Adduct-ion formation in trapped ion mobility spectrometry as a potential tool for studying molecular structures and conformations

Barbara M. Zietek¹ · Ynze Mengerink² · Jan Jordens² · Govert W. Somsen¹ · Jeroen Kool¹ · Maarten Honing³

Received: 15 November 2017 / Accepted: 4 December 2017 / Published online: 15 December 2017
© The Author(s) 2017. This article is an open access publication

Abstract

Recent developments in the field of ion mobility spectrometry provide new possibilities to explore and understand gas-phase ion chemistry. In this study, hyphenated trapped ion mobility spectrometry-mass spectrometry (TIMS-MS) was applied to investigate analyte ion mobility as function of adduct ion formation for twelve pharmaceutically relevant molecules, and for tetrahydrocannabinol (THC) and its isomer cannabidiol (CBD). Samples were introduced by direct infusion and ions were generated with positive electrospray ionization (ESI+) observing protonated and sodiated ions. Measurements were performed with and without addition of cesium-, lithium-, silver- and sodium ions to the samples. For the tested compounds, metal adduct ions with the same m/z but with different mobility and collision cross section (CCSs) were observed, indicating different molecular conformations. Formation of analyte dimers was also observed, which could be associated with molecular geometry of the compounds. By optimizing the range and speed of the electric field gradient and ramp, respectively, the separation of THC and CBD was achieved by employing the adduct formation. This study demonstrates that the favorable resolution of TIMS combined with the ability to detect weakly bound counter ions is a valuable means for rapid detection, separation and structural assignment of molecular isomers and analyte conformations.

Keywords Trapped ion mobility spectrometry · Dimers · Adduct ions · Protomers · Molecular conformations

Introduction

Over recent times, ion-mobility spectrometry (IMS) has brought new insights into the behavior of gas-phase ions and complexes of small molecules, biomolecules and polymers [1–3]. The IMS separation mechanism, which is based on ion charge, size and shape, may facilitate the separation of isomers and recognition of different ion conformations. Additional structural identification often is achieved by

hyphenating IMS to (high-resolution) mass spectrometric (MS) detection. The past twenty years has seen rapid development of new IMS devices, in which a considerable improvement, especially in resolving power, was achieved [4–7]. Still, separation of structural isomers with IMS can be quite challenging.

A common approach to separate isomers, which exhibit similar collision cross section (CCS ($\text{\AA}^2$)), is the use of mono- and divalent cations, which form distinguishable adduct complexes with the target analytes, and has led to successful separations of isomeric carbohydrates, lipids, and peptides [8–13]. The improved resolution was due to mobility shifts in one of the isomeric forms as a result of conformational changes induced by cation adduct formation. Other common approaches to shift the mobility of ions involve the use of mixed carrier gases, shift reagents (SR) and/or changes in temperature of the buffer gas [14–17].

Huang & Dodds [9] and Clowers & Hill [13] reported unpredictable changes in conformations of analytes upon use of different adduct ions, which can depend on the structure of the analytes and the ionic radius of the cations. These molecular species could then result in improved mobility separation. Clowers & Hill [13] studied the separation of flavonoids

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s12127-017-0227-6>) contains supplementary material, which is available to authorized users.

✉ Jeroen Kool
j.kool@vu.nl

¹ Division of Bioanalytical Chemistry, Vrije Universiteit Amsterdam, De Boelelaan 1085, 1081, HV Amsterdam, The Netherlands

² DSM Materials Science Center, Urmonderbaan 22, 6167, MD Geleen, The Netherlands

³ M4I Institute Maastricht MultiModal Molecular Imaging, Faculty Health, Medicine & Life Sciences, Maastricht University, Universiteitssingel 50, 6229, ER Maastricht, The Netherlands

diglycosides isomers using dual gate-ion mobility quadrupole ion trap mass spectrometry. They showed multiple gas phase conformations for narirutin and hesperidin upon sodium, potassium and/or silver adduct formation. Similar observations were made for carbohydrates, where a trisaccharide maltotriose with cesium adduct showed more than one mobility peak [9]. In the latter study, a Waters Synapt G2 HDMS quadrupole time-of-flight hybrid mass spectrometer equipped with travelling-wave ion mobility (TWIM) cell was used. In both cases, the appearance of the new gas phase conformations of the molecules was explained as a result of different binding sites of the cation and the target compound.

