compromising high-pressure seal.
- Auto-adjusting design provides ZDV (zero dead volume) connection to any 10-32 female port.
- Guard column cartridges require EXP direct connect holder (cat.# 25808).
- For use with 3 or 5 µm Force LC columns. For 1.8 µm Force columns, use a 0.2 µm UltraShield filter.

Catalog No.	Product Name	Units
963450252-T	Force C18 Guard Cartridge, 5 x 2.1 mm EXP, 3-pk.	3-pk.
963450253-T	Force C18 Guard Cartridge, 5 x 3.0 mm EXP, 3-pk.	3-pk.

EXP Direct Connect Holder

Catalog No.	Product Name	Units
25808	EXP Direct Connect Holder for EXP Guard Cartridges, Includes Fitting & Ferrules	ea.

Thomson SINGLE StEP Standard Filter Vials with Screw-Top Caps

Catalog No.	Product Name	Cap Color	Material	Porosity	Units
27896	Thomson SINGLE StEP Standard Filter Vials with Screw-Top Caps, 0.2 µm, Nylon w/Preslit Cap, Black Cap, 100-pk.	Black	Nylon	0.2 µm	100-pk.
27897	Thomson SINGLE StEP Standard Filter Vials with Screw-Top Caps, 0.45 µm, Nylon w/Preslit Cap, Pink Cap, 100-pk.	Pink	Nylon	0.45 µm	100-pk.
27895	Thomson SINGLE StEP Standard Filter Vials with Screw-Top Caps, 0.2 µm, PES w/Preslit Cap, Gray Cap, 100-pk.	Gray	PES (polyethersul-fone)	0.2 µm	100-pk.
28307	Thomson SINGLE StEP Standard Filter Vials with Screw-Top Caps, 0.2 µm, PTFE w/preslit cap, Green Cap, 100-pk.	Green	PTFE (polytetrafluo-roethylene)	0.2 µm	100-pk.
28306	Thomson SINGLE StEP Standard Filter Vials with Screw-Top Caps, 0.45 µm, PTFE w/preslit cap, Blue Cap, 100-pk.	Blue	PTFE (polytetrafluo-roethylene)	0.45 µm	100-pk.
27894	Thomson SINGLE StEP Standard Filter Vials with Screw-Top Caps, 0.2 µm, PVDF w/Preslit Cap, Red Cap, 100-pk.	Red	PVDF (polyvinyldiflu-oride)	0.2 µm	100-pk.
27898	Thomson SINGLE StEP Standard Filter Vials with Screw-Top Caps, 0.45 µm, PVDF w/Preslit Cap, Yellow Cap, 100-pk.	Yellow	PVDF (polyvinyldiflu-oride)	0.45 µm	100-pk.

Patent No. 7,790,117