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Application Data Sheet

LC-MS
Liquid Chromatograph Mass Spectrometer

Analysis of 25-OH Vitamin D2 / D3 in Plasma and Serum by LCMS-8040 using ClinRep® LC-MS/MS Complete Kit MS7000

Anja Grüning[1] ,Dr. Johannes Engl[2]
[1] Shimadzu Europe GmbH [2]RECIPE Chemicals + Instruments GmbH

Introduction

Vitamin D measurement has become an important component in clinical assays largely because deficiency is associated with a number of disorders such as rickets, osteomalacia and osteoporosis. When serum concentration falls below 20 ng/mL osteoporosis can result, with normal levels ranging from 20-50 ng/mL. Developments in high pressure fast chromatography LC-MS/MS have now enabled on-line sample preparation methods and analysis times in less than 3 minutes.
In these experiments tandem mass spectrometry was used to quantify 25-OH Vitamin D2 / D3 using a kit containing standard compounds and pre-optimised method parameters.

Materials and methods

The LCMS-8040 triple quadrupole mass spectrometer was coupled to a Nexera UHPLC system. This was equipped with the switching valve FCV-32AH and configured for sample loading onto the column (pump SPE) and isocratic compound elution (pump MP). Compounds were measured using a commercially available test kit ClinMass® LC-MS/MS Complete Kit for 25-OH-Vitamin D2 / D3, MS7000 (RECIPE Chemicals + Instruments GmbH, Dessauerstraße 3, 80992 München, Germany). Chemical standards, control samples, analytical column and mobile phase solvents were provided by the kit. Sample preparation involved taking 50 µL of sample, adding to it 150 µL of precipitant solution (containing internal standard). Following incubation and centrifugation, 50 µL of supernatant was injected for analysis.
The LC-MS instrument was equipped with an atmospheric pressure chemical ionization source (APCI).

Analytical Conditions

UHPLC: Nexera UHPLC
Injection volume: 50 µL
Column temperature: 40° C
Mass spectrometer: LCMS-8040
Source conditions: Interface:350 ° C
Desolvation Line: 200 ° C
Heat Block: 200 ° C
Nebulizer Gas: 4.4 L/min
Drying Gas: 0 L/min
Interface voltage: 4.5 kV
Dwell time: 20 msec
Pause time: 3 msec
Ionization: Atmospheric pressure chemical ionization (APCI), positive mode.
Scan Type: Multiple-reaction-monitoring mode (MRM)

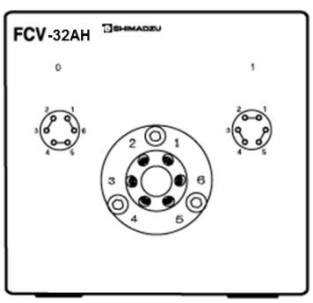

Fig. 1 The FCV-32AH six port valve was configured to switch between sample loading (pump SPE) and isocratic sample elution (pump MP).

Starting conditions:
Pump SPE: 0.1 mL/min
Pump MP: 0.5 mL/min
FCV: position 1

Table 1 LC parameters were chosen for rapid compound elution and fast analysis time. FCV valve position:
1 = sample loading and later column re-conditioning
0 = compound elution.

Time (min)	Pump / FCV FCV	Action	Setting
0.00	FCV	position 1	loading
0.00	Pump SPE	flow rate	0.1 mL/min
0.00	Pump MP	flow rate	0.5 mL/min
0.01	Pump SPE	flow rate	5 mL/min
0.75	FCV	position 0	elution
0.75	Pump SPE	flow rate	5 mL/min
0.85	Pump SPE	flow rate	0.1 mL/min
2.15	Pump SPE	flow rate	0.1 mL/min
2.20	FCV	position 1	conditioning
2.20	Pump SPE	position 1	1.5 mL/min
2.85	Pump SPE	flow rate	1.5 mL/min
2.90	Pump SPE	flow rate	0.1 mL/min
3.00	Pump SPE	flow rate	0. 1 mL/min

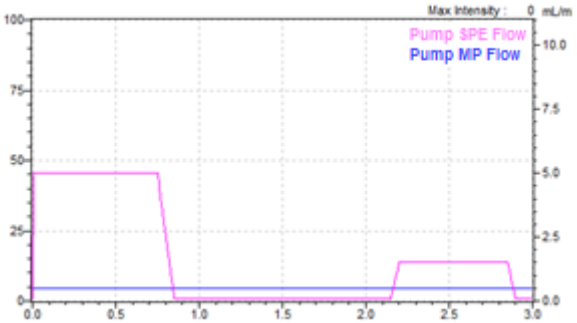

Fig. 2 LC time program during sample loading and elution.

