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Application News

SSI-LCMS-097

Liquid Chromatography Mass Spectrometry

Steroid Detection in Small Volume Blood by LC-MS/MS

Liquid Chromatograph Mass Spectrometer

LCMS-8060

■ Summary

This customized LC-MS/MS method demonstrates a significant correlation between steroid concentrations in fingerstick versus vein blood. Moreover, to accomplish sample cost reduction and safety efficiency, blood from finger stick could be used as a source for steroid measurement in clinical research.

■ Background

Liquid chromatography- tandem mass spectrometry (LC-MS/MS) has become a successful immunoassay substitute for steroid detection in clinical research laboratories. The LC-MS/MS platform provides superior specificity and the possibility to detect a vast number of hormones in a small amount of blood in just one acquisition. The sensitivity of the LC-MS/MS assay is equal or even lower than that of immunoassays. The aim of the present proof-of-concept study is to demonstrate the ability of LC-MS/MS to reach a higher level of sensitivity in samples using a small volume collection device for blood.

■ Method

An LC-MS/MS quantitative method was established on a Shimadzu 8060 triple quadrupole mass spectrometer for 16 steroids. Steroids were spiked in charcoal stripped FBS serum, which was tested negative for all hormones present in the study. Sample preparation was done by solid-phase extraction (SPE). After elution, samples were dried under nitrogen steam and reconstituted in a water/MeOH solution. Reconstitute was injected directly into the LC-MS system, which was operated in polarity switching mode (positive and negative modes). Concentrations of steroids from vein and capillary blood from 40 individuals were compared using the following LC-MS/MS assay.

LC run: Each sample was injected (10 uL) onto a Nexera HPLC system. Separation of compounds (including isobaric) was achieved on a Restek Raptor Biphenyl column (Bellefonte, PA), with a binary gradient beginning at 60% MPB. Total run time was 6 min, with column flow at 0.4 mL/min and oven temperature 35°C. Ammonium fluoride was used as a mobile phase modifier.

Steroid name	LOQ	AMR	CV % at LOQ	Matrix, %	Recovery, %
Cortisol, ug/dL	0.5	0.8- 20	12	105	74
Cortisone, ug/dL	0.125	0.18-5	14	106	73
21-Deoxycortisol, ng/dL	5	8 - 500	13	111	79
Estradiol, pg/ml	10	15-1000	17	115	81
Aldosterone, ng/dL	2.5	3-100	18	99	85
11-Deoxycortisol, ng/dL	12.5	15-500	15	103	83
Estrone, pg/ml	5	8-500	11	104	80
Corticosterone, ng/dL	25	30-1000	18	110	84
DHEA, ng/dL	15	15-1000	18	80	80
DHEA-S, ug/dL	12.5	15-500	10	82	75
17-OHP, ng/dL	10	15-1000	15	107	82
Testosterone, ng/dL	10	15-1000	18	114	79
DHT, ng/dL	5	8-1000	7.0	102	83
Androstendione, ng/dL	10	15-1000	19	118	88
11-DOC, ng/dL	2.5	4-500	16	113	87
Progesterone, ng/dL	12.5	15-2500	16	107	79

Table 1: Method validation parameters. Method was validated according to CLIA requirements; specific validation parameters are presented. Matrix, % – matrix effect. Recovery, % – compound recovery from SPE plate.

Quantitative method for steroids was established on an LCMS-8060 Shimadzu system and validated according to CLIA requirements. Calibrators and controls were spiked into FBS charcoal treated serum (Fisher), which was tested for any trace of hormones before use. Serum was also tested for steroid recovery in order to exclude possible effects of charcoal treatment. A correlation study was conducted on blood from the same individuals, collected from fingerstick and venipuncture.

SPE extraction: SPE plate was conditioned with MeOH followed by water. Plasma (200 uL) was diluted with 600 uL of water:IPA and 20 uL of internal standards(IS), vortexed and loaded onto the plate. Plate was washed through a series of solvent rinses prior to drying. Samples were eluted into fresh polypropylene plate, dried under nitrogen and diluted in 30 uL of MeOH. Plate was put on shaker for 10 min, 50 uL of water added to each well, and placed in autosampler.

