

TITLE: The study of neopterin retention characteristics in HILIC chromatography system with different types of stationary phases

SUMMARY:

This graduation thesis was dealing with the development of an analytical method for the identification and quantification of biologically active substances biopterin, neopterin and 7,8-dihydroneopterin by hydrophilic liquid interaction chromatography (HILIC) connected to fluorescence detection.

Neopterin represents an important and useful parameter for monitoring of the immune system activation, that accompanies different infectious and autoimmune diseases. Increased level of neopterin concentration can be observed in several types of malignant tumors. Recently the coherency between neopterin, 7,8-dihydroneopterin and intracellular oxidative stress and apoptosis of the cells has been demonstrated. Totally reduced form of biopterin serves as a cofactor of some enzymes in human organism.

HILIC is an option of high performance liquid chromatography (HPLC). Three chromatographic columns (LUNA HILIC, OBELISK R, OBELISK N) were tested at different chromatographic conditions. Mobile phase consisted of mixture of aqueous component (ammonium acetate buffer, acetic acid, water) and water miscible organic component (acetonitrile).

The mixture of acetonitrile and 100mM ammonium acetate buffer (pH=4,8) offers a chromatogram with baseline separated peaks of biopterin and neopterin, the peak of 7,8 dihydroneopterin doesn't match standard requirements (on chromatographic column OBELISK N). Quantitation limits of biopterin and neopterin were 1 nmol/l. Detection limits were 0,3 nmol/l. The best results were achieved with chromatographic column LUNA HILIC. The mixture of acetonitrile and 100mM ammonium acetate buffer (pH=6,8) offers a chromatogram with baseline separated peaks of tested substances. Quantitation limits of biopterin and neopterin were 1 nmol/l. Detection limits were 0,3 nmol/l. Quantitation limit of 7,8-dihydroneopterin was 25 nmol/l. Detection limit was 7,6 nmol/l.

KEYWORDS:

Biopterin, neopterin, 7,8-dihydroneopterin, HPLC, HILIC