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Application News

Liquid Chromatograph Mass Spectrometer LCMS-8060

Analysis of Methylmalonic acid in Serum / Plasma Using RECIPE® ClinMass® LC-MS/MS Complete Kit System with Fully Automated Sample Preparation LC/MS/MS System

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User Benefits

- Full solution provided by Shimadzu and RECIPE®
- Fully automated sample preparation
- Verified method for RECIPE® ClinMass® LC-MS/MS Complete Kit, advanced Methylmalonic Acid in Serum / Plasma / Urine

■ Introduction

Vitamin B12 is an essential nutrient and plays an important role for the normal functioning of the human organism.

The coenzyme B12 participates in two metabolic key positions. One of these reactions is the vitamin B12-dependent conversion of Methylmalonyl-coenzyme A (CoA) to Succinyl-CoA. In cases of Vitamin B12 deficiency Methylmalonyl-CoA accumulates and Methylmalonic acid (MMA) is subsequently released .

Accordingly, vitamin B12 deficiency results in quantitative accumulation of MMA in blood and urine. This occurs already in the early stages of insufficiency, i.e., when vitamin B12 levels still appear "normal", making MMA a sensitive, early biomarker for intracellular, functional vitamin B12 deficiency.

The determination of MMA can be performed from serum, plasma, and urine.

Serum samples are generally used for MMA determination, as this matrix is used for parallel cobalamin level tests. The advantage of determination from serum therefore is the sample availability and the liability (nutrition seems to have less influence on the MMA level).

The advantage of determination from urine however lies in the significantly higher MMA levels, which facilitate the analyses. But in this case, creatinine is also necessary because the MMA/creatinine ratio is required for data interpretation.

RECIPE®'s fully validated analytical method provides the quantification of Methylmalonic acid (Table 1 and 2) in serum, plasma and urine using LC-MS/MS.[1] Sample preparation is simple, rapid, and analogous for the different biological matrices. By addition of the Shimadzu CLAM (Clinical Laboratory Automated sample preparation Module) in front of the LC-MS/MS system (Figure 1) the required sample preparation could be fully automated which achieves results on a fast and high-precision analytical workflow.

To prove that the automated sample preparation leads to reliable results a method verification procedure was evaluated according to the CLSI Guidelines EP06-A, EP15-A3, EP17-A2.

Fig. 1 CLAM LCMS TQ

■ Materials and Methods

Fast, sensitive and robust LC-MS/MS systems provide the basis for routine analysis in clinical laboratories. For the described verification, a Shimadzu CLAM-2040 coupled with a Nexera X3 UHPLC system and a LCMS-8060 triple-quadrupole mass spectrometer was used.

MMA in serum and urine was verified using the RECIPE® ClinMass® LC-MS/MS Complete Kit, advanced Methylmalonic Acid in Serum / Plasma / Urine (order no. MS5100). The ClinCal® Serum Calibrator Set lyophilised, for Methylmalonic Acid (order no. 5013) and ClinChek® Serum Control lyophilised, for Methylmalonic Acid (order no. MS5082) from RECIPE®, were used as serum samples. As urine sample the 3PLUS1® Multilevel Urine Calibrator Set Methylmalonic Acid, lyophilized (order no. 64029) and MassCheck® Methylmalonic Acid Urine Controls, lyophilisiert (order no. 0316) from CHROMSTYSTEMS® were used.

Lyophilized, matrix-based calibrator and control samples were reconstituted, aliquoted and stored until use. Then the samples were loaded directly into the CLAM-2040. It was programmed to perform protein precipitation using Precipitant P including internal standards followed by filtration and sample collection. The sample is then transported using an arm from the CLAM-2040 to the LC without human intervention for LC-MS/MS analysis.

Due to overlapped sample preparation (Figure 2) and analysis the throughput was one complete analysis each 3min. Analytical conditions are listed in Table 1 and 2. The optimized MRM transitions are summarized in Table 3.

Table 1 Analytical conditions

Mass Spectrometer	: LCMS-8060
Ionization	: Electrospray Ionization (ESI), positive
Interface Voltage	: -1.5 kV
Heating Gas	: 10 L/min
DL Temp.	: 200 ˚C
Interface Temp.	: 350 ˚C
Nebulizing Gas	: 3 L/min
Drying Gas	: 5 L/min
Heat Block	: 300 ˚C
CID	: 270 kPa
UHPLC	: Nexera X3
Column Oven	: 40 ˚C
Injection Volume	: 10.0 µL
Flow rate	: 0.6 mL/min
Time Programme	: Binary gradient

Table 2 Analytical conditions (suite)

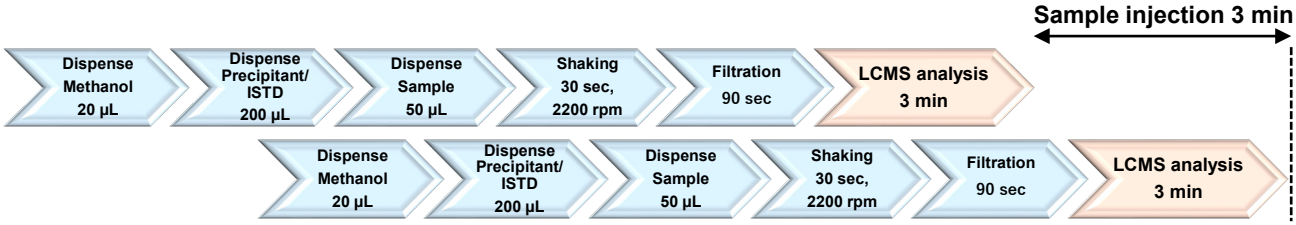
Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
Initial	95	5
0.30	95	5
0.31	70	30
0.60	70	30
0.61	30	70
1.30	30	70
1.31	0	100
1.40	0	100
1.41	95	5
3.00	Stop	

