SUPPORTING INFORMATION

COLLISION CROSS SECTION (CCS) DATABASE: AN ADDITIONAL MEASURE TO CHARACTERIZE STEROIDS

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Section 'Sample analysis'

Different mobile phases were used according to the ionization conditions (positive or negative mode). In positive mode, mobile phase (A: water containing 0.1% (v/v) acetic acid, B: acetonitrile containing 0.1% (v/v) acetic acid) was supplied at 0.175 mL/min under isocratic mode (85/15 (%, v/v) A/B), except for steroid esters characterization. A gradient program was established for the analysis of steroid esters because cross contamination was observed under isocratic conditions (gradient program: 0 min, 60% (v/v) B; 1.5 min, 60% (v/v) B; 2 min, 95% (v/v) B; 2.5 min, 95% (v/v) B; 3 min, 60% (v/v) B; 3.5 min, 60% (v/v) B). In negative mode, which was only evaluated for non-esters estrogens, mobile phase (A: water, B: acetonitrile) was supplied at 0.175 mL/min under isocratic conditions (85/15 (%, v/v) A/B). In all cases, 5 µL of each standard solution were injected. Analyses lasted 2 min for runs under isocratic conditions whereas analyses accomplished under gradient conditions required of 3.5 min.

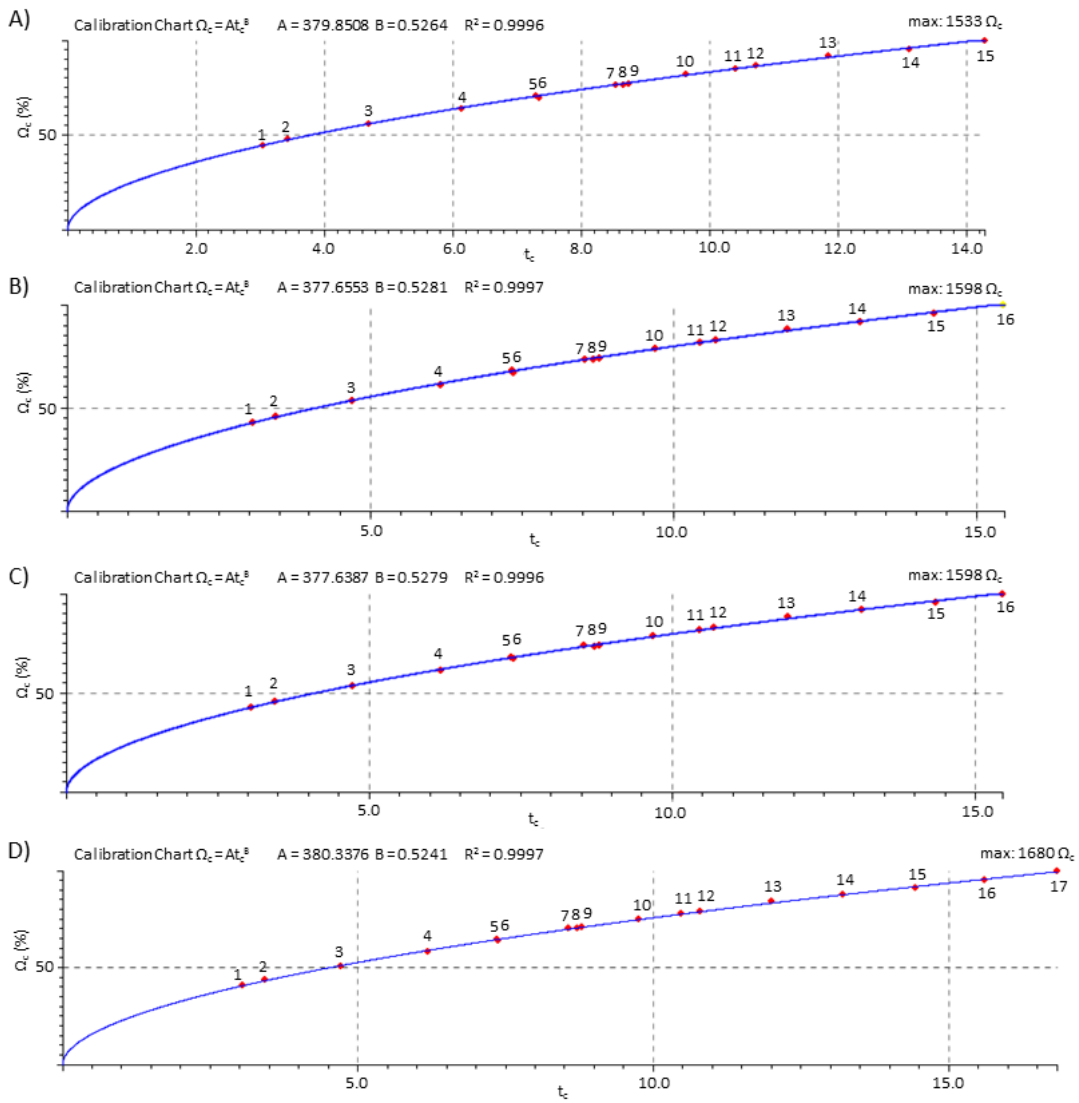

Figure S1. Calibration curves resulted from the CCS calibration (positive mode) of TWIMS system and obtained on the 1st (A), 35th (B), 61st (C) and 89th (D) day of the investigation period. Ω_c and t_c represent the corrected CCS (Å) and corrected drift time (milliseconds), respectively[1,2,3]. Experimental conditions: wave velocity, 1000 m/s; wave height: 40 V. Calibration curves were obtained using the Major Mix IMS/ToF Calibration Kit (Ref. 186008113) provided by Waters (Manchester, UK). Reference compounds according to the supplier: 1, caffeine (*m/z* 195.0882, CCS = 138.2 Å); 2, sulfaguanidine (*m/z* 215.0603, CCS = 146.8 Å); 3, sulfadimethoxine (*m/z* 311.0814, CCS = 168.4 Å); 4, Val-Tyr-Val (*m/z* 380.2185, CCS = 191.7 Å); 5, verapamil (*m/z* 455.2910, CCS = 208.8 Å); 8, terfenadine (*m/z* 472.3216, CCS = 228.7 Å); 9, leucine-enkephalin (*m/z* 556.2771, CCS = 229.8 Å); 11, reserpine (*m/z* 609.2812, CCS = 252.3 Å); and 6 (*m/z* 516.2782, CCS = 211 Å), 7 (*m/z* 587.3153, CCS = 228 Å), 10 (*m/z* 658.3524, CCS = 243 Å), 12 (*m/z* 729.3895, CCS = 256 Å), 13 (*m/z* 800.4266, CCS = 271 Å), 14 (*m/z* 871.4637, CCS = 282 Å), 15 (*m/z* 942.5008, CCS = 294 Å), 16 (*m/z* 1013.5379, CCS = 306 Å), and 17 (*m/z* 1084.58, CCS = 321.5 Å) correspond to singly protonated polyalanine ions.

