LC-MS/MS Study of 25-OH Vitamin D2 and D3 with Perkin Elmer Vitamin D kit Using both Derivatized and Non-derivatized Methods

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Background: The major physiological function of Vitamin D is to maintain blood calcium and phosphorus levels within the normal range, and maintain metabolic functions that are essential for most life processes. Vitamin D exists in two forms, vitamin D2 and D3. 25-OHase enzyme converts Vitamins D2 and D3 to 25-OH Vitamin D in the liver. Quantification of these metabolites is widely used as a means of assessing vitamin D deficiency status because of their clinical significance in a variety of disorders, which lead to alterations in the concentrations of circulating hormones. Measurement of serum concentrations of 25-OH vitamin D2 and D3 have been performed for differential diagnosis of hypo- and hypercalcemic disorders in metabolic bone disorders [1]. LC-MS/MS technology has demonstrated superior sensitivity, selectivity, and robustness for simultaneously detecting these Vitamin D metabolites in complex biological matrices such as serum or plasma. This work presents a fast, reliable, and accurate LC-MS/MS method on an IONICS 3Q 120 triple quadrupole mass spectrometer for studying 25-OH Vitamin D2 and D3 for research purposes with Perkin Elmer Vitamin D kit using both derivatized and non-derivatized methods.

Methods: The Perkin Elmer Vitamin D kit is intended for quantitative determination of 25-OH Vitamin D2 and D3 in human serum and plasma samples. This kit uses a combined solvent extraction and protein precipitation method to isolate 25-OH Vitamin D2 and D3 from serum and plasma samples. It can be used in two alternative ways; non-derivatized, or derivatized. The sample preparation procedure was followed as per instructions included with the kit. The calibrators are isotope labeled as $^{2}H_{6}$-25-OH Vitamin D2 and $^{2}H_{6}$-25-OH Vitamin D3. The calibrators have 6 levels ranging from 5.4 to 164.5 ng/mL for $^{2}H_{6}$-25-OH Vitamin D2 and 4.6 to 139 ng/mL for $^{2}H_{6}$-25-OH Vitamin D3, respectively. Three levels of QC standards were provided, 10, 42.3, and 87 ng/mL for $^{2}H_{6}$-25-OH Vitamin D2, and 8.7, 35.8, and 73.6 ng/mL for $^{2}H_{6}$-25-OH Vitamin D3, respectively. The IONICS 3Q 120 mass spectrometer was equipped with a heated coaxial flow ion source and “Hot Source-Induce Desolvation” interface for the best

ionization and sampling efficiencies. Electrospray ionization was used for this analysis. A Shimadzu Prominence XR UFLC system was used. The column was from Kinetex (C18, 100 x 2.1 mm, 1.7 um). The injection volume was 10 □L. A gradient method was created with a flow rate of 0.3 mL/min and a total LC cycle time of 4.5 minutes.

Results:

In a 4.5-minute LC run, good chromatogram peak shapes were obtained for both $^{2}H_{6}$-25-OH Vitamin D2 and D3. No carryover was detected in a blank injection immediately following the upper level calibration sample. All calibration curves used a linear weighting regression of 1/x. The calibration curves showed good linearity with a coefficient R^2>0.993 for both $^{2}H_{6}$-25-OH Vitamin D2 and D3 with non-derivatized and derivatized methods (Figure 1), respectively. At the lowest levels for both $^{2}H_{6}$-25-OH Vitamin D2 and D3, the accuracies were between 97-102% and CVs were < 10%. For the lowest level in the kit, the S/N ratios of $^{2}H_{6}$-25-OH Vitamin D2 were about 70 and 110 for non-derivatized and derivatized, respectively; the S/N ratios of $^{2}H_{6}$-25-OH Vitamin D3 were about 40 and 92 for non-derivatized and derivatized (Figure 2), respectively. For $^{2}H_{6}$-25-OH Vitamin D2 and D3 in the QC samples with both non-derivatized and derivatized analysis, the accuracies were between 93-109% and the CVs were < 9%.

Conclusion:

A rapid, accurate, and reproducible LC-MS/MS research method was developed on IONICS 3Q 120 mass spectrometer for evaluating the Perkin Elmer Vitamin D kit. The results in this study demonstrated that using a simple protein precipitation sample preparation method for both non-derivatized and derivatized samples, good quantitation results were obtained. Calibration curves showed good linearity with coefficients R^2>0.993. At the lowest levels, the accuracies were between 97-102% and CVs were < 10%. The S/N ratio results at the lowest levels also indicate that the expected LLOQs for $^{2}H_{6}$-25-OH Vitamin D2 and D3 would be at least 4 times lower (~ 1 ng/mL) for non-derivatized and about 10 times lower (~ 0.5 ng/mL) for derivatized methods. Therefore, this LC-MS/MS method with IONICS 3Q 120 mass spectrometer is capable to provide high enough sensitivity, accuracy and reproducibility for quantifying 25-OH Vitamin D2 and D3 with Perkin Elmer Vitamin D kit.

