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Validation of a 13 steroids panel with the Chromsystem kit by LC-MS/MS.

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Background:

It's important for clinicians to obtain an accurate and precise dosage of steroid hormones. For this purpose, liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS) has become an essential tool for small molecule quantification due to its high sensitivity, specificity, excellent reproducibility and the ability to perform simultaneous analysis. The aim of our work was the validation of the MassChrom® for Steroids in Serum/Plasma kit by LC-MS/MS (Chromsystems). This method comprises the detection and quantification of aldosterone(ALDO), cortisol(COR), cortisone, cortsicosterone, 11-deoxycortisol(S), androstenedione,(AND), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate(DHEAS), dihydrotestosterone(DHT), estradiol (E2), 17 α-hydroxyprogesterone (HYP), progesterone and testosterone(TST) as total steroids.

Materials and Methods:

➢Until now Radio-Immunoassays(RIA) or ELISA were used for the determination of some of the mentioned compounds.

➢Validation of the new methodology has been carried out using an Sciex 6500 triple quadrupole MS/MS(Framingham, MA, USA) equipped with LC-30A Nexera UHPLC system (Shimadzu Co., Kyoto, Japan) (Fig.1).
➢The 13 steroids were separated in 2 panels.

➢For sample preparation, to 500 µl serum/QC/calibrator both Internal standard Mix and Extraction buffer were added before performing an extraction in a 96-well solid phase extraction(SPE) plate. The procedure was validated by testing 3 levels in triplicate during 3 different days.

➢Statistical analysis was performed using the Enoval validation software (Arlenda, Mariakerke, Belgium).

➢***Results:***

➢The evaluation of the mentioned kit was carried out at the following concentrations (µg/L) for each steroid.
➢Panel 1(Fig. 2): ALDO(0.025-3.08), cortisol(10.2-288), cortisone(1.03-38.9),corticosterone (0.52-48.2) and S (0.09-13.9).
➢Panel 2 (Fig. 3): AND(0.18-14), DHEA(0.97-55.9), DHEAS(105-5975), DHT(0.06-1.34), E2(0.04-4.94), HYP (0.1-15.1),progesterone (0.17-25.6) and TST(0.05-11.8).
➢The mean recoveries values did not differ significantly from 100% while the precision, as CV%, was below 10% for both the intraday and interday variability except for corticosterone(11%).
➢The developed method was shown to be linear (R2>0.99) for all steroids in serum.
➢The limit of detection(LOD) and limit of quantification(LOQ) were calculated with the lowest concentration tested.

Figure 1: Qtrap 6500

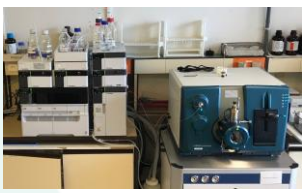


Figure 2: Chormatogram exemple for panel 1 with the control level II

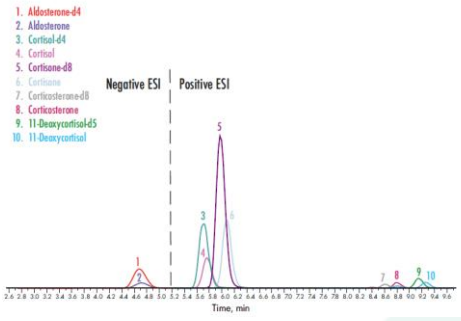
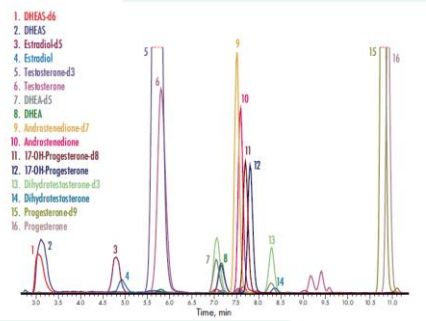


Figure 3: Chormatogram exemple for panel 2 with the control level II



Conclusions:

The method based on LC-MS/MS using the MassChrom® Kit has been satisfactory validated and meets the requirements to be applied in routine.