

## Vitamin D Analysis Using the ACQUITY UPLC H-Class PLUS System

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### GOAL

Successful analysis of vitamin D in foods using an ACQUITY™ UPLC™ H-Class PLUS System with Xevo™ TQ-S micro.

### BACKGROUND

Vitamin D is a fat-soluble vitamin that promotes calcium absorption and maintains adequate serum calcium and phosphate concentrations to enable normal mineralization of bone.<sup>1</sup> The most common vitamin Ds are ergocalciferol (vitamin D<sub>2</sub>) and cholecalciferol (vitamin D<sub>3</sub>). Small amount of vitamin Ds (D<sub>2</sub> and D<sub>3</sub>) may exist as previtamin Ds, which are also bioactive. The previtamin Ds and vitamin Ds can reversibly isomerize to each other. Recently, the U.S. Food and Drug Administration (FDA) revised the food labeling regulations and required vitamin D content on the nutrition or supplement facts labels for all conventional food and dietary supplements products.<sup>2</sup> The change in labeling regulation is aimed to promote vitamin D awareness among consumers.

New vitamin D analysis method has been developed on an ACQUITY UPLC H-Class System coupled with Xevo TQ-S micro Mass Spectrometer.<sup>3</sup> In this new method,

The ACQUITY UPLC H-Class PLUS System with Xevo TQ-S micro is a high performance and reliable analytical system for accurate analysis of vitamin D in foods.

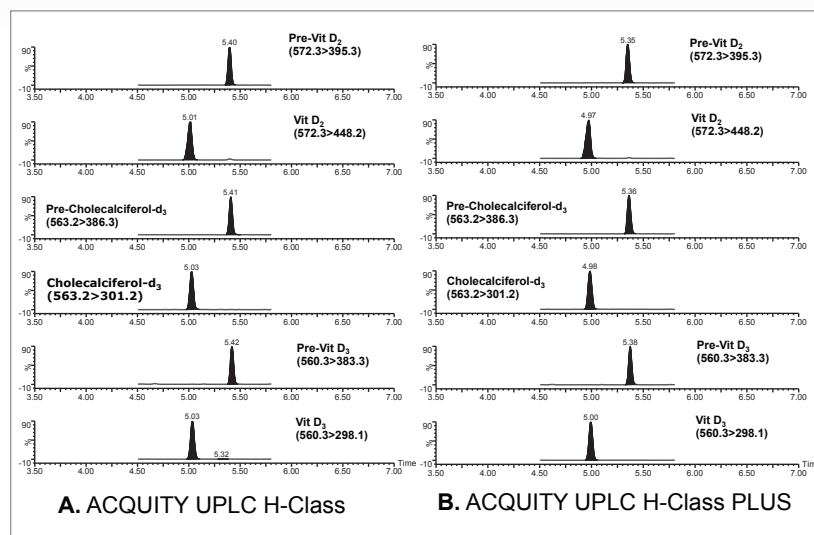


Figure 1. Chromatograms of vitamin D standards acquired on (A) ACQUITY UPLC H-Class and (B) ACQUITY UPLC H-Class PLUS.

the previtamin Ds and vitamin Ds are individually determined, which is an improvement to the existing vitamin D method<sup>4</sup> in which only the vitamin Ds, not the previtamin Ds, are determined. In this technology brief, we demonstrate that the ACQUITY UPLC H-Class PLUS System performs equivalently to the original ACQUITY UPLC H-Class System in providing a robust and reliable solution for the accurate analysis of vitamin Ds in food.

