ABSTRACT

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Title of GraduationThesis: Optimalization of sample preparation step for UHPLC-MS/MS analysis of atorvastatin, rosuvastatin and their metabolites

The purpose of this graduation thesis was the optimalization of UHPLC-MS/MS method for the determination of concentrations of atorvastatin, rosuvastatin and their metabolites in biological material, and then development and optimalization of a MEPS (microextraction by packed sorbent) method for sample preparation of biological material and validation of this method.

Acquity BEH C18 column (50 x 2.1 mm, 1.7 μm, Waters) was used for the separation of the analytes. Electrospray ionization was performed in both negative and positive ion mode. Triple quadrupole mass analyser was used for detection. Precursor ions and fragment ions were chosen for each statin. Collision energy and cone voltage were optimized for all analytes individually. Quantification of analytes was performed using the SRM (selected reaction monitoring).

Protonated molecule $[M+H]^+$, which was measured in the positive ion mode, was chosen as a precursor ion for rosuvastatin lactone. Ion $[M-H]^-$, which was measured in the negative mode, was selected for the other statins, because it was the most intense ion in the mass spectrum.

These conditions were used for the development and optimalization of MEPS method.
Following solid phases were tested - C18, C8 and M1. C8 sorbent provided the best results.
Washing and elution reagents consisting of acetonitrile and ammonium acetate in various ratios were tested. The mixture of acetonitrile and 0.1 M ammonium acetate pH 4.5 in a ratio of 95:5 was chosen as elution agent. A mixture of acetonitrile and 0.01M ammonium acetate pH 4.5 in ratio of 5:95 was chosen as washing agent. The method was applied to the biological material after optimalization. In this case it was a human serum.

The method was validated. The linearity, accuracy, precision and selectivity of the method were verified. The limit of detection and quantification and matrix effects were verified too. The method was linear. Correlation coefficients were determined in the range from 0.9982 to 0.9998. Limit of detection (LOD) ranged from 0.15 to 0.75 nmol / l and limit of quantification (LOQ) from 0.5 to 2.5 nmol / l.

Key words: MEPS, UHPLC-MS/MS, rosuvastatin, rosuvastatin lactone, N-desmethyl rosuvastatin, atorvastatin, atorvastatin lactone, p-hydroxyatorvastatin, o- hydroxyatorvastatin