Simultaneous Measurement of 18 Steroids in Human or Mouse Serum by LC-MS/MS to Profile the Classical and Alternate Pathways of Androgen Synthesis

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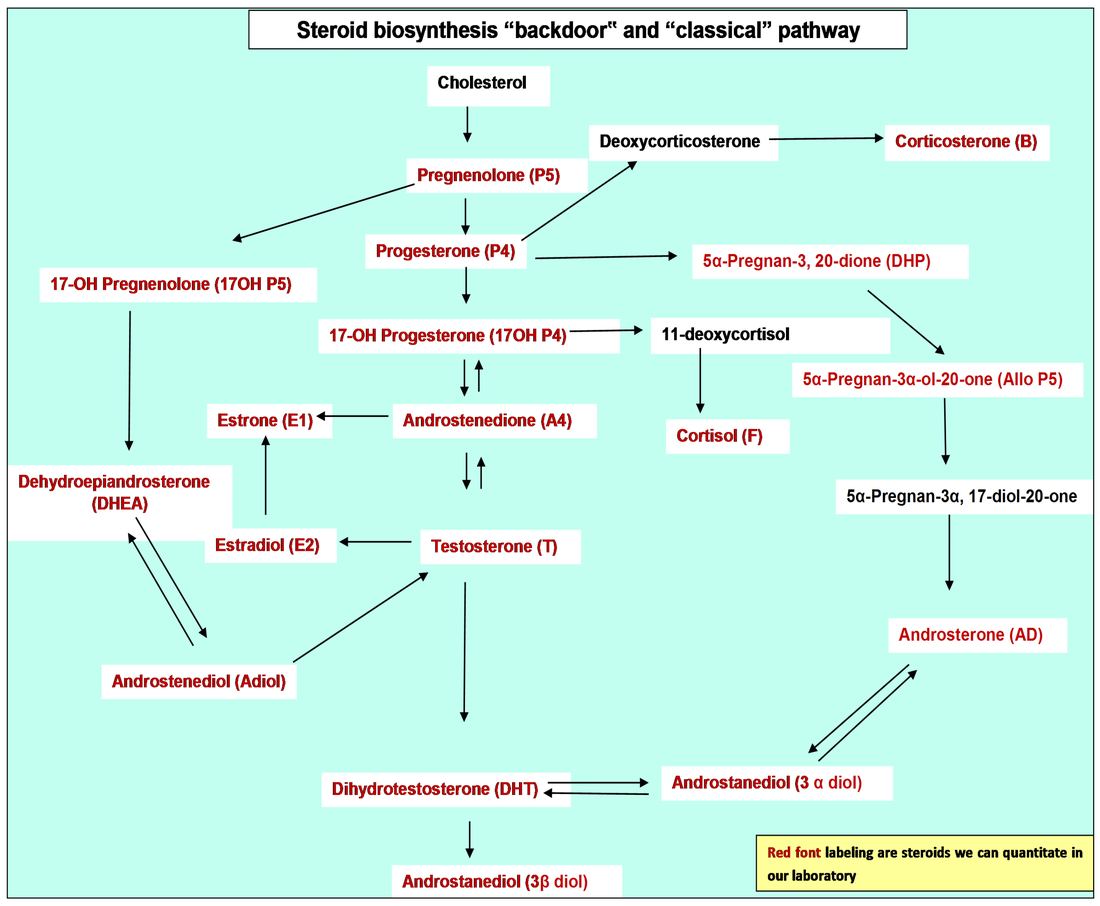
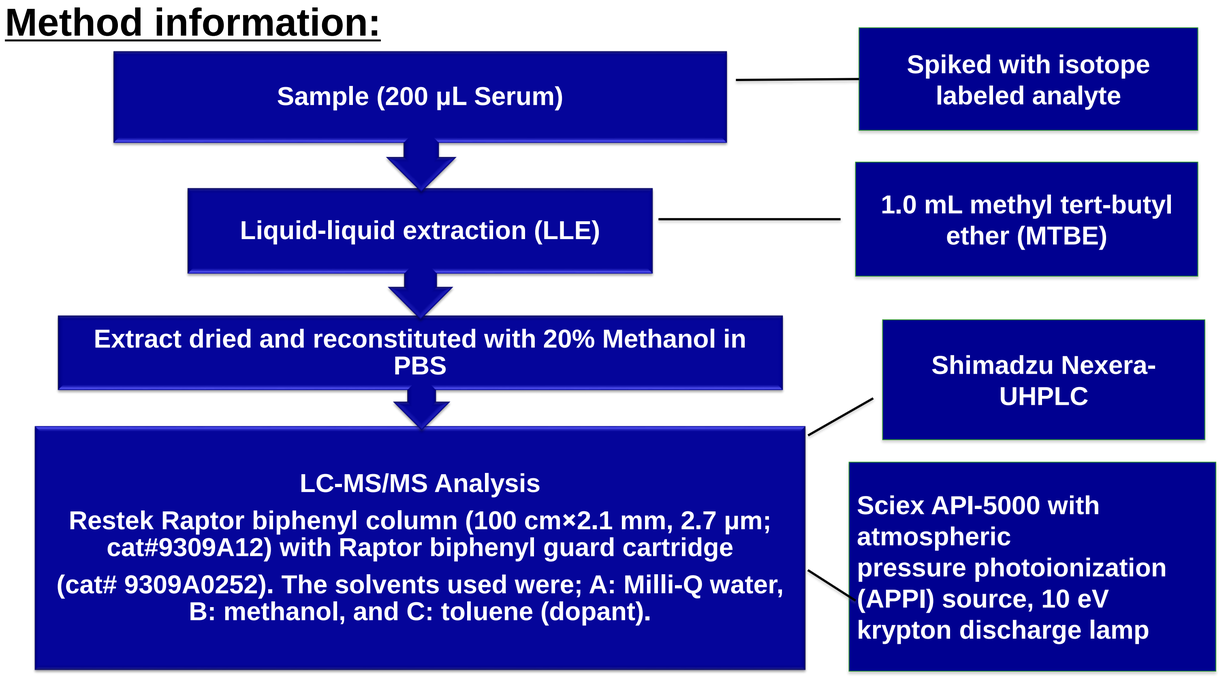
Introduction

