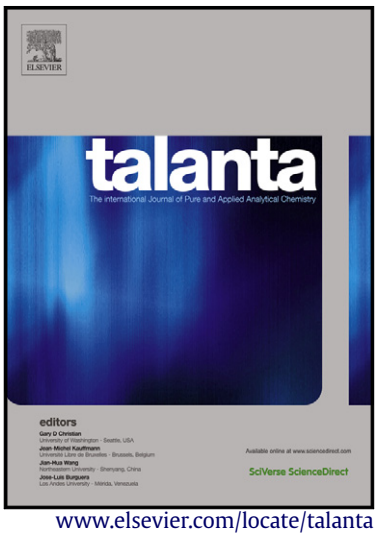

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E. Martín Tornero, I. Durán Merás, A. Espinosa-Mansilla

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HPLC determination of serum pteridine pattern as biomarkers

E. Martín Tornero, I. Durán Merás, A. Espinosa-Mansilla*

Department of Analytical Chemistry, University of Extremadura, Avd. de Elvas, s/n, 06006 Badajoz. Spain

*Corresponding author: Tel.: +34 924289376; fax:+34924274244.

E-mail addresses: nuncy@unex.es (A. Espinosa-Mansilla)

ABSTRACT

Pteridinic derivatives are important biomolecules considered as biomarkers for several diseases, especially in cancer and infectious pathologies. A newly fluorimetric - HPLC method for the analysis of nine pteridines in human serum has been reported. Two analytical columns composed by C18 porous and fused core particles were assayed and the results compared. Fused core particle column allow us adequate separation, in only one run and in 15 min. Acid precipitation step of the proteins and clean-up process with an Isolute ENV+ (hydroxylated polystyrene-divinylbenzene copolymer) cartridge of the serum samples have been optimized. Analytes were determined by fluorimetric detection, exciting at 272 nm and measuring the fluorescence emission at 410 nm for isoxanthopterin, at 465 nm for xanthopterin, and at 445 nm for the analysis of the other pteridines. Detection limits between 0.07 and 0.61 ng mL^{-1} were calculated according with Clayton criterium. Intraday precision varied from 1.2 to 5.3 and interday precision between 1.2 and 7.4, both expressed as RSD (%). External standard and standard addition calibrations were compared in the analysis of serum samples. The pteridine amounts in serum (expressed as ng mL^{-1}± confidence interval) were: 3.69±1.78; 1.35±0.24; 0.46±0.14; 0.54±0.24; 0.84±0.55; 2.10±0.51 and 0.23±0.11 for XAN, NEO, MON, ISO, BIO and 6HMPT, respectively using external standard method. Comparable results were obtained by standard addition method. It is noticeable that 7BIO was not detected in the healthy serum samples analyzed.