Recently, Morrison, Bendiak, & Clowers [18] investigated the influence of cation adduct formation with tetrasaccharides using atmospheric pressure dual-gate drift ion mobility coupled to a Thermo linear ion trap mass spectrometer. They noticed that for some glycans the coordination of a metal ion lead to formation of dimers, which was observed as a second mobility peak appearing at the same nominal mass as the monomeric form of the analyte. The dimers were formed at a very low cation concentration, and adducts of bivalent metals were more prone to form dimers than adducts of monovalent cations.

In IMS devices which use high electric fields for the separation of ions, such as TWIMS, heating of ions may result in analyte fragmentation, as observed for e.g. *p*-methoxybenzylpyridinium (m/z 200) [19] and a leucine enkephalin dimer (m/z 1112) [20]. Ion heating may also cause disintegration of ion adducts and dimers in the mobility cell. Trapped ion mobility spectrometry (TIMS), a recent IMS development commercialized by Bruker Daltonics (Bremen, Germany), operates at low electric field, preventing ion heating, thus offering optimal conditions for studying non-covalent complexes and relatively loosely bound adducts. Additionally, TIMS offers mobility separations with up to a resolving power (R) of 400 [4], which was successfully applied in fast separations of isobars and isomers [4, 21, 22].

During exploring TIMS experiments in our laboratory in which the ortho, meta and para isomers of dimethylphthalate were separated, we observed various sodium adduct ions (data not shown). The main objective of the present study was to gain a more fundamental understanding of adduct-ion formation in TIMS and to further evaluate this phenomenon to distinguish isomeric analytes which could not be separated as protonated molecule. Fourteen pharmaceutically relevant compounds with a molecular weight ranging from 270 to 645 Da were used as test compounds, systematically studying several cationic counter ions. The effect of adduct-ion formation with the target analytes on the appearance of new conformations and dimers, was investigated by TIMS, and correlations with the analyte molecular structures were made. Additionally, gas phase separation of isobaric tetrahydrocannabinol (THC) and cannabidiol (CBD) by TIMS was studied employing cation adduct formation.

Materials and methods

Chemicals

Amoxicillin (AMX), carvedilol (CVD), alpha-naphthoflavone (ANF), lincomycin hydrochloride (LM), amiodarone hydrochloride (AMD), fluvoxamine (FVX), rutaecarpine (RUT), loperamide hydrochloride (LOP), ketoconazole (KET), tolbutamide (TBM), sodium chloride (NaCl) and formic acid (FA) were obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands). Cetirizine (CTZ) was kindly provided by DSM Materials Science Center. Ellipticine was a kind gift from Dr. Jan Commandeur from the Division of Molecular and Computational Toxicology (VU Amsterdam, The Netherlands). Tetrahydrocannabinol (THC) and cannabidiol (CBD) were obtained from Echo Pharmaceuticals (Weesp, The Netherlands). Lithium chloride (LiCl), silver nitrate (AgNO_3) and acetonitrile (ACN) of LC-MS grade, were from Merck (Darmstadt, Germany), and cesium chloride (CsCl) was from Riedel de Haen (Zwijndrecht, The Netherlands). Acetic acid was from J.T.Baker (Deventer, The Netherlands). High purity water of MS grade was used for samples and solvents preparation.