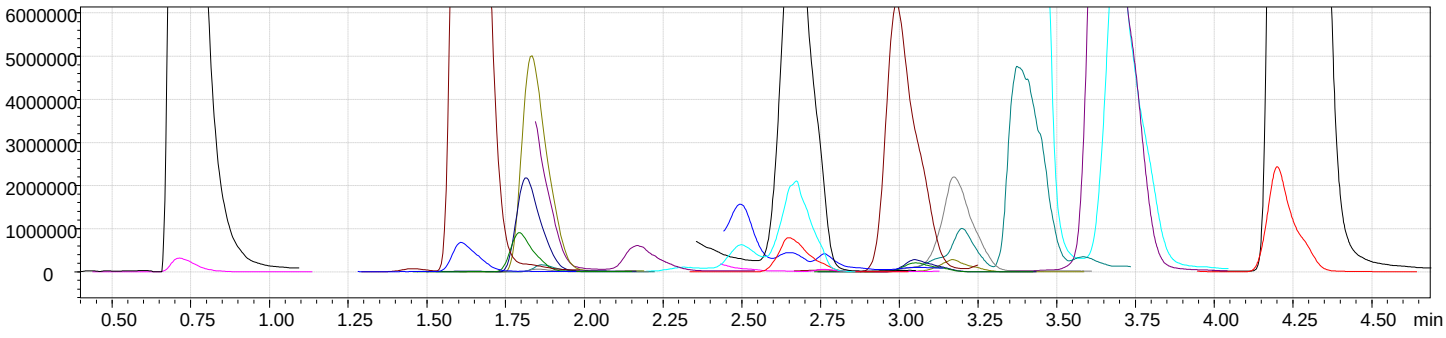

Figure 1: TIC for all 16 steroids and 6 internal standards.

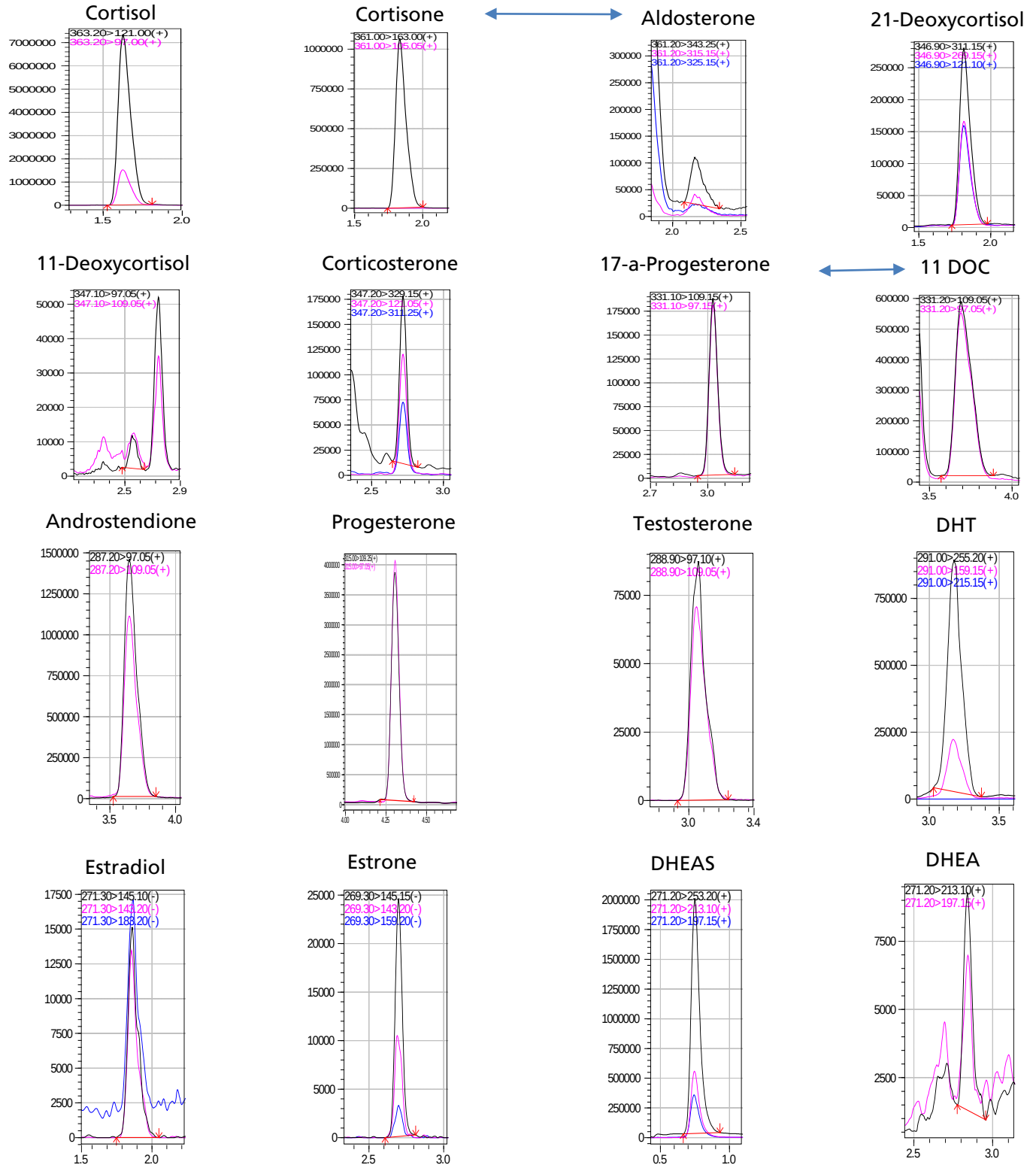

Figure 2: MS chromatograms demonstrate baseline separation for isobaric pairs.

MS settings: ESI was used as the MS ionization source with a probe voltage of 4kV. LabSolutions Connect™ software was used to automatically optimize nitrogen gas flows (nebulizing and drying gases), desolvation line (DL) temperature, heat block temperature, and ESI interface settings. The LabSolutions™ MRM Optimization Wizard was used to optimize collision energy (CE).