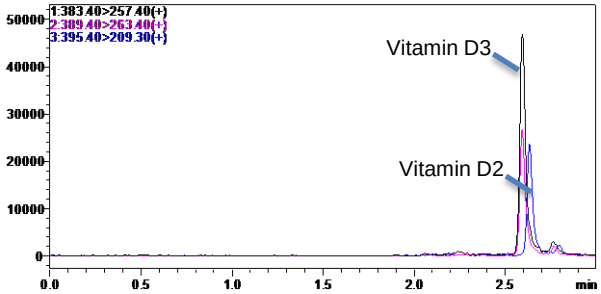

Fig. 3 LC-MS separation of 25-OH Vitamin D2 / D3 and deuterated standard in less than three minutes by isocratic chromatography.

Table 2 25-OH-Vit D optimised MRM transitions, retention times (RT). T/I = target or internal standard.

Compound		Formula	MRM1	MRM2	RT
Vitamin D3	T	C27H44O	383>257	383>211	2.59
Vitamin D2	T	C28H44O	395>269	395>209	2.63
D6-Vitamin D3	I	C27H38D6O	389>263	389>211	2.59

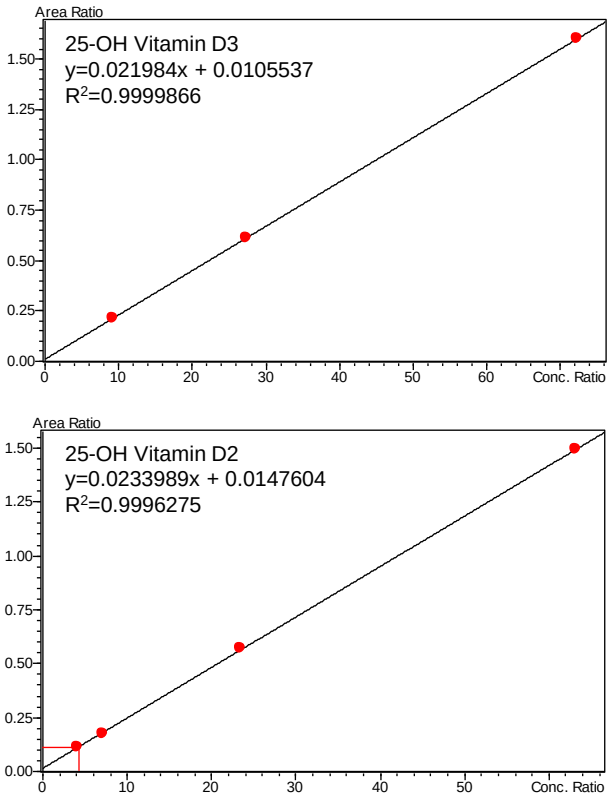

Fig. 4 Calibration curves for 25-OH Vitamin D3 (9.29-72.3 ng/mL) and 25-OH Vitamin D2 (4.07-63.2 ng/mL).

Results

The rapid elution of vitamin D3 and D2 by isocratic chromatography produced excellent peak shape and accuracy with elution in under three minutes (Fig. 3). Calibration curves showed good linearity in the clinically relevant concentration range (Fig. 4). Measurement of three human serum test samples, known to be deficient in vitamin D, were measured at 8.4, 9.6 and 5.5 ng/mL 25-OH vitamin D3 and below the detection limit for 25-OH vitamin D2.

Conclusion

The application of the clinical kit for vitamin D3 & D2 in Serum and Plasma proved simple to implement and successfully confirmed vitamin deficiency in three patient samples.

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