Sample preparation

Stock solutions of twelve pharmaceuticals (Fig. 1) were prepared in DMSO with the exception of RUT, which was dissolved in water. The concentration of the stock solutions for AMX and ELL, were 10 and 40 mM respectively, and for the other pharmaceuticals 20 mM. The working concentrations of the pharmaceuticals were 2 μM for all compounds except for AMX, which was prepared at 5 μM , diluted in 80% ACN, and except for AMX and CVD, which were diluted in 30% ACN. Stock solutions of cesium chloride, sodium chloride, lithium chloride and silver nitrate were prepared at concentration of 20 mM in water and used at a final concentration of 50 μM . Stock solutions of THC and CBD (structures are depicted in Fig. 1) were used as received (3.2 mM in methanol). Depending on the analytical question the two compounds were diluted to working concentrations of 2, 0.2, or 0.02 μM in 80% acetonitrile with addition of 0.1% acetic acid or 20 μM cesium chloride, sodium chloride, lithium chloride or silver nitrate.

TIMS operational mode

In this study a prototype TIMS-MS instrument (Bruker Daltonics, Bremen, Germany) was used. The principles and operation of TIMS have been extensively described in previous studies [5, 23, 24]. Briefly, in conventional IMS devices, ions travel through a drift tube driven by an electric field, and collide with stationary gas molecules. In TIMS, ions are

Fig. 1 Molecular structures of the twelve pharmaceuticals and the two cannabinoid isomers (CBD and THC) analysed in this study, including information on the molecular weight and CCS of the $[M + H]^+$ forms, experimentally determined in this study

trapped in an electric field gradient and a concurrent gas flow [24]. The TIMS device consists of three regions: entrance funnel, mobility analyzer and exit funnel (Fig. S1). Positive ions which are generated using e.g. electrospray ionization (ESI), enter the funnel via a glass ion-transfer capillary and are pushed into the direction of the analyzer section due to an

applied DC potential and gas flow. In the analyzer, a weak axial electric field gradient (EFG) is applied, and ions are trapped and positioned according to their mobility when the electric force balances the collision force by the constant gas flow. As the highest electric field is located near the exit and the lowest electric field near the entrance of the analyzer, ions

with lower mobility (i.e. larger charge and/or CCS) will occupy positions more near the exit, whereas the higher mobility ions are in more near the entrance. Additional radial trapping of ions near the axis of the analyser tunnel is ensured by applying RF voltages. In the analyzer, the ions are collected and trapped for a selected time, the so-called trapping time (t_{trap}). After trapping, the EFG is gradually decreased at a selected rate as determined by the voltage ramp (V_{ramp}) and ramp time (t_{ramp}). As the electric field decreases and the gas flow is unchanged, the ions leave the analyzer at an elution voltage $V_{elution}$ characteristic for their mobility (K), and are subsequently transferred to the quadrupole high-resolution time-of-flight mass spectrometer [24]. The mobility (K) in TIMS can be described as

$$K = \frac{v_g}{E} = A \left(\frac{1}{(V_{elution} - V_{out})} \right) \quad (1)$$

Where v_g is the gas velocity, E is the electric field at which an ion elutes, A is the calibration constant that can be obtained from measuring calibration solution with known CCS. The difference between the $V_{elution}$ (elution voltage) and V_{out} (voltage applied to the last electrode in the analyser section in TIMS) defines the mobility K of an ion.

TIMS-MS parameters

All analyte solutions were analyzed by direct infusion at a flow rate of 180 $\mu\text{L/h}$ using a KDS100 syringe pump (KD Scientific Inc., MA, USA). TIMS settings depended on the analytical question. For most pharmaceuticals, the EFG was from -20 to -120 V using 1000 TOF pulses per accumulation and 500 accumulations, whereas for ELL and AMD it was -5 to -160 V and -20 to -150 V, respectively with 1000 TOF pulses per accumulation and 500 accumulations. For the analysis of the two isobaric compounds THC and CBD, initially, the EFG was from 0 to -165 V, enabling analysis of ions within a large mobility range. For isomer separation studies, a narrower EFG was used (from -30 to -80 V) to improve the resolving power of TIMS. The resolving power in TIMS can be improved by reducing the EFG range and to a lesser extent by increasing the number of TOF pulses, which determines the V_{ramp} and t_{ramp} , respectively. In both of the analyses (with a narrow and wide EFG range) 600 TOF pulses per IMS scan with 400 accumulations and 20 repetitions were used. Nitrogen was used as buffer gas at temperature of 300 K, and a default pressure of 1.7 mbar. The mobility resolving power was calculated following equation: $R = CCS/\Delta CCS$.

