Supporting Information

Multidimensional Separations of Intact Phase II Steroid Metabolites Utilizing LC-Ion Mobility-HRMS

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Comments on LC-IM-MS instrument settings and data presented in this work

In this supporting information, we provide tables for steroids, RPLC settings, source settings, DTIMS settings, figures for the novel/synthesized AAS investigated in human urine, LC-IM-HRMS isomer analysis, and conformational space analysis.

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Table S1. AASs separated by isomer groups, neutral formulas, neutral exact mass, adduct exact mass, retention time (RT) using the 15 minute method, elution order annotation, RT CV%, collision cross sections (Å^2), and CCS CV% (n = 3 technical replicates over 3 different days at 5 µg/mL). The color coordination is for isomer types described throughout this manuscript.

AAS	Formula	Exact Mass	$[M-H]^-$	Elution #	RT (min)	RT CV%	CCS	CCS CV%
Epi-THMT S3	$C_{20}H_{34}O_5S$	386.213	385.205	22	9.5	0.2	200.7	0.3
Methenolone M1 G	$C_{26}H_{38}O_8$	478.257	477.249	18	6.4	0.2	217.6	0.1
7-Keto DHEA-3 S	$C_{19}H_{26}O_6S$	382.145	381.137	1	1.4	2.1	195.0	0.2
Bolasterone M1 G	$C_{27}H_{44}O_8$	496.304	495.296	19	6.4	0.5	212.2	0.1
EpiNandrolone S	$C_{18}H_{26}O_5S$	354.150	353.142	7	3.1	2.3	189.3	0.1
Boldenone G	$C_{25}H_{34}O_8$	462.225	461.218	5	2.8	2.3	217.4	0.1
Prednisolone 21-S	$C_{21}H_{28}O_8S$	440.151	439.143	3	2.1	3.2	196.9	0.1
Mesterolone M2 G	$C_{26}H_{42}O_8$	482.288	481.280	17	6.3	0.3	220.0	0.1
Nandrolone G	$C_{24}H_{34}O_8$	450.225	449.218	6	2.8	2.5	215.4	0.1
Stanozolol 1'N – G	$C_{27}H_{40}N_2O_7$	504.284	503.276	10	4.1	1.4	231.0	0.1
Stanozolol 3'OH G	$C_{27}H_{40}N_2O_8$	520.279	519.271	14	5	0.6	229.9	0.1
Constitutional Isomers								
16α-hydroxy DHEA 3-S	$C_{19}H_{28}O_6S$	384.161	383.153	2	1.9	1.0	199.1	0.1
11-Ketoetiocholanolone S	$C_{19}H_{28}O_6S$	384.161	383.153	4	2.5	1.8	188.4	0.1
5α-androstan-3β-ol-16-one S	$C_{19}H_{30}O_5S$	370.181	369.174	13	4.7	1.6	197.3	0.1
Epitestosterone S	$C_{19}H_{28}O_5S$	368.166	367.158	9	4.1	2.1	193.0	0.03
Mesterolone M1 G	$C_{26}H_{40}O_9$	480.272	479.265	20	7.3	0.3	217.8	0.1
Drostanolone M1 G	$C_{26}H_{40}O_8$	480.272	479.265	21	8.5	0.3	218.7	0.1
Stereoisomers								
Prasterone S (3α)	$C_{19}H_{28}O_5S$	368.166	367.158	12	4.6	1.9	194.7	0.1
Prasterone S (3β)	$C_{19}H_{28}O_5S$	368.166	367.158	8	3.9	1.1	196.4	0.2
Epiandrosterone S	$C_{19}H_{30}O_5S$	370.181	369.174	11	4.5	2.1	197.4	0.1
Androsterone S	$C_{19}H_{30}O_5S$	370.181	369.174	16	5.5	0.6	195.7	0.1
Etiocholanolone S	$C_{19}H_{30}O_5S$	370.181	369.174	15	5.4	1.2	196.2	0.1

