Steroidal Hormone Analysis in Patient plasma and serum samples by Tandem Mass Spectrometry (Acquity UPLC-XEVO TQD)

Taposh G, Bhaskar K, Veeranjaneyulu P, Tirupateswar rao B, Rajesh PMN, Anil Kurup
Waters India Applications Laboratory, , Bangalore, 560058

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

ABSTRACT

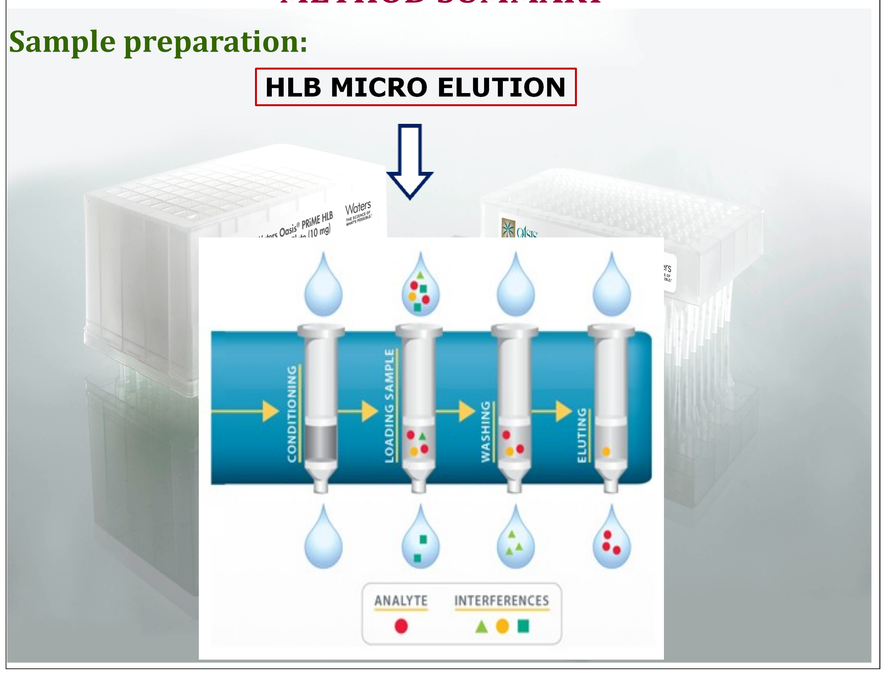
Quantitation of endogenous steroids is important in the diagnosis of several endocrine disorders. Steroid hormones like11-deoxycortisol, testosterone, androstenedione, DHEA and 17-hydroxyprogesterone etc. are synthesized from cholesterol. These hormones shows different physiological effects even at very low concentrations (nano Moles– Pico Moles levels) and their determination at Pico mole level can be a challenge for clinical researchers.

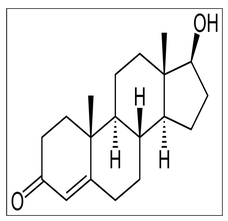
OBJECTIVE

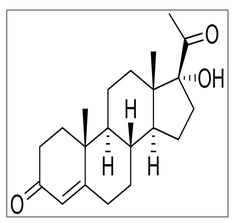
The objective of the study to develop a simple LC-MS/MS method to estimate five steroids simultaneously in picogram level with simple sample extraction procedure from plasma and serum and to overcome the challenges of sensitivity, selectivity, specificity and reproducibility.

