Quantitative Analysis of Methylmalonic Acid in Plasma Using SOLAμ Plates for Sample Preparation and a TSQ Endura Triple Quadrupole Mass Spectrometer for Clinical Research

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Key Words
MMA, plasma, triple quadrupole mass spectrometer, TSQ Endura, SOLAμ

Goal
To demonstrate a sensitive, robust, reliable quantitative analysis of methylmalonic acid in plasma for clinical research.

Application Benefits
- Wide quantitative analytical range from 25 to 100,000 nM
- Simple, economical, easily automated sample preparation method
- Confident analyte identification with ion ratio confirmation
- Robust method without matrix effects
- Can be implemented on dual- and four-channel LC systems

Introduction
Liquid chromatography-mass spectrometry (LC-MS) analytical methods are widely used for quantitation of methylmalonic acid (MMA) in clinical research laboratories. The quantitation of MMA poses several challenges, including (i) the need for a simple sample preparation method; (ii) achievement of low limits of quantitation; and (iii) separation from the naturally occurring structural isomer succinic acid. Here we present an analytical method that successfully addresses all of these requirements.

Methods
Calibrators and Quality Controls
Because it is difficult to obtain MMA-free plasma, calibration standards in the range of 25 to 100,000 nM were prepared in 2% acetonitrile. Pooled plasma containing MMA at 110 nM was used as Level 0 Quality Control. Level 1 (260 nM) and Level 2 (583 nM) Quality Controls were purchased from RECIPE™ Chemicals and Instruments GmbH (ClinChek™ control kit P/N MS5082).

Sample Preparation
Plasma samples, calibrators, and QCs (all 100 µL aliquots) spiked with internal standard (d_3-MMA) were processed by solid phase extraction using Thermo Scientific™ SOLAμ™ WAX 96-well plates (P/N 60209-005). Analytes were eluted from the extraction plate with 70 µL of 1% ammonium hydroxide, and the eluent was neutralized with 30 µL of 10% formic acid. A 15 µL aliquot of eluent was analyzed by LC-MS.

Liquid Chromatography
A 3.5-minute chromatographic method with a Thermo Scientific™ Accucore™ RP-MS column (2.6 µm, 100 x 2.1 mm, P/N 17626-102130) at room temperature was performed using a Thermo Scientific™ Dionex™ UltiMate™ 3000RS liquid chromatography pump with OAS autosampler. Mobile phases consisted of 0.4% formic acid in water and 0.1% formic acid in methanol (Fisher Chemical Optima™ grade) for phases A and B, respectively. This chromatographic method separated MMA from its endogenous structural isomer, succinic acid.

Mass Spectrometry
Compounds were detected on a Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer equipped with an Ion Max™ source and a heated electrospray (HESI) sprayer operated in negative ionization mode. Two SRM transitions each for MMA and its internal standard were monitored for quantitation and confirmation (Table 1).

Table 1. SRM transitions acquisition parameters.

Analyte	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	RF Lens (V)	Ion Function
MMA	117.1	73.3	10	79	Quantifier
MMA	117.1	55.3	25	79	Qualifier
MMA-D_3	120.1	76.3	10	79	Quantifier
MMA-D_3	120.1	58.3	25	79	Qualifier

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Method Performance Evaluation

The limits of quantitation (LOQ) and linearity ranges were determined by collecting calibration curve data. Method precision was evaluated by running replicate calibration curve standards and QCs on three different days. Method accuracy was assessed by calculating %recovery for QC samples and for ten donor plasma samples spiked with 200 nM of MMA. Matrix effects were evaluated by spiking internal standard into SPE-processed plasma samples from eight different donors and calculating recovery against the same internal standard amount spiked into SPE-processed water.

Data Analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ software. The average ion ratios calculated for analyte confirmation were about 4% and were within the allowed 50% (relative) of average calculated from the calibrators.

Results and Discussion

Limits of quantitation were defined as the lowest concentrations that had back-calculated values within 20% and ion ratio within the specified range. Using these criteria, the limit of quantitation for MMA was 25 nM. The upper limit of the calibration curve was 100,000 nM. Figure 1 shows a representative calibration curve, along with quantifying and qualifying ion chromatograms for the lowest calibration standard. Quadruplicate calibration standards' precision was better than 5%, and accuracy was within ±9%.