These software tools allow for analyte conditions to be automatically optimized. LabSolutions™ software was used for quantitation.

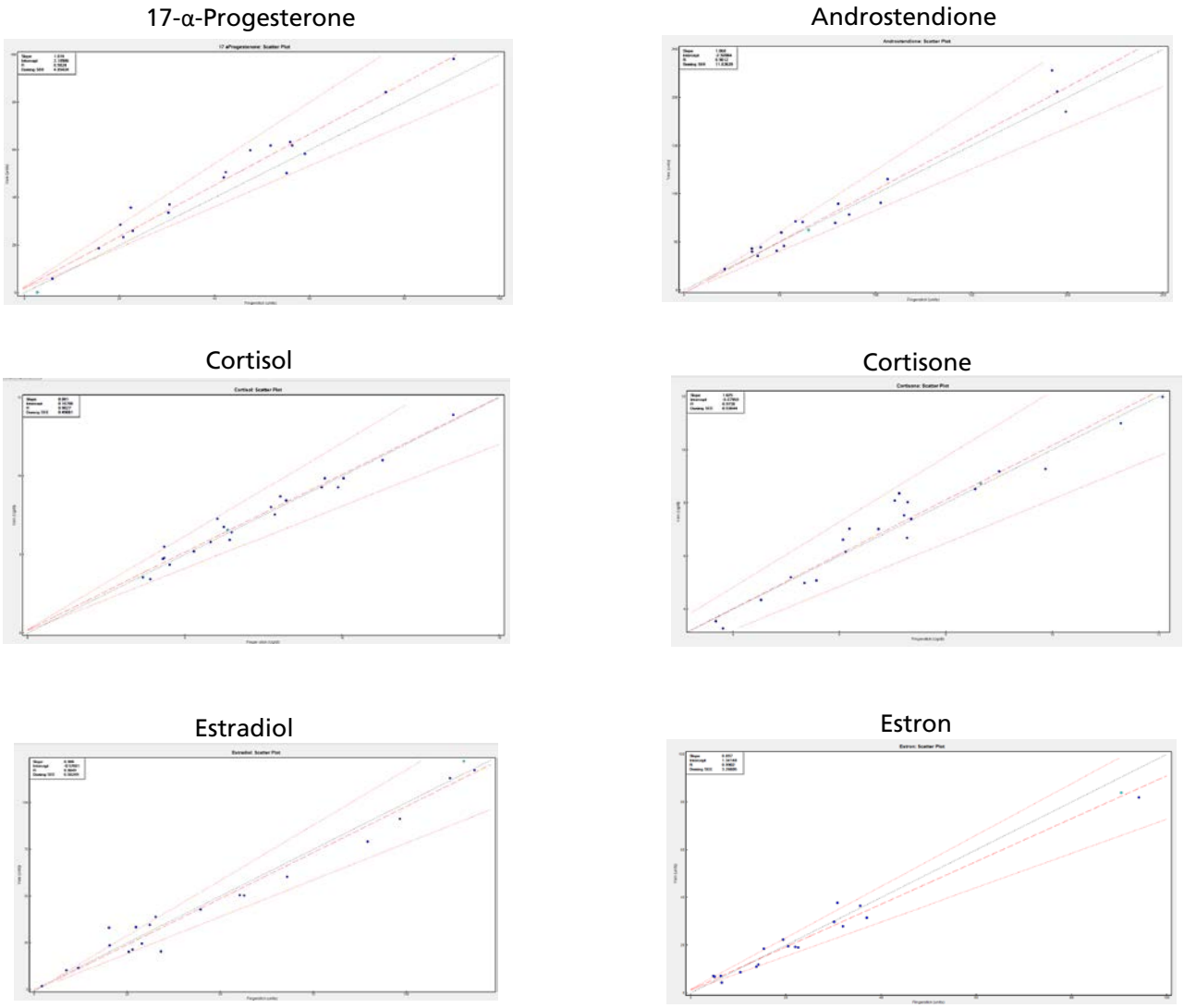

Figure 3: Steroid concentrations in small volume versus vein blood (method comparison by Deming regression). Graphs represent selected hormones.

■ Results and Discussion

This method was validated and demonstrated acceptable accuracy (>90%) and precision (CV<15%). Linearity ranges for all compounds in this study covered normal published reference ranges. LOQs were comparable with results reported for classical immunochemistry assays. Method performance was confirmed by cross-validation with a set of peer-group samples. Hormone concentration from vein and capillary blood demonstrated significant correlation, coefficient values (R^2) > 0.98.

■ Conclusion

The current sensitivity level for steroid detection demonstrated by this LC-MS/MS assay allows for a potential substitute to the classical immunochemistry assay. The hormone concentrations in capillary blood (fingerstick) were nearly identical to conventional plasma venipuncture levels. Fingerstick could be an excellent source of blood for hormone testing due to convenience, cost, and timing of collection. Low level steroid concentrations can be detected from small volume blood obtained from fingerstick.

ULTRA FAST MASS SPECTROMETRY


LCMS-8040


LCMS-8045


LCMS-8050


LCMS-8060


LCMS-2020


LCMS-IT-TOF

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