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Simultaneous determination of vitamin D-2, D-3 and their 25-hydroxy metabolites in human plasma by ultra-performance liquid chromatography

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A simple, precise and rapid ultra-performance liquid chromatography (UPLC) method for simultaneous determination of vitamin D-2 (VD-2), vitamin D-3 (VD-3), 25-hydroxyvitamin D-2 [25 (OH) VD-2], and 25-hydroxyvitamin D-3 [25 (OH) VD-3] in human plasma was developed and validated. Using n-dodecanophenone as an internal standard (IS), separation was achieved on Acquity UPLC C18 column. The mobile phase (gradient elution mode) consists of methanol, acetonitrile and water (pH=3.0); the eluents were monitored by photodiode array detector wavelength set at 265 nm. Plasma samples were deproteinized with a mixture of methanol and 2-proponol, then extracted with hexane. After evaporation, the residue was dissolved in methanol: Water (50: 50, v/v), centrifuged and then clear solution was injected on the system. The relationship between the concentration of VD-2, VD-3, 25 (OH) VD-2, 25 (OH) VD-3 in plasma and their peak area ratio to the IS were linear over the range of 2.5-100 ng/mL. The precision and accuracy of the method was found to be within acceptable limits. Mean extraction recoveries of VD-2, VD-3, 25 (OH) VD-2, and 25 (OH) VD-3 from plasma were over 80%. The method was used for the determination of vitamin D level in plasma obtained from healthy subject participated in the study.

Biography

Syed N Alvi has obtained his PhD in Chemistry from Osmania University, Hyderabad, India in 2001. He is currently a Scientist at King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia. He has published more than forty papers in journals of international repute. His research interest includes method development and validation and application for pharmacokinetic and bioequivalence studies.

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