

A Selective Method for Separation of Methylmalonic Acid using the Luna® Omega 3 µm C18 Column

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Overview

Methylmalonic acid (MMA) is a small dicarboxylic acid. Since this molecule is hydrophilic, it can present chromatographic challenges both in achieving adequate retention under reversed phase conditions as well as resolution from the isomeric/isobaric species such as succinic acid. In this application note, we present an LC-MS/MS method to analyze MMA by utilizing the Luna Omega 3 µm C18 column in three different dimensions.

A mixture of 100 ng/mL of MMA and D3-MMA, 800 ng/mL of SA in diluent (1:2 of acetonitrile:0.1% formic acid in water) was prepared from a 1 mg/mL stock solution of each compound, then directly injected into LC-MS/MS system.

We have built on a previous method that was employed to separate succinic acid from MMA. In this method, succinic acid was well-resolved chromatographically from MMA. This was achieved by using a UHPLC column that contains both a positively charged ligand for retention of acidic compounds and a C18 ligand for added selectivity. The different column dimensions allowed for resolution between succinic acid and MMA at longer run times with at least 0.4 min between the two.

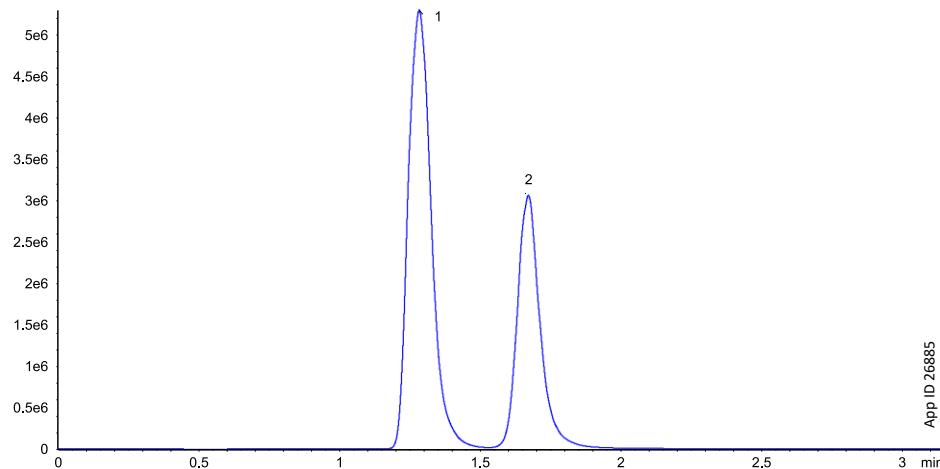
LC-MS/MS Conditions

Column: Luna Omega 3 µm C18
Dimension: 100 x 2.1 mm
150 x 2.1 mm
150 x 3.0 mm
Part No.: [00D-4784-AN](#)
[00F-4784-AN](#)
[00F-4784-Y0](#)
Pressure (bar): 400 (100 x 2.1 mm)
400 (150 x 2.1 mm)
500 (150 x 3.0 mm)
Mobile Phase: A: 0.5 % Formic Acid in Water
B: 0.5 % Formic Acid in Methanol
Gradient: See Chromatograms
Flow Rate: See Chromatograms
Injection Volume: 10 µL
Temperature: 20 °C
LC System: Agilent® 1260 Infinity
Detection: LC-MS/MS
Detector: 4000 QTRAP® (SCIEX®)

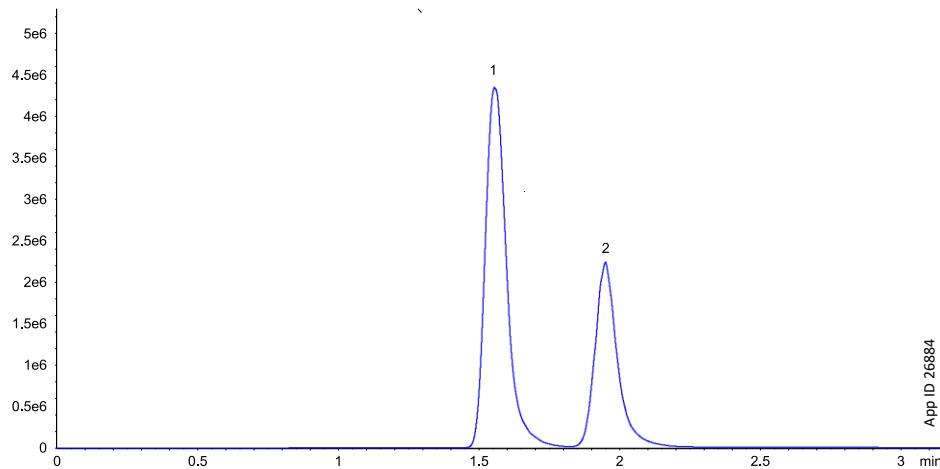
MRM Transitions

Peak No.	Analyte	Q1 (m/z)	Q3 (m/z)
1	Succinic Acid	117.1	73.1
1	Succinic Acid, confirming	117.1	99.1
2	Methylmalonic Acid	117	73.1
2	Methylmalonic Acid-D3	120	76.1

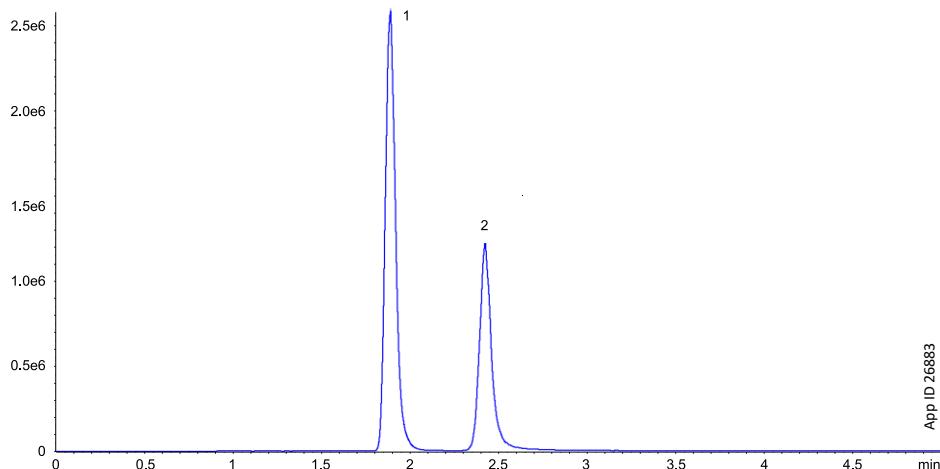


Luna® Omega 3 μ m C18, 100 x 2.1 mm

Time (min)	%B	Flow Rate (mL/min)
0.2	20	0.25
0.7	40	0.25
1.2	40	0.25
1.4	20	0.25
3.4	20	0.25

Luna Omega 3 μ m C18, 150 x 2.1 mm

Time (min)	%B	Flow Rate (mL/min)
0.2	20	0.3
0.7	40	0.3
1.2	40	0.3
1.4	20	0.3
3.4	20	0.3

Luna Omega 3 μ m C18, 150 x 3.0 mm

Time (min)	%B	Flow Rate (mL/min)
0.2	15	0.6
4.2	65	0.6
4.7	65	0.55
4.9	15	0.55
6.9	15	0.55



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