

# ABSTRACT

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Title of Doctoral Thesis **Using of modern separation methods for clinical practice**

This work is focused on the development of methods for the determination of vitamin A and E in biological materials using liquid chromatography.

The theoretical background describes bioanalytical approaches to the determination of analytes from complex matrices. Sample preparation before analysis, chromatographic separation approaches and the validation of bioanalytical methods are discussed. The structure and biological function of target analytes are characterized.

The experimental part contains three sections, two of them focus on the development of new chromatographic methods for the determination of vitamin A and E in biological samples such as serum, lipoprotein layers and breast milk. The aim is to find the most suitable conditions for the chromatographic separation of target analytes that will be used for clinical trials in University Hospital in Hradec Králové. The third section is concerned with monitoring the stability of vitamins in breast milk as well as the influence of pasteurisation and storage on levels of the target vitamins.

1. A comparison was made of different types of UHPLC stationary phases (monolithic columns of 2nd generation, columns filled with porous particles and core-shell particles) for the simultaneous determination of retinol and  $\alpha$ -tocopherol in serum and breast milk. The new method must be applicable to the routine determination of analytes in clinical laboratory. The second generation monolithic column Chromolith HighResolution had the best separation efficiency of all tested columns for the intended purpose.
2. The study of the stability of retinol and  $\alpha$ -tocopherol in human breast milk was conducted. The influence of pasteurisation and storing ( $-27\text{ }^{\circ}\text{C}$ ) on levels of liposoluble vitamins was evaluated. It was proved that neither the pasteurisation procedure nor low temperature storing have an effect on these essential compounds during the shelf life (12 weeks). These findings are significant because of the importance of a sufficient supply of vitamins to premature babies for their proper growth and development.
3. A new method for the simultaneous determination of retinol,  $\alpha$ - and  $\gamma$ -tocopherol using internal standard and fluorescence detection was developed. This method is suitable for the determination of target analytes in serum and lipoprotein fractions in patients treated by apheresis and for determination of the efficiency of the processes. A short analysis time is favorable for the method to be used in large batches in a clinical laboratory.