Development of a Simple and Rapid LC-MS/MS Method for Quantitative Determination of Vitamin A and Vitamin D in Serum

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Vitamin A and D are essential micronutrients that support numerous physiological functions. Vitamin D plays a key role in promoting the absorption of calcium and phosphorus, which are critical for the development and maintenance of healthy bones and teeth. It helps prevent bone-related disorders such as osteoporosis, especially in older adults. Furthermore, vitamin D is known to modulate immune system activity and has been linked to anti-inflammatory effects in various clinical studies.

Vitamin A, is essential for maintaining visual function, particularly under low-light conditions, and helps prevent night blindness. It also contributes to skin tissue repair, promotes healthy skin, reduces the incidence of acne, and plays a role in supporting immune responses by enhancing the body's defense against infections. Given their vital functions, accurate monitoring of serum Vitamin A and Vitamin D levels is important for clinical assessment and early detection of potential deficiencies or imbalances.

In this study, we established a method for the quantitative analysis of Vitamin D, specifically 25-(OH) D2 and D3 and Vitamin A in human serum using the Liquid Chromatography - Mass Spectrometry (LC-MS/MS), an advanced analytical technique renowned for its high sensitivity and specificity. The developed method delivers accurate, precise, and reproducible results, thereby improving the reliability and efficiency of clinical diagnostics.

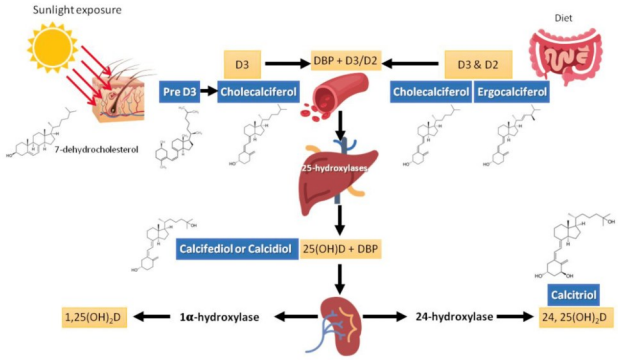

Figure 1 The Mechanism of Vitamin D in the Body and the Measurement of Vitamin D in the Form of 25-(OH)D2/D3

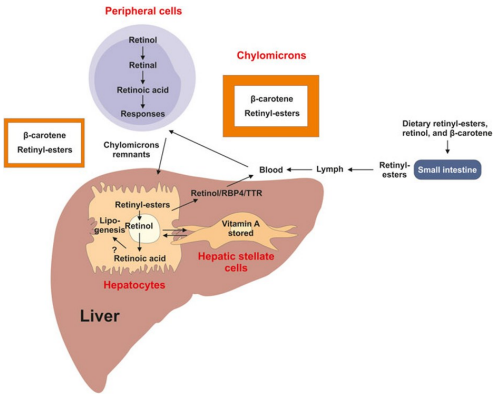

Figure 2. Mechanism of Vitamin A Metabolism in the Human Body and Its Quantification in Serum as Retinol.

Desorption, Clean-up and LC Conditions

Analytical Column	Accucore™ C18 (2.1x100mm) 2.6μm
Column temperature	40 °C
Mobile phase	A: 0.1% Formic acid in H2O B: 0.1% Formic acid in Methanol
Data acquisition mode	Selected ion monitoring (SRM)

MS Conditions

Ion source type	APCI
vaporizer temperature	350°C
Ion Transfer tube Temp	325°C
Spray voltage	4800 V
Sheath gas and auxiliary	45 and 5
Collision gas (argon) pressure	1.5 mTorr.

SRM transition and Collision Energy of Compound

Compound	Precursor (m/z)	Product (m/z)	CE(V)
25-Hydroxyvitamin D2	395.3	269.2	19
		377.3	14
25-Hydroxyvitamin D3	383.2	257.2	13
		365.3	12
Retinol	269.2	83.0	15
		93.0	15
25-Hydroxyvitamin D3-d6 (ISTD)	389.3	263.3	11

Sample preparation

Sample preparation was carried out using a protein precipitation technique. A 100 µL aliquot of serum was spiked with 5 µL of internal standard solution, followed by the addition of 40 µL of a protein-precipitating reagent. The mixture was vortexed thoroughly to ensure complete mixing, then centrifuged at 4,000 g for 10 minutes at room temperature. After centrifugation, 100 µL of the clear supernatant was carefully transferred into a sample vial. A 5 µL portion of this prepared extract was injected into the LC-MS system for analysis.