Experimental CCS and K_0 calculations

The experimental reduced mobility (K_0) and CCS (Ω) of compounds and complexes were calculated from the

measured mobilities ($1/K_0$) using Compass Mobility Calculator of the Bruker Compass Data Analysis software (version 5.0). The reduced mobility K_0 is the mobility K , normalized for pressure (P (torr)) and temperature (T (K)) in the mobility tube. The mobility axis was calibrated using tuning mix (Agilent Technologies, Santa Clara, Ca, USA), which comprises compounds with known CCS. CCS Calculations were according to Eq. 2.

$$\Omega = \frac{(18\pi)^{1/2}}{16} \frac{ze}{(k_B T)^{1/2}} \left[\frac{1}{m_i} + \frac{1}{m_b} \right]^{1/2} \frac{1}{K_0 N} \quad (2)$$

Where ze is the charge of the ion, k_B the Boltzman's constant, N is the number density of the buffer gas, T is the gas temperature, and m_i and m_b are the molecular mass of the ions and buffer gas, respectively.

LC-MS of THC and CBD

In order to better understand the dimerization of THC and CBD observed, and to compare the dimer formation of both compounds (as discussed in the results section), solutions of THC and CBD (5 μL injected) were analyzed by LC-MS using an Agilent Technologies 1200 series LC system (Waldbronn, Germany) equipped with an Acquity UPLC BEH C18 (2.1 $\times$ 50 mm; particle size, 1.7 μm) analytical column (Waters, Milford, MA). Seven different concentrations between 0 and 20 μM were prepared from THC and CBD stock solutions (3.2 mM in methanol), by serial dilution in 80% ACN. Mobile phases A and B were pure water and ACN, respectively. The gradient used started with 20% B for 1 min, rose to 95% B in 8 min, was isocratic at 95% B for 4 min, and in 0.1 min decreased to 20% B. Column equilibration was set for the next 7.9 min at 20% B. The total run time was 18 min at a flow rate of 0.3 ml/min and the column oven was set to temperature of 40 $^\circ\text{C}$. To form silver adducts, post-column addition of a silver nitrate in water (250 μM) was performed in a flow ratio of 1:4 leading to a final concentration of silver ions of 50 μM . MS detection was performed with a Micro-TOF II instrument (Bruker Daltonics, Bremen, Germany) employing ESI+ with a needle voltage of 4.5 kV and a drying gas temperature and flow rate of 240 $^\circ\text{C}$ and 8 L/min, respectively.

Results and discussion

Separation and structural identification of isobaric and isomeric molecules and their conformations can be a challenging task. Adduct-ion formation has shown to specifically influence molecular conformation, potentially allowing separation of isomeric compounds by TIMS. In the present work, the cation-adduct formation of twelve pharmaceutically relevant

compounds and its effect on molecular conformation and dimer formation was studied by measuring their mobilities using TIMS-MS. Additionally, TIMS separation of the two isomers THC and CBD was investigated using their cation adducts.

