A Five-Minute Analysis that Separates 25-hydroxyvitamin D from its C3 Epimer

Katherine Rogers[1], Lisa Calton[2], Robert Wardle[2], Joe M. El-Khoury[1,3]

[1]Yale-New Haven Hospital, 55 Park Street, New Haven, CT 06510
[2]Waters Corporation, 34 Maple Street, Milford, MA 01757
[3]Yale University School of Medicine, 20 York Street, New Haven, CT 06510

Vitamin D, the sunshine vitamin, exists in two biologically active forms, vitamin D2 and vitamin D3. Measurement of its metabolite, 25-hydroxyvitamin D (25OHD) in serum/plasma is useful for the assessment of a patient's vitamin D status. For accurate assessment of a patient's vitamin D status both 25OHD3 and 25OHD2 must be measured equally. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is regarded as the "gold standard" for measurement of 25OHD. However, LC-MS/MS methods are susceptible to interference from the C3 epimer of 25OHD3, which has been found to be present in adults as well as infants and may lead to falsely overestimating a patient's vitamin D status. A few LC-MS/MS methods have been developed to separate this interference, but these methods suffer from long chromatography and/or sample preparation, making them less useful for routine clinical use. We have developed and fully validated a simple, fast (5 min) and interference-free liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to measure 25OHD2 and 25OHD3 in blood.

Sample preparation involved protein precipitation followed by solid-phase extraction. Liquid chromatography (LC) was performed on a Waters I-Class UPLC system. Total chromatography run time was 5 min. Tandem mass spectrometry was performed on a Waters Xevo-TQD. Two transitions are monitored for each analyte and the ion ratio is used as an additional quality metric.

Chromatograms showing 25OHD2, 25OHD3, C3-epi-25OHD3, and their isotope-replaced internal standard are displayed in Figure 1.

The method is free from ion suppression, carryover and interference in both matrices. The assay is linear from 4 to 311 ng/mL and 5 to 311 ng/mL for 25OHD2 and 25OHD3, respectively. Recovery was evaluated by measuring spiked vitamin D-stripped serum diluted to 9 different levels and ranged from 90% to 105% and 98% to 110% for 25OHD2 and 25OHD3, respectively. Precision was evaluated by repeatedly measuring 3 different levels twice a day for 5 days using spiked patient samples. Total CV was less than 7.2% for both analytes. Method comparison (n=52) was performed with an LC-MS/MS method from a reference laboratory. The methods showed excellent agreement. NIST SRM 972 was also measured to assess accuracy and our method showed excellent agreement with assigned values.

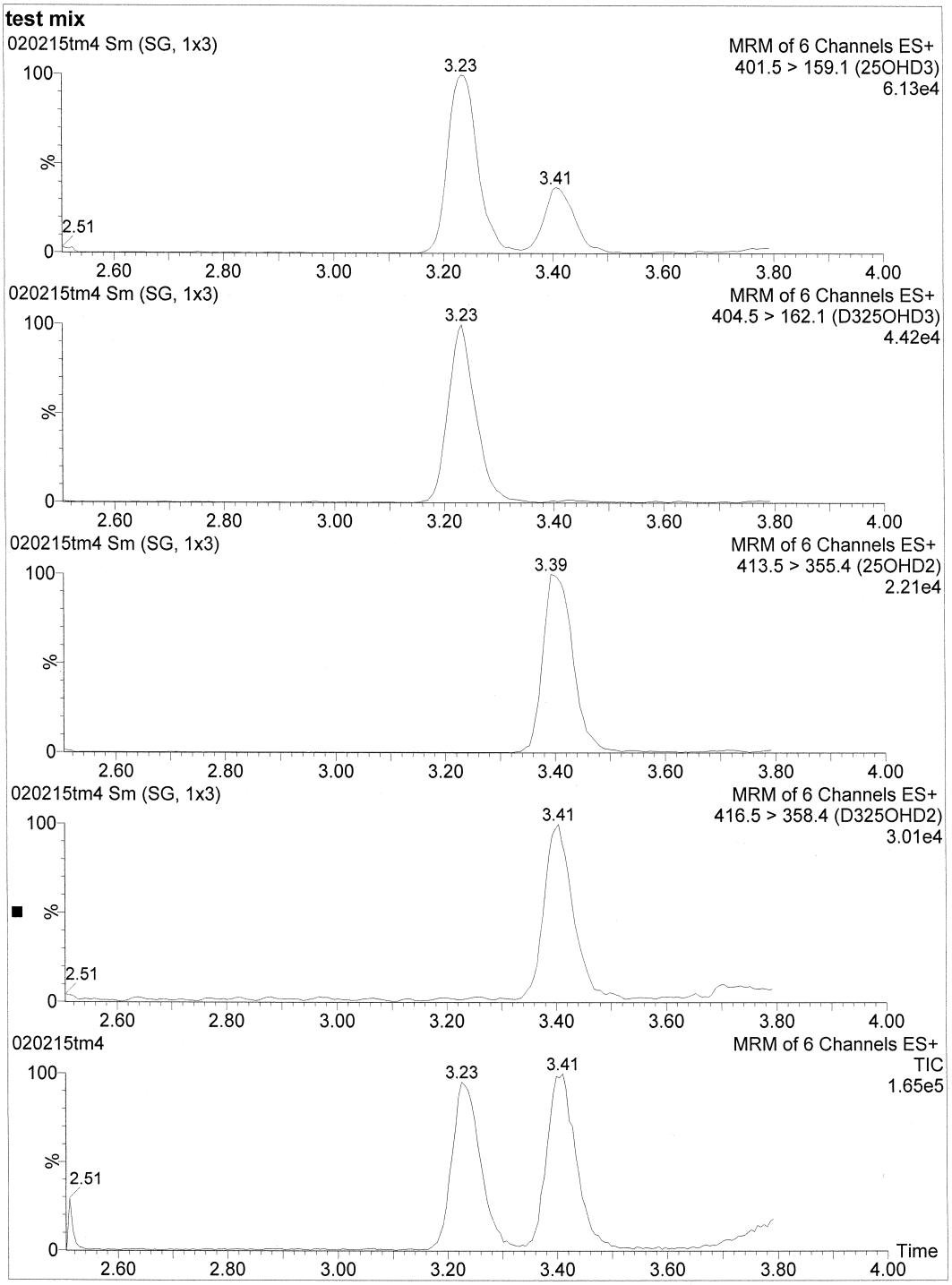

Figure 1: Representative method chromatograms showing from top to bottom: 1) 25OHD3 (RT: 3.23 min, 30 ng/mL), 2) D3-25OHD3, 3) 25OHD2 (30 ng/mL), 4) D3-25OHD2 and 5) Total ion chromatogram. The peak at RT 3.41 min in top panel represents the C3-epi-25OHD3 (10 ng/mL).