(A) Retinol at **5.45 minutes**,

(B) 25-Hydroxyvitamin D2 at **5.31 minutes**,

(C) 25-Hydroxyvitamin D3 at **5.27 minutes**, and

(D) Internal standard (25-Hydroxyvitamin D3-d6) at **5.26 minutes**.

sample 100 µl + Internal standard 10 µl + protein precipitant 40 µl

↓

Vortex

↓

Centrifuge at 4,000 g for 10 minutes

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Aliquot 100 µl into vial

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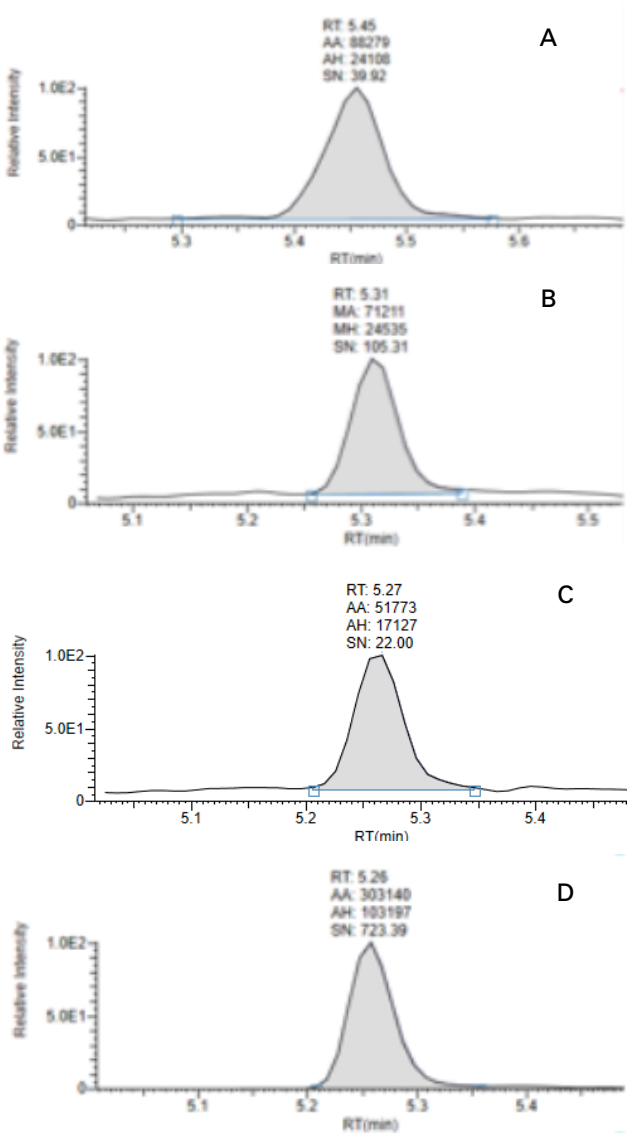
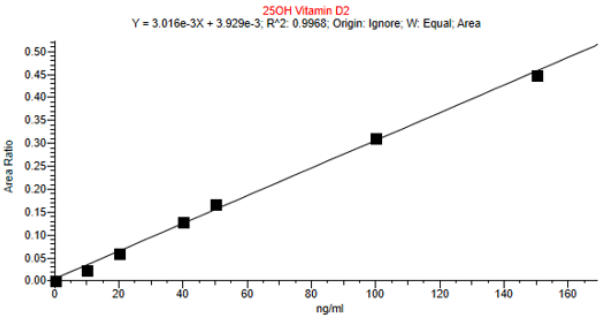
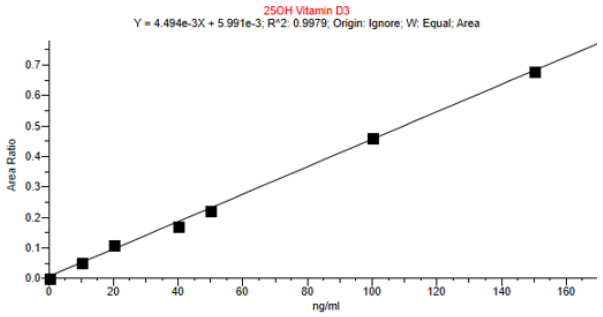
Inject into LC-MS system 5 µl

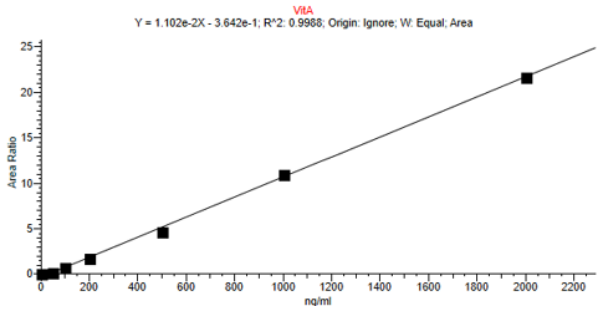
Results : Chromatogram



Results : Calibration Curve







Acknowledgements

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Conclusion

An in-house method for the simultaneous quantification of Vitamin A and Vitamin D in human serum was successfully developed using liquid chromatography–mass spectrometry (LC-MS). This approach offers a cost-effective solution with the capability to measure three analytes in a single run. The method requires only 100 µL of serum and employs a simple and rapid protein precipitation protocol for sample preparation. With a total run time of just 6 minutes, the method enhances analytical throughput, offering high efficiency, speed, and convenience.