A Multi-Assay for the Quantification of Seven Steroid Hormones and Precursors in Serum with LC-MS/MS

Leila Zamani[1], Anna Lindahl[1], Magnus Axelson[1], Inga Bartuseviciene[1]
[1]Clinical chemistry, Karolinska University Hospital, Sweden

Introduction

The analysis of steroid hormones is an important part of the diagnostic workup of many conditions caused by disorders in the hypothalamic-pituitary-adrenal (HPA) axis and gonads. Examples include Cushing's syndrome characterized by cortisol excess, and congenital adrenal hyperplasia resulting from enzyme deficiencies leading to altered production of glucocorticoids, mineralocorticoids and sex steroids, female hyperandrogenism and male hypogonadism.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a highly specific and sensitive methodology suited for rapid analysis of multiple analytes in a clinical laboratory. The simultaneous detection of multiple steroid hormones in one sample has the advantage of increased precision and cost-efficiency.

Objective

To develop an LC-MS/MS multi-assay for the quantification of seven endogenous steroid hormones and precursors in serum: 17α-hydroxyprogesterone, androstenedione, testosterone, dihydrotestosterone, cortisol, cortisone and 11-deoxycortisol, for use in our routine clinical laboratory.

Method

Automated sample preparation was performed using a robotic liquid-handling system (Microlab STARLet, Hamilton). Serum samples (200 µl) were mixed with deuterated internal standards and phosphoric acid followed by hydrophilic-lipophilic solid phase extraction (Oasis HLB, Waters). The steroids in a 20 µl aliquot of eluate were then separated by reversed phase C18 ultra-high performance LC (Acquity UPLC HSS T3 column, 1.8 µm, 2.1x150 mm, Waters) with mobile phase A: 0.2% FA in water and B: ACN using a linear gradient of 35-70% B in 13 min. Positive mode MS (Xevo TQS, Waters) was used for detection with a total runtime of 20 min per sample.

Quantification was performed based on one-point calibration using one quantifying transition and a second qualifying transition for identity confirmation of each analyte.

Steroid-free serum (Golden West Biological) was used for method validation.

Results and discussion

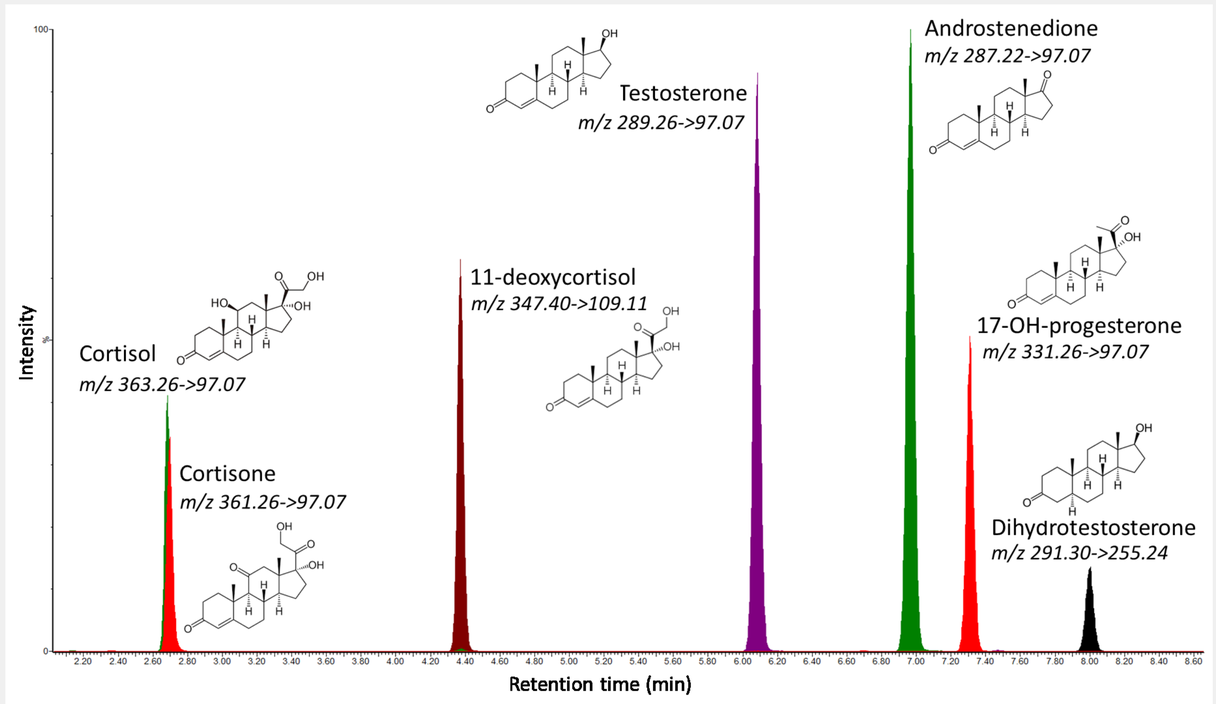
The limit of quantification was in the low nmol-range or below for all seven compounds, with acceptable linearity (R^2 > 0.99) within their respective measuring range, which included each reference range. The total coefficient of variation of the assay was <7%.