The following AASs are grouped by supplier where the common name corresponds to **Table S1** and the chemical name is provided in the parentheses. Epitestosterone S (4-androsten-17α-ol-3-one sulphate), 7-Keto DHEA-3 S (5-androsten-3β-ol-7,17-dione sulphate), 16α-hydroxy DHEA S (5-androsten-3β, 16α-diol-17-one-3 sulphate), Prednisolone 21-S (1,4-pregnadien-11,17,21-triol-3,20-dione 21-sulphate), 11-Ketoetiocholanolone S (5β-androstan-3α-ol-11,17-dione sulphate), Prasterone S (3β) (5-androsten-3β-OL-17-one sulphate), Epiandrosterone S (5α-androstan-3β-ol-17-one sulphate), Prasterone (3α) S (5-androsten-3α-OL-17-one sulphate), 5α-androstan-3β-ol-one S, Etiocholanolone S (5β-androstan-3α-ol-17-one sulphate), and Androsterone S (5α-androstan-3α-ol-17-one sulphate) were purchased from Steraloids Inc. (Newport, RI, USA). Drostanolone M1 G (2α-methyl-5α-androstan-3α-ol-17-one-3-β-D-glucuronic acid), Methenolone M1 G (1-methylene-5α-androstan-3α-ol-17-one-3-β-D-glucuronic acid), Mesterolone M1 G (1α-methyl-5α-androstan-3α-ol-17-one-3β-D-glucuronic acid), Mesterolone M2 G (1α-methyl-5α-androstan-3α,17β-diol-3-β-D-glucuronic acid), Boldenone G (1,4-adrostadien-17β-diol-3-one-17-β-D-glucuronic acid), Bolasterone M1 G (7α,17α-dimethyl-5β-androstan-3α,17β-diol-3-β-D-glucuronic acid), Stanozolol 3'OH G (5α-androstan-[3,2-c] pyrazole-3',17β-diol-17α-methyl-3'-β-glucuronic acid, Nandrolone G (4-estren-17β-ol-3-one-17-β-D-glucuronic acid), Epinandrolone S (17α-sulfoxy-4-estren-3-one), 19-norandrosterone D4 G (2,2,4,4-d4-5α-Estran-3α-ol-17-one-3-β-D- glucuronic acid), Testosterone D3 S (16,16,17α-d3-17β-sulfoxy-androst-4-en-3-one) were purchased from The National Measurement Institute of Australia (NMIA). Epi-THMT S3 (3α-sulfoxy-17β-methyl-5β-androstan-17α-ol) was a kind gift from the Institute Hospital del Mar d'Investigacions Mèdiques (IMIM) (Barcelona, Spain). Stanozolol 1'N-G (5α-androstan-[3,2-c] pyrazole-3',17β-diol-17α-methyl-1´N-glucuronic acid) was provided by Seibersdorf Laboratories (Austria).

Table S2: LC-IM-HRMS parameters used to collect the steroid data in our work. Source settings for the Agilent Jet Stream ESI and chromatographic conditions were optimized specifically for this study using Agilent 1290 I (LC) and 6560 (DTIMS-HRMS) instruments.

RPLC Settings

Parameter	Value	Units
Column	Waters ACQUITY BEH C18	2.1 x 75 mm (1.7 µm)
Temperature	45	Celsius
Injection Volume	10	µL
Flow Rate	0.400	mL/min
Mobile Phase (A)	H_2O	1 mM ammonium formate/ 0.1% formic acid
Mobile Phase (B)	MeOH	1 mM ammonium formate/ 0.1% formic acid

RPLC Settings

Time (min)	%B
0	45
1	45
9.5	70
10.5	100
12	100
13	45
15	45

Source Settings

Parameter	Value	Units
Gas Temp	300	Celsius
Drying Gas	12	L/min
Nebulizer	20	psi
Sheath Gas Temp	300	Celsius
Sheath Gas Flow	12	L/min
Capillary Voltage (VCap)	2000	V
Nozzle Voltage	500	V

RPLC Settings

Time (min)	%B
0	50
1	50
5.5	100
7	100
8	50
10	50

DTIMS-MS Single Pulse Settings

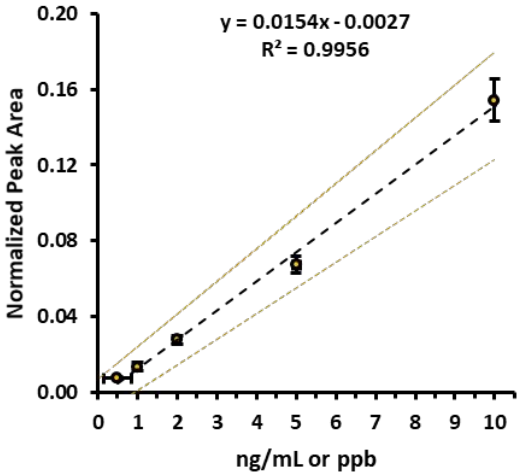
Parameter	Value	Units
Mass Range	50-1700	m/z
Trap Fill Time	20000	µs
Trap Release Time	180	µs
Frame Rate	0.9	Frames/sec
IM Transient Rate	14	IM Transients/Frame
Max Drift Time	60	ms
TOF Transient Rate	600	Transients/IM Transients
Drift Tube Entrance	-1474	V
Drift Tube Exit	-224	V
Rear Funnel Entrance	-217.5	V
Rear Funnel Exit	-45	V