### THE SOLUTION

The ACQUITY UPLC H-Class PLUS System with Xevo TQ-S micro provides a quick and reliable analytical approach for accurate measurement of vitamin Ds and previtamin Ds in foods. The detection of previtamin Ds eliminates a major source of error when the situation occurs that non-negligible amounts

ACQUITY UPLC H-Class					
	Number of injections	RT (avg.)	RT (% RSD)	N. theoretical plate (avg.)	N. theoretical plate (% RSD)
Vitamin D <sub>3</sub>	6	5.037	0.07%	66120	3.5%
Pre-Vit D <sub>3</sub>	6	5.422	0.06%	96830	1.6%
Cholecalciferol-d <sub>3</sub>	6	5.029	0.00%	68578	2.8%
pre-Cholecalciferol-d <sub>3</sub>	6	5.411	0.06%	97538	3.2%
Vitamin D <sub>2</sub>	6	5.013	0.04%	61767	5.5%
Pre-Vit D <sub>2</sub>	6	5.400	0.06%	93657	2.8%

ACQUITY UPLC H-Class PLUS					
	Number of injections	RT (avg.)	RT (% RSD)	N. theoretical plate (avg.)	N. theoretical plate (% RSD)
Vitamin D <sub>3</sub>	6	4.994	0.06%	68001	2.3%
Pre-Vit D <sub>3</sub>	6	5.375	0.00%	95119	1.9%
Cholecalciferol-d <sub>3</sub>	6	4.985	0.05%	69031	4.7%
pre-Cholecalciferol-d <sub>3</sub>	6	5.364	0.00%	93302	3.0%
Vitamin D <sub>2</sub>	6	4.972	0.04%	52147	9.1%
Pre-Vit D <sub>2</sub>	6	5.350	0.06%	90478	2.6%

Table 1. Chromatographic retention times and column efficiencies of vitamin D standards acquired on an ACQUITY UPLC-H Class and an UPLC H-Class PLUS System.

of vitamin D<sub>3</sub> thermally isomerizes to previtamin D<sub>3</sub> during the manufacturing, transportation, or storage of food products.

The vitamin D standards (cholecalciferol, ergocalciferol, the stable isotope labeled cholecalciferol-d<sub>3</sub>, and their previtamin forms) were analyzed on both the ACQUITY UPLC H-Class and the ACQUITY UPLC H-Class PLUS System. As displayed in Figure 1, the chromatographic separation acquired on both systems was comparable. The repeatability of the retention time (RT) and the column efficiency (number of theoretical plate) for six replicated injections are shown in Table 1. The relative standard deviations (% RSD) in the RT and in the number of theoretical plate are less than 0.06% and 9.1%, respectively with the ACQUITY UPLC H-Class PLUS System, and less than 0.07% and 5.5%, respectively with the ACQUITY UPLC H-Class System. These RSD values from

both systems are comparable. The RTs with the H-Class PLUS System are on average 0.045 min shorter than those with the H-Class. This small difference in RT does not affect the measurement of vitamin D<sub>3</sub>.

## SUMMARY

The ACQUITY UPLC H-Class PLUS System and the Xevo TQ-S micro Mass Spectrometer provide a robust and reliable analytical system for the vitamin D analysis in foods. The ACQUITY UPLC H-Class PLUS System performance is equivalent to the ACQUITY UPLC H-Class System in terms of reproducibility in RT and column efficiency. This ensures accurate and reliable determination of vitamin D<sub>3</sub> in foods on the ACQUITY UPLC H-Class PLUS System coupled with the Xevo TQ-S micro Mass Spectrometer.

## References:

1. Vitamin D Fact Sheet for Health Professionals, National Institutes of Health. Website: <https://ods.od.nih.gov/factsheets/VitaminD-HealthProfessional/> visited May 1, 2018
2. Food labeling: Revision of the nutritional and supplement facts labels, FDA, Federal Register/Vol. 81, No. 103/Friday, May 27, 2016/Rules and Regulations.
3. Determination of vitamin D and previtamin D in food products, Waters application note [720006064en](#), July 2017, Waters corporation, 34 Maple Street, Milford, MA, USA.
4. AOAC Official Method 2016.05, Official Methods of Analysis of AOAC INTERNATIONAL (2016) 20th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA.

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