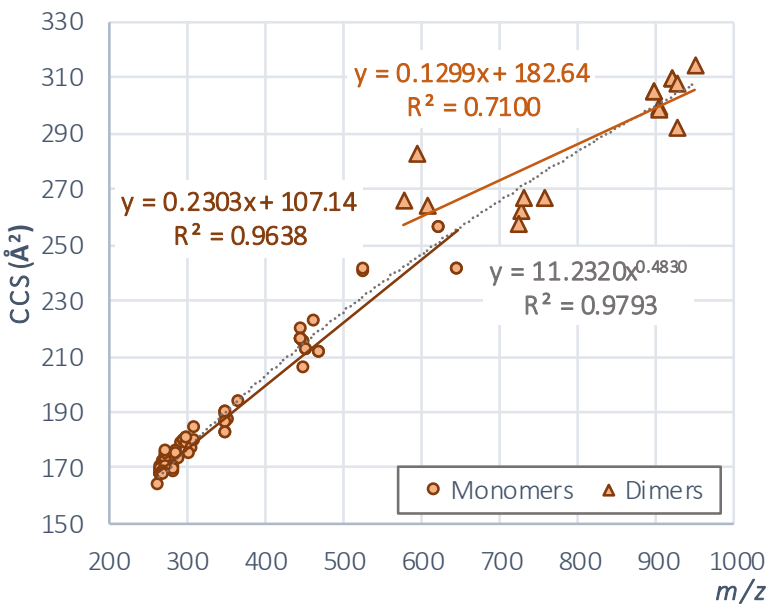

Figure S2. Representation of CCS vs *m/z* for singly charged ions resulted from the ionization of estrogens (n = 53) under ESI(-) conditions.

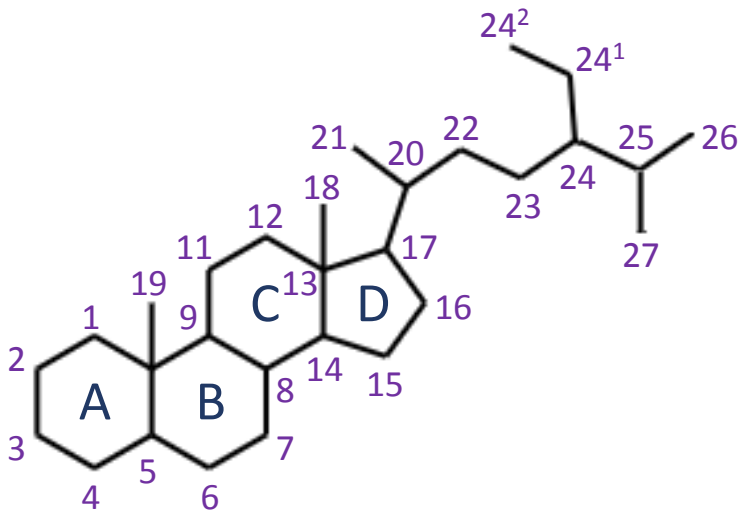

Figure S3. Steroid skeleton. The nomenclature of steroids can be found in "Nomenclature of steroids (recommendations 1989)[4]".

