

ABSTRACT

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Title of Doctoral Thesis: Modern separation techniques for the analysis of biological material in clinical research

This doctoral thesis deals with development and validation of a chromatographic determination of liposoluble vitamins and with optimization of biological materials preparation. Clinical monitoring of these vitamins, especially vitamin D in different biological materials is one of the most discuss theme in the last decade.

In the theoretical part of this dissertation the target analytes - vitamins A, E and D with their biological activity are characterized - as well as the biological material used in the Research Laboratory, especially a detailed description of the composition of breast milk, its irreplaceable role in neonatal nutrition and description of breast milk processing in Milk Bank. This part is followed by a comprehensive chapter dealing with the treatment of the biological matrix before analysis. The most commonly used sample pretreatment techniques such as extraction methods are described here, with a great description of new trends in these methods that have been developing in recent years. The theoretical part concludes analytical determination using separation chromatographic methods with emphasis on HPLC and UHPLC.

An important part of this dissertation is a review publication dealing with the determination of vitamin D and its metabolites in human breast and cows' milk. There are discussed circuits from importance of vitamin D and its metabolism through pretreatment of milk to analytical determination using modern instrumental techniques, including the combination of ultra-high performance liquid chromatography with tandem mass detection.

The experimental part is divided into two thematic areas. The first part deals with the preparation of the biological matrix - serum and human milk - and the development of chromatographic methods. The developed and published UHPLC-MS/MS method for the determination of 25-hydroxy derivatives of vitamin D in human serum with a simple sample preparation suitable for routine use is described herein. The second method deals with the

development of a suitable extraction procedure of vitamin D from human milk to subsequent chromatographic analysis with MS detection.

Another part of the dissertation deals with analytical determination of lipophilic vitamins A, E, D and inflammatory markers of neopterin, kynurenin, tryptophan and their applications in medicine. A study conducted in collaboration with Tissue Bank, where the stability of retinol and α -tocopherol in human milk during pasteurization and storage was monitored. Further studies address changes in antioxidant capacity in the treatment of age-related macular degeneration and familial hypercholesterolaemia. The last round is the study of neopterin as a predictive marker in oncological patients in cooperation with Teaching Hospital Olomouc.