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Comprehensive analysis of vitamin D analogues: Rapid LC-MS/MS method for quantification of eight metabolites and two epimers

Iltaf Shah
Kingston University, UK

Abstract

Despite numerous studies, little progress has been made in associating vitamin D levels or supplementation with the many diseases to which it is linked. One reason may be that current assays are ineffective and focus on a minority of metabolites. The aim of this study was to develop a comprehensive LC-MS/MS assay to rapidly quantify ten forms of vitamin D.

The assay uses dynamic multiple reaction-monitoring (Dyn-MRM) technology to facilitate rapid analysis of 10 analogues of vitamin D with limits of detection and ranges (ng/mL) as follows: Vitamin D2 [0.019, 0.2-39.7], Vitamin D3 [0.019, 0.2-38.5], 3-epi-25OHD2 [0.02, 0.04-40.2], 3-epi-25OHD3 [0.021, 0.04-41.3], 24R,25$(OH)_2$D3 [0.021, 0.04-41.7], 25OHD2 [0.010, 0.2-41.3], 25OHD3 [0.010, 0.2-40.2], 1,α25$(OH)_2$D2 [0.004, 0.006-0.416], 1α25$(OH)_2$D3 [0.020, 0.04-40.2] and 23R,25$(OH)_2$D3 [0.004, 0.006-0.42]. The accuracy of the assay was assessed with Chromsystem and the vitamin D external quality assessment scheme (DEQAS) and it was applied to 20 healthy samples from Seralab. The results matched those of the DEQAS samples for key vitamin D analytes, whilst providing quantitative data for the additional vitamin D forms. The total run time of the analysis with the new assay is less than 7 minutes. In conclusion, this assay is the first to quantify ten forms of vitamin D simultaneously in human sera including the 23R,25$(OH)_2$D3 form. The significant improvement in run time, coupled with a single extraction protocol, makes this rapid assay more efficient for clinical use. This assay will be instrumental in future research fully exploring the roles of all forms of vitamin D in vivo.

Biography

Iltaf Shah has completed his PhD and postdoctoral studies from Kingston University School of Pharmacy and Chemistry. He is a lecturer and Research Scientist at Kingston University London. He has published more than 22 papers in reputed journals and scientific conferences and he has teaching and research interest in the analysis of vitamins, drugs of abuse and antioxidants in human body matrices using HPLC, GCMS and LC-MS/MS.