Abstract

In this work the new HPLC method for simultaneous determination of neopterin, tryptophan, kynurenine and creatinine was developed and validated.

For separation of analytes two monolithic columns (Chromolith Speed ROD RP-18e, 50 x 4.6 mm and Chromolith Performance RP-18e, 100 x 3.0 mm) were connected together. In front of this connection Chromolith Guard RP-18e, 10 x 4.6 mm disc for better separation and serum proteins elimination was used. Neopterin and tryptophan were monitored using fluorescent detection. Neopterin was monitored at 353 nm excitation and 438 nm emission wavelength, for tryptophan 245 nm excitation and 404 emission wavelength was used. The detection of creatinine and kynurenine was carried out with help of diode array detector at wavelength 235 nm for creatinine and 230 nm for kynurenine.

As the mobile phase 15 mmol/l phosphate buffer at pH 4.51 was used with the flow rate 1 ml/min in time 0 - 3 minutes, then the flow rate was changed to 2.3 ml/min. The total time of analysis was 9.5 minutes including the column equilibration. The injection volume of sample was 1 µl.

This method was developed and validated using the standards of analytes and also in human serum.