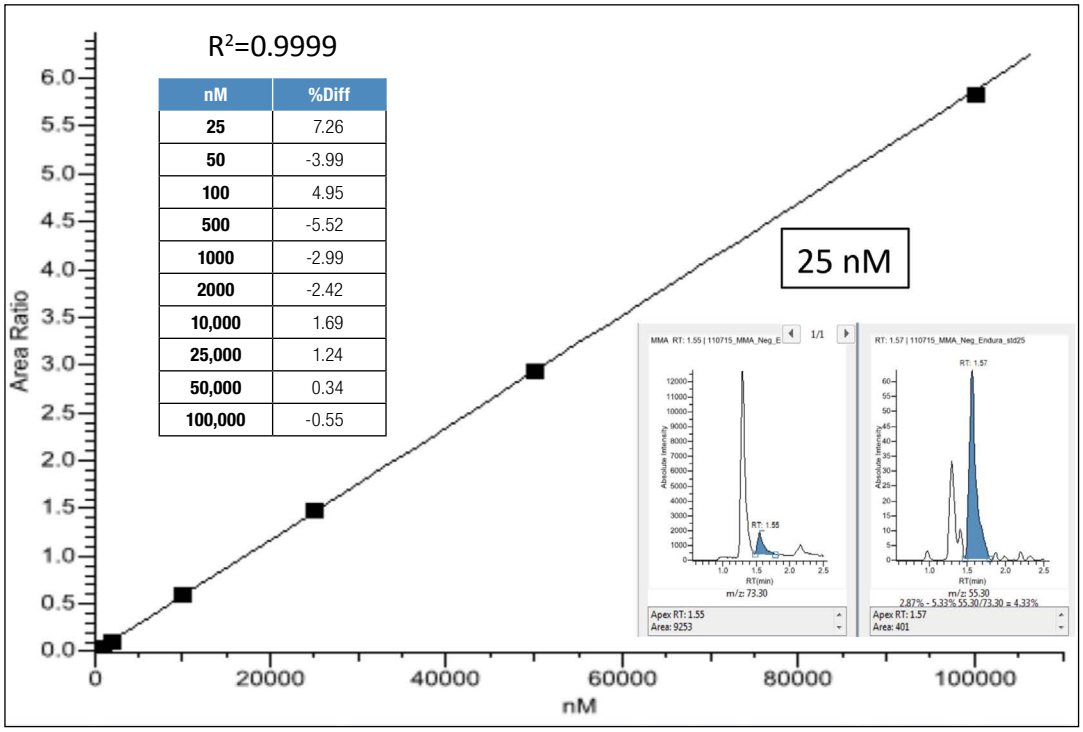

Figure 1. Representative calibration curve.

Method accuracy calculated as % recovery of QC and spiked donor samples ranged from 90% to 104% and from 96.8% to 107% for QCs and spiked donor samples, respectively. This shows that the use of the surrogate matrix for the calibration curve produces accurate results for samples in plasma matrix.

Intra-assay precision for all QC levels was better than 5.1% (Table 2), and inter-assay precision was better than 6.4% (Table 3). Figure 2 presents chromatograms of quantifying and qualifying ions in plasma QC samples and also shows the chromatographic separation of the structural isomer, succinic acid.

Table 2. Intra-assay precision and accuracy.

	QC Level 0	QC Level 1	QC Level 2
Concentration (nM)	110	260	583
Precision (%RSD)	<5.1	<2.0	<0.9
Accuracy (average %recovery)	89.7–101	91.8–95.3	96.9–104

Table 3. Inter-assay precision and accuracy.