Conformational changes in small molecular ions upon cation adduct formation

Twelve compounds exhibiting different numbers of rotational bonds, double bonds and functional groups (Fig. 1) were selected to study their ionization behavior and possible changes in structural conformation and shape in the gas phase by TIMS. Solutions of the test compounds in 0.1% acetic acid were infused directly into the ESI-TIMS-MS system and the protonated ($[M + H]^+$) species of the analytes were observed. In the cases of, LM, TBM, KET and AMX, additional peaks were observed in the recorded mobilograms (Fig. 2a–d). Considering that in our study only pure compounds were used, these additional mobility peaks do not represent isomers but indicate multiple structural conformations, probably caused by different position of the proton on the analyte molecule. In the case of LM (Fig. 2a), the two protonated species were visible only after applying a narrow EFG (–70 to –50 V) and a higher accumulation number (1200) per scan. Compounds/isomers which differ in the proton position are referred to as protomers. Lalli et al. [25], who used aniline as a model compound to study protomers present in the gas phase using ESI (+)-TWIM-MS, observed the presence of two species in the mobilogram which represented the N-protonated and ring-protonated aniline. The authors suggested that the different protonation sites caused a different charge distribution in the molecule and thus interaction with the carbon dioxide buffer gas, resulting in different CCS values for the protomers. As in our study, nitrogen was used as buffer gas, interactions with the protonated species seem unlikely. We presume the protonation site might affect the rotational freedom of specific bonds and/or influence the equilibrium of tautomeric forms (as occur in AMX, TBM and LM) causing a difference in CCS and thus mobility.

In addition to protonated molecules, the usually observed sodium adducts were encountered in the acquired electrospray mass spectra. Interestingly, also in the mobilograms of several sodiated analytes multiple mobility peaks were observed. Cation adducts indeed are known to be able to alter structural conformation of gas phase ions as shown for example, for carbohydrates [9, 26]. Therefore, we studied the effect of several cations on the IM behavior of the test analytes by mixing the compounds with salts of alkali (LiCl and CsCl) and transition metals ($AgNO_3$). The metal ions tend to form non-covalent adducts with target analytes via electrostatic interactions, such as ion-dipole interactions. Silver ions also have affinity towards double bonds, as has been employed, for

example, in the LC separation and MS detection of fatty acids (FA) and triacylglycerols (TGA) [27, 28].

For six of the pharmaceuticals, more than one conformation upon cation adduct formation was observed. These were AMX ($+Na^+$, $+Ag^+$, $+Li^+$), RUT ($+Ag^+$), AMD ($+Ag^+$), TBM ($+Na^+$, $+Ag^+$, $+Li^+$), LOP ($+Ag^+$) and ANF ($+Na^+$). The mobilograms for the ions for which multiple conformations were observed are shown in Fig. 2. For most of these compounds (AMX, RUT, AMD, LOP and TBM) an additional conformation was observed upon addition of silver ions, which could be due to the earlier mentioned affinity of silver to double bonds (Fig 2e–i). When the compound possesses multiple double bonds and has high density of π electrons, binding of a silver ion can occur at more than one site in a molecule, and formation of multiple conformations can result [29]. Indeed, AMX, RUT, AMD, TBM and LOP, comprise at least three double bonds. It should be noted that KET and FVX, which have double bonds, do not show detectable multiple conformations in IM, indicating that silver affinity is compound specific. In addition, other ionic interactions obviously can play a role in the induction of multiple conformations. Other cations that also lead to the appearance of multiple mobility peaks were sodium (in the case of TBM, ANF and AMX, Fig. 2J–L, respectively) and lithium (TBM and AMX, Fig. 2I, m, respectively).

As mentioned earlier, we expected rotational bonds to facilitate the formation of other conformations upon cation binding. Interestingly, multiple conformations were also observed in planar compounds, i.e. RUT and ANF (Fig. 2f, k, respectively). However, it is not clear what causes the change in the shape of the molecule in the gas phase, since in the case of RUT the additional conformation was observed only upon cation adduct formation with silver. This could suggest that the size of the silver ion, which has an ionic radius of 1.15 Å [30], has a considerable impact on the overall shape of the complex, causing the mobility shift even in absence of rotating bonds. The behavior could also be explained by silver residing on different positions. Depending on the position, silver ion could cause changes in the shape of the molecule and consequently changing its CCS.

