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Application News

Liquid Chromatograph Mass Spectrometer LCMS-8060

Analysis of 25-OH-Vitamin D2/D3 in Plasma/Serum 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, 25-OH-Vitamin D2/D3 in Plasma / Serum

■ Introduction

The term "vitamin D" means a group of steroids that possess antirachitic activity. Clinically relevant members of this group are vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). These vitamins are subsequently metabolised in the liver into 25-OH-vitamin D. And finally, by further hydroxylation in the kidney, the biologically active form of the vitamin (1,25-$(OH)_2$-vitamin D) is formed.

Vitamin D plays an important role for the incorporation of calcium into the bone matrix. Vitamin D deficiencies (hypovitaminosis D) therefore interfere with bone mineralisation. As a result, the bones maintain soft and twist under mechanical stress. The deficiency leads to rickets in children and osteomalacia in adults. In contrast, a vitamin D intoxication (hypervitaminosis D) leads to various symptoms such as nausea, vomiting, diarrhea, headache, polydipsia and polyuria all associated with hypercalcemia.

So, for the assessment of the vitamin D status, the concentration of the primary metabolites (25-OH-vitamin D) is used. The main reason for this is a long metabolite half-life (3 weeks) that gives an indication of vitamin D stores over long periods. In addition, the plasma/serum concentrations of 25-OH-vitamin D are approximately 1000-fold larger than for 1,25-$(OH)_2$-vitamin D.

RECIPE®'s fully validated analytical method provides the quantification of 25-OH-Vitamin D2/D3 (Table 1 and 2) in serum/plasma using LC-MS/MS.[1]. 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 with an APCI source was used.

25-OH-Vitamin D2 and 25-OH-vitamin D3 in serum were verified using the RECIPE® ClinMass® LC-MS/MS Complete Kit, 25-OH-Vitamin D2/D3 in Plasma / Serum (order no. MS7000). The ClinCal® Serum Calibrator Set lyophilised, for 25-OH-Vitamin D2/D3 (order no. 7013) and ClinChek® Serum Control lyophilised, for 25-OH-Vitamin D2/D3 (order no. MS7082) from RECIPE® 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.

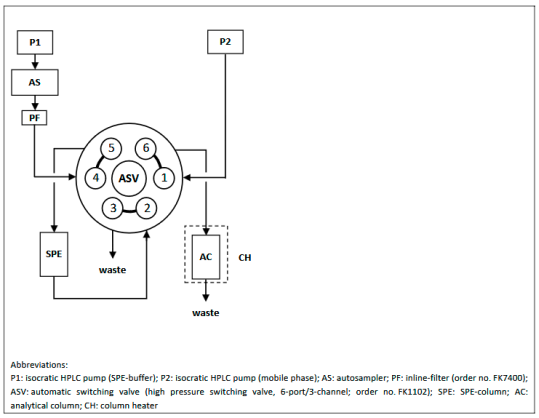
Due to overlapped sample preparation (Figure 2) and analysis the throughput was one complete analysis each 5 min. Analytical conditions are listed in Table 1 and 2. The optimized MRM transitions are summarized in Table 3.

Table 1 Analytical conditions

Mass Spectrometer	: LCMS-8060
Ionization	: Atmospheric pressure chemical ionization (APCI), positive
Interface Voltage	: 5 kV
DL Temp.	: 250 ˚C
Interface Temp.	: 450 ˚C
Nebulizing Gas	: 2 L/min
Drying Gas	: Off
Heat Block	: 250 ˚C
CID	: 270 kPa

UHPLC	: Nexera X3 with SPE on-line
Column Oven	: 40 ˚C
Injection Volume	: 40.0 µL
Flow rate	: 0.5 mL/min
Time Programme	: Binary gradient