DTIMS-MS Ion multiplexing Settings

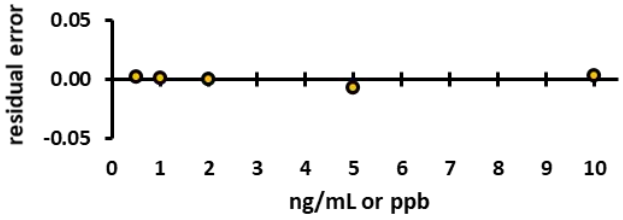
Parameter	Value	Units
Mass Range	50-1700	m/z
Trap Fill Time	3900	µs
Trap Release Time	180	µs
Frame Rate	0.9	Frames/sec
IM Transient Rate	18	IM Transients/Frame
Max Drift Time	60	ms
TOF Transient Rate	600	Transients/IM Transients
Drift Tube Entrance	-1474	V
Drift Tube Exit	-224	V
Rear Funnel Entrance	-217.5	V
Rear Funnel Exit	-45	V

Figure S1. Anabolic androgenic steroid investigated in human urine (top table). A calibration curve (left) showing the lower dynamic range from 0.5 ppb to 10 ppb with the gold dashed lines representing the 95% confidence interval. Standard error bars (n = 3 intraday technical replicates) were used to demonstrate variation in both peak area and concentration. A table is provided to the right of the calibration curve to provide expected concentrations, normalized peak areas, standard deviations, calculated concentrations, and analytical figures of merit (e.g., precision by %CV, accuracy by %bias, and LOQ). Also, a residual error versus concentration plot was provided to evaluate the validity of the linear regression model.

Analyte	LOQ (ng/mL)	Formula	Exact m/z	Adduct	Exp. m/z	Mass error (ppm)	IM drift time (ms)	CCS (Å^2)	CCS %diff standard vs sample
Epi-THMT S3 (human urine sample)	1	$C_{20}H_{31}O_4S$	367.194	$[M\text{-}H_2O\text{-}H]^-$	367.193	-3.5	28.56	200.8	0.3