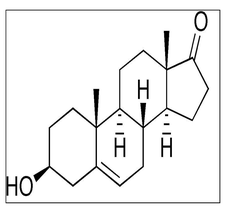
METHOD SUMMARY

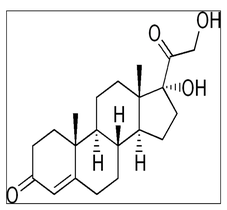
Sample preparation:

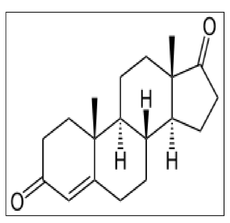



TESTOSTERONE


17-HYDROXYPROGESTERONE


DIHYDROEPIANDROSTERONE


11- DEOXYCORTISOL


ANDROSTENEDIONE

MS Conditions:

MS System	: Xevo TQD
Mode	: ESI +Ve
Cone gas	: 50 L/Hr
Desolvation Temp	: 600 °C
Source Temp	: 150 °C
Desolvation gas flow	: 1000 L/hr

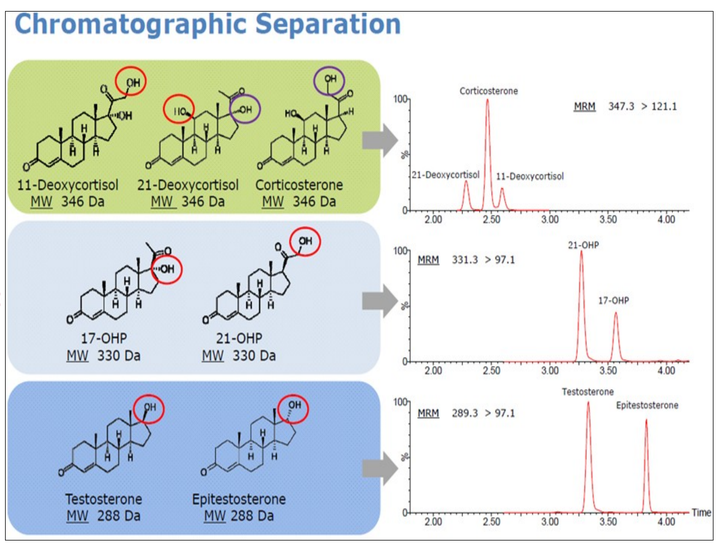
Data processed through **TargetLynx** application manager.

UPLC WITH XEVO TQD

MRM CONDITIONS

Compound	MRM	Dwell (secs)	Cone Voltage (V)	CE (eV)
Testosterone	289.23>96.94	0.02	38	25
$^{13}C_3$ -Testosterone	292.23>99.93	0.02	38	25
Androstenedione	287.17>96.94	0.02	38	25
$^{13}C_3$-Androstenedione	290.21>99.93	0.02	38	25
11-DeoxyCortisol	347.3>97.1	0.02	32	25
11-DeoxyCortisol IS	352.3>100.1	0.02	32	25
17-HydroxyProgesterone	331.3>97.1	0.02	30	30
17-HydroxyProgesterone (IS)	339.3>100.1	0.02	30	25
Dehydroepiandrosterone (DHEA)	367.2>80	0.02	45	70
DHEA (IS)	277.2>257.3	0.02	45	30

Chromatographic Separation



LINEARITY RANGES

Compound	Linearity range
Testosterone	50.0 pg/ml to 10.0 ng/ml
Androstenedione	50.0 pg/ml to 10.0 ng/ml
11-DeoxyCortisol	50.0 pg/ml to 10.0 ng/ml
17-HydroxyProgesterone	50.0 pg/ml to 10.0 ng/ml
DHEA	250.0 pg/ml to 50.0 ng/ml

