Abstract

Electrochemical detection of sterols is difficult. This thesis aims to study the voltammetric behaviour of selected oxysterols, i.e. 25-hydroxycholesterol and 7-ketocholesterol on the boron-doped diamond electrode. Further, an HPLC method with spectrophotometric and electrochemical detection was developed for separation of selected sterols. The study was performed using cyclic and differential pulse voltammetry. 0.1 mol l^{-1} perchloric acid in acetonitrile (water content 0.55 %) was chosen as the most suitable electrolyte. 25-hydroxycholesterol provides anodic signal at the potential of +1500 mV and 7-ketocholesterol at +1900 mV in that media. Electrochemical reduction of 7-ketocholesterol was also studied. However, the observed cathodic signal isn´t reproducible. The stability of oxysterols in the presence of perchloric acid was confirmed by UV-VIS spectroscopy. Calibration dependence of oxysterols was measured using differential pulse voltammetry and resulted in the limit of detection of 0.82 μmol l^{-1} for 25-hydroxycholesterol and 0.66 μmol l^{-1} for 7-ketocholesterol.

In the second part of the thesis, an HPLC method was developed for the separation of a sterol mixture on a reversed-phase C18 column using a mobile phase containing 50 mmol l^{-1} sodium perchlorate in acetonitrile with 0% or 6% water content. This method enables the separation of cholesterol, lanosterol, 7-ketocholesterol, 25-hydroxycholesterol, 27-hydroxycholesterol, and 7α-hydroxycholesteroland 7β-hydroxycholesterol in the mixture. The separation of the latter two compounds is enabled by their dehydration using perchloric acid prior injection. It leads to formation of products with longer retention times than the original compounds and separation from the other sterols in the mixture.The detection limits for amperometric detection at +2.0 V on the boron-doped diamond electrode ranged from 0.82 μmol l^{-1} to 29.33 μmol l^{-1}, and for spectrophotometric detection at 200 nm they ranged from 0.13 μmol l^{-1} to 4.99 μmol l^{-1}. The developed electroanalytical methods represent instrumentally simple and sensitive alternatives to separation methods with MS detection commonly used for the detection of cholesterol, its precursors and oxidation products.

Key words:

25-hydroxycholesterol, 7-ketocholesterol, boron-doped diamond electrode, cholesterol, high performance liquid chromatography, oxysterols, voltammetry.