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Analysis of 25-OH Vitamin D2/D3 in Serum by LC-MS/MS with full-automated sample preparation

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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.
LC-MS/MS has become essential tool for monitoring the concentration of Vitamin D2/D3 in biological samples due to its high level of sensitivity and specificity; however, manual sample preparation often involves several complicated steps which can introduce error into the results. Additionally, the time consuming nature of the sample preparation and the large number of samples makes LC-MS/MS a less desirable method. Automated sample preparation has been shown to eliminate human error, as well as increase laboratory efficiency, making LC-MS/MS a feasible method to incorporate in the clinic.

In this study, we investigated the ability to analyze for 25-OH Vitamin D2/D3 by LC-MS/MS (LCMS-8050, Shimadzu) using automated sample preparation (CLAM-2000, "For research use only. Not for use in clinical diagnostics" Shimadzu) to process large sample sets (Fig. 1). The CLAM-2000 has the ability to perform a variety of steps appropriate for automated sample preparation by LC-MS/MS including precipitation, filtration, heating, shaking, and pipetting. This system is seamlessly integrated with the LC-MS/MS system requiring no human involvement after loading the biological samples into the sample chamber. We validated the automated method by using a kit containing standard compounds.

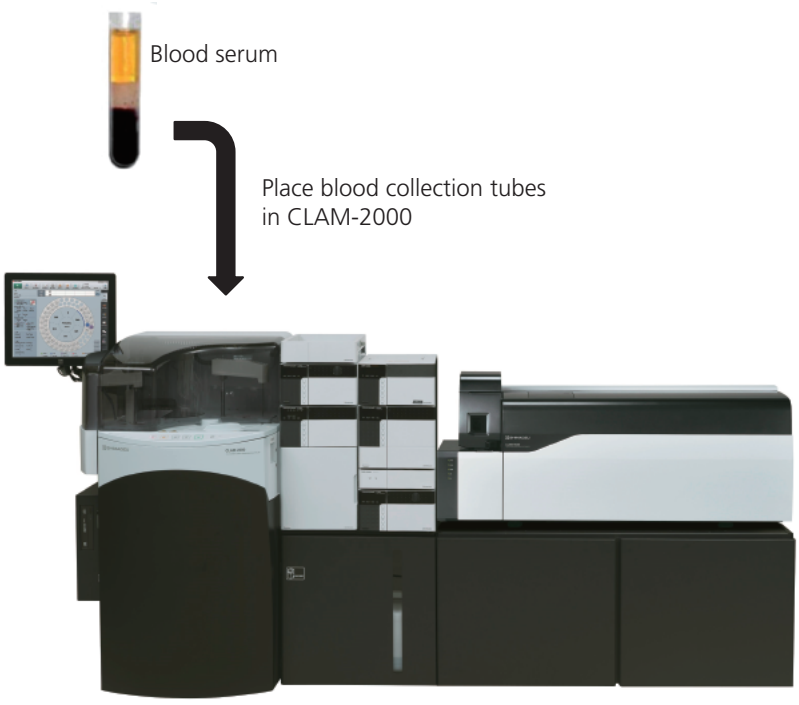

Fig. 1 CLAM-2000 and LCMS-8050 system

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Method

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). Calibrators, control samples, analytical column and mobile phase solvents were provided by the kit. These calibrators and controls were loaded directly into the CLAM-2000 for sample processing.
The CLAM-2000 was programmed to perform protein precipitation using precipitant solution followed by filtration and sample collection. Sample preparation involved taking 30 µL of sample, adding to it 90 µL of precipitant solution (containing internal standard). Following filtration, the filtrated sample is then transported using an arm from the CLAM-2000 to the HPLC for LC-MS/MS analysis and no human intervention was required (Fig. 2). The LC-MS/MS instrument was equipped with an atmospheric pressure chemical ionization source (APCI).