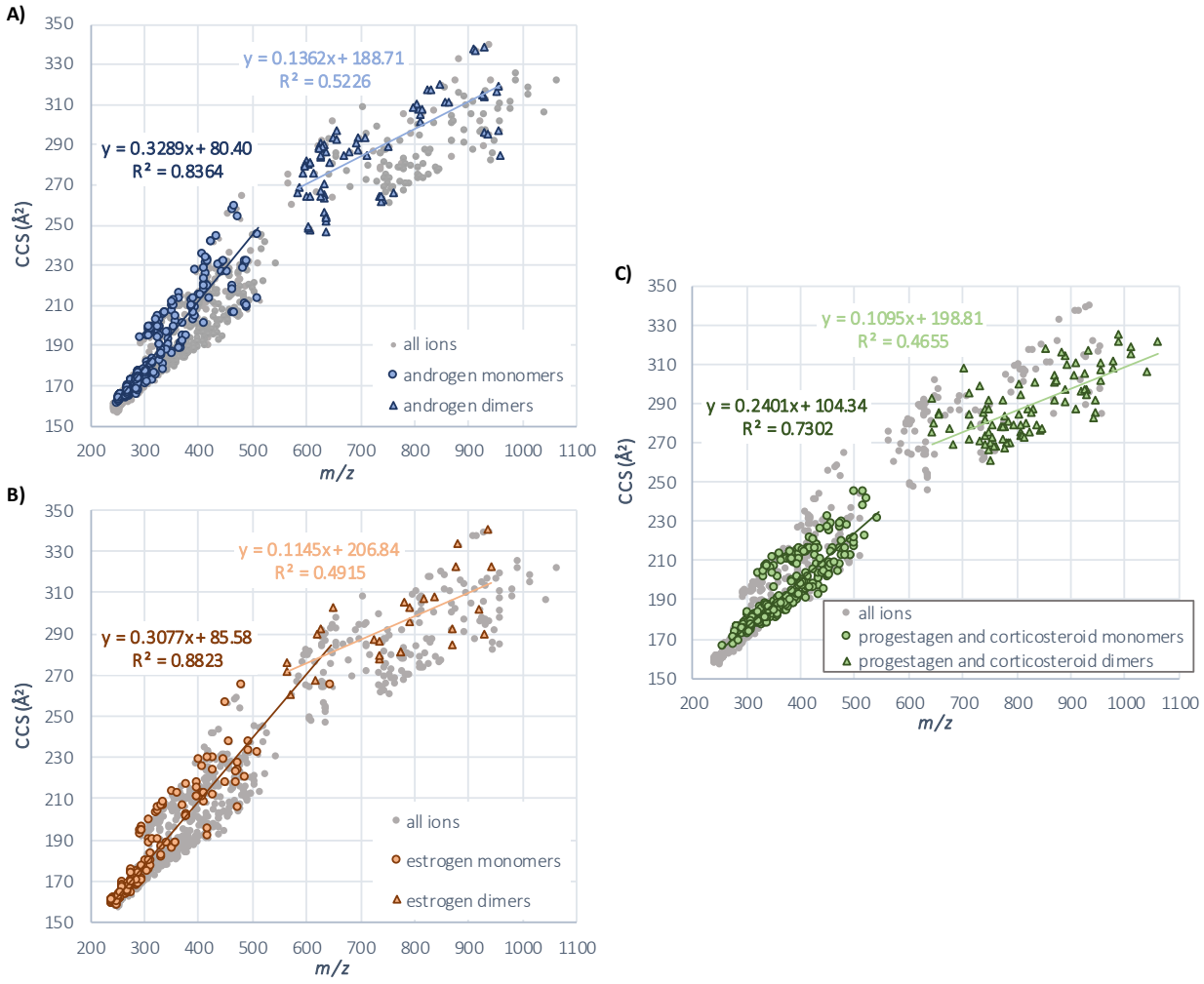

Figure S4. Linear correlation between CCS and *m/z* for singly charged monomers and dimers resulted from the positive ionization of: A) androgens, B) estrogens and C) progestagens and corticosteroids.

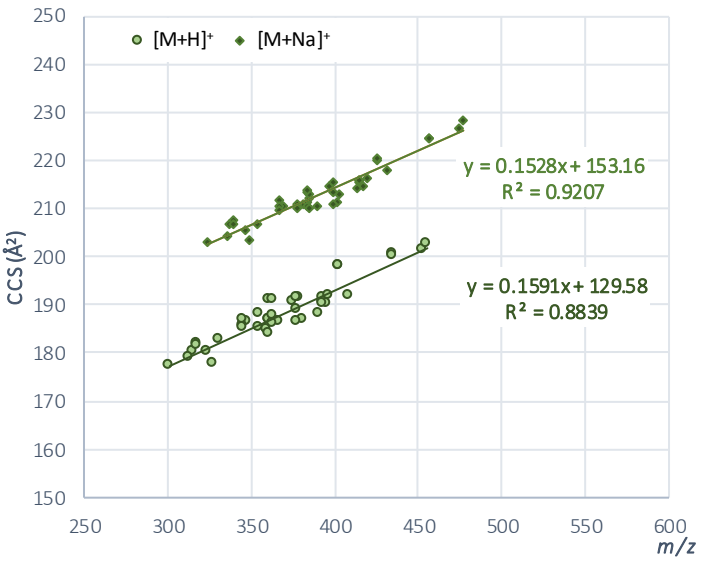

Figure S5. Representation of CCS vs *m/z* progestagens and corticosteroids which result in both $[M+H]^+$ and $[M+Na]^+$ when positive ionization is applied. A t-test ($\alpha = 0.05$) was conducted in order to discard compounds leading to statistical outliers.

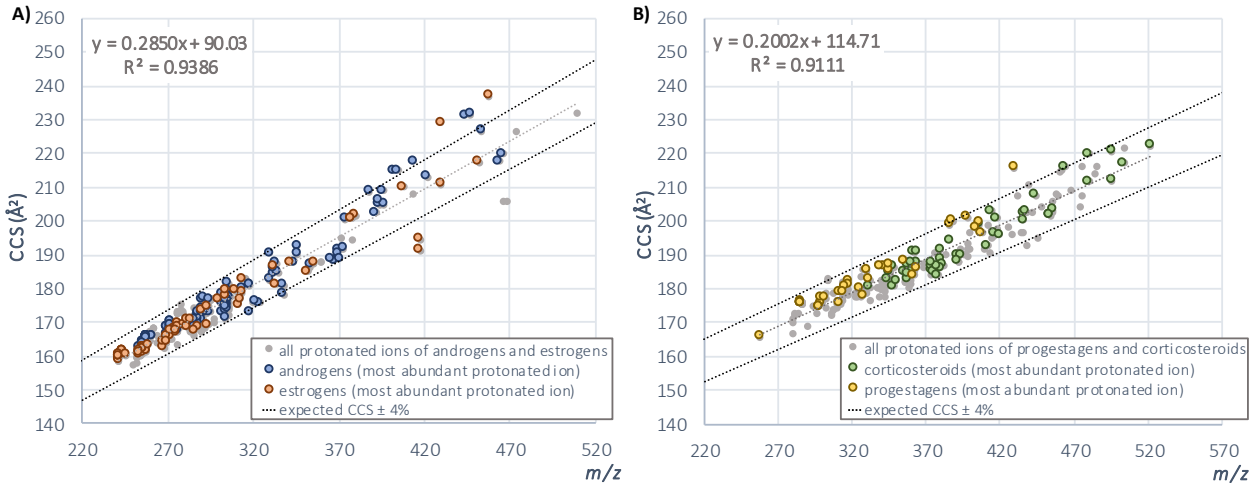

Figure S6. Representation of CCS vs *m/z* for: A) the most abundant protonated ion (i.e. $[M+H]^+$, $[M-H_2O+H]^+$, $[M-2H_2O+H]^+$) observed for each androgen and estrogen under ESI(+) conditions, and B) the most abundant protonated ion (i.e. $[M+H]^+$, $[M-HCl+H]^+$, $[M-H_2O+H]^+$) observed for each progestagen and corticosteroid under ESI(+) conditions. Other protonated ions of each steroid have also been included and are indicated as grey points.

