

VITAMIN D METABOLISM AND ANALYSIS OF VITAMIN D₂/D₃ AND THEIR METABOLITES BY LIQUID CHROMATOGRAPHY WITH TANDEM MASS SPECTROMETRY (LC-MS/MS) IN MULTIPLE SPECIES

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Abstract

Low vitamin D status is associated with musculoskeletal diseases, increased mortality, and a range of other diseases. Vitamin D₃ is a vitamin that is generated by UV irradiation of 7-dehydrocholesterol and is present in oil-rich fish such as salmon, mackerel, and herring. Vitamin D₂ is obtained from the UV irradiation of the sterol ergosterol and is found naturally in sun-exposed mushrooms. As vitamin D₃ circulates through the body, metabolism begins by hydroxylation of the vitamin form into the 25-hydroxyvitamin D₃ [25OHD₃], which is commonly assessed for vitamin D status. 25OHD₃ is further metabolized into the active hormone 1 α ,25(OH)₂D₃. The predominant circulating form and best indicator of vitamin D status is total 25OHD, which is the total of 25OHD₃ and 25OHD₂. Sera or plasma are the standard biological specimens used for measuring the circulating 25OHD concentration. We developed an assay to measure 25OHD₃ and 25OHD₂ using liquid chromatography-tandem mass spectrometry (LC-MS/MS). LC-MS/MS offers high sample volume throughput, high sensitivity, and robust accurate determination of individual analogs of vitamin D based on molecular weight, with low total CV and low error around mean bias. This methodology can be used to assess vitamin D status in mammals, fish, birds, and reptiles.