Table 3 MRM transitions and parameters of the analyte and isotope-labelled substance

Analyte / IS	Quantifier MRM		Dwell Time	CE
	Precursor (m/z)	Product (m/z)	msec	(V)
MMA	117.2	73.1	25	12
MMA-d3	120.2	76.1	25	12

Analyte / IS	Qualifier MRM		Dwell Time	CE
	Precursor (m/z)	Product (m/z)	msec	(V)
MMA	117.2	55.1	25	12
MMA-d3	120.2	58.9	25	11

Fig. 2 Scheme fully automated sample preparation and analysis



■ Results

The trueness was determined by 4-fold analysis of two different quality control (QC) samples in a single analysis sequence. The results (precision in CV% and deviation from the target in % Bias) are summarized in Table 4. The acceptance criteria of CV<15% (<20% near LLOQ) and Bias ±20% were fulfilled.

To determine the precision intraday two different levels of QC samples were prepared in 8-fold and analysed in a single analysis sequence. And for the interday precision, the 2 QC samples were prepared in 5-fold and analysed in a single analysis sequence on 3 days. The intra and interassay precision for each level is summarized in Table 5 and 6. The acceptance criteria of CV<15% (<20% near LLOQ) was fulfilled.

For determination of the linearity and the lower limit of quantification (LLOQ) in serum, several dilutions of ClinCal® Serum Calibrator Set lyophil. for Methylmalonic Acid, Level 0 – 3, (order no. MS5013, RECIPE® ,Germany) were prepared to obtain 10 levels in 3-fold and analyzed in a single analysis sequence.

For their determination in urine, several dilutions of 3PLUS1® Multilevel Urine Calibrator Set Methylmalonic Acid, lyophilisiert, (order no. 64029, CHROMSYSTEMS, Germany) were prepared to obtain 9 levels in 3-fold and analyzed in a single analysis sequence.

The results for linearity evaluation and for the LLOQ are summarized in Table 7. The acceptance criteria used to define LLOQ were the precision with a CV<20% and the Bias ±20%. The criteria for linearity were the precision with a CV<15% and the Bias ±15%.

Table 4 Trueness of measurement in serum and urine

Analyte	Sample	Target value (mg/L)	Measured value (mg/L); Mean (n=4)	CV (%)	Bias (%)
Methylmalonic acid	Serum Control Level I	31.9	29.6	2.3	-7.4
	Serum Control Level II	69.5	65.3	1.2	-6.1

Analyte	Sample	Target value (mg/L)	Measured value (mg/L); Mean (n=4)	CV (%)	Bias (%)
Methylmalonic acid	Urine Control Level I	1080	992	1.3	-8.1
	Urine Control Level II	3570	3078	0.9	-13.8

Table 5 Intraassay results [CV%]

Analyte	Sample	Measured value (mg/L); Mean (n=8)	CV (%)
Methylmalonic Acid	Serum Control Level I	29.8	2.8
	Serum Control Level II	67.8	4.9

Analyte	Sample	Measured value (mg/L); Mean (n=8)	CV (%)
Methylmalonic Acid	Urine Control Level I	995	1.4
	Urine Control Level II	3085	1.2

Table 6 Interassay results [CV%]

Analyte	Sample	Measured value (mg/L); Mean (n=5)	CV (%)
Methylmalonic Acid	Serum Control Level I	30.1	3.0
	Serum Control Level II	65.5	2.2

Analyte	Sample	Measured value (mg/L); Mean (n=5)	CV (%)
Methylmalonic Acid	Urine Control Level I	994	2.1
	Urine Control Level II	3054	1.5

Table 7 Linearity evaluation, including LLOQ / LOD and CV and Bias at the LLOQ

Analyte	Matrix	Linear Range (mg/L)	R^2	LLOQ (mg/L)	LOD (mg/L)	CV (%)	Bias (%)
MMA	Serum / Plasma	8.77 - 324	0.999	8.77	2.92	1.9	12.9
MMA	Urine	433 - 4760	0.999	433	144	0.2	6.1

■ Conclusion

The ClinMass® LC-MS/MS Complete Kit, advanced Methylmalonic Acid in Serum / Plasma / Urine (order no. MS5100) was successfully verified on the CLAM-2040 with the analytical system LCMS-8060 from Shimadzu.

Methylmalonic Acid passed the acceptance criteria for accuracy (trueness, precision) and linearity in serum and urine matrices.

The lower limit of quantification (LLOQ) was below published clinical reference ranges.

■ References

1. Instruction Manual, ClinMass® LC-MS/MS Complete Kit, advanced Methylmalonic Acid in Serum / Plasma / Urine, RECIPE® Chemicals + Instruments GmbH

05-SCA-210-081-EN First Edition: Dec. 2024

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