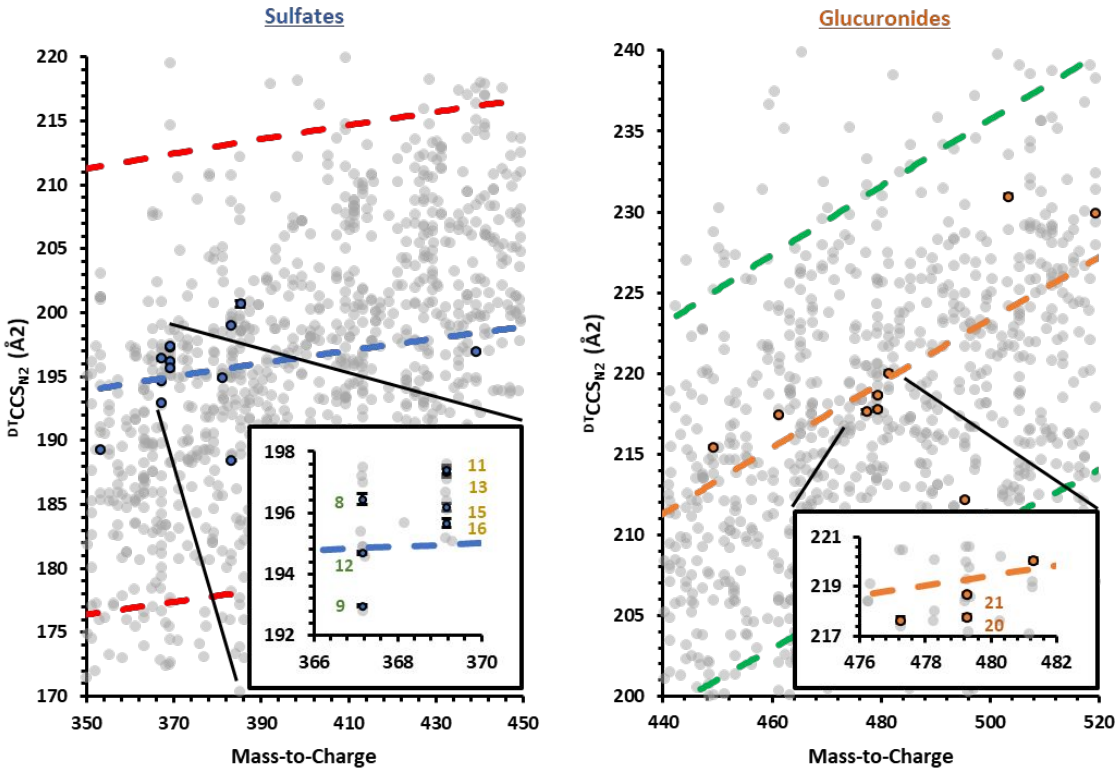


[expected] (ppb)	normalized peak area	%CV	[calculated] (ppb)	%bias	
0.5	0.0076	8.5%	0.67	-34%	LOD
1.0	0.0137	15%	1.06	-6.4%	LOQ
2.0	0.0279	7.6%	1.99	0.7%	
5.0	0.0673	6.6%	4.55	9.1%	
10	0.1541	7.2%	10.2	-1.8%	



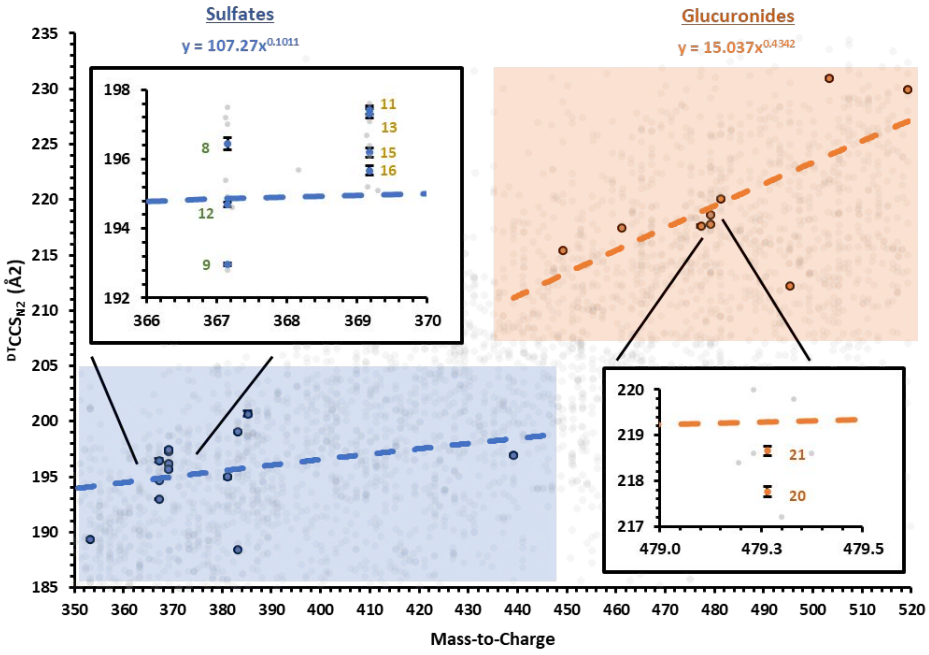
AAS in Human Urine. To evaluate the fitness for purpose of our LC-IM-HRMS method, urine samples spiked with Epi-THMT S3 were tested after the SPE extraction protocol described in the Methods section (**Figure S1**). This metabolite was assessed at several low concentration levels to demonstrate analytical figures of merit, such as limits of detection and quantification limits in human urine, shown in **Figure S1**. In addition to ppb level quantification/linearity, high mass measurement accuracy, and retention time alignment afforded by our instrument setup, observed CCS values for Epi-THMT S3 in the human urine samples are in good agreement with CCS values from neat standards analyzed on different days (typically <0.5% difference) as shown in **Figure S1**. These results illustrate the potential benefits of adding the IMS separation to current analytic workflows in routine testing laboratories performing urine analyses, especially for anti-doping purposes.

