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Application of Group I Metal Adduction to the Separation of Steroids by Traveling Wave Ion Mobility Spectrometry

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Abstract

Steroids represent an interesting class of small biomolecules due to their use as biomarkers and their status as scheduled drugs. Although the analysis of steroids is complicated by the potential for many isomers, ion mobility spectrometry (IMS) has previously shown promise for the rapid separation of steroid isomers. This work is aimed at the further development of IMS separation for the analysis of steroids.

Here, traveling wave ion mobility spectrometry (TWIMS) was applied to the study of group I metal adducted steroids and their corresponding multimers for five sets of isomers. Each set of isomers had a minimum of one dimeric metal ion adduct that exhibited a resolution greater than one (i.e., approaching baseline resolution). Additionally, ion-neutral collision cross sections (CCSs) were measured using polyalanine as a calibrant, which may provide an additional metric contributing to analyte identification. Where possible, measured CCSs were compared to previously reported values. When measuring CCSs of steroid isomers using polyalanine as the calibrant, nitrogen CCS values were within 1.0% error for monomeric sodiated adducts and slightly higher for the dimeric sodiated adducts. Overall, TWIMS was found to successfully separate steroids as dimeric adducts of group I metals.

Keywords: Steroids, Metal ion adduction, Isomer discrimination, Ion mobility spectrometry, Collision cross section

Introduction

Steroids are an important biomolecular class, specifically given their roles in cellular signaling [1–3]. Interest in the development of new methods for steroid analysis has recently increased due to the potential of steroids as biomarkers for a variety of diseases and their misuse to enhance performance in sports [4–8]. Steroids present a complex analytical challenge due to the large number of isomers with similar structures that are not readily distinguished by chromatography without extensive analysis times or the need for chemical derivatization [9, 10]. The broad dissemination of commercial instruments for ion mobility spectrometry (IMS) coupled to mass spectrometry (MS) has provided an additional, orthogonal separation technique that offers the unique ability to separate ions based on differences in the gas-phase structures prior to MS analysis [11, 12].

Several types of IMS have been applied to small molecule analysis including drift tube IMS (DTIMS), traveling wave IMS (TWIMS), and high-field asymmetric waveform IMS (FAIMS). IMS analysis of steroids was initially performed by combining liquid chromatography (LC) with FAIMS or TWIMS [5, 8, 13–18]. When coupled to LC, FAIMS, a spatial mobility separator, provides the ability to increase the signal-to-noise ratio of steroids and decrease the possibility of false positive results arising from signals with similar retention times but very different mobility features [5, 8]. TWIMS, a temporal mobility separator, provided complementary analysis to FAIMS in the ability to select a feature by retention time, drift time, and mass-to-charge ratio (m/z), allowing

for increased confidence in identification and quantification of steroids [16]. The preceding evidence has shown that IMS in combination with LC and MS provides a useful approach for steroid analysis.

More recently, IMS-MS has been explored as a stand-alone technique for steroid analysis. For example, Ahonen et al. used derivatization with *p*-toluenesulfonyl isocyanate (PTSI) to obtain partial or full separation of three pairs of stereoisomeric steroids through TWIMS [19]. Subsequent DTIMS analyses of native steroids as sodiated multimers were reported in two articles by Chouinard et al. [20, 21]. They reported the successful separation of five different steroid species as sodiated dimer adducts in nitrogen. These experiments were complemented by performing monomer and dimer analysis with additional cation adducts on androsterone and epiandrosterone. Their results showed that alkaline earth metals did not greatly increase the separation compared to the sodium adduct, whereas transition metals increased resolution but resulted in lower signal-to-noise ratios.

