ABSTRACT

This dissertation thesis is focused on development and optimization of high-performance liquid chromatography (HPLC) and tandem mass spectrometry (LC-MS/MS) methods, and its utility for diagnosis of inherited metabolic diseases.

The first thematic part describes a comprehensive laboratory approach to diagnostics of patients with hereditary xanthinuria by determination of specific markers and enzyme activity. For this purpose HPLC method with diode array detection for measurement of hypoxanthine, xanthine, allopurinol and oxypurinol in urine and plasma and HPLC method with fluorimetric detection for analysis of pterin and isoxanthopterin in plasma were employed. These methods were successfully applied in clinical practice to ascertain two patients with hereditary xanthinuria type I.

The second thematic part aims at developing and clinical application of new LC-MS/MS method for simultaneous determination of total homocysteine (tHcy), methionine (Met) and cystathionine (Cysta) in dried blood spots (DBS) and plasma. The results demonstrated the clinical utility of this method for detection of patients with homocystinuria and possibility to distinguish between defects in the remethylation and transsulfuration pathways of homocysteine metabolism. Due to ease of DBS collection and sample transportation this method is suitable for monitoring of tHcy and Met concentrations of homocystinuria patients and also as a rapid screening procedure for detecting patients with hyperhomocysteinemia applied in newborn screening of Met disorders. During the study unique and unpublished data of Cysta concentrations in DBS was obtained.