Figure S2. Conformational space analysis showing CCS values for the phase II steroids investigated using LC-IM-HRMS with neat standards. Included is a blue dashed trendline, fit to a power function, representing the best fit line of the sulfated data and the orange trendline represents glucuronide data. Also shown are red dashed lines representing ±10% deviation from the best fit line for sulfated data and green dashed lines representing ±10% deviation from the best fit line for glucuronide data. Measured phase II steroids were within ±10% of the best fit line. Error bars represent standard errors and are for most values within the scale of the marker (*n* = 3 technical replicates over 3 different days at 5 µg/mL). The gray data points represent ~5000-9000 entries from blank human urine.



Separation of isomeric AAS via LC-IM-HRMS can also be observed in **Figure S2**. The data in **Figure S2** exemplify the presence of unique mass-mobility correlations with ± 10% deviation from the best fit line for 13 sulfate AASs and for 9 glucuronide AASs. This differentiation demonstrates the structural properties of AAS and lays the groundwork for using CCS measurements as an identifier in untargeted and/or targeted AAS studies.[2] Taken together, LC-IM-HRMS data strengthen the confidence in identifying AAS in a complex biological matrix and offers a characterization strategy of potentially unknown or known structurally similar steroids or steroid-like compounds in urine as chromatographically coeluting interferences.

Figure S3. Conformational space analyses showing CCS values for the phase II steroids investigated using LC-IM-HRMS with neat standards. Included is a blue dashed trendline, fit to a power function, representing the best fit line of the sulfate data and the orange trendline represents glucuronide data. The boxes are for isomer groups. Error bars represent standard errors and are for most values within the scale of the marker (n = 3 technical replicates over 3 different days at 5 µg/mL). The gray data points represent ~5000-9000 entries from blank human urine.



Mass-Mobility Correlations. This study's primary objectives were to determine CCS values of known AAS standards and develop correlations between mass and CCS values for two of the main AAS phase II metabolite groups (sulfonic acid and glucuronic acid). The fused ring sterol core, common to these AAS, has no rotational freedom. Therefore, the variability in CCS values is introduced by ketones, double bonds, a pyrazole ring, and stereochemistry of proton, methyl, hydroxyl, sulfate, and glucuronide functional groups. AASs cluster in CCS vs. *m/z* conformational space based on their structural similarity (**Figure S3**). The AAS sulfate-conjugates exhibited the smaller CCS values, likely resulting from this group possessing a smaller sulfonic acid functional group than the glucuronides. The IMS resolving power was able to demonstrate different CCS values between most compounds within isomer sets (color coordinating in **Table 1** and **Figure 1**). The AAS glucuronide-conjugates exhibited larger CCS values, likely because of the larger glucuronic acid functional groups.

Utilizing the CCS values reported here, we can develop correlations similar to those used by Picache et al. in the Unified CCS Compendium by mapping expected mobility-mass space for AAS phase II metabolites.[2] Our AAS data is plotted using a power fit. This correlation is representative of AAS phase II metabolites (sulfate and glucuronide). By reporting an AAS correlation generated from standard reference materials (**Figure S3**) and using it to identify known and unknown AAS in human samples CCS values in future studies, we can increase AAS annotation confidence.[3] Utilizing the information gained from the trendline classification of AAS phase II metabolites shows great promise for identifying new and emerging AASs by plotting their *m/z* and CCS values. All values fall within ± 10% of the calculated correlation, demonstrating its use for potentially identifying AAS phase II metabolite unknowns.

Figure S4. (A) LC-IM-MS analyses showing an LC chromatogram of 11 isomeric phase II AASs in the liquid phase. (B) LC-IM-MS analyses showing experimental DTIMS traces (ms) of 11 isomeric phase II AASs in the gas phase.

