

ABSTRACT

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Title of the diploma thesis: Difficulty of urinary neopterin and creatinine determination using high-performance liquid chromatography with focus on clinical practice

This diploma thesis deals with the determination of neopterin and creatinine in urine samples by high performance liquid chromatography. The theoretical part summarizes information about the substances and their analysis. It also includes a review of methods published in the literature. The method of high-performance liquid chromatography including types of stationary phases and chromatographic principles used in the experimental part of this work is briefly introduced.

The separation properties of six chromatographic columns for the determination of neopterin and creatinine in urine were verified in the experimental part of the thesis. Columns were compared by the following parameters: repeatability, resolution, peak symmetry, and column efficiency. The composition of the mobile phase was optimized for selected columns. The mobile phase was a phosphate buffer with a concentration of 15 mmol/l and pH 6.5 in most cases. Creatinine detection was performed by a Photodiode Array Detector at 235 nm. Neopterin was detected by a Fluorescent Detector at an excitation wavelength of 353 nm and an emission wavelength of 438 nm. The most suitable was a YMC Triart C-18, 150 x 4.6 mm, 3 μ m chromatographic column. This column achieved satisfactory results in the most evaluated parameters.