ABSTRACT

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Title of the Diploma Thesis: Development and optimization of new chromatographic method for determination of retinol in human urine

This diploma thesis is based on the method development for determination of retinol and creatinine using HPLC. The aim of this project was to find optimal HPLC-MS/MS conditions for clinical research.

Experiment was carried out using UHPLC set Nexera with mass spectrometer LCMS-8030, (Shimadzu, Japan). Three stationary phases were tested. The chromatographic separation was achieved using a Kinetex 2.6 µm PFP 100A 100x4.6 mm (Phenomenex, USA). The used mobile phase consisted of acetonitrile (with addition of formic acid 0.005 M) and ammonium acetate (pH 6.69) in the ratio 78:22 (v/v). The temperature was maintained at 25°C, flow rate was set at 0.5 ml/min and 3 µl of sample was injected. After optimization of separation conditions, the method was applied to urine samples, and simple sample preparation procedures were tested

In these days is the work on method finishing and the new method will be used for determination of renal damage in clinical research and practice. The inclusion of creatinine for urine dilution correction is very beneficial mainly in combination with noninvasive determination in urine.

Keywords: creatinine, retinol, UHPLC-MS, urine, renal damage