

LC/MS Quantitative Analysis of Water Soluble Vitamins in Blood

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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 water soluble vitamins- vitamin B1 (thiamine, thiamine pyrophosphate), vitamin B2 (riboflavin), vitamin B3 (nicotinic acid and nicotinamide), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxal 5-phosphate, pyridoxine, pyridoxal), vitamin B7 (biotin), folic acid, 5-methyltetrahydrofolate and vitamin B12 (cyanocobalamin). 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 water 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. 100 μ L of human blood was used for the analysis of the water soluble vitamins and the sample preparation involved a simple protein crash for the water soluble vitamins in buffer. Various columns were evaluated and an Agilent Poroshell 120 SB-Aq, 100 x 2 mm, 2.7 μ m with water:methanol containing 0.1% formic acid and 5 mM ammonium formate gradient achieved baseline chromatographic separation of the water 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 water 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 water soluble vitamins in blood. The sample preparation is quick and easily applied for high throughput analysis.