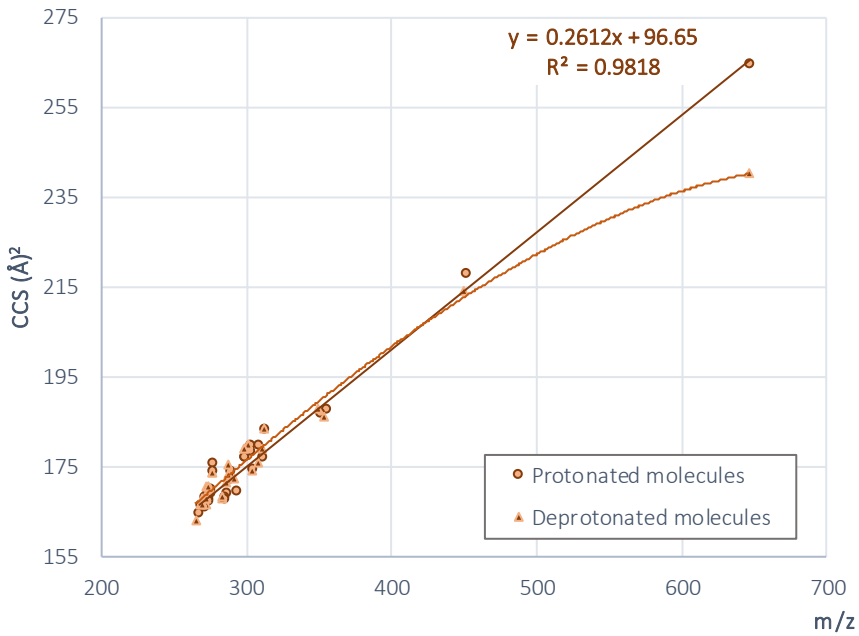

Figure S7. Representation of CCS vs *m/z* for estrogens presenting both $[M+H]^+$ and $[M-H]^-$ species.

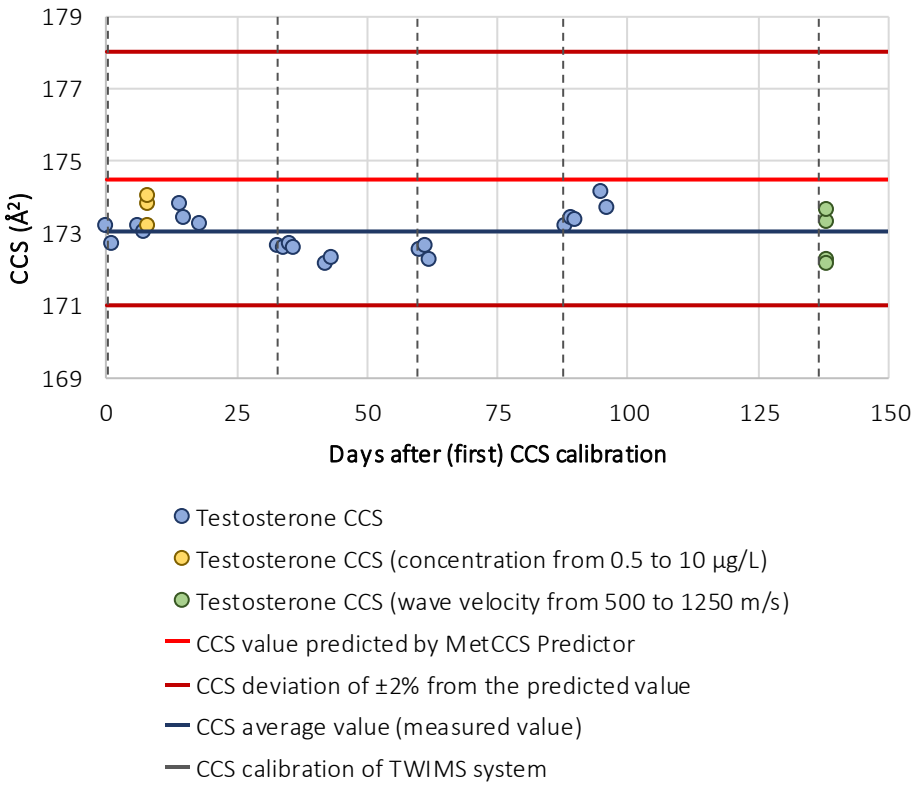

Figure S8. Reproducibility and accuracy of the CCS of testosterone protonated molecule.

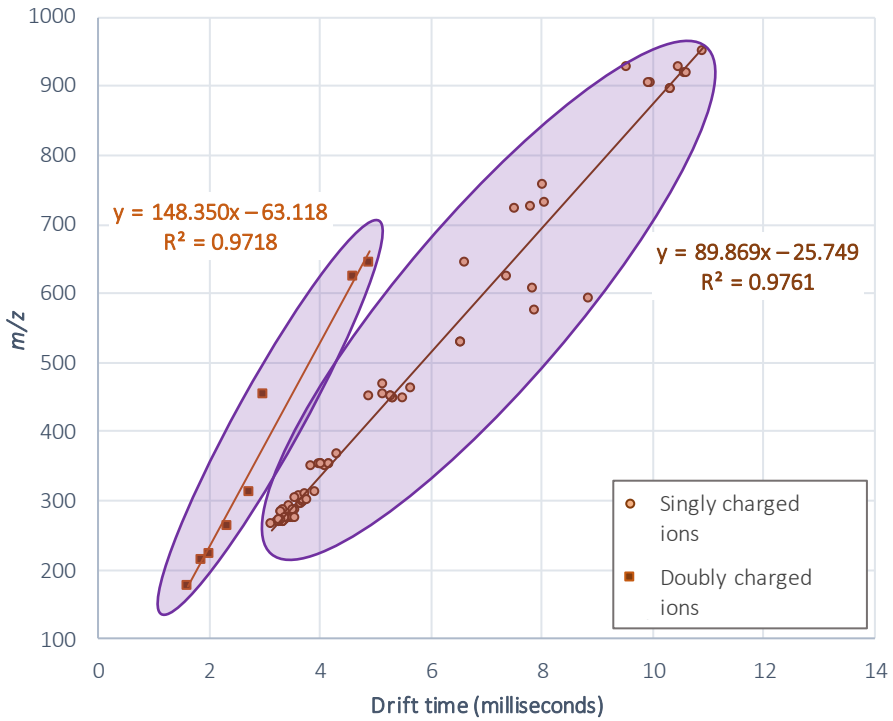

Figure S9. Separation of estrogen singly charged and doubly charged ions. IMS conditions: wave velocity, 600 m/s and wave height, 40 V. Negative ionization conditions.

