

SUPPLEMENTARY MATERIAL TO ACCOMPANY:

**LIQUID CHROMATOGRAPHY-ION MOBILITY SPECTROMETRY-MASS SPECTROMETRY ANALYSIS OF
MULTIPLE CLASSES OF STEROID HORMONE ISOMERS IN A MIXTURE**

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Table S1. Names, molecular weights, and structures of the steroids studied here.

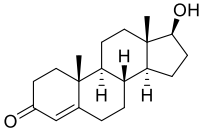
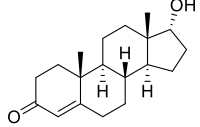
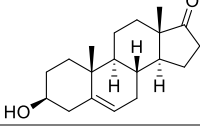
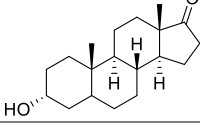
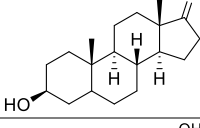
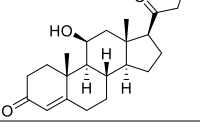
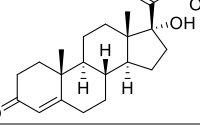
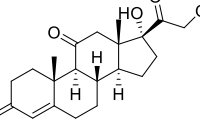
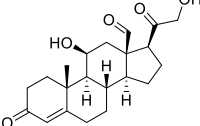
Name	Average Molecular Weight (g/mol)	Structure
Testosterone	288.43	
Epitestosterone	288.43	
DHEA	288.43	
Androsterone	290.44	
Epiandrosterone	290.44	
Corticosterone	346.47	
11-Deoxycortisol	346.47	
Cortisone	360.45	
Aldosterone	360.45	

Table S2. Gradient for the method that achieves the elution of all steroids in 11 minutes.

Time	Flow Rate ($\mu\text{L}/\text{min}$)	%Water	%Methanol
Initial	13	50	50
6.5	13	50	50
8.5	13	0	100
18.0	13	0	100
20.0	13	50	50
20.5	13	50	50

Table S3. Gradient for the method that achieves the elution of all steroids in 12 minutes.

Time	Flow Rate ($\mu\text{L}/\text{min}$)	%Water	%Methanol
Initial	9	80	20
4.0	9	40	60
7.0	9	0	100
22.5	9	0	100
24.5	9	70	30
25.0	9	70	30

Table S4. Gradient for the method that achieves the elution of all steroids in 14 minutes.

Time	Flow Rate ($\mu\text{L}/\text{min}$)	%Water	%Methanol
Initial	11	55	45
7.0	12	53	47
11.5	12	0	100
22.5	12	0	100
24.5	11	55	45
25.0	11	55	45

Table S5. Average CCSs (Ω) and standard deviations (SD; $n = 4$) for the lithiated dimeric steroid species. These are compared to literature CCSs and standard deviations (SD; $n = 4$) from Rister *et al.* [1]. These collision cross sections are evaluated with respect to their relative error (% error between literature values and those reported here) and compared *via t*-test.

	<i>m/z</i>	Ω (\AA^2)	SD	Lit. Ω (\AA^2)	Lit. SD	% Error	t_{calc}	t_{crit} (99%)
Testosterone	583.43	283.8	2.0	282.3	2.5	0.52	0.43	5.841
DHEA	583.43	272.7	2.2	271.8	2.6	0.32	0.23	
Epitestosterone	583.43	275.7	1.6	275.3	2.7	0.14	0.12	
Androsterone	587.47	253.7	1.9	255.4	1.7	-0.67	0.79	
Epiandrosterone	587.47	273.5	1.9	274.5	2.0	-0.35	0.38	
11-Deoxycortisol	699.44	295.1	2.1	290.2	0.2	1.69	3.35	
Corticosterone	699.44	305.6	2.1	307.0	1.9	-0.46	0.52	

REFERENCES

- [1] A.L. Rister, T.L. Martin, E.D. Dodds, Application of Group I Metal Adduction to the Separation of Steroids by Traveling Wave Ion Mobility Spectrometry, *J. Am. Soc. Mass Spectrom.*, 30 (2018) 248-255.