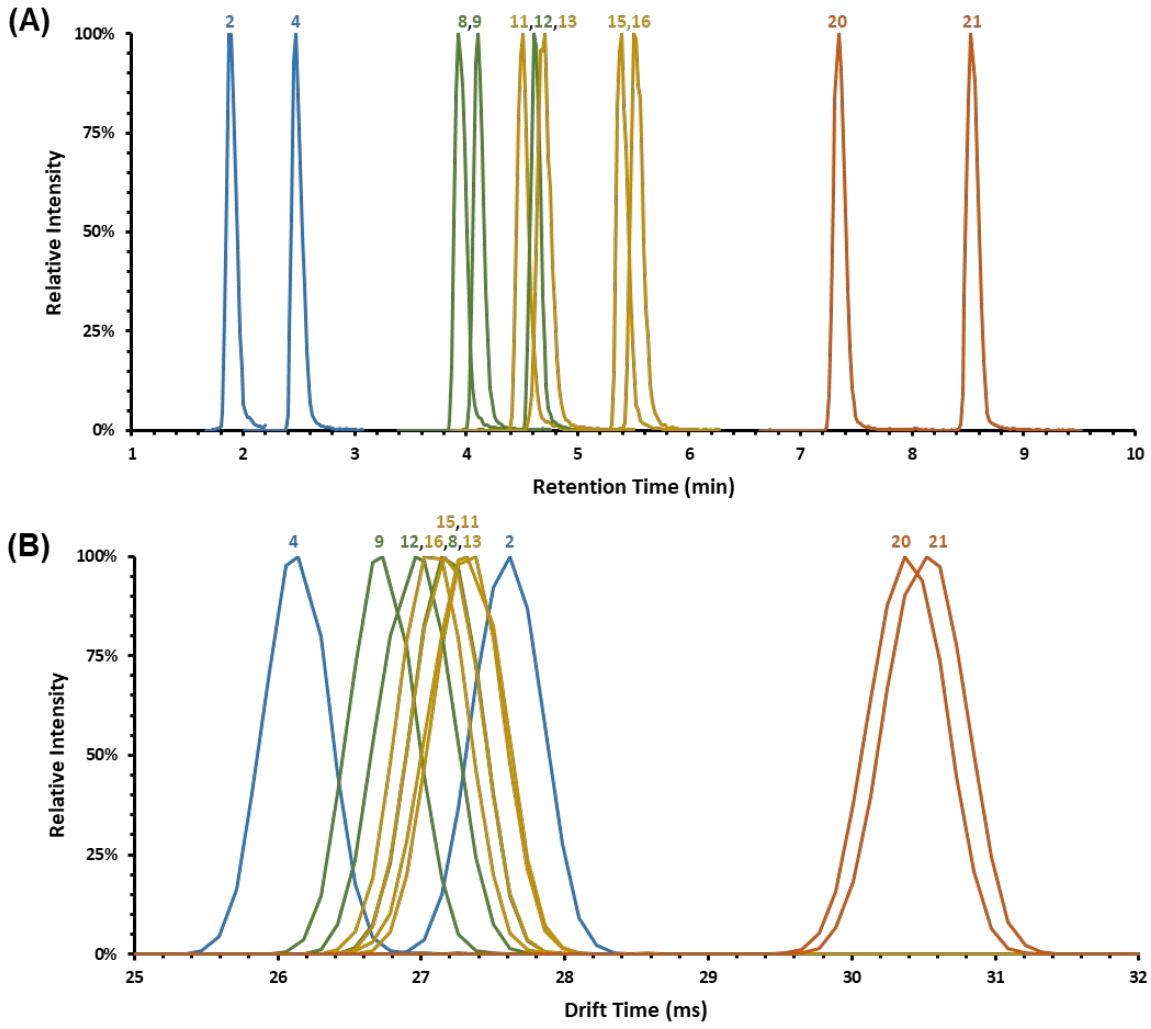


Table S3. P-values for sulfate isomeric retention times and collision cross section values ((2) 16alpha-hydroxy DHEA 3-S and (4) 11-Ketoetiocholanolone S), calculated by t-tests.

	Sulfate Isomers RT values				
Column B	(4) 11-Ketoetiocholanolone S				
vs.	vs.				
Column A	(2) 16alpha-hydroxy DHEA 3-S				
Unpaired t test					
P value	< 0.0001				
P value summary	****				
Significantly different? (P < 0.05)	Yes				
One- or two-tailed P value?	Two-tailed				
t	df	t=20.88 df=4			
How big is the difference?					
Mean ± SEM of column A	1.853 ± 0.01074 N=3				
Mean ± SEM of column B	2.434 ± 0.02567 N=3				
Difference between means	0.5810 ± 0.02782				
95% confidence interval	0.5037 to 0.6582				
R square	0.9909				
F test to compare variances					
F	DFn	Dfd	5.71	2	2
P value	0.2981				
P value summary	ns				
Significantly different? (P < 0.05)	No				

	Sulfate Isomers CCS values				
Column B	(4) 11-Ketoetiocholanolone S				
vs.	vs.				
Column A	(2) 16alpha-hydroxy DHEA 3-S				
Unpaired t test					
P value	< 0.0001				
P value summary	****				
Significantly different? (P < 0.05)	Yes				
One- or two-tailed P value?	Two-tailed				
t	df	t=77.12 df=4			
How big is the difference?					
Mean ± SEM of column A	199.1 ± 0.1202 N=3				
Mean ± SEM of column B	188.5 ± 0.06667 N=3				
Difference between means	-10.60 ± 0.1374				
95% confidence interval	-10.98 to -10.22				
R square	0.9993				
F test to compare variances					
F	DFn	Dfd	3.25	2	2
P value	0.4706				
P value summary	ns				
Significantly different? (P < 0.05)	No				

Table S4. P-values for glucuronide isomeric retention times and collision cross section values ((20) Mesterolone M1 G and (21) Drostanolone M1 G), calculated by t-tests.