Steroid measurement by LC–MS/MS now widely accepted as the method of choice for quantifying endogenous steroids including bioactive androgens, as well as their precursors and metabolites. The alternate, or backdoor, pathway of DHT synthesis has recently been recognized as proving a testosterone-independent route for DHT synthesis bypassing the classical pathway. We developed a rapid and versatile liquid chromatography–tandem mass spectrometry (LC–MS/MS) method to simultaneously quantify key steroids in human or mouse serum involved in either the classical or backdoor androgen synthesis pathways.



Method information:

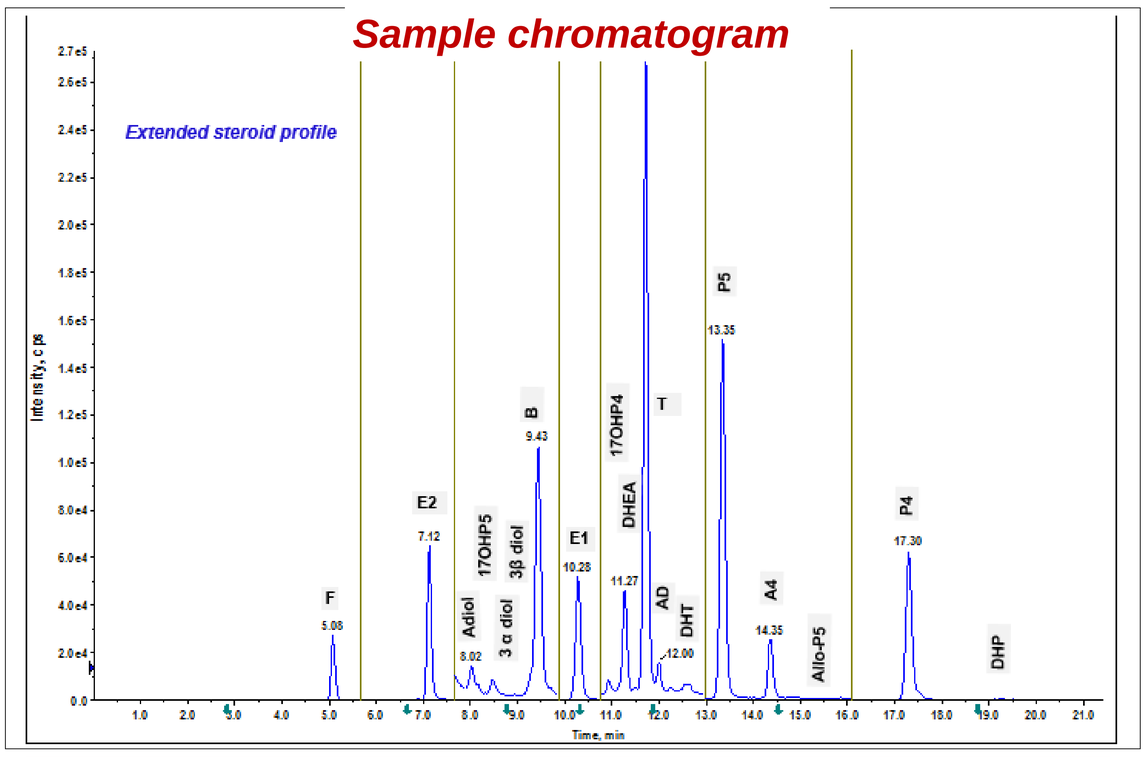


MRM settings: Optimized settings for MRM transitions of steroids quantified. The declustering potential (DP), entrance potential (EP) and exit potential (CXP) was 80, 10 and 12 in positive mode and -80, -10 and -12 in negative mode, respectively, for all analytes.

	Steroid	Ionization mode	RT (min)	Precursor Ion	MRM transition (Q1 → Q3)	CE
1	F	+ APPI	5.04	[M + H]+	363.2 → 121.3	35
	d4-F	+ APPI	4.99	[M + H]+	367.3 → 121.3	45
2	E2	−APPI	6.87	[M − H]−	271.0 → 145.0	− 57
	d4-E2	−APPI	6.87	[M − H]−	275.0→ 147.0	− 57
3	3α Diol	+ APPI	9.12	$[M - 2H_2O + H]^+$	257.0 → 161.0	28
	d3-3α Diol	+ APPI	9.06	$[M - 2H_2O + H]^+$	260.0 → 164.0	28
4	3β Diol	+ APPI	8.83	$[M - 2H_2O + H]^+$	257.0 → 161.0	28
	d3-3βDiol	+ APPI	8.80	$[M - 2H_2O + H]^+$	260.0 → 164.0	28
5	17-OHP5	+ APPI	8.35	$[M - 2H_2O + H]^+$	297.2→ 104.9	55
