Development of a Rapid LCMS Method for Steroids in Plasma

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Steroids are important to the human body for normal biological activity. Steroid measurement is essential when evaluating disorders such as congenital adrenal hyperplasia (CAH), Cushing's disease and polycystic ovarian disease[1]. Traditional methods to measure steroids have been immunoassays, however, these assays lack specificity, are affected by matrix interferences and could take up to a week to be completed. More recently, liquid chromatography mass spectrometry (LC-MS/MS) has become the industry standard in evaluating steroids due to the specificity, precision and sensitivity of the system. This presentation will focus on a rapid six minute method to analyze 16 different steroids and 3 internal standards in plasma at picogram per milliliter levels using reversed phase liquid chromatography with LC-MS/MS detection.

A mixture of the steroids, including three internal standards, have been analyzed in neat solution using the LCMS 8050 triple quadrupole mass spectrometer (Shimadzu Corporation, Kyoto, Japan). The steroids analyzed include estrone, progesterone, testosterone, 17β-estradiol, 17α-ethynylestradiol, aldosterone, 11-deoxycortisol, 11-deoxycorticosterone, cortisol, cortisone, 17-hydroxyprogesterone, androstenedione, DHEA, DHEA sulfate, and 5-dihydrotestosterone. The compounds were ionized via heated electrospray ionization (hESI) utilizing rapid polarity switching. MRM transitions for each analyte were optimized and analytes were separated using reversed phase chromatography. A representative chromatogram for the 19 steroids is shown in **Figure 1**.

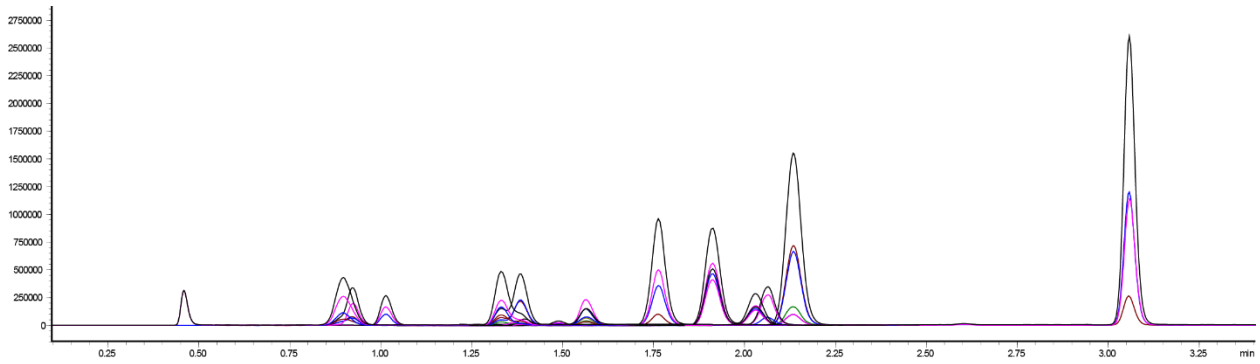

Figure 1. Representative chromatogram for 19 steroids analyzed in the neat solution on the LCMS-8050.

The linearity was investigated over a 9 point calibration curve (125 ng/mL – 80 pg/mL) with correlation coefficients for all compounds greater than 0.995. The limit of quantitation (LOQ) for two steroids, progesterone and estrone was 180 fg on column. This method panel will be analyzed in plasma where the detection limits, peak area reproducibility and linearity will be assessed.

[1]Guo, T., Taylor, R.L., Singh, R.J., Soldin, S.J.., **Clinica Chemica Acta** 372 (2006) 76-82