

ABSTRACT

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Title of Diploma Thesis: Analysis of steroids from the group of androgens and glucocorticoids using UHPSFC-MS/MS

The goal of this study was to develop and optimize an analytical method for the separation of 37 steroids mixture by the ultra-high performance supercritical fluid chromatography hyphenated to mass spectrometry (UHPSFC–MS/MS). Analysis was carried out using Waters Acquity UPC² system coupled to Xevo TQ-XS mass spectrometer.

In the first step it was necessary to consistently characterize the precursor ions of each analyte. Then, the Product ion scan was carried out and proper SRM transitions were chosen. Steroids are isomeric and isobaric substances that often provide the same precursor ions ($[M+H]^+$, $[M+H-H_2O]^+$, eventually $[M+H-2H_2O]^+$), or similar fragmentation spectra. This is complicated by the fact that most intense ion of the spectrum is not always $[M+H]^+$, but also $[M+H-H_2O]^+$ ion which is simultaneously isobaric m/z for a completely different compound. After careful selection of SRM transitions, the conditions of the ion source were optimized for both positive and negative modes.

In the next step, the screening of 18 stationary phases was carried out. The gradient elution involved CO₂ (solvent A) and 5 – 40 % MeOH + 0.1% NH₄OH (solvent B) in 5 minutes with isocratic step hold for 1 minute at 40 % of solvent B. The chromatographic conditions were set as follows: temperature 40 °C, flow-rate 1.5 mL/min and ABPR pressure 150 bar. The most successful separation was obtained with Torus 1-AA and GreenSep Naphthyl HC columns, that become subject of following optimization. According to different elution strength, the 5 mobile phases (MeOH, MeOH:ACN 1:1, EtOH, IPA, IPA:ACN 1:1) were tested in several gradient conditions, with various time of analysis. Further, the temperature optimization was carried out in the range 22 – 50 °C and the effect of gradient curves 2, 4, 6, 8 and 10 was measured. The best selectivity of separation was achieved using the Torus 1-AA (100 x 3.0 mm, 1.7 μm) with CO₂ and MeOH (solvent B) in the linear gradient elution from 1 to 20 % of solvent B in 13 minutes, and 2 minutes of equilibration in combination with the temperature of analysis 22 °C.

Keywords: androgens; glucocorticoids; steroids; UHPSFC-MS/MS; method development; optimization

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