Abstract

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Title: ***The application of chromatographic methods for separtion metabolites of cholesterol***

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The proposed diploma thesis deals with the development and validation of analytical method for free and esterified cholesterol separation from blood serum and adrenal tissue samples. This method is neccessary due to the different metabolism of free and esterified cholesterol in the body and it enables the research of these particular pathways using stable isotope tracers. The solid phase extraction method was chosen, optimised and validated with following results: precision values 2.14% for serum cholesterol ester, 6.98% for tissue cholesterol esters, 2.91% for serum free cholesterol, 7.48% for tissue free cholesterol, limit of detection 1 ⌈mol/l and linear range 1-25 mmol/l were found. The stability of the derivative was tested for 30 days at temperatures 4 °C and -25 °C. The derivative was found to be stable for the whole time period in both temperatures. Laboratory temperature was not tested due to a high volatility of the solvent used. The developed method was used in experimental studies evaluating the influence of dietary cholesterol and septic shock to cholesterol synthesis rate.