Simultaneous Measurement of 18 Steroids in Human or Mouse Serum by LC-MS/MS to Profile the



Classical and Alternate Pathways of Androgen Synthesis

<u>Reena Desai, †</u>, D. Tim Harwood, ‡ David J. Handelsman† **†ANZAC** Research Institute, University of Sydney, Sydney, NSW 2139, Australia **‡Cawthron Institute, Nelson 7010, New Zealand**



Poster number: 23h

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:



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

Analytes	LOD ng/ml	LOQ ng/ml
Т	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)
В	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)

		Spiked wi	Spiked with isotope		Nominal	Within Day (n=5) Between day (n=15)			Recoveries					
Sample (200 µL Serum)		labeled	labeled analyte Steroid		concentration	Accuracy Precision Accuracy Precision		Human	Mouse					
		1					(ng/mL)	0/	9/ CV	0/	9/ C)/	serum	serum	Equation fit
						E 2	0.02	% 05	%CV 10	[%] 0	12 %CV	% 96	% 9.4	Lincor
		V		1.0 mL meth	nvl tert-butvl		0.02	95		92	10	00	04 97	Linear
	Liquid-liquid	extraction (Ll	LE)	ether (MTBE)		0.08	103	5	08	7	80	80	
					······//	F1	0.4	99	6	93	8	85	85	Linear
							0.02	108	8	105	11	92	87	Linear
							0.00	100	4	105	7	93	89	
Extract	dried and reconst	tituted with 20	0% Methanol in			т	0.4	110	8	104	6	97	96	Quadratic
		PBS		Shimad	zu Nexera-		1.6	106	6	104	3	95	93	Quadratic
					HPLC		8	108	2	94	5	93	92	
		Y				DHT	0.4	92	13	89	11	86	90	Linear
							1.6	108	9	101	10	92	94	
	LC-MS/N	IS Analysis		Sciev APL-500	0 with		8	109	7	103	9	97	88	
Restek Ra	ptor biphenyl col	umn (100 cm	×2.1 mm, 2.7 μm;	Sciex API-500	Sciex API-5000 with		0.2	108	11	103	8	94	94	Linear
cat#93	09A12) with Rapto	or biphenyl g	uard cartridge	atmospheric			0.8	111	7	105	5	99	92	
(cat# 9309/	A0252). The solve	nts used wer	e: A: Milli-Q water.	pressure phot	toionization		4	108	5	102	9	92	92	
	B: methanol, and	C: toluene (d	lopant).	(APPI) source	e, 10 eV	3β diol	0.2	95	12	98	9	93	94	Linear
		`		krypton disch	arge lamp		0.8	109	8	105	5	102	89	
							4	111	6	103	2	92	91	
RM setting	S: Optimized sett	ings for MRM	I transitions of ster	roids quantified. The	e declusterina	DHEA	0.8	101	7	114	6	90	98	Quadratic
tential (DP), e	ntrance potential	(FP) and exit	potential (CXP) wa	as 80, 10 and 12 in r	positive mode		3.2	108	6	114	3	89	98	
d -80 -10 and	-12 in negative m	ode respecti	ively for all analyte	$a \in OO, \ io \ a i a \ i \ge in r$			16	103	4	111	3	91	97	
						F	8	111	12	108	13	89	86	Quadratic
Steroid	Ionization mo	de RT (min)	Precursor Ion	MRM transition	CE		32	110	9	105	12	99	89	
				$(01 \rightarrow 03)$			160	109	13	109	11	94	90	
-		5.04	FRA - 117 -		25	B	4	89	11	92	12	94	91	Quadratic
		5.04	[M + H]+	$363.2 \rightarrow 121.3$	35		16	88	11	85	9	98	90	
d4-F	+ APPI	4.99	[M + H]+	367.3 → 121.3	45		80	97	8	85	7	92	87	
