ABSTRACT

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Title of Doctoral Thesis:

The development of HILIC method for UHPLC-MS/MS determination of pteridins, a comparison of selectivity of various stationary phases

This graduation thesis was dealing with the development of HILIC method for the identification and quantification of biologically active substances neopterin, biopterin, 7,8-dihydroneopterin and 7,8-dihydrobiopterin by ultra-high performance liquid chromatography coupled to the mass spectrometry detector of triple quadrupole type.

Three chromatographic columns (BEH Glycan, BEH Amide and BEH HILIC) were tested. Several mobile phases and their influence on the separation of target analytes were tested. Mobile phase consisted of aqueous component (acetic and formic acid, ammonium formate and acetate and ammonim hydroxide of low concentration) and acetonitrile.

On the chromatographic column BEH Glycan following best mobile phases were evaluated: 1mM ammonium acetate pH= 3.8 with acetonitrile in the ratio 30:70 and 1mM ammonium acetate pH= 6.8 with acetonitrile in the ratio 28:72. Two mobile phases composed of 1mM ammonium acetate pH= 4.8 with acetonitrile in the ratio 23:77 and 1mM ammonium acetate pH= 6.8 with acetonitrile in the ratio 28:72 offered the best results on the chromatographic column BEH Amide. The chromatographic column BEH HILIC was assessed as unsuitable, because it did not allow to achieve the separation of pteridins.

System suitability test, linearity of the method and its sensitivity were measured at the selected optimal conditions. The methods are linear (for the BEH Glycan r= 0.9915 to 0.9999, for the BEH Amide r= 0.9987 to 0.9999). The amide column show higher sensitivity (LOD of NEO and BIO was 0.22 to 0.91 nmol/l, LOD of NH2 and BH2 was 113.94 to 916.67 nmol/l, LOQ of NEO a BIO was 0.74 to 3.00 nmol/l, LOQ of NH2 and BH2 was 376.00 to 3025.00 nmol/l).

KEYWORDS:

Neopterin, biopterin, 7,8-dihydroneopterin, 7,8-dihydrobiopterin, HILIC, UHPLC, mass spectrometry, BEH Glycan, BEH Amide, BEH HILIC