A SIMPLE AND RAPID METHOD FOR STEROID PROFILING BY TWO DIMENSION - LIQUID CHROMATOGRAPHY – TANDEM MASS SPECTROMETRY (2D-LC-MS/MS): TOWARD ROUTINE APPLICATION

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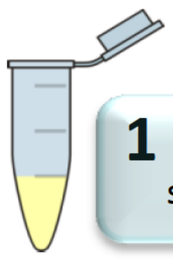
INTRODUCTION

Steroid testing has a central role in clinical decision-making and in research studies on diseases such as hypercortisolism, female hyperandrogenism, male hypogonadism or in inborn disorders of steroid synthesis. Recently, LC-MS/MS proved its superiority to routine immunoassays (IA) in accurately and sensitively measuring low level steroids. However, the replacement of automated IA with LC-MS/MS platforms is currently limited by the need for extraction procedures requiring operator handwork, large sample volume and long runtime.

Aiming at improving LC-MS/MS practicability, we developed a 2D-LC-MS/MS method for the simultaneously determination of serum cortisol (F), testosterone (T), 17OHpregnenolone (OHp), androstenedione (A) and 17OHprogesterone (OHP) based on minimal sample preparation and short runtime.

METHOD

BENCH-WORK



1 50µl serum

2 Protein precipitation (PP): 100µl precipitating solution + Internal Standards (IS)

3 Vortex and centrifugation

4 Dilution with mobile phase

INSTRUMENTS

5 Injection of 90µl

2D CHROMATOGRAPHY

TOTAL RUN TIME: 15min

1. **ON LINE PURIFICATION** on perfusion column: 2.0min
2. **SEPARATION** on RP-C18 column: 7.5min
3. **CLEAN-UP and RIEQUILIBRATION**: 5.5min

ESI - MS/MS

	Ion mode	Target Ion	Reference Ion	IS
F	+	363.3/121.1	363.3/267.3	d4-F
T	+	289.1/97.1	289.1/109.0	13C2-T
OHp	-	331.2/287.1	331.2/313.1	13C3-E1
A	+	287.0/97.1	287.0/109.1	d5-A
OHP	+	331.1/97.1	331.1/109.1	d8-OHP

RESULTS

LC-MS/MS METHOD VALIDATION AND PERFORMANCE

	Calibration curve in surrogate matrix (BSA 4%)								SENSITIVITY in blank serum matrix			
	Linearity		Limit of Quantification				Limit of Detection					
	Range (ng/ml)	R^2	Conc. (ng/ml)	Accuracy %	CV %	S/N	fg on column	S/N	Conc. (ng/ml)	Accuracy %	CV %	S/N
F	0.488 - 500.0	0.9998	0.4883	109.9	3.5	273.1	183	8.8	0.4883	96.7	6.0	151.5
T	0.020 - 20.0	0.9993	0.0195	104.3	11.8	13.4	73	7.0	0.0195	113.7	11.6	11.2
OHp	0.781 - 100.0	0.9994	0.7813	104.0	13.2	16.2	1465	5.3	0.7813	100.8	12.8	14.5
A	0.039 - 20.0	1.0000	0.0391	104.4	12.6	10.1	73	3.3	0.0781	106.3	9.3	16.3
OHP	0.049 - 50.0	0.9997	0.0488	112.8	15.1	10.3	183	5.5	0.0977	96.4	14.7	16.4

IMPRECISION

duplicate measurements

	Intra-assay CV%	Inter-assay CV%
F	4.3	6.2
T *all*	7.6	5.4
T *<1ng/ml*	0.5	3.4
T *>1ng/ml*	6.0	9.1
OHp	15.6	17.0
A	7.6	4.5
OHP	4.3	3.2

METHOD COMPARISON STUDY: The novel LC-MS/MS assay (PP) was compared with an established LC-MS/MS assay (1) requiring 900µl of serum processed by PP followed by solid phase extraction (PP+SPE).

Duplicate measurements of F, T, A and OHP on 17 de-identified sera were performed by both methods; results were compared by Passing & Bablok regression. Slope and intercept coefficients (95%CI) and R values (fig. 1) revealed that the novel LC-MS/MS provided results non different from the established LC-MS/MS assay.