In most cases of multiple conformations only one additional peak was observed, which had a lower mobility and thus a higher CCS than the primary conformation of the molecule. Here we define the primary conformation as the one which has a mobility closest to the protonated form. This means that the second conformation has a more open or elongated structure. The two planar molecules RUT and ANF (Fig. 2f, k, respectively) appear to follow the common trend, in which the second conformation of the molecule is more open than the primary one. Interestingly, in the protonated species the abundance of the peak with the higher mobility (smaller CCS) tends to be lower in intensity than the peak with the lower mobility. A different behavior was observed in the analytes

Fig. 2 Extracted ion mobigrams (EIM) of cation adducts of target analytes showing multiple conformations. X-axis shows the inverse reduced mobility value $1/K_0$ ($\text{V}\cdot\text{s}/\text{cm}^2$), and the y-axis represents the signal intensity of the mobility peak. The peaks annotated with a lower letter are (a) the primary mobility, (b) the additional conformation mobility, (d) the mobility of the dimer which fragmented to its monomer. **a**: protonated lincomycin $[\text{LM} + \text{H}]^+$; **b**: protonated tolbutamide $[\text{TBM} + \text{H}]^+$; **c**: protonated ketoconazole $[\text{KET} + \text{H}]^+$; **d**: protonated amoxicillin $[\text{AMX} + \text{H}]^+$; **e**: amoxicillin with silver adduct

$[\text{AMX} + \text{Ag}]^+$; **f**: rutaecarpine with silver adduct $[\text{RUT} + \text{Ag}]^+$; **g**: amiodarone with silver adduct $[\text{AMD} + \text{Ag}]^+$; **h**: loperamide with silver adduct $[\text{LOP} + \text{Ag}]^+$; **i**: tolbutamide with silver adduct $[\text{TBM} + \text{Ag}]^+$; **j**: tolbutamide with sodium adduct $[\text{TBM} + \text{Na}]^+$; **k**: alpha-naphthoflavone with sodium adduct $[\text{ANF} + \text{Na}]^+$; **l**: amoxicillin with sodium adduct $[\text{AMX} + \text{Na}]^+$; **m**: tolbutamide with lithium adduct $[\text{TBM} + \text{Li}]^+$; **n**: amoxicillin with lithium adduct $[\text{AMX} + \text{Li}]^+$ (star) indicates measurement for which a different electric field gradient was used

upon cation adduction, where the first peak (higher K_0 and smaller CCS) was observed to be more abundant. This means that the conformation of the ion complex which was observed

to have a higher K and lower CCS was present in the gas phase at a higher concentration than the one with a lower K and higher CCS.

Changes in the mobility of molecular ions after formation of cation adduct complexes

The overall data obtained in the adduct formation experiments are outlined in Fig. 3, which shows the experimental CCS of the different adducts and their conformations. Except for TBM, the protonated form of the analysed compounds was always observed, and thus the averages and standard deviation (SD) of the determined K_0 and CCS values of the protonated forms were calculated and used as a measure of precision of the mobility measurement. For TBM, sodiated species were used. The protonated forms of the compounds showed to have a stable CCS values in each experiment with a very small SDs in the range of 0.2–0.5 Å². The positive result of the precision measure allowed comparison of the CCS and mobility values of the different cation adduct ions obtained in the different experiments. The averages of measured $1/K_0$, K_0 and CCS values together with the SD for the protonated species of individual compounds are presented in Table S1. For most of the tested compounds, the CCS of the adducts showed dependence on the mass of the cation. That is, CCS values of the metal adducts increased with increasing ionic radius of the cation (i.e. in the order H⁺, Li⁺, Na⁺, Ag⁺ and Cs⁺). Yet, this trend was not clearly observed for AMX, LOP, RUT, TBM and CTZ. On the contrary those compounds were observed to have multiple conformations. For example, [RUT + Ag]⁺ was observed to have three different conformations, with CCS values of 181.9, 187.4 and 195.2 Å², respectively. The RUT silver adduct with the lowest CCS (181.9 Å²) has a lower CCS value than the [M + Li]⁺. The reduced mobility values and corresponding CCSs of the different adducts of all compounds can be found in Table S2. The lack of a positive correlation between the m/z and CCS in some of the ions has already been observed in other studies, for example those on isomeric carbohydrates [8, 9]. As indicated by Seo et al. [26] several features of metal cations can impact the way they bind to a molecule and thus influence the overall shape of the metal-ion complex.