Through IMS, ion-neutral collision cross section (CCS) values can be measured. These CCSs are intrinsic properties of the analyte and drift gas collision partners and are potentially useful for the identification of unknowns. Although these may be directly calculated in DTIMS experiments, they must be calibrated by a series of analytes of known CCS values in TWIMS experiments [22, 23]. Many calibrant series have been reported in both positive and negative modes for a variety of biomolecule classes, but the most common calibrant for small molecule analysis is polyalanine [24–31]. Calibrated CCSs obtained from TWIMS measurements may be subject to errors introduced by differences in the chemical and physical characteristics of the calibrants and analytes; however, current research is addressing ways to decrease the error of calibrated CCSs, including calibrant/analyte charge state matching [31]. Despite some uncertainty, calculated and calibrated CCS values are potentially useful for small molecule analysis and identification. Steroid CCS values have been reported in both positive and negative ion modes and for monomers as well as multimers. Zheng et al. published deprotonated, protonated, and sodiated CCS values for various steroids [32]. Chouinard et al. calculated the monomer and dimer CCS values for a variety of steroids [20]. These values can be utilized to identify steroid isomers by ion mobility, and they can also be compared to CCSs obtained from theoretical modeling to determine the gas-phase structures [21].

This study furthers the body of work summarized above by examining the separation provided by TWIMS for seven pairs of steroid isomers, structures shown in **Figure 1**, as alkali-metal adducts. These results demonstrate that in some cases, metal adducted dimers enabled enhanced separation of steroid isomers compared to the corresponding monomers or sodiated dimers previously reported. Additionally, CCS values are reported for the various steroid species analyzed. Finally, an examination on the accuracy of the calibrated CCS values was performed by comparison with the sodiated monomer and dimer species previously measured [20].

Experimental

Solution Preparation

Polyalanine, testosterone, androsterone, α -estradiol, β -estradiol, epi-androsterone, cortisone, corticosterone, 11-deoxycortisol, lithium acetate, sodium acetate, potassium acetate, rubidium acetate, cesium acetate, and water were purchased from Sigma-Aldrich (St. Louis, MO, USA). Aldosterone, dehydroepiandrosterone (DHEA), methanol, and acetonitrile were purchased from Fisher Scientific (Pittsburg, PA, USA). Polyalanine was prepared at 12.5 $\mu\text{g/mL}$ in 50% acetonitrile and 0.1% formic acid. Steroid solutions contained 50 μM of the given steroid and 100–150 μM of lithium acetate, sodium acetate, potassium acetate, rubidium acetate, or cesium acetate.

Ion Mobility Spectrometry-Mass Spectrometry

All IMS-MS experiments were performed on a Waters Synapt G2-S HDMS quadrupole time-of-flight mass spectrometer (Milford, MA, USA) which contains a TWIMS separator positioned between the quadrupole and time-of-flight mass analyzers. The samples were ionized by nanoelectrospray ionization (nESI) using emitters that were formed from Pyrex melting point capillaries (Corning, NY, USA) with the aid of a vertical micropipette puller (David Kopf Instruments, Tujunga, CA). Approximately 10 μL of the solution filled the emitters that were then placed on a custom built nESI source stage, where a platinum wire was placed in contact with the solution. Capillary voltages

Figure 1. Structures of the steroids measured: α -estradiol, β -estradiol, estriol, testosterone, DHEA, epitestosterone, epiandrosterone, androsterone, 11-deoxycortisol, corticosterone, aldosterone, and cortisone

were optimized between 0.9 and 1.5 kV, where the sample cone voltage and source temperature were kept constant at 10.0 V and 80 °C, respectively. The TWIMS experiment settings for nitrogen gas flow, wave height, and wave velocity were set at 60 mL/min, 40 V, and 600 m/s, respectively. Data was analyzed through DriftScope 2.7 and MassLynx 4.1 (Waters). This data was further visualized using Igor Pro 7 (WaveMetrics, Lake Oswego, OR) and SigmaPlot 13 (Systat, Chicago, IL, USA).