	d3-17-OHP5	+ APPI	8.30	$[M - 2H_2O + H]^+$	300.3→ 105.2	55
6	B	+ APPI	9.45	[M + H]+	347.2 → 121.2	30
	d8-B	+ APPI	9.38	[M + H]+	355.2 → 125.1	35
7	Adiol	+ APPI	7.88	$[M - 2H_2O + H]^+$	255.3→ 91.1	68
	d3-Adiol	+ APPI	7.82	$[M - 2H_2O + H]^+$	258.3→ 91.1	61
8	E1	−APPI	10.09	[M − H]−	269.1 → 144.9	− 53
	d4-E1	−APPI	10.02	[M − H]−	273.2 → 147.1	− 53
9	T	+ APPI	11.66	[M + H]+	289.0 → 109.0	35
	d3-T	+ APPI	11.56	[M + H]+	292.0 → 109.0	35
10	DHT	+ APPI	12.61	$[M - 2H_2O + H]^+$	273.0 → 123.0	31
	d3-DHT	+ APPI	12.51	$[M - 2H_2O + H]^+$	276.0 → 123.0	31
11	DHEA	+ APPI	11.08	$[M - 2H_2O + H]^+$	253.1→ 197.1	30
	d2-DHEA	+ APPI	11.03	$[M - 2H_2O + H]^+$	255.2→ 197.1	30
12	17- OHP4	+ APPI	11.69	[M + H]+	331.3 → 97.1	37
	d8-17- OHP4	+ APPI	11.62	[M + H]+	339.4 → 100.2	37
13	AD	+ APPI	11.93	$[M - 2H_2O + H]^+$	255.3→ 199.1	29
	d4-AD	+ APPI	11.85	$[M - 2H_2O + H]^+$	259.3→ 203.4	28
14	A4	+ APPI	13.27	[M + H]+	287.1 → 97.1	34
	d3-A4	+ APPI	13.15	[M + H]+	290.1 → 100.1	34
15	P5	+ APPI	14.26	$[M - 2H_2O + H]^+$	281.1→ 171.1	35
	d4-P5	+ APPI	14.16	$[M - 2H_2O + H]^+$	285.1→ 175.1	35
16	** AlloP5	+ APPI	14.55	$[M - 2H_2O + H]^+$	305.5 → 135.2	28
17	P4	+ APPI	17.34	[M + H]+	315.3 → 97.1	34
	d9-P4	+ APPI	17.14	[M + H]+	324.3 → 100.1	34
18	** DHP	+ APPI	18.27	[M + H]+	317.3 → 123.2	32

*** For DHP the internal standard d9-P4 and for AlloP5 the internal standard was d4-P5 used for quantitation purposes as the deuterated internal standards for these 2 compounds were not available.*

