

## **LC/MS Quantitative Analysis of Fat Soluble Vitamins in Blood**

**Lauren Frick, Mauricio Marques, Rory M Doyle**

**Agilent Technologies, Inc., Wakefield, MA, Sao Paulo, Brazil and Wilmington, DE**

Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC/MS/MS) are suited for rapid analysis of multiple analytes. A highly sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of the relevant fat soluble vitamins- vitamin A (retinol, retinal and retinoic acid), vitamin D (25-hydroxy-vitamin D3 and D2), vitamin E (alpha-, beta-, gamma- and delta- tocopherol and tocotrienols) and vitamin K (phylloquinone). These compounds are essential nutrients required for normal physiological functioning that either cannot be synthesized at all or in necessary amounts but can be toxic at high levels. Therefore, a simple and accurate quantitative analytical method was developed to measure these fat soluble vitamins in human blood using a simple offline sample preparation.

An Agilent 6460 QQQ LC/MS with Agilent Jet Stream (AJS) technology in positive electrospray mode and an Agilent Infinity 1260 HPLC system were utilized for this analysis. 500 µL of serum was used for the analysis of the fat soluble vitamins and the sample preparation involved a simple protein crash followed by a simple liquid-liquid extraction for the fat soluble vitamins in buffer. Various columns were evaluated and an Agilent Poroshell 120 PFP, 100 x 2 mm, 2.7 µm with water:methanol containing 0.1% formic acid gradient achieved baseline chromatographic separation of the fat soluble vitamins. 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 Recipe and UTAK controls and serum and blood adult samples.

Good linearity and reproducibility were obtained for all the fat soluble vitamins across their respective ranges. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were achieved at well below their respective ranges. The intra- and inter-day CVs were < 15% and the calibration curves displayed linearity with an  $R^2 > 0.998$  respectively for all the vitamins.

A sensitive, simple, specific and accurate liquid chromatography- tandem mass spectrometry analytical method was developed and verified for the measurement of fat soluble vitamins in blood. The sample preparation is quick and easily applied for high throughput analysis.