Collision Cross Section Calibration and Resolution Calculations

TWIMS drift times were calibrated to CCS values by following previously established procedures [33–35] with slight modifications as described elsewhere [36, 37]. Briefly, a series of singly protonated polyaniline ions was used for CCS calibration. These ions have been widely used for CCS calibration [24–31] and have been well-characterized by DTIMS and TWIMS. Their prior characterization includes the determination of nitrogen CCS values by DTIMS, as reported by Bush et al. [25]. The arrival time distributions (ATDs) of the polyanilines were measured by TWIMS, and the corresponding drift times were determined using routines in IGOR Pro 7. This was accomplished by applying a Gaussian fit to each ATD and using this fit to determine the centroid drift time. These drift times were then corrected for irrelevant transit times as previously described [36, 37], providing the dependent variable for the calibration curves. The independent variable was obtained by taking accepted polyaniline CCS values [25] and normalizing for ion charge state and collision partner reduced mass as previously described [36, 37]. For each set of unknown measurements made, a calibration curve was first generated by fitting the corrected drift time vs. normalized CCS data with a quadratic polynomial function. This allowed analyte drift times to be converted to the corresponding nitrogen CCSs. New calibration curves were constructed for each day the analysis was performed. The ATDs for ions of unknown CCS were processed in an identical manner to those of the calibration standards, and the resulting centroid drift times were used in concert with the appropriate calibration curve to calculate the unknown CCSs.

For the calculation of resolution values, the Gaussian fitting of adjacent ATDs was used to obtain both the centroid time (t) and the

width of the peak at half maximum (w_{FWHM}). The peak resolution (R_s) was then calculated in a fashion typically used in separation science via Eq. (1), where $w_{FWHM, avg}$ corresponds to the average of both half maximum peak widths:

$$R_s = \frac{2.35\Delta t}{4w_{FWHM, avg}} \quad (1)$$

Here, the resolution is described in terms of both ATDs, which is perhaps more relevant to assessment of isomer separation than the single-peak resolving power definition often used in ion mobility spectrometry (e.g., $t/\Delta t$ or $\Omega/\Delta\Omega$) [23].

Results

Lithium Steroid Adduction

ATDs of lithium adducted monomers and dimers for various steroid isomers are shown in **Figure 2**. The dimer species had higher drift times than those of the monomer species due to their increased size. More intriguingly, the dimers showed improved separation through IMS analysis compared to the monomer species. Therefore, dimer adduction with lithium, as has been previously shown with sodium, is a possible avenue of separation of structurally similar steroid isomers.

These ATDs illustrated the ability for lithium adduction to aid in separation of specific steroid dimer isomer pairs on a millisecond time scale. The separation of lithium-dimer adducts varied depending on the steroid isomer pair. The carbon-3 epimers, androsterone, and epi-androsterone (Figure 2b), exhibited baseline separation only as lithium adduct due to the sodium adduct containing multiple features (Figure S1b in the Supplementary Material). By contrast, corticosterone/11-deoxycortisol and testosterone/DHEA showed only a partial separation (Figure 2a, c). Additionally, aldosterone and cortisone remained overlapped as lithium monomer and dimer species. In this case, the separation was compromised due to the formation of multiple conformers of aldosterone, potentially due to a mixture of the aldehyde and hemi-acetal forms (Figure 2d). While lithium adduction provided

Figure 2. Arrival time distributions for the monomeric and dimeric lithium adducts of corticosterone and 11-deoxycortisol (a); androsterone and epiandrosterone (b); testosterone, epitestosterone, estriol, and DHEA (c); and aldosterone and cortisone (d)

enhanced separation for three steroid isomer sets, additional metal adducts could further enhance the separation of other steroid isomers, especially in terms of reducing the number of conformers formed.