E2	-APPI	6.87	[M – H]–	271.0 → 145.0	- 57	170H P5	0.1	109	9	112	7	102	90	Linear
d4-E2	-APPI	6.87	[M – H]–	275.0→ 147.0	- 57		0.4	92	6	89	11	98	88	
3α Diol	+ APPI	9.12	[M – 2H ₂ O + H] ⁺	257.0 → 161.0	28		2	91	3	94	2	91	90	
d3-3α Diol	+ APPI	9.06	[M – 2H ₂ O + H] ⁺	260.0 → 164.0	28	170H P4	0.2	94	13	97	9	103	88	Linear
3β Diol	+ APPI	8.83	[M – 2H ₂ O + H] ⁺	257.0 → 161.0	28		0.8	96	11	92	11	104	89	
d3-3βDiol	+ APPI	8.80	[M – 2H ₂ O + H] ⁺	260.0 → 164.0	28		4	89	7	85	9	89	88	
17-OHP5	+ APPI	8.35	[M – 2H ₂ O + H] ⁺	297.2 → 104.9	55	A 4	0.1	107	8	102	11	99	90	Linear
d3-17-OHP5	+ APPI	8.30	[M – 2H ₂ O + H] ⁺	300.3 → 105.2	55		0.4	107	6	92	9	97	106	
В	+ APPI	9.45	[M + H]+	347.2 → 121.2	30		2	108	4	98	2	96	89	
d8-B	+ APPI	9.38	[M + H]+	355.2 → 125.1	35	AD	0.1	111	4	107	8	89	94	Linear
Adiol	+ APPI	7.88	[M – 2H₂O + H]⁺	255.3→ 91.1	68		0.4	106	8	102	11	90	97	
d3-Adiol	+ APPI	7.82	[M – 2H₂O + H]⁺	258.3 → 91.1	61		2	101	3	97	4	88	98	
E1		10.09	[M – H]–	269.1 → 144.9	- 53	P4	0.8	101	11	114	7	100	102	Quadratic
d4-F1		10.02	[M – H]–	$273.2 \rightarrow 147.1$	- 53		3.2	108	2	105	9	99	100	
T		11.66	[M + H]+	$270.2 \rightarrow 147.1$	35		16	105	6	98	7	92	108	
42 T		11.56		$203.0 \rightarrow 109.0$	25	P5	0.1	92	8	85	9	103	107	Linear
03-1 DUT		11.50		$292.0 \rightarrow 109.0$	35		0.4	103	10	112	11	92	98	
		12.61	$[M - 2H_2O + H]$	$273.0 \rightarrow 123.0$	31		2	109	7	97	6	91	88	
d3-DHT	+ APPI	12.51	$[W - 2H_2O + H]^{+}$	$276.0 \rightarrow 123.0$	31	Adiol	0.4	90	12	94	9	92	90	Linear
DHEA	+ APPI	11.08	$[M - 2H_2O + H]^2$	253.1→ 197.1	30		1.6	95	7	98	6	96	93	
d2-DHEA	+ APPI	11.03	[M – 2H ₂ O + H]⁺	255.2 → 197.1	30		8	105	3	103	5	94	97	
17- OHP4	+ APPI	11.69	[M + H]+	331.3 → 97.1	37	AlloP5	0.1	89	7	86	9	85	83	Linear
d8-17- OHP4	+ APPI	11.62	[M + H]+	339.4 → 100.2	37		0.4	101	7	97	8	88	86	
AD	+ APPI	11.93	[M – 2H ₂ O + H] ⁺	255.3 → 199.1	29		2	101	2	93	5	94	92	
d4-AD	+ APPI	11.85	[M – 2H ₂ O + H] ⁺	259.3 → 203.4	28	DHP	0.2	95	4	98	7	86	85	Linear
A4	+ APPI	13.27	[M + H]+	287.1 → 97.1	34		0.4	93	9	89	11	88	87	
d3-A4	+ APPI	13.15	[M + H]+	290.1 → 100.1	34		2	90	3	94	6	86	102	
P5	+ APPI	14.26	[M – 2H ₂ O + H] ⁺	281.1 → 171.1	35	Conclu	sione							
d4-P5	+ APPI	14.16	[M – 2H ₂ O + H] ⁺	285.1→ 175.1	35						-		,	
** AlloP5	+ APPI	14.55	[M – 2H₂O + H]⁺	$305.5 \rightarrow 135.2$	28	The met	hod is suffic	ently se	ensitive,	specific	and repr	oducible	to meet	t the quality
PA	+ APDI	17 34	[M + H]+	315.3 07.1	34	criteria f	or routine la	boratory	v applica	tion for a	accurate	quantita	tion of 1	8 steroid
		17.54		$324.2 \rightarrow 37.1$	34	concent	rations in ma	le or fer	nale ser	um from	humans	or mice	for the	
** 010		17.14		$324.3 \rightarrow 100.1$	34	comprol	hansiva nrafi	lina and	ronon	Inthosis	and mot	aholiem	nathway	18
	+ APPI	18.27	[M + H]+	317.3 → 123.2	32	compren	iciisive piuli	my and	vyen sj	y11118515	anu met	anonsin	pauwdy	3