1. M.F. Holick, W.O.Song, G.R. Beecher, R.R.Eitenmiller, Modern Analytical Methodoliges in Fat and water soluble Vitamins, John Wiley & Sons, Inc. New York, P.51.

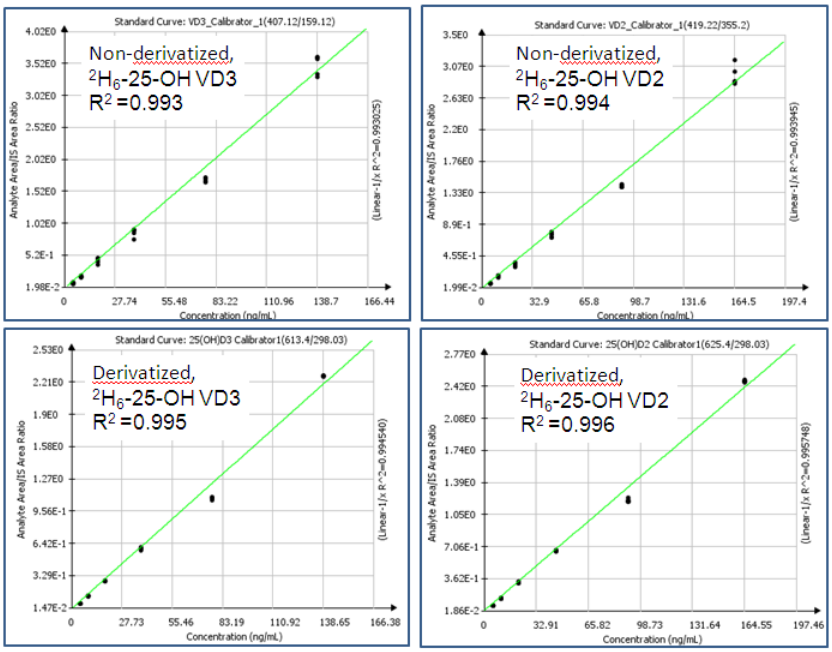

Figure 1. Calibration curves for both $^2H_6$-25-OH VD2 and VD3 with non-derivatized and derivatized analysis. Good linearity was obtained with R^2>0.993.

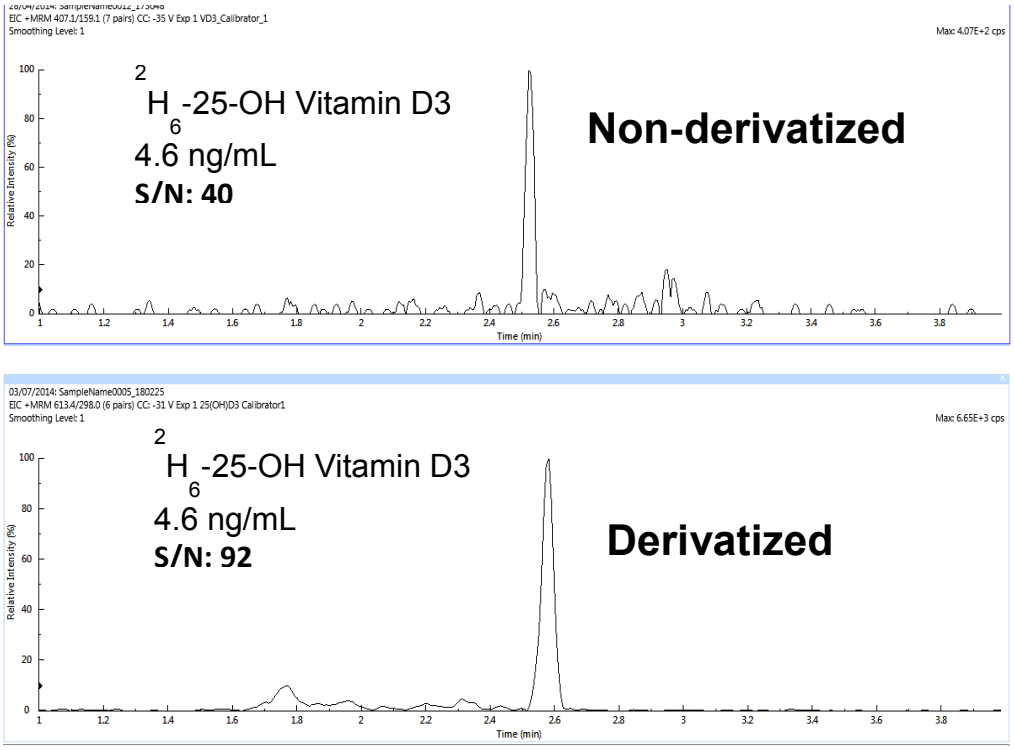

Figure 2. Examples of chromatogram for $^2H_6$-25-OH Vitamin D3 with non-derivatized and derivatized analysis at the lowest level (4.6 ng/mL) in the Perkin Elmer kit.