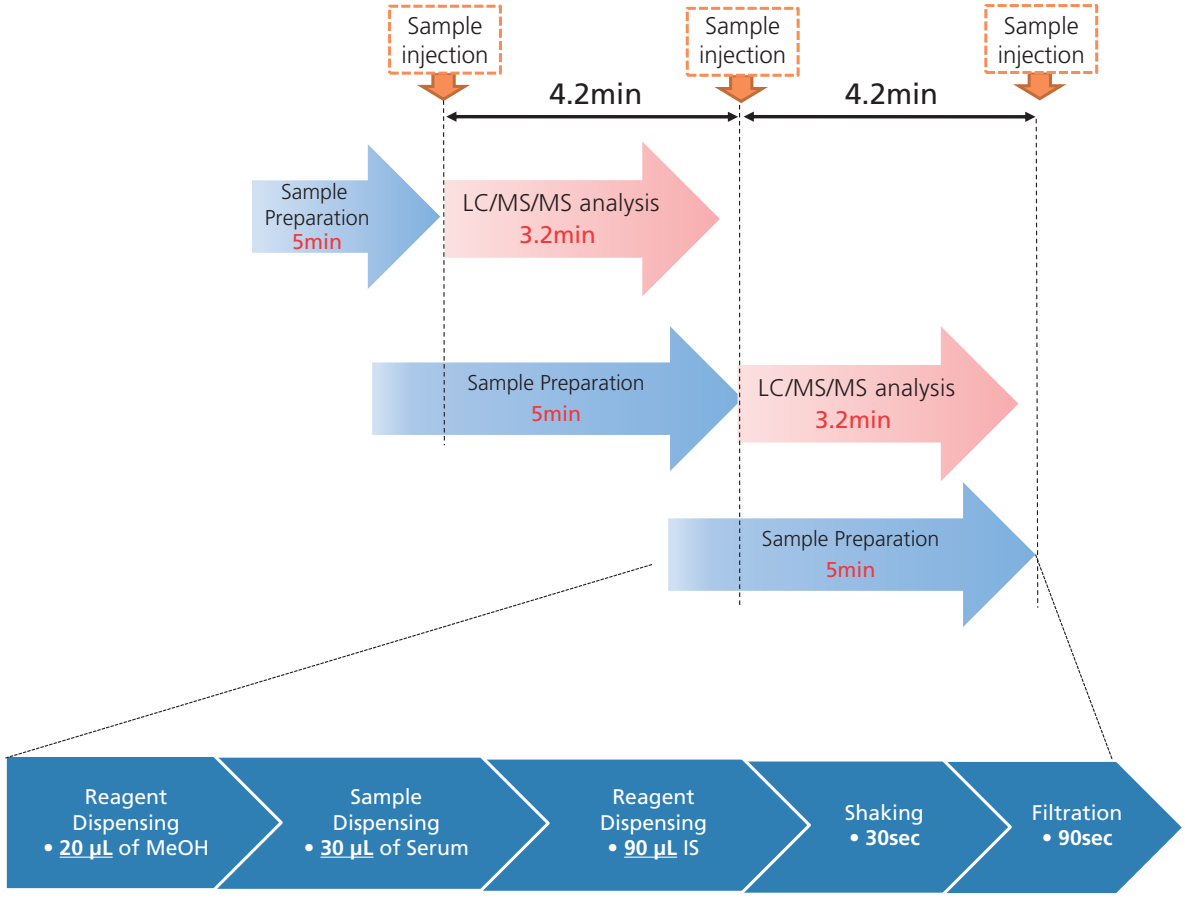

Fig. 2 Analytical Flow with Parallel Processing

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Table 1 Analytical Condition

[LC] NexeraX2 System	
Column Temp.	: 40 °C
Injection Volume	: 30 µL
[MS] LCMS-8050	
Ionization	: APCI Positive
Source conditions	:
Nebulizer Gas	: 1.5 L/min
Interface temperature	: 375 °C
Desolvation Line	: 225 °C
Heat Block temperature	: 250 °C
Drying Gas	: Off
Scan Type	: MRM

Result and discussion

Linearity, Precision

The calibration curves showed good linearity (R^2>0.999) over a clinical relevant range of 4.10 - 68.5 µg/L for 25-OH Vitamin D2 and 4.68 - 77.3µg/L for 25-OH Vitamin D3 (Fig 3). The reproducibility (N=7) at three concentrations, including LLOQ, of each compounds was excellent (CV<6.5%). Different day reproducibility (N=7) for 3 days at three concentrations as well (CV<7.2%) (Table 2).

