Abstract

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Title of Graduation Thesis:

Development of UHPLC-MS/MS method for the determination of statins and their metabolites

This graduation thesis deals with development of the method for the determination of statins and their metabolites by ultra-high performance liquid chromatography coupled to tandem mass spectrometry. First, the chromatographic conditions were optimized. Next, the parameters of mass spectrometer were optimized. And finally, repeatability, linearity and sensitivity of the method were assessed.

Chromatographic column BEH C18 (50 x 2.1 mm, 1.7 μm) was used for the analysis. The choice of column was performed using UV spectrophotometric detector. The mobile phase of the gradient elution consisted of 0.1 mM ammonium acetate (A) and acetonitrile (B). The initial ratio of mobile phase A:B was set at 70:30.

The optimization of the mass spectrometry parameters started with the selection of precursor ions using direct infusion. Thereafter, the ion source parameters of the mass spectrometer were optimized. The ion transitions were optimized after the selection of product ions. The optimization of ion transitions was extended by the evaluation of the mobile phase additives influence on the ionization process. The above mentioned steps were measured in both positive and negative ionization modes. The resulting ionization mode for each compound was chosen after comparison of calibration curve linearity and sensitivity.

Finally after optimization of all important conditions, the sensitivity (LOQ = $1x10^{-9}$ g/ml – $1x10^{-8}$ g/ml), linearity (15 analytes: $r^2 \geq 0,9990$; lovastatin acid and pravastatin lactone: $r^2 > 0,9900$) and repeatability (RSD < 1 % for retention time, RSD < 10 % for peak areas) of the final method was measured.

The keywords: atorvastatin, *o*-hydroxyatorvastatin, *p*-hydroxyatorvastatin, atorvastatin lactone, fluvastatin, fluvastatin lactone, lovastatin, lovastatin acid, pitavastatin, pitavastatin lactone, pravastatin, pravastatin lactone, rosuvastatin, rosuvastatin lactone, N-desmethylrosuvastin, simvastatin, simvastatin acid, UHPLC-MS/MS, statins