Table Analyzed	Glucuronide Isomers RT values				
Column B	(21) Drostanolone M1 G				
vs.	vs.				
Column A	(20) Mesterolone M1 G				
Unpaired t test					
P value	< 0.0001				
P value summary	****				
Significantly different? (P < 0.05)	Yes				
One- or two-tailed P value?	Two-tailed				
t	df	t=69.96 df=4			
How big is the difference?					
Mean ± SEM of column A	7.366 ± 0.01131 N=3				
Mean ± SEM of column B	8.540 ± 0.01239 N=3				
Difference between means	1.173 ± 0.01677				
95% confidence interval	1.127 to 1.220				
R square	0.9992				
F test to compare variances					
F	DFn	Dfd	1.2	2	2
P value	0.9092				
P value summary	ns				
Significantly different? (P < 0.05)	No				

Table Analyzed	Glucuronide Isomers CCS values				
Column B	(21) Drostanolone M1 G				
vs.	vs.				
Column A	(20) Mesterolone M1 G				
Unpaired t test					
P value	0.0101				
P value summary	*				
Significantly different? (P < 0.05)	Yes				
One- or two-tailed P value?	Two-tailed				
t	df	t=4.596 df=4			
How big is the difference?					
Mean ± SEM of column A	217.8 ± 0.1453 N=3				
Mean ± SEM of column B	218.6 ± 0.1202 N=3				
Difference between means	0.8667 ± 0.1886				
95% confidence interval	0.3431 to 1.390				
R square	0.8408				
F test to compare variances					
F	DFn	Dfd	1.461	2	2
P value	0.8125				
P value summary	ns				
Significantly different? (P < 0.05)	No				

Table S5. P-values for sulfate isomeric retention times and collision cross section values ((8) Prasterone S (3beta), (9) Epitestosterone S, (12) Prasterone S (3alpha)), calculated by ANOVA followed by Tukey's corrections for multiple comparisons. ns stands for not significant.

Number of families	1	Sulfate Isomers RT values						
Number of comparisons per family	3							
Alpha	0.05							
Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary	Adjusted P Value			
(8) Prasterone S (3beta) vs. (9) Epitestosterone S	-0.1185	-0.2841 to 0.04709	No	ns	0.1635			
(8) Prasterone S (3beta) vs. (12) Prasterone S (3alpha)	-0.6146	-0.7802 to -0.4490	Yes	****	< 0.0001			
(9) Epitestosterone S vs. (12) Prasterone S (3alpha)	-0.4961	-0.6494 to -0.3428	Yes	****	< 0.0001			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
(8) Prasterone S (3beta) vs. (9) Epitestosterone S	3.88	3.999	-0.1185	0.05795	3	4	2.892	8
(8) Prasterone S (3beta) vs. (12) Prasterone S (3alpha)	3.88	4.495	-0.6146	0.05795	3	4	15	8
(9) Epitestosterone S vs. (12) Prasterone S (3alpha)	3.999	4.495	-0.4961	0.05365	4	4	13.08	8

Number of families	1	Sulfate Isomers CCS values						
Number of comparisons per family	3							
Alpha	0.05							
Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary	Adjusted P Value			
(8) Prasterone S (3beta) vs. (9) Epitestosterone S	3.467	2.932 to 4.001	Yes	****	< 0.0001			
(8) Prasterone S (3beta) vs. (12) Prasterone S (3alpha)	1.733	1.199 to 2.268	Yes	***	0.0001			
(9) Epitestosterone S vs. (12) Prasterone S (3alpha)	-1.733	-2.268 to -1.199	Yes	***	0.0001			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
(8) Prasterone S (3beta) vs. (9) Epitestosterone S	196.4	193	3.467	0.1743	3	3	28.13	6
(8) Prasterone S (3beta) vs. (12) Prasterone S (3alpha)	196.4	194.7	1.733	0.1743	3	3	14.07	6
(9) Epitestosterone S vs. (12) Prasterone S (3alpha)	193	194.7	-1.733	0.1743	3	3	14.07	6

Table S6. P-values for sulfate isomeric retention times and collision cross section values ((11) Epiandrosterone S, (13) 5alpha-androstan-3beta-ol-16-one S, (15) Etiocholanolone S, and (16) Androsterone S), calculated by ANOVA followed by Tukey's corrections for multiple comparisons. ns stands for not significant.

