SEX HORMONE AND STEROID PRECURSOR MEASUREMENT BY SIMPLE AND RAPID LC-MS/MS: COMPARISON WITH CURRENT ROUTINE IMMUNOASSAYS

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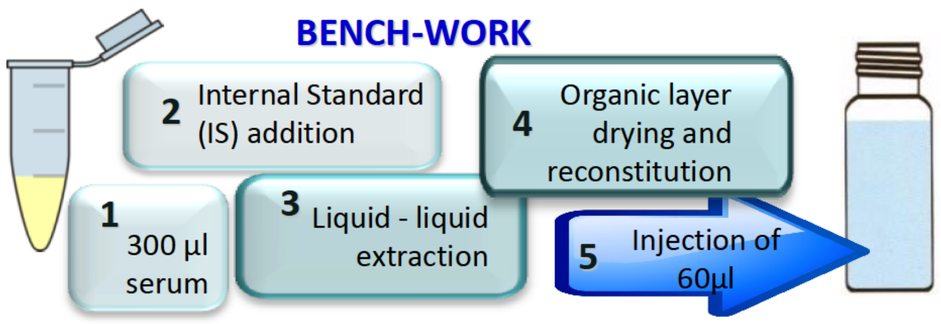
INTRODUCTION

Sex steroid testing has a central role in clinical decision-making and in research studies on diseases such as female hyperandrogenism, male hypogonadism or in inborn disorders of steroid synthesis. In the last decade, LC-MS/MS clearly displayed its analytical superiority over routine immunoassays (IA) in accurately and sensitively determining serum testosterone and in assessing large androgen profiles, thus promoting studies on the re-characterization of androgen imbalance diseases. Nonetheless, the measurement of other sex steroids and precursors is still challenging, and is available only in experienced research laboratories. Our aim was to develop a simple-prep and rapid LC-MS/MS method for the simultaneous measurement of estrone (E1), estradiol (E2), dihydrotestosterone (DHT) and 17OHpregnenolone (OHp).

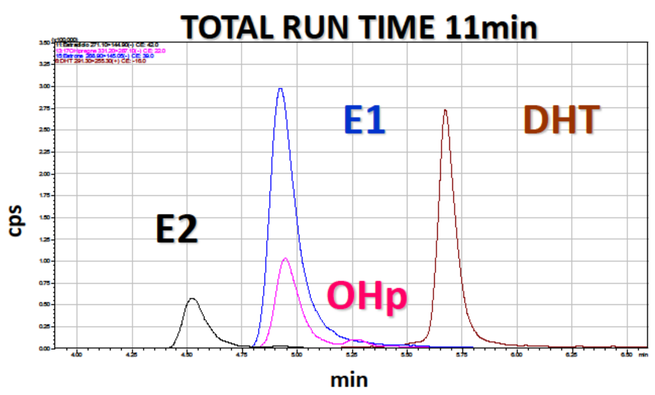
MATERIALS AND METHODS

BENCH-WORK



INSTRUMENTS

2D CHROMATOGRAPHY



0 – 1.3 min ON LINE SAMPLE PURIFICATION | 1.3 – 5.9 min SEPARATION C6-phenyl column | 5.9 – 11 min CLEAN-UP and RE-EQUIL

MASS-SPEC COMPOUND-DEPENDENT PARAMETERS

	Retention Time	ESI	MS/MS transitions		
	min	ion mode	Target	Reference	IS
E2	4.48	-	271.1/144.9	271.1/182.9	^{13}C3-E2
E1	4.88	-	268.9/145.1	268.9/145.1	^{13}C3-E1
OHp	4.91	-	331.2/287.1	331.2/313.1	^{13}C3-E1
DHT	5.65	+	291.3/255.3	291.3/159.1	d3-DHT