					(Q1 → Q3)	
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	2	-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 ₂ O + H]⁺	257.0 → 161.0	28
d3-	-3α Diol	+ APPI	9.06	[M – 2H ₂ O + H]⁺	$\textbf{260.0} \rightarrow \textbf{164.0}$	28
4 3β	Diol	+ APPI	8.83	[M – 2H ₂ O + H]⁺	257.0 → 161.0	28
d3-	-3βDiol	+ APPI	8.80	[M – 2H ₂ O + H] ⁺	260.0 → 164.0	28
5 17-	-OHP5	+ APPI	8.35	[M – 2H₂O + H]⁺	297.2 → 104.9	55
d3-	-17-OHP5	+ APPI	8.30	[M – 2H ₂ O + H]⁺	300.3 → 105.2	55
6 B		+ APPI	9.45	[M + H]+	347.2 → 121.2	30
d8-	-В	+ APPI	9.38	[M + H]+	355.2 → 125.1	35
7 Adi	liol	+ APPI	7.88	[M – 2H ₂ O + H]⁺	255.3→ 91.1	68
d3-	-Adiol	+ APPI	7.82	[M – 2H ₂ O + 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-	з-т	+ APPI	11.56	[M + H]+	292.0 → 109.0	35
10 DH	IT	+ APPI	12.61	[M – 2H ₂ O + H]⁺	273.0 → 123.0	31
d3-	-DHT	+ APPI	12.51	[M – 2H ₂ O + H]⁺	276.0 → 123.0	31
11 DH	IEA	+ APPI	11.08	[M – 2H ₂ O + H] ⁺	253.1→ 197.1	30
d2-	-DHEA	+ APPI	11.03	[M – 2H ₂ O + 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 ₂ O + H]⁺	255.3→ 199.1	29
d4-	-AD	+ APPI	11.85	[M – 2H ₂ O + H]⁺	259.3 → 203.4	28
14 A4	L ·	+ APPI	13.27	[M + H]+	287.1 → 97.1	34
d3-	S-A4	+ APPI	13.15	[M + H]+	290.1 → 100.1	34
15 P5	5	+ APPI	14.26	[M – 2H ₂ O + H] ⁺	281.1 → 171.1	35
d4-	-P5	+ APPI	14.16	[M – 2H ₂ O + H] ⁺	285.1→ 175.1	35
16 ** A	AlloP5	+ APPI	14.55	[M – 2H ₂ O + H] ⁺	305.5 → 135.2	28
17 P4	L .	+ APPI	17.34	[M + H]+	315.3 → 97.1	34
d9-	-P4	+ APPI	17.14	[M + H]+	324.3 → 100.1	34
18 ** C	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.

This work is published in Jan 2019 : Clinical Mass spectrometry Journal https://doi.org/10.1016/j.clinms.2018.12.003