ABSTRACT

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Title of Doctoral Thesis: Evaluation of properties of new HILIC stationary phases for pteridins using UHPLC-FD method

The subject of this thesis was to evaluate the characteristics of three new types of HILIC stationary phases for the identification and quantification of biologically active substances biopterin, neopterin, dihydrobiopterin and dihydroneopterin by UHPLC with fluorescence detection.

Neopterin is used as a marker of immune system activation and inflammatory diseases. Its early detection in urine or plasma may indicate a pathological immune activity. Elevated concentration of neopterin is described in viral or bacterial diseases, in autoimmune diseases in HIV infection or in malignant tumors. 7,8 - dihydroneopterin is able to prevent proteins and lipoproteins from oxidative damage.

Hydrophilic Interaction Liquid Chromatography (HILIC) is a chromatographic method that can be used to improve retention of very polar compounds. It uses a polar stationary phase and mobile phase containing a certain amount of water and polar solvents. UHPLC is the most advanced separation technology to develop a pressure of about 100 MPa. It uses columns with small particles (< 2 μm), which contribute to the improvement of the separation efficiency and resolution.

Overall the three chromatographic columns were tested (BEH AMIDE, BEH GLYCAN, BEH HILIC) at different chromatographic conditions, where the pH and the concentration and the percentage of aqueous content of mobile phase components were changed. BEH HILIC did not provide sufficient retention and selectivity for the separation of four pteridines under any tested conditions. Conversely BEH AMIDE provided a strong retention for all analytes, especially at high pH values (9.8). However, at this high pH the selectivity of separation for the pairs neopterin – dihydroneopterin and biopterin – dihydrobiopterin was significantly reduced, leading to very long analysis time. The best separation of all four pteridines, lasting up to eight minutes, was achieved with 50mM ammonium acetate buffer at pH range 4.8 - 7.8.

Keywords:

HILIC, UHPLC-FD, biopterin, neopterin, dihydrobiopterin, dihydroneopterin, 5,6,7,8 - tetrahydrobiopterin, BEH AMIDE, BEH GLYCAN, BEH HILIC