TECHNICAL NOTE 73518

Quantification of methylmalonic acid in human plasma or serum for use in clinical research

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Keywords: Methylmalonic acid, plasma, serum, mass spectrometry, TSQ Quantis

Application benefits

- Increased accuracy of method by implementing a comprehensive ClinMass® kit for sample preparation
- Fast acquisition time allows for increased productivity
- Robust, sensitive hardware enables increased confidence in data

Goal

Implementation of an analytical method for the quantification of methylmalonic acid in human plasma or serum on a Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer for clinical research.

Introduction

Reduced function of methylmalonyl CoA mutase enzyme results in increased serum methylmalonic acid (MMA) concentrations. Increased MMA concentrations can be used as a strong indicator of vitamin B_{12} deficiency.

Since the 1980s, various analytical methods for the measurement of MMA in biological fluids have been reported. These include numerous approaches for sample preparation (e.g., ultrafiltration, solid-phase or liquid-liquid extraction) and analytical measurement (e.g., GC/MS, electrophoresis, and LC-MS/MS). However, the analysis of MMA in serum can be challenging due to the low molecular weight of dicarboxylic acid, its hydrophilic, non-volatile nature, and the low concentration at which it is typically present. Compared to GC-MS, LC-MS/MS methods offer relatively shorter run times and better sensitivity—two critical advantages that can benefit every laboratory focused on conducting large cohort studies.

In this report, an analytical clinical research method for the quantification of methylmalonic acid in human plasma or serum is reported. Plasma or serum samples were extracted by offline internal standard addition and protein precipitation. Extracted samples were injected onto a Thermo Scientific™ Transcend™ UHPLC system connected to a TSQ Quantis triple quadrupole mass spectrometer. Detection was performed by selected reaction monitoring (SRM) using d_3-methylmalonic acid as the internal standard. Method performance was evaluated using the MS5100 ClinMass® Complete Kit, advanced, for Methylmalonic Acid in Serum / Plasma from RECIPE® Chemicals + Instruments GmbH (Munich, Germany), in terms of linearity of response within the calibration range, carryover, accuracy, and intra- and inter-assay precision.

Experimental

Sample preparation

Reagents included four calibrators (MS5013 batch #1152) and two controls (MS5082 batch #1159) from RECIPE, covering a concentration range of 220 to 1404 nM. A sample of 50 µL of plasma or serum was mixed with 200 µL precipitation solution containing the internal standard, vortex-mixed, and centrifuged for 5 min at 10,000 x g. The supernatant was transferred to a clean plate or vial.

Liquid chromatography

Liquid chromatography separation was achieved using a Transcend UHPLC system by gradient elution using the mobile phase and analytical column provided by RECIPE. Details of the analytical method are reported in Table 1. Total runtime was 3.0 minutes. Injection volume was 10 µL.

Mass spectrometry

Analyte and internal standard were detected by SRM on a TSQ Quantis triple quadrupole mass spectrometer with heated electrospray ionization source operated in negative ion mode. Two SRM transitions for each analyte were included in the acquisition method for quantification and confirmation, respectively. Mass spectrometric conditions are reported in Table 2.

Method evaluation

The method performance was evaluated in terms of linearity of response within the calibration range, carryover, accuracy, and intra- and inter-assay precision. Carryover was calculated in terms of percentage ratio between peak area of the highest calibrator and a blank sample injected just after it. Analytical accuracy was evaluated in terms of percentage bias between nominal and average back-calculated concentrations using the two different levels of RECIPE Kit quality control samples prepared and analyzed in replicates of five on three different days. Intra-assay precision for each day was evaluated in terms of percentage coefficient of variation (%CV) using the same two controls in replicates of five (n=5). Inter-assay precision was evaluated as the %CV on the full set of samples (control samples at two levels in replicates of five prepared and analyzed on three different days).

Data analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ 4.1 software.

Table 1. Liquid chromatography method description

	Time (min)	Flow rate (mL/min)	A (%)	B (%)
Gradient profile	0.00	0.6	100	0
	0.30	0.6	100	0
	0.31	0.6	70	30
	0.60	0.6	70	30
	0.61	0.6	40	60
	1.30	0.6	40	60
	1.31	0.6	0	100
	1.40	0.6	0	100
	1.41	0.6	100	0
	3.00	0.6	100	0
Injection volume	10 µL	Column temperature		40 °C

Table 2. Mass spectrometric parameters

Parameter	Value
Source type	Heated electrospray ionization (H-ESI II)
Vaporizer temperature	350 °C
Capillary temperature	350 °C
Spray voltage (negative mode)	1000 V (negative mode)
Sheath gas	45 AU
Sweep gas	0 AU
Auxiliary gas	5 AU
Data acquisition mode	Selected-reaction monitoring (SRM)
Collision gas pressure	1.5 mTorr
Cycle time	0.250 s
Q1 mass resolution (FWMH)	0.7
Q3 mass resolution (FWMH)	1.2

Results and discussion

The method proved to be linear in the calibration range covered by the calibrators with a correlation factor (R^2) always above 0.999. A representative chromatogram of both methylmalonic acid and d_3-methylmalonic acid at the lowest calibration level is reported in Figure 1. A representative calibration curve is reported in Figure 2.