Sodium and Potassium Adduct Separation

While lithium showed great potential as a charge carrier for the separation of steroids, sodium and potassium adducts also showed the greatest potential for different steroid isomer pairs. The ATDs for four pairs of steroid isomers are presented in **Figure 3**. In the ATDs for corticosterone and 11-deoxycortisol, increased separation was seen for the dimers as sodium (Figure 3a) and potassium (Figure S1c of the Supplementary Material) adducts compared to the dimer as a lithium adduct (Figure 2a). However, all three adducts showed reasonable separation for this isomer pair. On the other hand, the potassium dimer for carbon-17 epimers, α -estradiol, and β -estradiol (Figure 3b), provided the only valuable separation of the isomers, which is largely due to multiple features appearing with lithium and sodium adduction.

Furthermore, the testosterone series showed altered behavior as sodiated dimer adducts (Figure 3c) than as lithiated dimer adducts. Where epitestosterone and DHEA were similarly overlaid, testosterone was further distinguished from both isomers. As monomers, estradiol, testosterone, and epitestosterone were fully overlapped. On the other hand, DHEA was partially distinguished from the other isomer sodiated monomers. The overlap of lithiated and sodiated adducts could prove useful to enhance the separation of these four testosterone isomers and isobars.

The case of aldosterone and cortisone was surprising due to the high number of mobility features observed both as lithium (Figure 2d) and sodium adducts (**Figure S1a** of the Supplementary Material). With potassium adduction, only the dimer of aldosterone showed more than a single conformer (Figure 3d). In this case, the ATD of cortisone was uniquely positioned between the two conformers of aldosterone, allowing for the possible separation for these compounds as their dimer species. The monomer species also showed partial separation of the major feature although peak tailing could cause difficulty with quantification of cortisone. Ultimately, aldosterone and cortisone as potassium adducts were the only species exhibiting a trimer form with significant signal-to-noise ratio. The potassium adducted trimers of

Figure 3. Arrival time distributions for the sodiated monomers and dimers of corticosterone and 11-deoxycortisol (a); the potassiated monomers and dimers of α -estradiol and β -estradiol (b); the sodiated monomers and dimers of testosterone, epitestosterone, DHEA, and estriol (c); and the potassiated monomers, dimers, and trimers of aldosterone and cortisone (d)

aldosterone and cortisone showed near-baseline resolution despite some peak tailing of aldosterone.

Resolution of Steroid Isomer Pairs

Using Eq. (1), resolution between each of the isomer pairs was calculated. A scatter plot of resolution between dimer species vs. molecular weight of the neutral steroid is presented in **Figure 4** (values are available in **Table S1** of the Supplementary Material). The resolution values show that for each set of isomer pairs, which have molecular weights ranging from 272 to 360 Da, there was at least a single dimer species, either as a lithium, sodium, or potassium adduct, that had a resolution value greater than 1.0, which could be used for quantitation of the steroids. Specifically, the lithiated dimers of the androsterone isomers showed the highest resolution ($R_s = 3.19$) throughout the other adducts (i.e., sodium and potassium) or isomer pairs (i.e., testosterone and epitestosterone, and aldosterone and cortisone) studied here.

The testosterone series (288 Da) showed resolution above 1.5 for sodiated and lithiated dimer adducts for specific species. Higher resolution values were seen when comparing testosterone/epitestosterone or testosterone/DHEA. On the other hand, both lithium and

Figure 4. Graph of resolution values vs. molecular weight of the isomeric dimers of lithiated (blue circle), sodiated (red diamond), and potassiated (green square) adducts. The dashed lines represent resolution cutoffs of 1.0 (long dashed) and 1.5 (short dashed)

sodium dimer adducts provided very low resolution values between epitestosterone and DHEA, which correlated to their overlapped ATDs. For this series, estriol monomer and dimer adducts were resolved in the m/z dimension and thus resolution values are not shown for the IMS dimension.

Isomer pairs, both above and below 288–290 Da, show dimers with potassium adducts that offered the highest resolution. For estradiol isomers (272 Da), the potassium adduct was the only one to provide sufficient resolution for this series. Surprisingly, the corticosterone and 11-deoxycortisol isomer pair (346 Da) could be resolved as lithium, sodium, and potassium dimer adducts. Contrastingly, the lithiated dimer adduct resolution was reduced for the aldosterone/cortisone isomer pair (360 Da). In this case, the lithium dimer adduct formed multiple overlapping conformers for both species minimizing the potential for separation of these species. Overall, these results showed that under optimum conditions, all steroid isomer pairs studied have a resolution greater than 1.0 with interesting trends dependent on the molecular weight of the steroids, as well as the adduct used.

