Abstract

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Title of Diploma Thesis: Development UHPLC-MS/MS method and sample preparation procedure for the determination of steroid compounds in rat plasm

The diploma thesis deals with the development of the UHPLC-MS/MS method for the analysis of 37 steroids in plasma and optimization of sample preparation using PP.

The analytes include structurally very similar substances with different numbers of hydroxyl or ketonic functional groups, isomeric and mutually isobaric compounds. Since these substances cleave water molecules very easily, precursor ions with one m/z for different substances can be found in the MS spectrum. For these reasons, careful and detailed characterization of the precursors, subsequent scanning of the product ions, and selection of additional SRM transitions were essential.

Separation of critical compounds with the same m/z for precursor ions was of importance. The optimization started with the screening of 7 columns using gradient elution of the mobile phase ACN/0.1 % FA in the range of 5 - 98% ACN. Based on the results, an optimization of the mobile phase gradient was performed. The Cortecs C18 column was optimal. Most of the analytes eluted in a gradient of 35 – 60 % ACN. The duration of the analysis was 18 minutes with 2 minute column equilibration.

Subsequently, the protein precipitation was optimized. The MODDE software was used to design and evaluate the experiment. The following factors were tested: the type and volume of the precipitating agent, time of precipitation and of centrifugation. Acetonitrile in a volume of 1000 µl per 100 µl of plasma was chosen as a suitable precipitating agent. The precipitation time was 10 minutes and the precipitate was centrifuged for 5 minutes at 8 °C and 14,000 RPM. Finally, verification of certain validation parameters was demonstrated.

Key words: steroids, UHPLC-MS / MS, protein precipitation, plasma, optimization