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Application Data Sheet

LC-MS
Liquid Chromatograph Mass Spectrometer

Determination of Methylmalonic Acid in Serum, Plasma and Urine by LCMS-8030 using RECIPE ClinMass® Complete Kit MS5000

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Introduction

Measurement of methylmalonic acid (MMA) is used as a specific diagnostic marker for the group of disorders known collectively called as methylmalonic acidemias.
The metabolic pathway involves methylmalonyl-coenzyme A (CoA) being converted into succinyl-CoA. Vitamin B_{12} is also needed for this conversion. Therefore measurement of MMA can be used to diagnose a number of genetic disorders in this pathway and is elevated in 90-98% of patients with B_{12} deficiency.
Typically the concentration of MMA is low and normally requires off-line extraction before analysis, however in these experiments a sensitive method was developed requiring minimal pre-treatment with just 10 µL sample injection to achieve suitable detection.

Materials and methods

The LCMS-8030 triple quadrupole mass spectrometer was coupled to a Nexera UHPLC system. MMA was measured using a commercially available test kit ClinMass® Complete Kit for Methylmalonic Acid in Serum, Plasma and Urine, MS5000 (RECIPE Chemicals + Instruments GmbH, Dessauerstraße 3, 80992 München, Germany). Chemical standards, control samples, analytical column and mobile phase solvents were provided by the kit. 100 µL of sample was added to 400 µL of precipitant solution (containing internal standard). Following centrifugation 10 µL of supernatant was analysed.
For analysis the $[M-H]^-$ ion was measured and used as the precursor ion (negative electrospray ionization).

Analytical Conditions

UHPLC: Nexera UHPLC
0.8 mL/min starting at 20% B
Injection volume: 10 µL
Column temperature: 30° C
Mass spectrometer: LCMS-8030
Source conditions: Desolvation Line: 275 ° C
Heat Block: 225 ° C
Nebulizer Gas: 3 L/min
Drying Gas: 20 L/min
Interface voltage: -2.25 kV
Dwell time: 20 msec
Pause time: 3 msec
Ionization: Electrospray ionization (ESI), negative mode.
Scan Type: Multiple-reaction-monitoring mode (MRM)

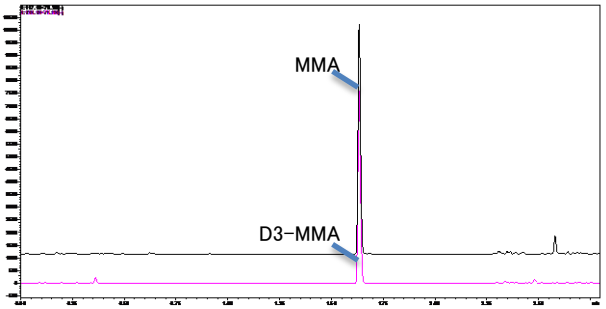

Fig. 1 LC-MS separation of MMA and deuterated standard in under three minutes by fast chromatography.

Table 1 LC parameters were chosen for rapid compound elution and fast analysis time.

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)	Flow rate (mL/min)
0	80	20	0.8
0.01	10	90	0.8
1.6	10	90	0.8
1.61	80	20	0.8
2.3	80	20	0.8
2.31	80	20	1.4
2.75	80	20	1.4
2.8	80	20	0.1

Table 2 MMA optimized MRM transitions, retention time (RT). T/I = target or internal standard

Compound		Formula	MRM1	MRM2	RT
MMA	T	C4H6O4	117.1>73.2	117.1>55.2	1.63
D3−MMA	I	C4H3D3O4	120.1>75.9	–	1.63

Results

The rapid separation of MMA produced good peak shape and was eluted in less than two minutes.
The calibration curve showed good linearity over a clinically relevant range 26.1-177 μg/L (Fig. 2).
Two patient samples were anlaysed and measured in duplicate yielding MMA concentrations of 26.7 μg/L and 65.7 μg/L, respectively. The replicate analyses achieved good analytical reproducibility at less than 5% relative standard deviation.

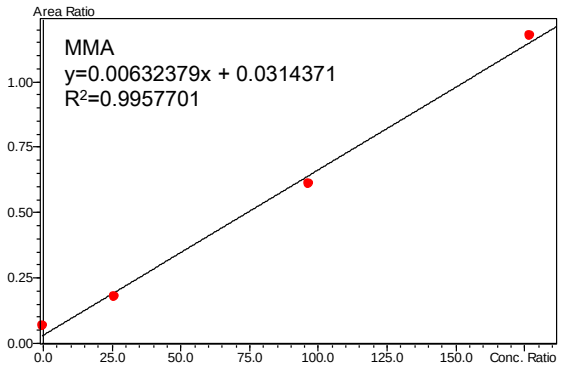

Fig. 2 Calibration curve for methylmalonic acid (concentraion range 26.1-177 μg/L).

Conclusion

The application of the clinical ClinMass® Complete Kit for Methylmalonic Acid in Serum, Plasma and Urine proved simple to implement and showed good sensitivity and linearity in a clinically relevant concentration range.

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