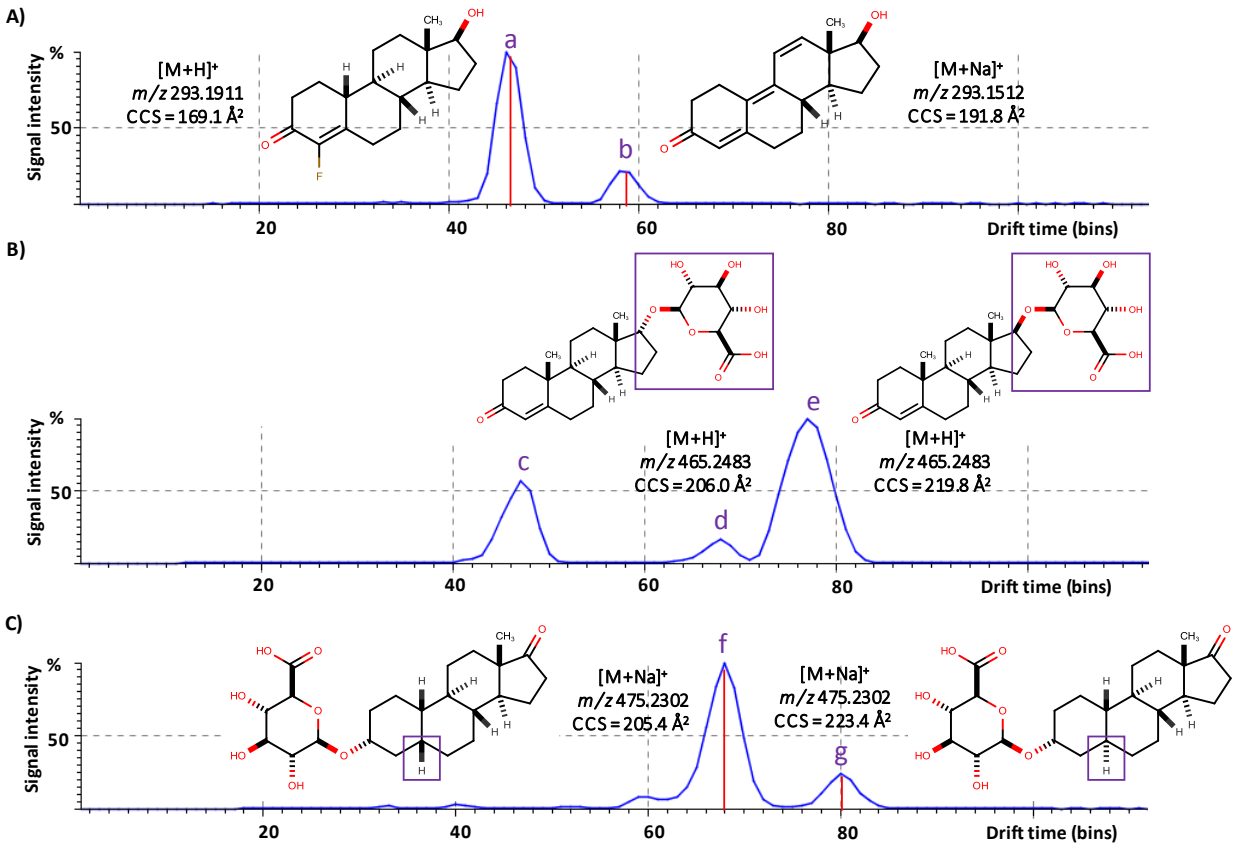

Figure S10. Ion mobility spectra related to the separation of: A) 4-fluoro-19-nortestosterone (a) and trenbolone (b), B) epitestosterone glucuronide (d), testosterone glucuronide (e), and molecular fragmentations of both steroid glucuronides (c), and C) 19-noretiocholanolone glucuronide (f) and 19-norandrosterone glucuronide (g). IMS conditions: wave velocity, 1000 m/s; wave height, 40V. Quadrupole resolution: 12.5. ESI positive conditions.