Number of families	1	Sulfate Isomers RT values						
Number of comparisons per family	6							
Alpha	0.05							
Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary	Adjusted P Value			
(11) Epiandrosterone S vs. (13) 5alpha-androstan-3beta-ol-16-one S	-0.2079	-0.3525 to -0.06323	Yes	**	0.0052			
(11) Epiandrosterone S vs. (15) Etiocholanolone S	-0.9292	-1.074 to -0.7846	Yes	****	< 0.0001			
(11) Epiandrosterone S vs. (16) Androsterone S	-1.073	-1.218 to -0.9283	Yes	****	< 0.0001			
(13) 5alpha-androstan-3beta-ol-16-one S vs. (15) Etiocholanolone S	-0.7213	-0.8659 to -0.5767	Yes	****	< 0.0001			
(13) 5alpha-androstan-3beta-ol-16-one S vs. (16) Androsterone S	-0.8651	-1.010 to -0.7204	Yes	****	< 0.0001			
(15) Etiocholanolone S vs. (16) Androsterone S	-0.1437	-0.2883 to 0.0008919	No	ns	0.0516			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
(11) Epiandrosterone S vs. (13) 5alpha-androstan-3beta-ol-16-one S	4.385	4.593	-0.2079	0.04871	4	4	6.035	12
(11) Epiandrosterone S vs. (15) Etiocholanolone S	4.385	5.314	-0.9292	0.04871	4	4	26.98	12
(11) Epiandrosterone S vs. (16) Androsterone S	4.385	5.458	-1.073	0.04871	4	4	31.15	12
(13) 5alpha-androstan-3beta-ol-16-one S vs. (15) Etiocholanolone S	4.593	5.314	-0.7213	0.04871	4	4	20.94	12
(13) 5alpha-androstan-3beta-ol-16-one S vs. (16) Androsterone S	4.593	5.458	-0.8651	0.04871	4	4	25.12	12
(15) Etiocholanolone S vs. (16) Androsterone S	5.314	5.458	-0.1437	0.04871	4	4	4.173	12

Number of families	1	Sulfate Isomers CCS values						
Number of comparisons per family	6							
Alpha	0.05							
Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary	Adjusted P Value			
(11) Epiandrosterone S vs. (13) 5alpha-androstan-3beta-ol-16-one S	0.1333	-0.5418 to 0.8084	No	ns	0.9187			
(11) Epiandrosterone S vs. (15) Etiocholanolone S	1.233	0.5582 to 1.908	Yes	**	0.0017			
(11) Epiandrosterone S vs. (16) Androsterone S	1.767	1.092 to 2.442	Yes	***	0.0001			
(13) 5alpha-androstan-3beta-ol-16-one S vs. (15) Etiocholanolone S	1.1	0.4249 to 1.775	Yes	**	0.0036			
(13) 5alpha-androstan-3beta-ol-16-one S vs. (16) Androsterone S	1.633	0.9582 to 2.308	Yes	***	0.0003			
(15) Etiocholanolone S vs. (16) Androsterone S	0.5333	-0.1418 to 1.208	No	ns	0.1289			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
(11) Epiandrosterone S vs. (13) 5alpha-androstan-3beta-ol-16-one S	197.4	197.3	0.1333	0.2108	3	3	0.8944	8
(11) Epiandrosterone S vs. (15) Etiocholanolone S	197.4	196.2	1.233	0.2108	3	3	8.273	8
(11) Epiandrosterone S vs. (16) Androsterone S	197.4	195.7	1.767	0.2108	3	3	11.85	8
(13) 5alpha-androstan-3beta-ol-16-one S vs. (15) Etiocholanolone S	197.3	196.2	1.1	0.2108	3	3	7.379	8
(13) 5alpha-androstan-3beta-ol-16-one S vs. (16) Androsterone S	197.3	195.7	1.633	0.2108	3	3	10.96	8
(15) Etiocholanolone S vs. (16) Androsterone S	196.2	195.7	0.5333	0.2108	3	3	3.578	8

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