Collision Cross-Section Measurements

Nitrogen CCSs for the monomer and dimer species with varying adducts for corticosterone and 11-deoxycortisol, epiandrosterone and androsterone, aldosterone and cortisone, and testosterone, DHEA, epitestosterone, and estriol are shown in **Figure 5** (nitrogen values are available in **Tables S2–S4** of the Supplementary Material; a scatter plot showing the CCSs for the estradiols is available in **Figure S2** of the Supplementary Material). As expected, the dimers of all steroids have larger CCS than the monomer species. Surprisingly, the alterations of CCS values between steroid isomers as their metal adduct changes were different. For example, in the corticosterone and 11-deoxycortisol monomers (Figure 5a) and for the testosterone series monomers (Figure 5d), CCSs increased as the ionic radii of the metal increased. In contrast, androsterone and epiandrosterone (Figure 5b) monomer CCSs for potassium adducts were much smaller than those for the corresponding sodium or lithium adducts. The steroid dimer CCS values greatly changed when moving to different adducts. For corticosterone and 11-deoxycortisol (Figure 5a), the CCSs of the dimers decreased as the ionic radii of the metal adduct increased.

Figure 5. Graph of nitrogen collision cross sections vs. the adduct for corticosterone and 11-deoxycortisol (a); epiandrosterone and androsterone (b); aldosterone and cortisone (c); and DHEA,

However, the other steroids studied had no consistent trend in their dimer CCS across metal ionic radii. Interestingly, androsterone and aldosterone showed two conformers, each with a distinct CCS value, in adducts with the larger ionic radii metal.

Furthermore, due to the complex nature of TWIMS CCS calibration, the CCS values for the sodiated monomers and dimers of the steroids studied here were compared to the sodiated monomers and dimers published by Chouinard et al. measured on a DTIMS instrument [20]. We found that the calibrated and calculated CCS of the sodiated monomers, when measured using published nitrogen CCSs for polyalanine, agreed within 1.0% error (on average, 0.2%). However, there was an increase in the error of the calibrated dimer CCS, where the maximum error is at 7.3% (on average, 4.9%). The trend here suggests that while polyalanine calibrates steroid monomers well, it may not be optimal for calibrating multimer species. These discrepancies may result from multimer species having different charge distribution with more possible degrees of freedom than polyalanine, which could have charged localized to a specific end of the peptide and forms an alpha helix or globular structure in the gas phase [38]. Overall, we see a good agreement of calibrated nitrogen CCS values for steroids with DTIMS literature values.

Conclusions

TWIMS has been shown to be a key analytical tool for the separation of steroids through group I metal adduction and the formation of multimers. Through TWIMS separation, five pairs of steroid isomers in their own optimal parameters could achieve a resolution value greater than 1.0. CCS values were also measured for all steroid ion species and could be further used in modeling or identification of steroid analytes. Importantly, the calibration error associated with the CCS values was also analyzed by comparing the obtained CCS values to previously reported DTIMS values [20]. The error shows that while polyalanine is a good calibrant for the monomer, it tends to overestimate the dimer CCS resulting in higher error. Ultimately, TWIMS has been shown to be useful in the separation and identification of steroid isomers. In future work, it is important to determine whether separation through dimer adducts is a useful technique when applied mixtures of steroid isomers.

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SUPPLEMENTARY MATERIAL TO ACCOMPANY:

**APPLICATION OF GROUP I METAL ADDUCTION TO THE SEPARATION OF STEROIDS BY
TRAVELING WAVE ION MOBILITY SPECTROMETRY**

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Figure S1. Arrival time distributions for the sodiated monomer and dimer of aldosterone and cortisone **(a)**; sodiated monomer and dimer of epiandrosterone and androsterone **(b)**; potassiated monomer and dimer of corticosterone and 11-deoxycortisol **(c)**.