A relatively long gradient was used to separate the quantifying transition of 11-deoxycortisol from a number of interferences. The gradient can however be optimized to shorten the analysis time.

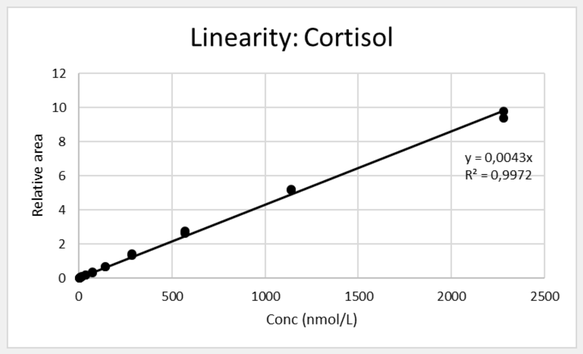
Verification in a larger cohort of clinical samples is ongoing and the method is planned to be in routine use at the end of 2019.

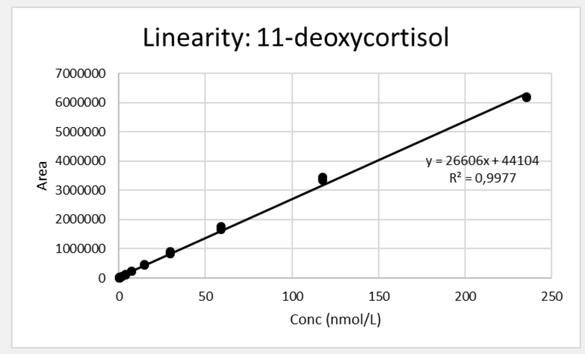
Conclusion

Our newly developed LC-MS/MS-based multi-assay of seven steroid hormones and precursors in serum will aid in the diagnostic process of disorders of the HPA axis and gonads. It also has the potential to be of use in patient follow-up care.