Table 2 a) HPLC configuration



b) Gradient

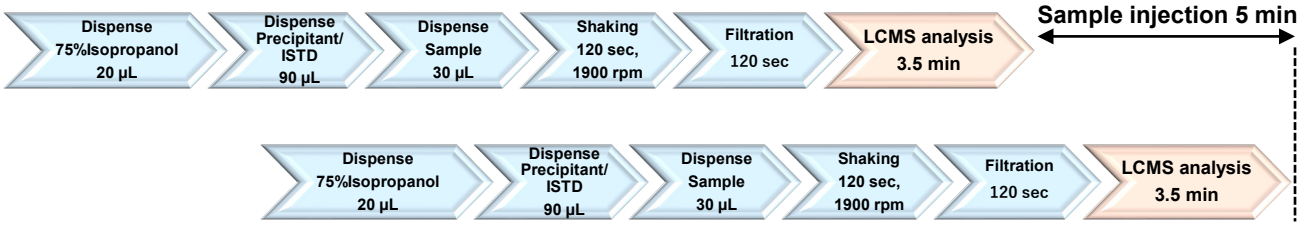
Time (min)	Pump 1 Flow rate (mL/min)	Pump 2 Flow rate (mL/min)	Valve position
Initial	0.1	0.5	load
0.01	5.0		
0.75	5.0	0.5	inject
0.85	0.1		
2.15	0.1		
2.20	2.0	0.5	load
2.85	2.0		load
2.90	0.1		
3.30	0.1		inject

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

Analyte / IS	Qualifier MRM		Dwell Time	CE
	Precursor (m/z)	Product (m/z)	msec	(V)
25-OH-Vitamin D2	395.0	269.1	20	-20
25-OH-Vitamin D3	383.1	211.2	20	-26
d_6-25-OH-Vitamin D3	389.1	211.2	20	-25

Analyte / IS	Quantifier MRM		Dwell Time	CE
	Precursor (m/z)	Product (m/z)	msec	(V)
25-OH-Vitamin D2	395.0	209.1	100	-25
25-OH-Vitamin D3	383.1	257.3	100	-16
d_6-25-OH-Vitamin D3	389.1	263.3	60	-17

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 intraday precision 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 25-OH-Vitamin D2/D3, Level 0 - 3, (order no. MS7013, RECIPE® ,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

Analytes	Sample	Target value (µg/L)	Measured value (µg/L); Mean (n=4)	CV (%)	Bias (%)
25-OH-Vitamin D2	Control Sample Level I	14.8	14.2	4.9	-4.1
	Control Sample Level II	42.7	44.6	3.8	4.4
25-OH-Vitamin D3	Control Sample Level I	20.7	19.8	8.9	-4.6
	Control Sample Level II	44.2	44.5	3.3	0.6

Table 5 Intraassay results [CV%]

Analytes	Sample	Measured value (µg/L); Mean (n=8)	CV (%)
25-OH-Vitamin D2	Control Sample Level I	14.1	5.4
	Control Sample Level II	45.1	5.4
25-OH-Vitamin D3	Control Sample Level I	19.6	6.8
	Control Sample Level II	44.5	4.9

Table 6 Interassay results [CV%]

Analytes	Sample	Measured value (µg/L); Mean (n=5)	CV (%)
25-OH-Vitamin D2	Control Sample Level I	14.0	6.4
	Control Sample Level II	44.0	4.2
25-OH-Vitamin D3	Control Sample Level I	19.7	7.7
	Control Sample Level II	44.6	4.2

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

Analytes	Linear Range (mg/L)	R^2	LLOQ (µg/L)	LOD (µg/L)	CV (%)	Bias (%)
25-OH-Vitamin D2	2.48 - 167	0.997	2.48	0.827	8.4	2.6
25-OH-Vitamin D3	2.41 - 162	0.998	2.41	0.803	12.3	-7

■ Conclusion

The ClinMass® LC-MS/MS Complete Kit, for 25-OH-Vitamin D2/D3 in Plasma / Serum (order no. MS7000) was successfully verified on the CLAM-2040 with the analytical system LCMS-8060 from Shimadzu.

25-OH-Vitamin D2/D3 passed the acceptance criteria for accuracy (trueness, precision) and linearity. The lower limit of quantification (LLOQ) was below published clinical reference ranges.

■ References

1. Instruction Manual, ClinMass® LC-MS/MS Complete Kit 25-OH-Vitamin D2/D3 in Plasma and Serum (on-line, automated on-line) , RECIPE® Chemicals + Instruments GmbH

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