

Abstract

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Title of Thesis: **Development of Stage tips-UHPLC-MS/MS method for determination of statins in human serum**

This diploma thesis deals with development of a method for determination of statins in human serum. Extraction by Stage tips pipette tips was chosen and optimized for their extraction. The measurement was performed by the ultra-high performance liquid chromatography coupled to triple quadrupole mass spectrometer. The extraction procedure and important mass spectrometer parameters were optimized. Previously developed LC method for determination of statins was used for the analysis.

The optimization of the mass spectrometer parameters was started by the selection of precursor ions, consequently the ion source parameters were tuned and finally appropriate ionization mode, SRM transition and collision energy for each analyte were chosen. The suitability of the LC-MS method was verified by the repeatability method ($RSD \leq 1$ for the retention time and $RSD \leq 10$ for the peak area), linearity (correlation coefficient $\geq 0,997$) and sensitivity (LOQ in the range of 5×10^{-10} - 1×10^{-9} g/ml).

Firstly, the optimization employing mixed standard solution (elution and washing solvent, sample and elution volume) was performed during Stage tips method development. Subsequently, the developed method was applied to spiked human serum samples. As the selected procedure provided a very low recovery for individual statins, more detailed optimization on the spiked human serum was performed. In further development steps it was found that commercial sorbent used Stage tips are not suitable for the extraction of statins from human serum. Due to this reason the laboratory produced sorbents were also tested. Although optimized extraction procedure was not suitable for all determined statins due to the low recovery, the most suitable procedure for majority of statins was selected and the suitability of laboratory produced sorbent was proved. Searching for the more suitable sorbent for all statins will be covered in another testing of laboratory produced sorbents.

Keywords: statins, UHPLC-MS/MS, Stage tips, extraction by the pipette tips, PT-SPE