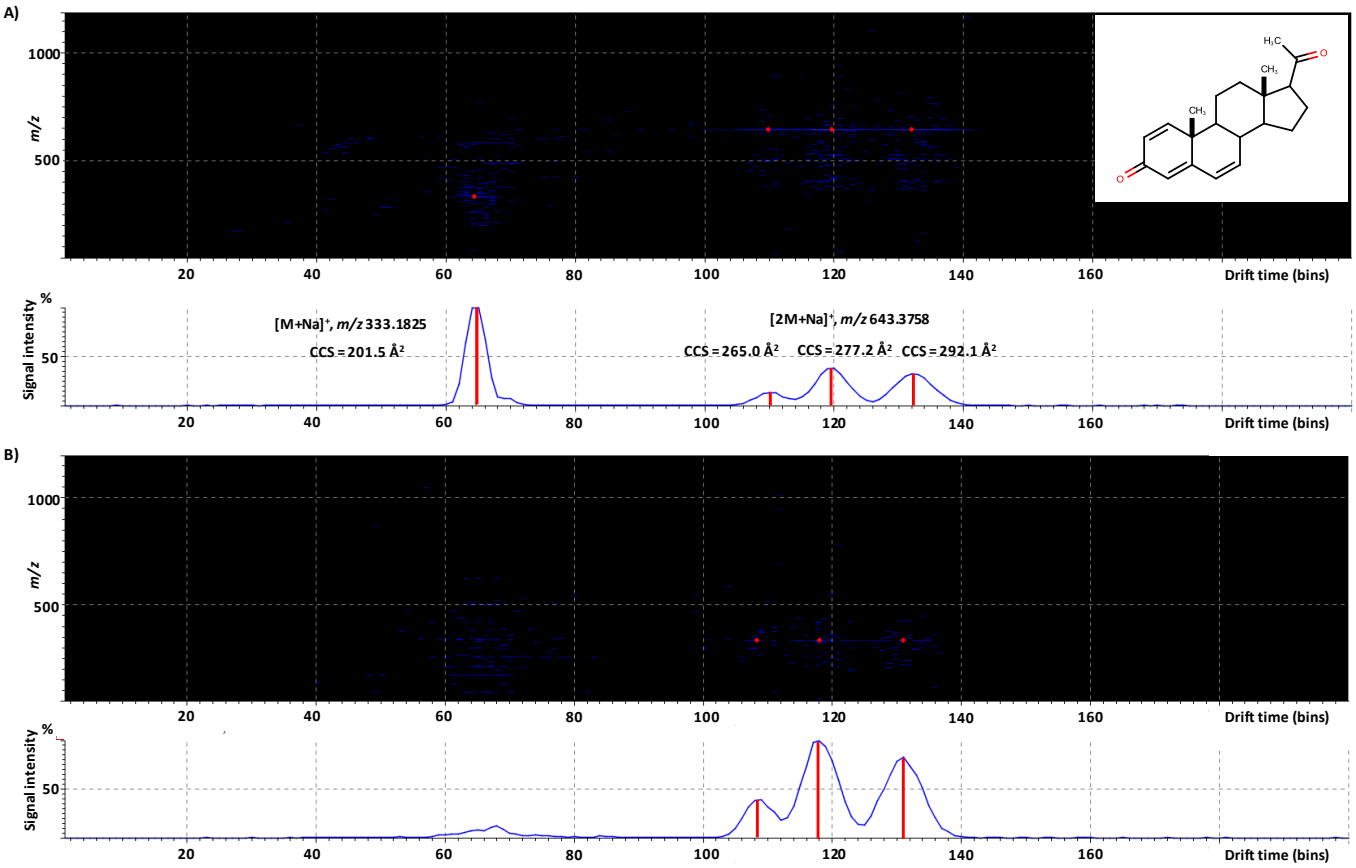

Figure S11. Representation of *m/z* vs drift time and ion mobility spectra obtained for the three $[2M+Na]^+$ species observed for 1,6-didehydroprogesterone. *m/z* 643.3758 was selected in the quadrupole and ion mobility separation was accomplished under a wave velocity of 1000 m/s and a wave height of 40 V. Under these conditions, the sodium adduct (*m/z* 333.1825) of this compound was also observed (A). In order to demonstrate that the three dimers are related to 1,6-didehydroprogesterone, collision induced dissociation (CID) experiments were carried out in the transfer (B). The application of 40 V in the transfer cell led to obtain only the $[M+Na]^+$ specie at the same drift time than previously showed by $[2M+Na]^+$ ions.

Table S1. Steroids with $[M+H]^+$ and $[M+Na]^+$ species exhibiting a CCS difference outside the range 22.8 ± 5.9 Å^2.

	CCS (Å^2)	**CCS (Å^2)**	**ΔCCS (Å^2) between both $[M+H]^+$ and $[M+Na]^+$ species**
testosterone glucuronide	219.8	229.9	10.1 Å^2
epitestosterone glucuronide	206.0	210.6	4.6 Å^2
boldenone glucuronide	217.6	228.5	10.9 Å^2
testosterone phenylpropionate	213.2	226.8	13.6 Å^2
19-nortestosterone phenylpropionate	210.2	223.2	13.0 Å^2
17-caproxyprogestorene	215.8	226.7	10.9 Å^2
beclomethasone dipropionate	222.3	231.0	8.7 Å^2
18-hydroxycortisol	188.6	200.4	11.8 Å^2
medroxyprogesterone acetate	199.9	214.0	14.1 Å^2
flugestone acetate	196.6	211.8	15.2 Å^2
megestrol acetate	198.8	214.7	15.9 Å^2
11-ketoestrone	167.8	176.5	8.7 Å^2
hydrocortisone hemissuciatinate	216.2	212.1	- 4.1 Å^2 ($CCS_{[M+Na]+} < CCS_{[M+H]+}$)
prednisolone 21-hemissuciatinate	215.1	214.2	- 0.9 Å^2 ($CCS_{[M+Na]+} < CCS_{[M+H]+}$)

Table S2. Comparison between the CCS (CCS_{exp}) experimentally measured and the CCS ($CCS_{expected}$) predicted from the regression curve (Figure 3A) for the sodium adducts of androgens and estrogens.

	Ion	CCS_{exp} (Å^2)	$CCS_{expected}$ (Å^2)	ΔCCS (%)	Is this molecule smaller or larger than expected?
Androgens					
epitestosterone sulfate	$[M-H+2Na]^+$	201.0	218.9	8.2	Smaller
etiocholanolone glucuronide	[M+Na]+	208.8	235.1	11.2	Smaller
	$[M-H+2Na]^+$	213.1	239.8	11.2	Smaller
epitestosterone glucuronide	$[M+Na]^+$	210.6	234.7	10.3	Smaller
testosterone decanoate	$[M+Na]^+$	257.8	230.0	12.1	Larger
testosterone benzoate	$[M+Na]^+$	231.3	219.4	5.5	Larger
boldenone benzoate	$[M+Na]^+$	228.6	218.9	4.4	Larger
testosterone cypionate	$[M+Na]^+$	243.9	223.6	9.1	Larger
stanolone benzoate	$[M+Na]^+$	232.5	219.8	5.8	Larger
testosterone benzoate d3	$[M+Na]^+$	231.8	220.0	5.4	Larger
testosterone 4-methylvalerate	$[M+Na]^+$	235.2	218.1	7.8	Larger
testosterone enanthate	$[M+Na]^+$	241.8	221.1	9.4	Larger
testosterone enanthate d3	$[M+Na]^+$	241.9	221.7	9.1	Larger
testosterone decanoate d3	$[M+Na]^+$	259.0	230.7	12.3	Larger
4-chlorotestosterone	$[M+Na]^+$	196.3	204.5	4.0	Smaller
6β-ol-etiocholanolone	$[M+Na]^+$	190.3	201.1	5.4	Smaller
epitestosterone glucuronide d3	$[M+Na]^+$	210.1	235.3	10.7	Smaller
boldenone undecylenate	$[M+Na]^+$	253.6	232.1	9.2	Larger
1-dehydroandrostanolone tetrahydropyranyl ether	$[M+Na]^+$	227.4	215.1	5.7	Larger
1-dehydroandrostanolone benzoate	$[M+Na]^+$	233.3	219.4	6.4	Larger
Estrogens					
4,6-estradien-17β-ol-3-one-17α-acetonitrile	$[M+Na]^+$	181.7	202.1	10.1	Smaller
estetrol	$[M+Na]^+$	190.0	200.6	5.3	Smaller
16α-hydroxyestrone	$[M+Na]^+$	187.5	196.8	4.7	Smaller
estradiol 3-glucuronide	$[M+Na]^+$	217.0	231.3	6.2	Smaller
19-noretiocholanolone glucuronide	$[M+Na]^+$	205.4	232.1	11.5	Smaller
estriol 3-glucuronide	$[M+Na]^+$	220.2	234.7	6.7	Smaller
19-nortestosterone benzoate	$[M+Na]^+$	228.4	216.4	5.5	Larger
19-nortestosterone decanoate	$[M+Na]^+$	256.1	227.0	12.8	Larger
19-nortestosterone laurate	$[M+Na]^+$	264.8	233.0	13.6	Larger
17β-estradiol diacetate	$[M+Na]^+$	200.6	211.7	5.2	Smaller
4-fluoro-19-nortestosterone	$[M+Na]^+$	189.9	198.1	4.2	Smaller
11-ketoestrone	$[M+Na]^+$	176.5	196.4	10.1	Smaller
17α-estradiol diacetate	$[M+Na]^+$	202.0	211.7	4.6	Smaller