Figure S2. Graph of nitrogen collision cross sections vs. the adduct for α -estradiol and β -estradiol, where the diamonds represent the dimer species and the circles represent monomer species.

Table S1. Resolution (R_s) values for steroid isomers as their lithium, sodium, and potassium dimer adducts.

Steroid	Adduct	R_s
Aldosterone	[2M+Li] ⁺	0.19
Cortisone		
Aldosterone	[2M+Na] ⁺	1.09
Cortisone		
Aldosterone	[2M+K] ⁺	1.69
Cortisone		
Androsterone	[2M+Li] ⁺	3.19
Epiandrosterone		
Androsterone	[2M+Na] ⁺	0.65
Epiandrosterone		
Androsterone	[2M+K] ⁺	0.22
Epiandrosterone		
β-Estradiol	[2M+Li] ⁺	0.08
α-Estradiol		
β-Estradiol	[2M+Na] ⁺	0.23
α-Estradiol		
β-Estradiol	[2M+K] ⁺	1.26
α-Estradiol		
Corticosterone	[2M+Li] ⁺	1.24
11-Deoxycortisol		
Corticosterone	[2M+Na] ⁺	1.96
11-Deoxycortisol		
Corticosterone	[2M+K'] ⁺	2.06
11-Deoxycortisol		
DHEA	[2M+Li] ⁺	0.03
Epitestosterone		
DHEA	[2M+Na] ⁺	0.04
Epitestosterone		
DHEA	[2M+Li] ⁺	1.64
Testosterone		
DHEA	[2M+Na] ⁺	1.99
Testosterone		
Testosterone	[2M+Li] ⁺	1.73
Epitestosterone		
Testosterone	[2M+Na] ⁺	2.07
Epitestosterone		

Table S2. Nitrogen collision cross section values for steroid monomer adducts with lithium, sodium, and potassium presented as average $\pm$ standard deviation ($n = 4$).

Adduct	MW	Li	Na	K
α-Estradiol	272.18	185.2 $\pm$ 2.1	176.9 $\pm$ 1.4	191.0 $\pm$ 2.5
β-Estradiol		187.9 $\pm$ 0.5	176.5 $\pm$ 0.9	192.0 $\pm$ 1.0
Testosterone	288.21	197.1 $\pm$ 1.8	197.3 $\pm$ 0.6	205.0 $\pm$ 2.3
DHEA		198.43 $\pm$ 3.2	195.5 $\pm$ 0.5	175.0 $\pm$ 1.7
Epitestosterone		198.5 $\pm$ 1.2	198.3 $\pm$ 0.6	205.4 $\pm$ 2.2
Estriol	288.17	187.1 $\pm$ 2.0	187.2 $\pm$ 0.6	197.5 $\pm$ 1.7
Androsterone	290.22	199.5 $\pm$ 2.2	196.6 $\pm$ 0.6	181.1 $\pm$ 1.4
Epiandrosterone		200.6 $\pm$ 2.4	197.9 $\pm$ 0.6	181.2 $\pm$ 1.8
Corticosterone	346.21	210.4 $\pm$ 2.3	211.1 $\pm$ 0.6	206.7 $\pm$ 2.4
11-Deoxycortisol		206.9 $\pm$ 1.3	211.3 $\pm$ 1.2	207.4 $\pm$ 3.0
Aldosterone	360.19	191.9 $\pm$ 3.8	196.2 $\pm$ 0.6	194.9 $\pm$ 4.0
Aldosterone		204.9 $\pm$ 3.9	211.8 $\pm$ 0.6	
Cortisone		210.8 $\pm$ 3.7	213.4 $\pm$ 0.6	208.7 $\pm$ 3.0
Cortisone		196.9 $\pm$ 2.9		

Table S3. Nitrogen collision cross section values for steroid dimer adducts with lithium, sodium, potassium, and rubidium presented as average $\pm$ standard deviation ($n = 4$).