CERTIFIED MATERIALS

Calibrators: Cerilliant® Analytical Reference Standards

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

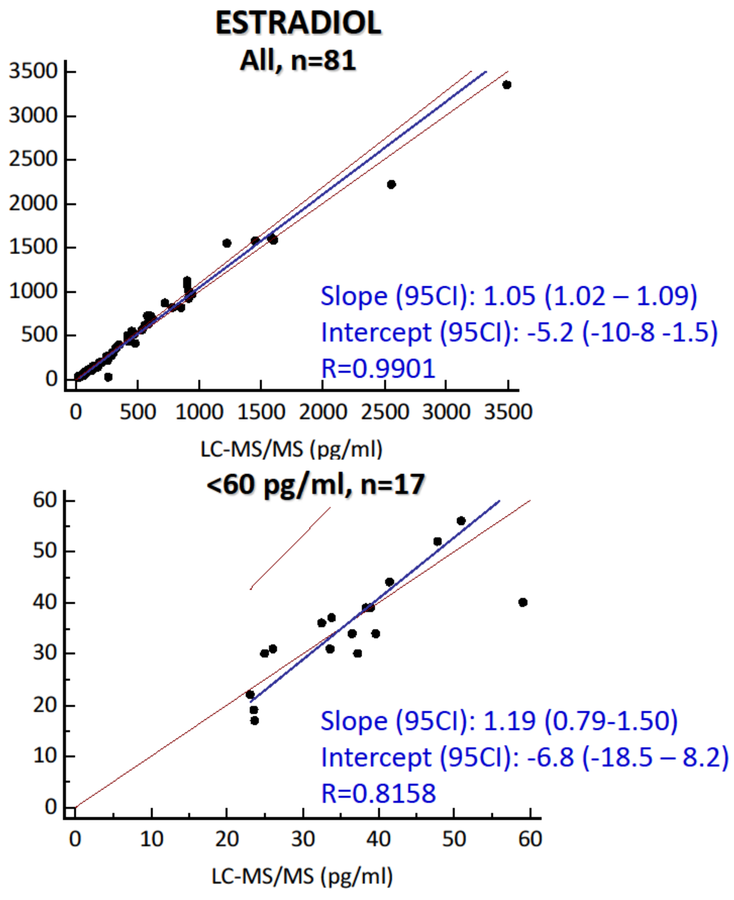
RESULTS

LC-MS/MS PERFORMANCE and VALIDATION

	Calibration range	Sensitivity in serum	Low level				Medium level				High level			
	pg/ml	pg/ml	range (pg/ml)	Intra-assay CV%	Inter-assay CV%	Accuracy (%)	range (pg/ml)	Intra-assay CV%	Inter-assay CV%	Accuracy (%)	range (pg/ml)	Intra-assay CV%	Inter-assay CV%	Accuracy (%)
E2	4.88 - 5000	9.77	< 40	6.3	4.0	102.7	200 - 350	3.5	4.2	96.0	1200 - 1600	2.2	7.4	99.3
E1	2.44 - 5000	4.88	< 40	6.8	5.6	90.3	150 - 350	1.8	5.4	90.1	1200 - 1600	1.1	5.1	96.6
OHp	9.77 - 80000	39.1	< 500	3.4	5.1	90.6	1000 - 1500	1.6	5.6	96.1	20000 - 25000	4.8	7.2	95.6
DHT	9.77 - 2500	39.1	< 160	3.1	10.2	100.6	200 - 700	8.9	4.8	82.9	1200 - 2500	5.9	6.4	90.1

REFERENCE INTERVALS: gender and age specific reference intervals were generated on 366 adult normal weight, healthy and drug free volunteers.

	FEMALES PRE-MENOPAUSAL								FEMALES MENOPAUSAL		MALES			
	All (n=130)		Follicular phase day 1 – 8 (n=30)		Ovulation day 13 – 16 (n=19)		Luteal phase day 21 – 26 (n=18)		(n=69)		Age: 18 - 44y (n=105)		Age: 45 - 80y (n=62)	
	Median	2.5 - 97.5 P	Median	2.5 - 97.5 P	Median	2.5 - 97.5 P	Median	2.5 - 97.5 P	Median	2.5 - 97.5 P	Median	2.5 - 97.5 P	Median	2.5 - 97.5 P
Age (y)	38.1	22.1 - 51.7	42.5	22.8 - 51.0	34.7	20.7 - 51.7	37.0	20.0 - 53.7	56.4	48.8 - 85.3	31.2	18.7 - 43.7	53.9	45.0 - 78.3
BMI (kg/m^2)	21.7	18.6 - 24.7	21.8	19.3 - 24.5	22.0	18.9 - 24.9	21.4	19. 6 - 23.9	22.9	19.1 - 24.7	23.0	18.7 - 24.9	23.7	20.5 - 24.9
E2 (pg/ml)	105.0	9.8 - 285.0	57.4	12.3 - 275.0	126.0	41.6 - 253.0	111.0	32.8 - 240.0	9.8	9.8 - 59.6	23.1	9.8 - 44.2	19.7	10.3 - 38.6
E1 (pg/ml)	64.0	15.6 - 143.0	41.2	21.1 - 120.0	86.6	44.9 - 168.0	65.6	31.2 - 125.0	18.8	9.6 - 51.5	32.2	18.2 - 59.0	31.6	17.4 - 53.0
OHp (ng/ml)	1.86	0.51 - 9.90	1.93	0.52 - 9.10	3.24	0.96 - 16.17	1.92	0.38 - 7.44	1.17	0.36 - 3.95	4.08	1.31 - 12.1	1.99	0.89 - 6.93
DHT (pg/ml)	68.0	39.1 - 180.0	77.8	39.1 - 161.0	71.9	39.1 - 176.0	66.9	39.1 - 184.0	39.1	39.1 - 126.0	295.0	68.0 - 651.0	314.0	85.1 - 575.0