Table S3. Comparison between the measured CCS (CCS_{exp}) and the predicted CCS (CCS_p) calculated by MetCCS Predictor[5] for some of the studied androgens and estrogens.

	$[M+H]^+$			$[M+Na]^+$			$[M-H_2O+H]^+$			$[M-H]^-$		
	CCS_{exp} (Å²)	**CCS_p (Å²)**	**ΔCCS (%)**	**CCS_{exp} (Å²)**	**CCS_p (Å²)**	**ΔCCS (%)**	**CCS_{exp} (Å²)**	**CCS_p (Å²)**	**ΔCCS (%)**	**CCS_{exp} (Å²)**	**CCS_p (Å²)**	**ΔCCS (%)**
Androgens												
testosterone	173.0	174.4	0.8	198.7	181.0	9.8						
androstanedione	176.9	176.7	0.1				167.6	170.9	1.9			
dehydroepiandrosterone	173.2	174.9	1.0				168.3	169.2	0.5			
methyltestosterone	177.7	178.7	0.6	203.3	185.2	9.8	171.8	173.3	0.9			
stanozolol	190.3	179.8	5.8									
stanolone / dihydrotestosterone	177.2	176.3	0.5				169.0	170.9	1.1			
boldenone	170.8	172.4	0.9	195.6	179.0	9.3	165.1	166.9	1.1			
androstenedione	172.6	173.1	0.3	197.5	180.2	9.6						
testosterone propionate	190.2	189.9	0.2	215.8	196.6	9.8						
oxymetholone	187.5	179.2	4.6				183.2	173.7	5.5			
fluoxymesterone	178.3	184.2	3.2	204.3	190.8	7.1						
dromostanolone	178.9	181.2	1.3				173.7	175.8	1.2			
bolasterone	181.0	182.8	1.0	206.4	189.2	9.1						
4-hydroxyandrostedione/ formestane	175.0	172.9	1.2				168.0	166.9	0.7			
calusterone	181.1	183.1	1.1	206.2	189.4	8.9						
4-chlorotestosterone/ clostebol	175.7	185.3	5.2	196.3	191.7	2.4						
Estrogens												
estriol	172.0	168.1	2.3	189.9	175.1	8.5	166.1	162.3	2.3	173.1	168.1	3.0
estradiol	167.9	161.8	3.8				162.1	155.9	4.0	170.5	169.1	0.8
estrone	166.2	164.4	1.1				160.3	158.0	1.5	168.0	170.5	1.5
estrone 3-sulfate	186.5	201.1	7.3				181.1	196.0	7.6	187.8	190.6	1.5
norethindrone/ norethisterone	176.8	174.0	1.6	202.3	180.6	12.0	169.0	168.6	0.2	178.9	174.9	2.3
19-nortestosterone decanoate	229.1	220.1	4.1	256.1	226.2	13.2						
19-nortestosterone phenylpropionate	210.2	207.8	1.2	223.3	213.9	4.4						
gestrinone	179.4	176.1	1.9	206.0	182.7	12.8	173.5	170.7	1.6	175.8	178.2	1.3
estetrol	173.9	175.3	0.8	190.0	181.8	4.5	169.9	169.9	0.0	173.9	170.4	2.1
19-norandrostendione	168.1	168.5	0.2				160.8	162.7	1.2	168.0	167.1	0.5
norethandrolone	178.0	179.2	0.7	203.5	185.7	9.6	172.1	173.8	1.0	180.0	176.9	1.8

Table S4. Comparison between the measured CCS (CCS_{exp}) and the predicted CCS (CCS_p) estimated by MetCCS Predictor[5] for some of the studied corticosteroids and progestagens.