	QC Level 0	QC Level 1	QC Level 2
Concentration (nM)	110	260	583
Precision (%RSD)	6.4	2.2	3.5
Accuracy (average %recovery)	96.7	93.1	99.7

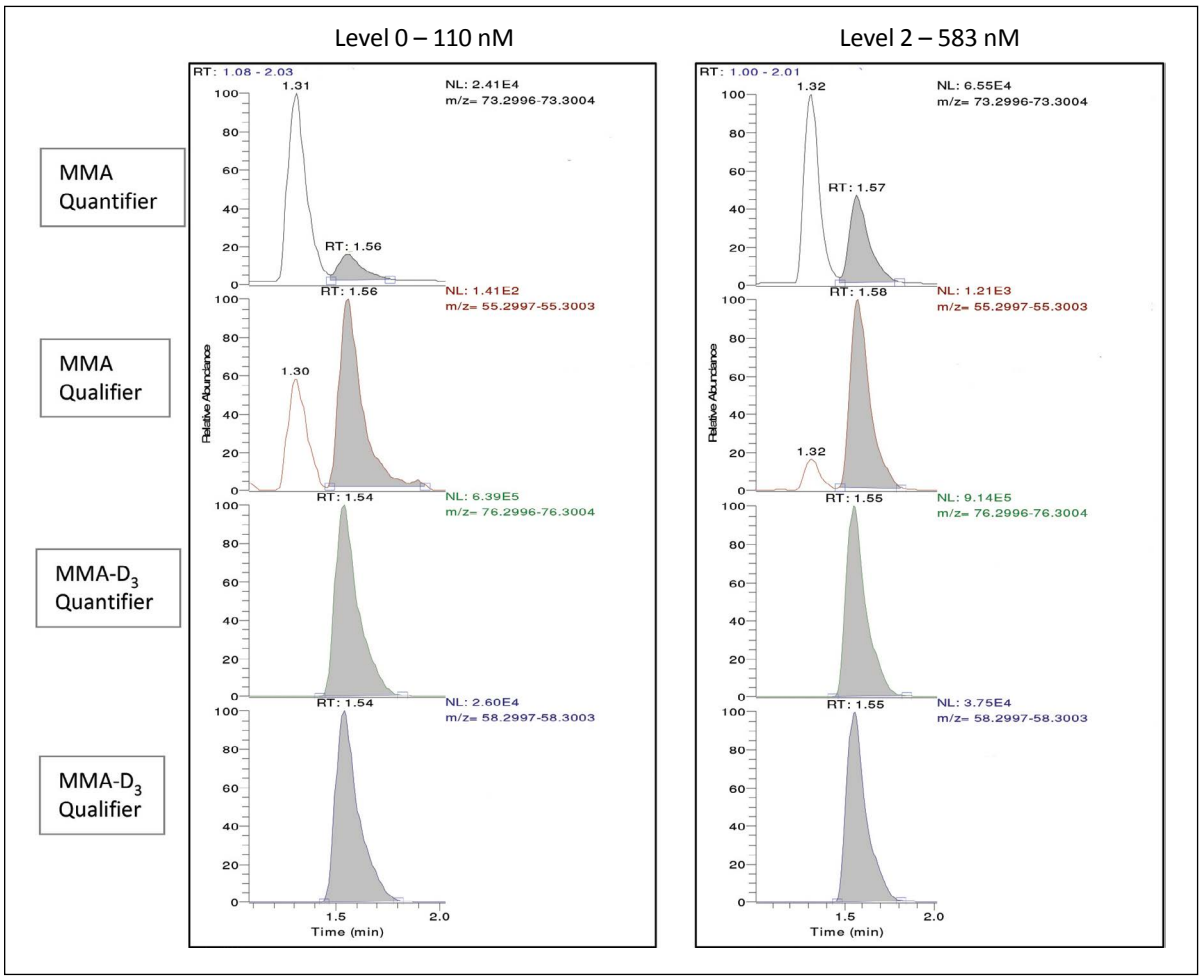

Figure 2. Quantifying and qualifying ions chromatographic peaks in QC samples.

Matrix effects were not observed. Recovery in eight donor samples, calculated as the ratio between internal standard peak area in matrix and internal standard peak area in neat solution, was within method analytical error and ranged from 89.7% to 107%.

Conclusion

We demonstrated a sensitive and robust method for quantifying MMA in plasma that provides a wide dynamic range, while employing simple and economical sample preparation. With the short run time and data acquisition window, this analytical method can be put into use on a 4-channel Thermo Scientific™ Transcend™ II LC system to address high-throughput analysis (50 samples/hour) requirement in clinical research.

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