

Simultaneous determination of methylmalonic acid and homocysteine in plasma by liquid chromatography tandem mass spectrometry

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Vitamin B12, also called cobalamin, is an essential nutrient normally involved in the metabolism of every cell of the human body. Cobalamin is necessary for hematopoiesis and central nervous system (CNS) development. It is involved in DNA synthesis fatty and amino acids metabolism. Cobalamin is utilized as a cofactor in two enzymatic reactions – in the conversion of methylmalonyl-CoA to succinyl-CoA and in the synthesis of methionine from homocysteine. Therefore, reduced cobalamin levels results in elevation of plasma homocysteine and methylmalonic acid concentrations, which makes them sensitive indicators of cobalamin status. Deficiency causes severe disorders, such as failure to thrive, peripheral neuropathy or macrocytic anemia.

In our laboratory a simply, fast and sensitive method for simultaneous determination of plasma levels of MMA and HCY by liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed. Samples were prepared without the step of derivatization. The separation of the analytes were achieved using hydrophilic interaction liquid chromatography (HILIC). HCY was determined by positive mode, while negative mode was used to establish MMA. Method validation as well as determination of plasma concentrations in patient samples were performed. This method fulfilled requirements for validation parameters for both analytes – linearity in the range 0.07 – 4.20 $\mu\text{mol/l}$ for MMA and 0.80 – 120 $\mu\text{mol/l}$ for HCY, LOD = 0.02 $\mu\text{mol/l}$ for MMA and 0.05 $\mu\text{mol/l}$ for HCY, LLQ = 0.07 $\mu\text{mol/l}$ (CV= 2.4 %) for MMA and 0.12 for HCY (CV= 3.8 %). The recoveries of MMA ranged from 104.6 % to 112.1 % and those for HCY ranged from 96.7 % to 104.5 %.

Presented method is simple, fast and ensures grater throughput of samples, method is reliable in terms of precision, the calibration curve provides linearity over the relevant range of MMA and HCY concentrations. Over more the method is verified by INSTAND External Quality Surveys with 100% success.