

ABSTRACT

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Title of diploma thesis: Analysis of arginine and its methylated derivatives in clinical samples using LC-MS/MS

Asymmetric dimethylarginine (ADMA) and its enantiomer, symmetric dimethylarginine (SDMA), are naturally occurring amino acids that are generated intracellularly from L-arginine. ADMA is an endogenous competitive inhibitor of nitric oxide synthase. Increased levels of ADMA and SDMA are associated with endothelial dysfunction, hypertension, cardiovascular and renal diseases. Both compounds are accumulating in chronic kidney disease (CKD) patients. The aim of this diploma thesis was to develop and validate a liquid chromatography with tandem mass spectrometry (LC-MS/MS) method for the determination of L-arginine, ADMA and SDMA in human plasma. Subsequently, LC-MS/MS method was compared with enzyme-linked immunosorbent assay (ELISA) and high-performance liquid chromatography with fluorescence detection (HPLC-FL) method. Chromatographic separation was performed on Dionex UltiMate 3000 UHPLC liquid chromatograph, followed by tandem mass spectrometric detection on amaZon SL mass spectrometer. L-Arginine- $^{13}\text{C}_6$, ADMA- d_6 and SDMA- d_6 were used as the internal standards. Sample preparation included precolumn derivatization by 1-butanol. Multiple reaction monitoring (MRM) in positive mode was used for analyte quantification. Developed LC-MS/MS method showed very good analytical properties meeting the conditions for its validation and it is comparable with the previously published methods of other authors. Plasma levels of ADMA and SDMA obtained by the LC-MS/MS method correlated very well with those obtained by ELISA and HPLC-FL method. Plasma levels of ADMA and SDMA were measured in plasma samples obtained from healthy volunteers ($n = 40$) and patients CKD ($n = 40$). Both ADMA and SDMA levels were significantly elevated in the patients with CKD.