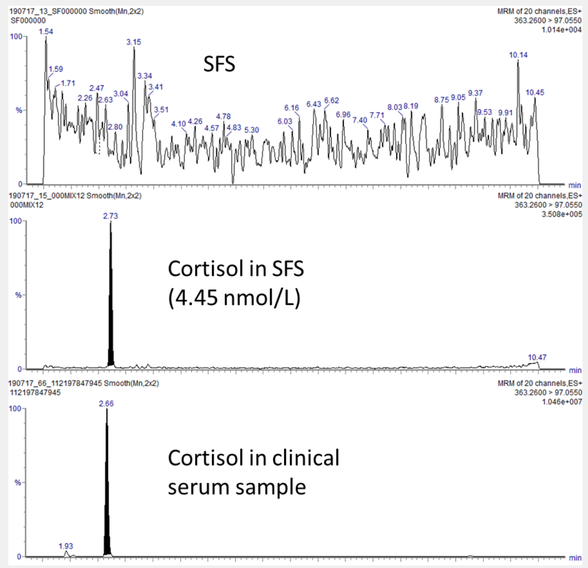


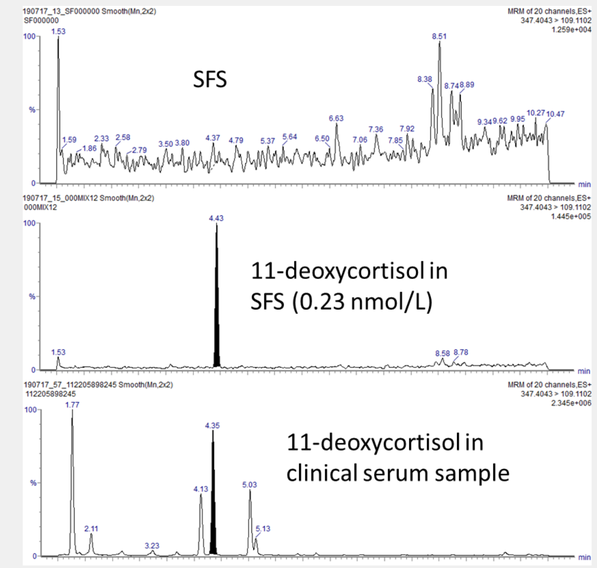
EICs (quantifying transition) in a pure standard compound mix of the seven steroid hormones and precursors analysed.



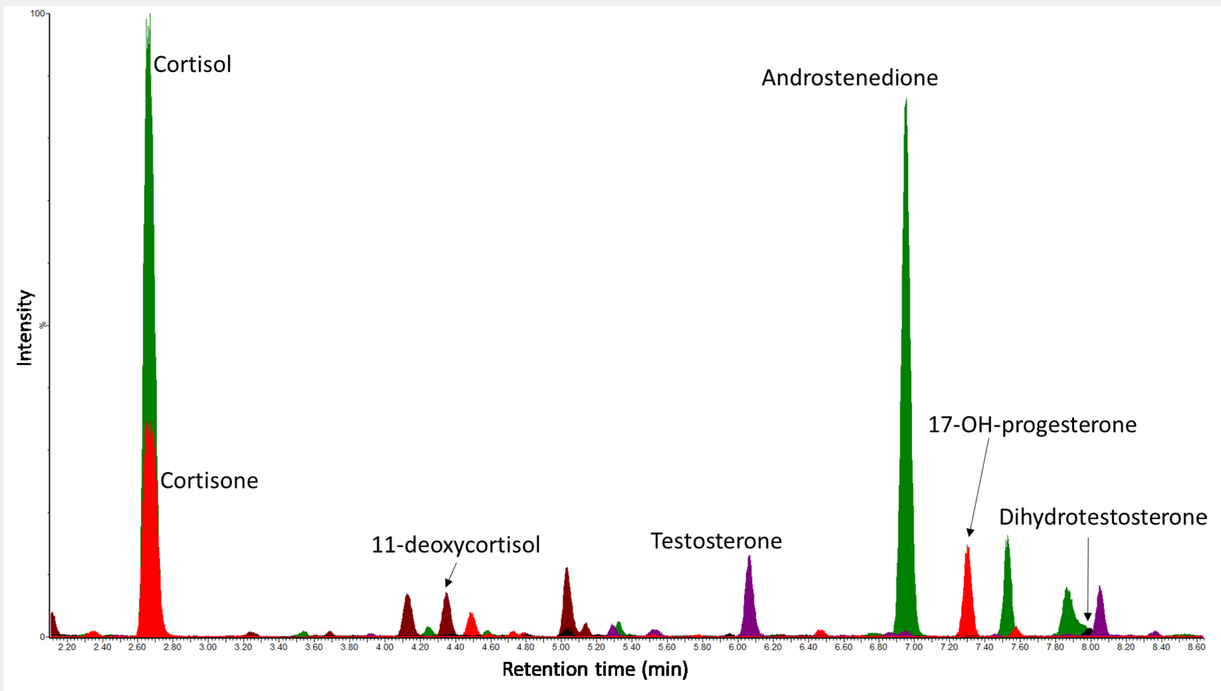


Linearity exemplified by cortisol (left) and 11-deoxycortisol (right) spiked into steroid-free serum (SFS).





Examples showing the quantifying transition for 11-deoxycortisol (left) and cortisol (right) in blank SFS (top), spiked at low amounts in SFS (middle) and in a clinical serum sample (bottom).



Example of EICs (quantifying transition) for all seven compounds in a clinical serum sample.

Karolinska University Hospital, Clinical chemistry
Anna Lindahl
E-mail: anna.lindahl@sll.se

Karolinska University Hospital, Clinical chemistry
Leila Zamani
E-mail: leila.zamani-fekri@sll.se

The Association for Mass Spectrometry: Applications to the Clinical Lab

KAROLINSKA UNIVERSITY HOSPITAL