No significant carryover was observed for both analytes, with no signal detected in the blank injected just after the highest calibrator.

The data demonstrated outstanding accuracy of the method with the percentage bias between nominal and average back-calculated concentration for the used control samples ranging between -1.0% and 0.8%. The %CV for intra-assay precision was always below 1.8%. The maximum %CV for inter-assay precision was 1.7%. Results are reported in Table 3.

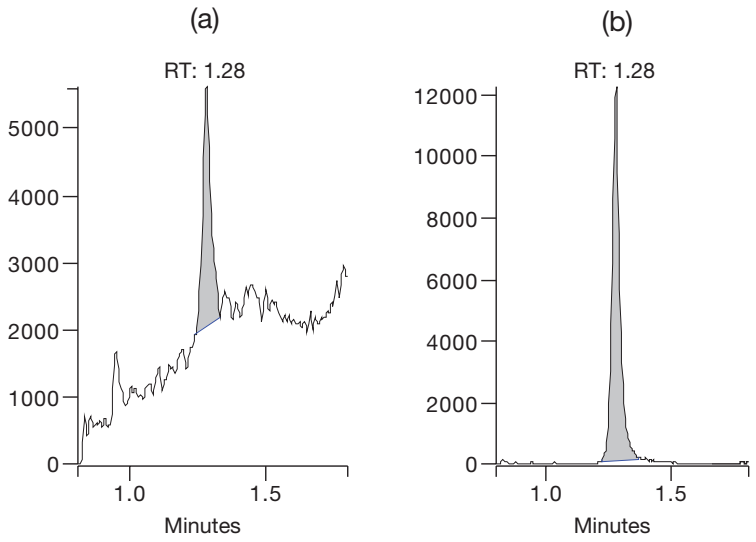

Figure 1. Representative chromatogram for (a) methylmalonic acid and (b) d_3-methylmalonic acid at the lowest calibration level

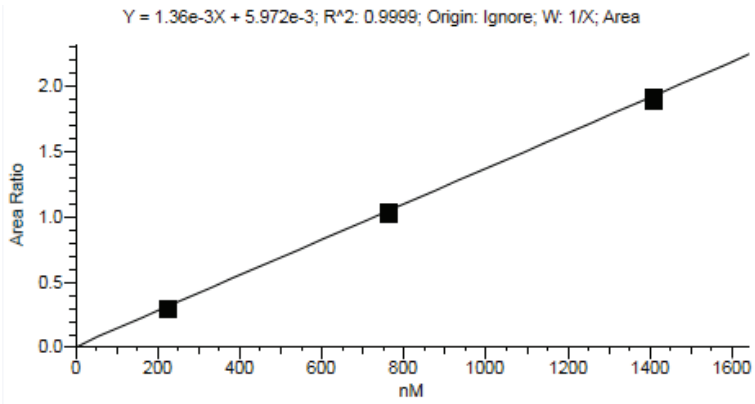

Figure 2. Representative calibration curve for methylmalonic acid (day 1)

Table 3. Analytical accuracy and intra- and inter-assay precision results for controls MS5082 – Level 1 and Level 2

		Level 1				Level 2			
		Nominal concentration (ng/mL)	Average calculated concentration (ng/mL)	Bias (%)	CV (%)	Nominal concentration (ng/mL)	Average calculated concentration (ng/mL)	Bias (%)	CV (%)
Accuracy		267	264	-1.0		577	581	0.8	
Intra-assay precision	Day 1		263		1.8		589		1.3
	Day 2		267		1.5		579		1.2
	Day 3		263		1.7		576		1.0
Inter-assay precision			264		1.7		581		1.5

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Conclusion

A robust, reproducible, sensitive and easy-to-implement liquid chromatography-tandem mass spectrometry method for clinical research for the quantification of methylmalonic acid in human plasma or serum using the MS5100 ClinMass Complete Kit Methylmalonic Acid in Plasma / Serum from RECIPE, was implemented and analytically validated on a Transcend UHPLC system connected to a TSQ Quantis triple quadrupole mass spectrometer. The described method meets research laboratory requirements in terms of sensitivity, linearity of response, accuracy, and precision.

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