Sample chromatogram



Method Validation – LOD, LOQ, Accuracy, Precision & Recovery

Analytes	LOD ng/ml	LOQ ng/ml
T	0.01 (0.035 nM)	0.025 (0.087 nM)
DHT	0.05 (0.172 nM)	0.1 (0.344 nM)
DHEA	0.05 (0.173 nM)	0.1 (0.347 nM)
A4	0.025 (0.087 nM)	0.05 (0.175 nM)
3α diol	0.05 (0.171 nM)	0.2 (0.684 nM)
3β diol	0.05 (0.171 nM)	0.2 (0.684 nM)
17OH-P4	0.05 (0.151 nM)	0.2 (0.605 nM)
P4	0.05 (0.159 nM)	0.1 (0.318 nM)
F	0.25 (0.690 nM)	1 (2.759nM)
B	0.1 (0.289 nM)	0.25 (0.722 nM)
17OH-P5	0.05 (0.150 nM)	0.1 (0.301 nM)
Adiol	0.05 (0.171 nM)	0.1 (0.342 nM)
AD	0.05 (0.172 nM)	0.1 (0.344 nM)
P5	0.05 (0.158 nM)	0.1 (0.316 nM)
AlloP5	0.05 (0.157 nM)	0.1 (0.314 nM)
DHP	0.1 (0.316 nM)	0.2 (0.632 nM)
E2	0.0025 (0.009 nM)	0.005 (0.018 nM)
E1	0.0025 (0.009 nM)	0.005 (0.018 nM)

Steroid	Nominal concentration (ng/mL)	Within Day (n=5)		Between day (n=15)		Recoveries		Equation fit
		Accuracy	Precision	Accuracy	Precision	Human serum	Mouse serum	
		%	%CV	%	%CV	%	%	
E2	0.02	95	10	92	13	86	84	Linear
	0.08	109	8	103	10	88	87	
	0.4	104	5	98	7	89	89	
E1	0.02	99	6	93	8	85	85	Linear
	0.08	108	8	105	11	92	87	
	0.4	109	4	106	7	93	89	
T	0.4	110	8	104	6	97	96	Quadratic
	1.6	106	6	101	3	95	93	
	8	108	2	94	5	93	92	
DHT	0.4	92	13	89	11	86	90	Linear
	1.6	108	9	101	10	92	94	
	8	109	7	103	9	97	88	
3α diol	0.2	108	11	103	8	94	94	Linear
	0.8	111	7	105	5	99	92	
	4	108	5	102	9	92	92	
3β diol	0.2	95	12	98	9	93	94	Linear
	0.8	109	8	105	5	102	89	
	4	111	6	103	2	92	91	
DHEA	0.8	101	7	114	6	90	98	Quadratic
	3.2	108	6	114	3	89	98	
	16	103	4	111	3	91	97	
F	8	111	12	108	13	89	86	Quadratic
	32	110	9	105	12	99	89	
	160	109	13	109	11	94	90	
B	4	89	11	92	12	94	91	Quadratic
	16	88	11	85	9	98	90	
	80	97	8	85	7	92	87	
17OH P5	0.1	109	9	112	7	102	90	Linear
	0.4	92	6	89	11	98	88	
	2	91	3	94	2	91	90	
17OH P4	0.2	94	13	97	9	103	88	Linear
	0.8	96	11	92	11	104	89	
	4	89	7	85	9	89	88	
A4	0.1	107	8	102	11	99	90	Linear
	0.4	107	6	92	9	97	106	
	2	108	4	98	2	96	89	
AD	0.1	111	4	107	8	89	94	Linear
	0.4	106	8	102	11	90	97	
	2	101	3	97	4	88	98	
P4	0.8	101	11	114	7	100	102	Quadratic
	3.2	108	2	105	9	99	100	
	16	105	6	98	7	92	108	
P5	0.1	92	8	85	9	103	107	Linear
	0.4	103	10	112	11	92	98	
	2	109	7	97	6	91	88	
Adiol	0.4	90	12	94	9	92	90	Linear
	1.6	95	7	98	6	96	93	
	8	105	3	103	5	94	97	
AlloP5	0.1	89	7	86	9	85	83	Linear
	0.4	101	7	97	8	88	86	
	2	101	2	93	5	94	92	
DHP	0.2	95	4	98	7	86	85	Linear
	0.4	93	9	89	11	88	87	
	2	90	3	94	6	86	102	

Conclusions:

The method is sufficiently sensitive, specific and reproducible to meet the quality criteria for routine laboratory application for accurate quantitation of 18 steroid concentrations in male or female serum from humans or mice for the comprehensive profiling androgen synthesis and metabolism pathways

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