RESULTS AND DISCUSSION

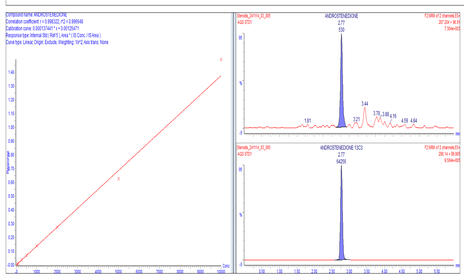


Figure 1: ***Linearity & Representative chrmatogram of Androstenedione.***

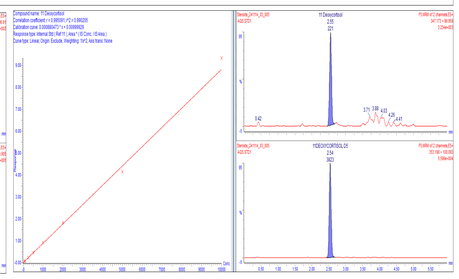


Figure 2: ***Linearity & Representative chromatogram of 11 Deoxycortisol.***

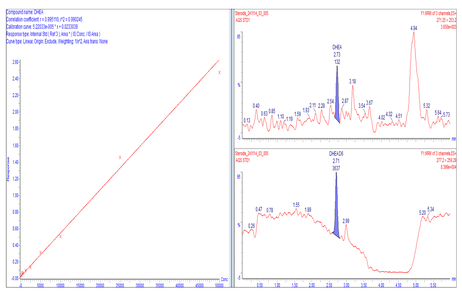


Figure 3: ***Linearity & Representative chromatogram of DHEA.***

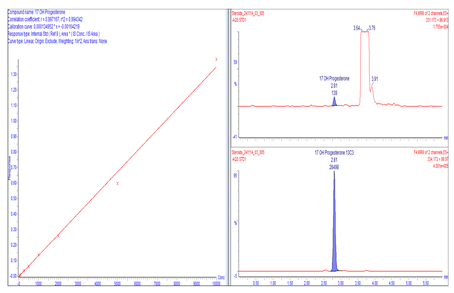


Figure 4: ***Linearity & Representative chromatogram of 17 OH Progesterone.***

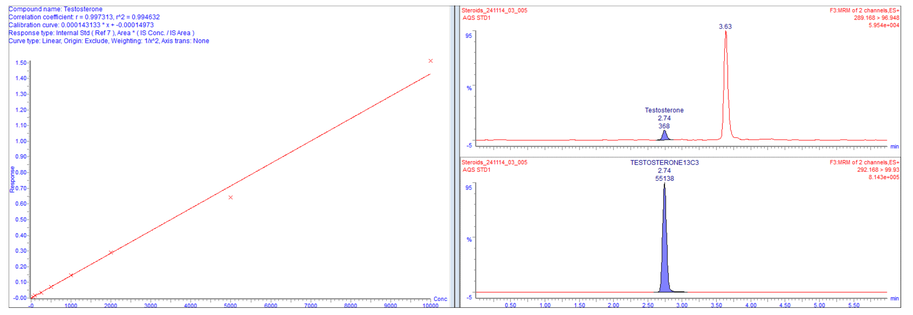


Figure 5: ***Linearity & Representative chromatogram of Testosterone.***

CONCLUSION

1) The method demonstrates simple and effective sample processing approaches with less matrix effect for all five steroids in plasma and serum.
2) The method demonstrates high recovery & reproducibility for all tested steroids in plasma and serum.
3) Phospholipids interference effectively removed using simple extraction procedure.
4) All steroid isomers were separated chromatographically with good reproducibility.

REFERENCES

1) Bock JL. The new era of automated immunoassay. American Journal of Clinical Pathology.2000;113:628–646. [PubMed].
2) Stanczyk FZ, Cho MM, Endres DB, Morrison JL, Patel S, et al. Limitations of direct estradiol and testosterone immunoassay kits. Steroids. 2003;68:1173–1178. [PubMed].
3) Ceglarek U, Kortz L, Leichtle A, Fiedler GM, Kratzsch J, Thiery J. Rapid quantification of steroid patterns in human serum by on-line solid phase extraction combined with liquid chromatography triple quadrupole linear ion trap mass spectrometry. Clinica Chimica Acta. 2009;401:114–118.doi:10.1016/ j.cca.2008.11.022 [PubMed].

ACKNOWLEDGMENT

The authors would like to thank Dr. Sujay Prasad (Chairman of Anand Diagnostics) and Mr. Prem Pal (Manager, Analytical Lab) for providing Reference standards and patient serum & plasma. samples.

Contact details: PMN_Rajesh@waters.com