	$[M+H]^+$			$[M+Na]^+$			$[M-H_2O+H]^+$		
	CCS_{exp} (Å²)	**CCS_p (Å²)**	**ΔCCS (%)**	**CCS_{exp} (Å²)**	**CCS_p (Å²)**	**ΔCCS (%)**	**CCS_{exp} (Å²)**	**CCS_p (Å²)**	**ΔCCS (%)**
Corticosteroids									
flumethasone	192.6	203.0	5.1	217.5	208.8	4.2			
prednisone	184.8	189.8	2.6	210.7	196.2	7.4	181.7	184.4	1.5
triamcinolone	190.0	197.9	4.0	214.8	203.4	5.6			
cortisol/ hydrocortisone	187.5	191.3	2.0	212.3	197.4	7.5			
flunisolide	202.5	214.8	5.7	227.0	220.6	2.9	198.0	210.1	5.8
budesonide	207.3	215.7	3.9	231.7	221.6	4.6	203.0	210.7	3.7
desonide	200.9	210.9	4.7	225.3	216.9	3.9	196.5	206.0	4.6
fludrocortisone	187.0	195.7	4.4	212.8	201.7	5.5			
fluorometholone	186.5	193.5	3.6	211.0	199.8	5.6			
methylprednisolone	190.4	193.6	1.7	214.5	199.6	7.5	187.1	188.5	0.7
fluocinolone acetonide	201.5	221.1	8.9	226.7	226.8	0.0			
flurandrenolide	202.8	216.3	6.2	228.2	222.0	2.8			
halcinonide	203.2	224.9	9.6	230.0	230.9	0.4			
amcinonide	217.1	237.8	8.7	241.6	243.3	0.7			
desoximetasone	189.0	193.5	2.3	213.3	199.7	6.8			
beclomethasone dipropionate	222.3	238.8	6.9	231.0	244.3	5.4	221.4	234.3	5.5
clobetasol propionate	209.0	222.2	5.9	227.5	228.1	0.3			
dexamethasone	191.6	197.5	3.0	215.8	203.5	6.0			
betamethasone	190.6	197.6	3.5	215.5	203.5	5.9			
prednisolone	186.8	189.4	1.4	211.2	195.6	8.0			
Progestagens									
medroxyprogesterone acetate	199.9	201.1	0.6	214.0	207.5	3.1			
medrysone	185.6	190.5	2.6	211.7	196.7	7.6	179.2	185.4	3.3
progesterone	180.4	181.9	0.8	206.7	188.7	9.5			
danazol	186.7	184.5	1.2						
medrogestone	186.8	190.6	2.0	213.3	197.3	8.1			
megestrol acetate	198.8	199.9	0.6	214.7	206.3	4.1			
17-caproxyprogesterone	215.8	216.9	0.5	226.7	223.1	1.6			

Table S5. Comparison between the measured CCS (CCS_{exp}) and the CCS obtained by another TWIMS instrument (CCS_{TWIMS})[6] and by DTIMS (CCS_{DTIMS})[7].

	Ion	CCS_{exp}(Å^2)	CCS_{TWIMS} (Å^2)	ΔCCS (%)	CCS_{DTIMS} (Å^2)	ΔCCS (%)
Androgens						
androsterone	$[M-H_2O+H]^+$	168.4			174.1	3.3
	$[M+Na]^+$	199.1			197.1	1.0
epiandrosterone/ *trans*-androsterone	$[M-H_2O+H]^+$	168.1			174.3	3.6
	$[M+Na]^+$	199.4			196.8	1.3
dehydroepiandrosterone	$[M+H]^+$	173.0			174.3	0.7
etiocholanolone	$[M-H_2O+H]^+$	169.1			167.7	0.8
epietiocholanolone	$[M-H_2O+H]^+$	168.3			167.2	0.7
testosterone	$[M+H]^+$	173.0	170.6	1.4	174.5	0.9
	$[M+Na]^+$	198.7			196.7	1.0
epitestosterone	$[M+H]^+$	173.3	170.7	1.5	174.7	0.8
	$[M+Na]^+$	198.6			196.4	1.1
testosterone propionate	[M+H]+	190.2	188.2	1.1		
Estrogens						
estradiol	$[M-H_2O+H]^+$	162.1			162.2	0.1
norethindrone acetate	[M+H]+	187.8	186.3	0.8		
norethindrone	[M+H]+	176.8	174.4	1.4		
norethynodrel	[M+H]+	176.7	174.6	1.2		
Corticosteroids						
cortisol	$[M+H]^+$	187.5	185.1	1.3		
methylprednisolone	$[M+H]^+$	190.4	187.7	1.4		
triamcinolone	$[M+H]^+$	190.0	186.8	1.7		
fluocinolone acetonide	$[M+H]^+$	201.5	199.7	0.9		
fluocinolone acetonide 21-acetate/ fluocinonide	$[M+H]^+$	212.0	229.9	7.8		
prednisolone 21-acetate	$[M+H]^+$	197.9	194.0	2.0		
prednisone	$[M+H]^+$	184.8	181.8	1.7		
hydrocortisone acetate	$[M+H]^+$	198.8	196.1	1.4		
dexamethasone 21-phosphate	$[M+H]^+$	204.2	200.6	1.8		
hydrocortisone hemissuccinate	$[M+H]^+$	216.2	212.4	1.8		
prednisolone 21-hemisuccinate	$[M+H]^+$	215.1	210.3	2.3		
amcinonide	$[M+H]^+$	217.1	214.9	1.0		
clobetasol propionate	$[M+H]^+$	209.0	205.1	1.9		
halcinonide	$[M+H]^+$	203.2	200.9	1.1		
dexamethasone	$[M+H]^+$	191.6	187.9	2.0		
betamethasone	$[M+H]^+$	190.6	186.7	2.1		
prednisolone	$[M+H]^+$	186.8	183.7	1.7		
cortisone	$[M+H]^+$	187.0	184.4	1.4	194.6	3.9
	$[M+Na]^+$	213.6			211.7	0.9
desonide	$[M+H]^+$	200.9	198.5	1.2		
cortexolone/11-deoxycortisol	$[M+H]^+$	186.4			190.4	2.1
	$[M+Na]^+$	210.4			210.7	0.1
Progestagens						
progesterone	$[M+H]^+$	180.4	177.8	1.5		
medroxyprogesterone acetate	$[M+H]^+$	199.9	197.2	1.4		
chlormadinone acetate	$[M+H]^+$	199.5	197.0	1.3		
melengestrol acetate	$[M+H]^+$	201.2	198.2	1.5		
17-hydroxyprogesterone	$[M+H]^+$	182.7	179.9	1.6	186.3	1.9
	$[M+Na]^+$	206.7			205.5	0.6
medrysone	$[M+H]^+$	185.6	183.2	1.3		
megestrol acetate	$[M-CH_3COOH+H]^+$	183.4	180.1	1.8		
pregnenolone	$[M+H]^+$	179.8			176.7	1.8

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