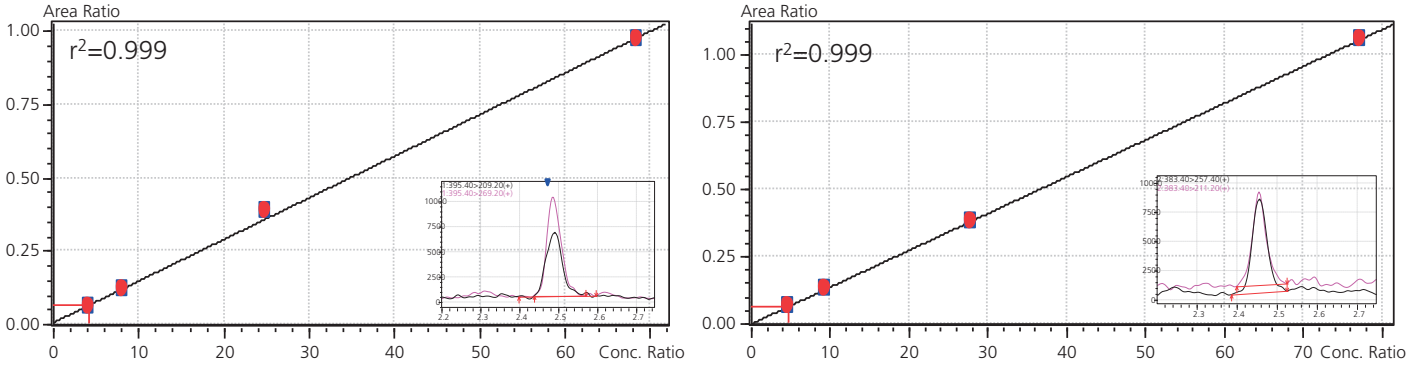

Fig. 3 Calibration Curves (L1-L4) and MRM Chromatograms (L1)

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Table 2 Intra-assay and Inter-assay Precision (* n=7 ; ** 3 days)

25-OH Vitamin D2

CV%	LOW (5.7µg/L)	Medium (17.1µg/L)	High (40.0µg/L)
Intra-assay*	4.4%	2.3%	1.1%
Inter-assay**	5.2%	4.7%	3.7%

25-OH Vitamin D3

CV%	LOW (6.3µg/L)	Medium (18.9µg/L)	High (43.0µg/L)
Intra-assay*	2.9%	5.1%	2.4%
Inter-assay**	5.1%	5.3%	5.2%

Data correlation with Manual method

Comparison of 25-OH Vitamin D3 concentration between manual sample preparation (following Recipe specifications) and automated sample preparation shows good agreement as highlighted by Passing and Bablok plot and scores (Fig. 4).

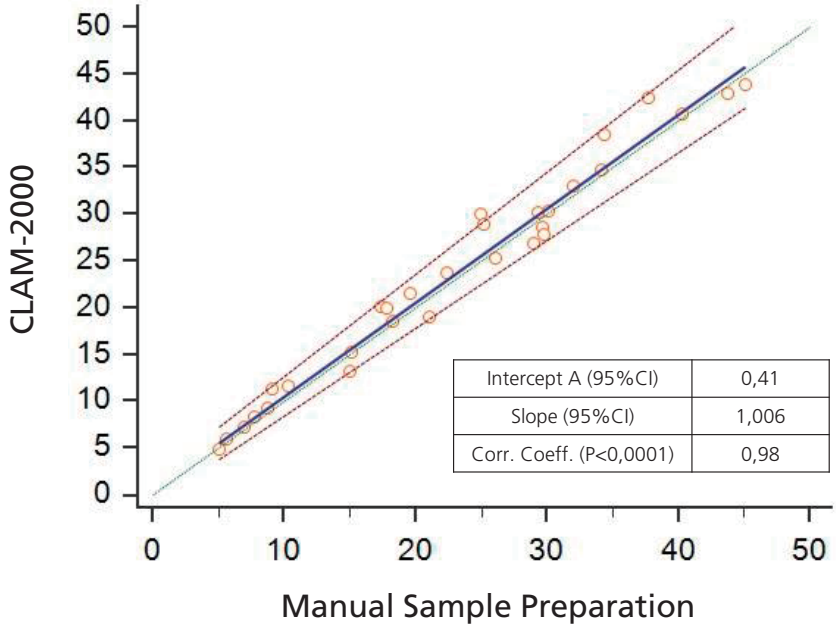

Fig. 4 Data correlation between Automatic sample preparation (CLAM-2000) and Manual method (n=30 human serum samples + 2 reference material, Recipe)

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Conclusion

- Fully Automated sample preparation procedure resulted suitable for the quantitation of 25-OH Vitamin D by elimination of all manual preparation steps.
- The automation of the method increases the analytical performance, reduces the risk for human operators and, due to the reduced reagent consumption, reduces also the cost of the analysis.

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