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Determination of 25-hydroxy vitamin D levels in type-2 diabetic patients by high performance liquid chromatography

Al Nemer
King Faisal Specialist Hospital & Research Center, Saudi Arabia

A reliable high performance liquid chromatography (HPLC) assay for simultaneous determination of 25-Hydroxyvitamin D-2 [25 (OH) VD-2], and 25-Hydroxyvitamin D-3 [25 (OH) VD-3] in human plasma was developed and validated. The analytes were extracted in hexane after precipitation with acetonitrile. The components of interest were efficiently separated on Zorbax C18 column. The mobile phase (gradient elution mode) consists of methanol, acetonitrile and water (pH = 3.0, with acetic acid); the eluents were monitored by photodiode array detector, with the wavelength set at 265 nm. The relationship between the concentration of 25(OH) VD-2, 25(OH) VD-3 in plasma and their peak area ratio to the benzopyrene (IS) was linear over the range of 5-100 ng/mL. Mean extraction recoveries of 25(OH) VD-2, 25(OH) VD-3 from plasma samples were over 80%. The method was successfully applied in monitoring the levels of 25-hdroxyvitamin D-2 and D-3 in samples obtained from Type-2 diabetic patients.

mbautista925@gmail.com