

Abstract

The aim of this diploma thesis was to compare the separative options of different types of chromatographic columns for the determination of neopterin, kynurenine, creatinine and uric acid in urine samples with the method that is currently used in the Research laboratory of the Department of Metabolic Care and Gerontology, Teaching Hospital in Hradec Kralove. Basic, already developed and validated method was High Performance Liquid Chromatography (HPLC) on reversed-phase column Gemini 5 μm C18 110 \AA , 150 x 3 mm, Phenomenex (Torrance, USA). Phosphate buffer with concentration of 15 mmol/l, pH 6,4 and flow rate 0,8 ml/min was used as a mobile phase. Diode array detector was used for detection of creatinine (235 nm) , kynurenine (230 nm) and uric acid (290 nm) Neopterin was monitored using fluorescent detection at 353 nm excitation and 438 nm emission wavelength.

All chromatographic systems were compared on the basis of the SST- System Suitability Test and were evaluated by the separation efficiency, peak resolution, asymmetry and other parameters.

Columns with the best parameters were selected for further optimization and validation. The resulting new modern HPLC method should be suitable for clinical use.