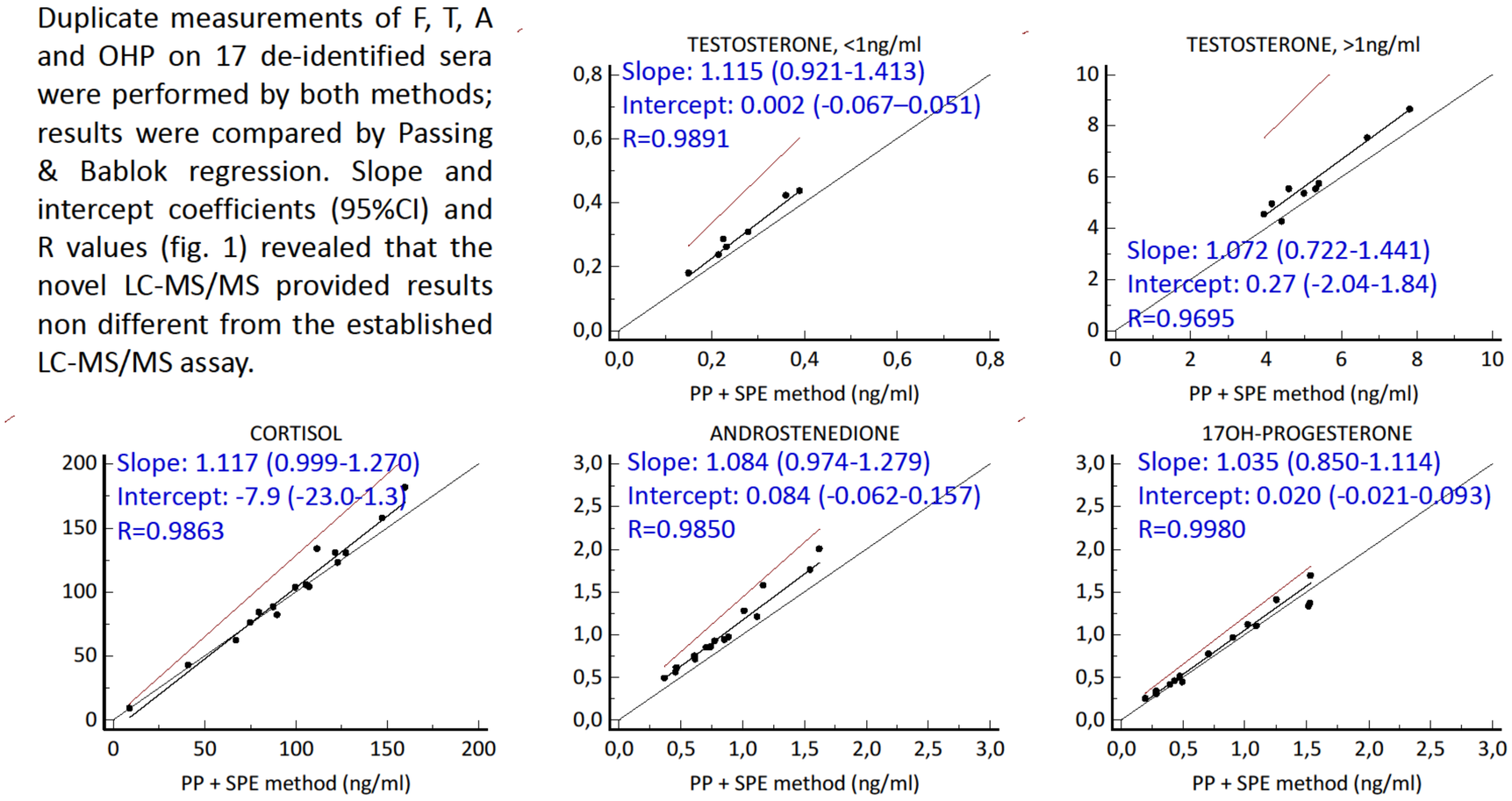


Figure 1: Passing & Bablok regression: novel (PP) vs established extraction-based (PP+SPE) LC-MS/MS methods.

ACCURACY

DGKL German Society for Clinical Chemistry and Laboratory Medicine — Reference Institute for Bioanalytics
Accredited to DIN EN ISO/IEC 17020

	Certified conc. (ng/ml)		Calculated conc. (ng/ml) (*Accuracy %*)	
	Sample A	Sample B	Sample A	Sample B
F	95.0	213.9	81.9 (*86.2*)	186.9 (*87.4*)
T	4.269	2.195	4.192 (*98.2*)	2.084 (*95.0*)
OHP	2.432	7.534	2.524 (*103.8*)	7.329 (*97.3*)

CONCLUSION

The novel 2D-LC-MS/MS method based on simple and rapid processing and analysis showed high sensitivity, accuracy and precision required to efficiently determine clinical relevant levels of the five steroids. Results provided for F, T, A and OHP are in perfect agreement with an established extraction-based LC-MS/MS method. The reduced bench-work and the overall performance make this assay well suited for application in routine settings.

Fanelli F, Belluomo I, Di Lallo VD, Cuomo G, De Iasio R, Baccini M, Casadio E, Casetta B, Vicennati V, Gambineri A, Grossi G, Pasquali R, Pagotto U. Serum steroid profiling by isotopic dilution-liquid chromatography-mass spectrometry: comparison with current immunoassays and reference intervals in healthy adults. Steroids, 2011;76:244-53.

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