Adduct	MW	Li	Na	K	Rb
α-Estradiol	272.18	239.4 ± 1.8	249.6 ± 1.5	250.9 ± 1.0	
β-Estradiol		243.9 ± 1.7	240.3 ± 0.8	259.4 ± 1.3	
Testosterone	288.21	282.3 ± 2.5	283.3 ± 0.4		
DHEA		271.8 ± 2.6	267.7 ± 0.6		
Epitestosterone		275.3 ± 2.7	270.5 ± 0.4		
Estriol	288.17	263.0 ± 1.9			
Androsterone	290.22	255.4 ± 1.7	251.8 ± 0.5	264.4 ± 4.4	
Epiandrosterone		274.5 ± 2.0	269.9 ± 0.7	269.3 ± 1.9	
Corticosterone	346.21	307.0 ± 1.9	303.0 ± 0.2	287.7 ± 1.9	284.5 ± 2.2
11-Deoxycortisol		290.2 ± 0.2	284.2 ± 2.5	273.7 ± 1.8	274.3 ± 2.3
Aldosterone	360.19	297.4 ± 3.4	273.3 ± 0.7	267.1 ± 3.1	
Aldosterone			296.3 ± 0.8	285.9 ± 2.9	
Cortisone		292.7 ± 3.8	286.9 ± 0.7	275.6 ± 2.6	

Table S4. Nitrogen sodiated monomer and dimer collision cross sections ($n = 4$) compared to previously measured collision cross sections by Chouinard *et al.* [1].

Species	Adduct	CCS (Å ²)	SD	Lit. CCS (Å ²)	Lit SD	% Error
DHEA	[2M+Na] ⁺	267.7	0.6	251.3	0.4	6.1
Epitestosterone	[2M+Na] ⁺	270.5	0.4	250.7	0.3	7.3
Testosterone	[2M+Na] ⁺	283.3	0.4	269.0	0.5	5.1
Corticosterone	[2M+Na] ⁺	303.0	0.2	289.9	0.6	4.3
Deoxycortisol	[2M+Na] ⁺	285.0	2.4	269.3	0.4	5.5
Androsterone	[2M+Na] ⁺	251.8	0.5	242.6	0.3	3.7
Epiandrosterone	[2M+Na] ⁺	265.3	2.5	256.3	0.3	3.4
Aldosterone	[2M+Na] ⁺	273.3	0.7	266.1	0.4	2.6
Cortisone	[2M+Na] ⁺	286.9	0.7	269.9	0.5	5.9
DHEA	[M+Na] ⁺	195.5	0.5	195.5	0.3	0.0
Epitestosterone	[M+Na] ⁺	198.3	0.6	196.4	0.3	1.0
Testosterone	[M+Na] ⁺	197.3	0.6	196.7	0.3	0.3
Corticosterone	[M+Na] ⁺	211.1	0.6	211.3	0.3	0.1
Deoxycortisol	[M+Na] ⁺	211.3	1.2	210.7	0.3	0.3
Androsterone	[M+Na] ⁺	196.6	0.6	197.1	0.2	0.2
Epiandrosterone	[M+Na] ⁺	197.9	0.6	196.8	0.2	0.6
Aldosterone	[M+Na] ⁺	196.2	0.6	197.7	0.3	0.7
Cortisone	[M+Na] ⁺	213.4	0.6	211.7	0.3	0.8

REFERENCES

1. Chouinard, C. D., Beekman, C. R., Kemperman, R. H. J., King, H. M., Yost, R. A.: Ion Mobility-Mass Spectrometry Separation of Steroid Structural Isomers and Epimers. *Int. J. Ion Mobil. Spectrom.* **20**, 31-39 (2017)