Quantitative Analysis of 25-Hydroxy-Vitamin D in Serum using LC/QQQ and LC/Q-TOF

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Liquid chromatography triple quadrupole (QQQ) and quadrupole-time-of-flight (Q-TOF) mass spectrometry (LC/MS/MS) are suited for rapid analysis of multiple analytes. The analysis of 25-hydroxy-vitamin D2 and D3 (25-OH-VD) has become ubiquitous in the measurement of vitamin D deficiency and supplementation monitoring. A highly sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of 25-hydroxy-vitamin D2 and D3 by QQQ and by Q-TOF. Using liquid-liquid extraction sample preparation technique and one dimensional (1D) chromatographic configurations achieves the required sensitivity and is capable of quantitating the compounds over their relevant dynamic range.

An Agilent 6460 QQQ and an Agilent 6530 Q-TOF with Agilent Jet Stream technology in positive electrospray mode and an Agilent Infinity 1260 HPLC system were utilized for this analysis. 150 µL of human serum was used for the analysis of the 25-OH-VD analytes. Various columns were evaluated and an Agilent Poroshell 120 EC-C18 50 x 2.1 mm, 2.7 µm with a water:methanol mixture containing 0.1% formic acid gradient achieved baseline chromatographic separation in less than 4 minute run time for both mass spectrometer configurations. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for each analyte and internal standard in positive mode and accuracy of the method was verified using reference materials from NIST (SRM 972), UTAK Controls and serum adult samples.

Good linearity and reproducibility were obtained with the concentration range of 1 ng/mL to 500 ng/mL for all 25-OH-VD analytes with a coefficient of determination $R^2 > 0.995$ for both configurations. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined to be at least 0.5 ng/mL and 1 ng/mL using both mass spectrometer configurations. Excellent reproducibility was observed for both compounds (CV < 15%) for all techniques.
A sensitive, simple, specific and accurate liquid chromatography QQQ and Q-TOF mass spectrometry analytical method was developed and verified for the simultaneous measurement of 25-OH-VD analytes in human serum.