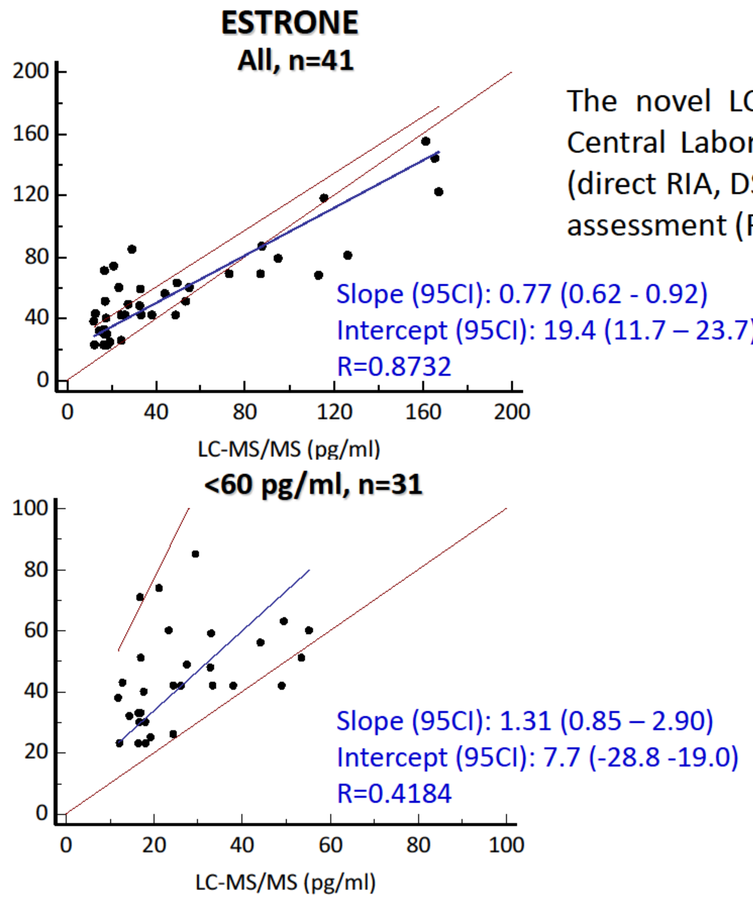


Figure 1: Passing & Bablok regression in the overall circulating range and in the lower circulating range.

LC-MS/MS vs IA COMPARISON STUDY

The novel LC-MS/MS assay was compared with IAs currently used by the Central Laboratory of S.Orsola – Malpighi Hospital of Bologna for routine E1 (direct RIA, DSL 8700 by Beckman Coulter) and E2 (ECLIA, Modular III by Roche) assessment (Fig. 1).

CONCLUSIONS

The proposed LC-MS/MS assay showed optimal performance in sensitively and accurately determining routinely-assayed estrogens, confirming the reliability of new-generation ECLIA by Roche for E2 and highlighting the severe unreliability of the direct RIA in determining E1. Our LC-MS/MS method further allowed the determination of important though not-routinely assayed DHT and 17OHpregnenolone. The low bench- and run-time required by the proposed LC-MS/MS assay, together with the reference intervals specific for age, gender and fertility status we provided, allow the immediate application of this powerful technique in research and in clinical settings, for the improvement of the characterization and of the diagnostic efficiency of female hyperandrogenism and male hypogonadism disorders.