Surprisingly, a substantial shift in the mobility between some of the ion adducts was observed for ELL, FVX and RUT. In order to assess the mobility shift, the mobility difference between the mobility value of the protonated species and the adduct of interest was calculated. The protonated species was taken as a reference. Mobility values of all complexes are reported in Table S2. For ELL, the difference in CCS for the silver-ion adduct [M + Ag]⁺ was 23.5 Å² (Fig. 3). For RUT, the difference for the [M + Ag]⁺, [M + Cs]⁺ and [M + Li]⁺ CCS was 17.9 Å², 72.8 and 24.2 Å², respectively (Fig. 3). In FVX, a clear mobility difference of 6.9 Å² was observed in the [M + Ag]⁺ and in LOP a difference of 11 Å² was observed between the [M + Cs]⁺ (Fig. 3). The largest difference in the

CCS induced by cationization of the twelve small molecular ions was observed in RUT and ELL, which are planar molecules with no freely rotating bonds. This could indicate that in the case of flexible molecules a cation adduct is capable of affecting the structure of the molecule in a way that the CCS does not significantly differ from the CCS of the protonated form. For the more rigid structures, the adducted cation increases the CCS of the analyte molecule.

Dimer formation in molecular ions with limited number of rotational bonds

Further analysis of the obtained TIMS data on the test compounds and their adducts, revealed a tendency of some adduct ions to form dimers. Interestingly, compounds with more planar structures and limited number of rotational bonds were observed to form dimers with cations more readily than the more flexible compounds used in this study. These planar compounds include ANF, RUT and ELL (Fig. 4a–g). More flexible compounds, in which dimer formation was also observed, include TMB (sodium adduct), FVX (cesium adduct), AMX (protonated species) and CVD (silver adduct) (Fig. 4h–k). In the case of the more flexible compounds the dimers show lower intensity than the monomers, as opposed to what was observed for the planar molecules. Only for the cesium adduct of FVX the mobility peak of the dimer [2 M + Cs]⁺ was observed to be more abundant than the monomer [M + Cs]⁺. Silver and sodium were the cations that most readily formed dimers with the selected compounds: [2 M + Ag]⁺ ions were observed for RUT, FVX, ANF, CVD, ELL, TBM, and [2 M + Na]⁺ ions for ANF, FVX, TBM and CVD. Figures 4a–f show extracted-ion mobilograms (EIM) of RUT and ANF, respectively. The formation of dimers in the case of ELL is less clear, as ELL showed to form adducts (both monomers and dimers) only with silver. A dimer of ELL [2 M + Ag]⁺ was observed which additionally supports our finding that the planar shape of molecular ions can influence the formation of dimers.

Dimers in the case of isomers: THC and CBD

While studying dimer formation in TIMS, interesting observations were made with two structural isomers, i.e. THC and CBD (Fig. 1). Although very similar, the two compounds are known to evoke a very different pharmacological effect [31]. Interestingly, they also behave differently in the gas phase when it comes to the formation of dimers with cations. In order to study dimer formation of THC and CBD, three different concentrations (2, 0.2, and 0.02 μM) were mixed with acetic acid or salt solutions containing Ag⁺, Li⁺ or Cs⁺. The solutions were analysed by infusion ESI-TIMS-MS. In order to enable measurement of both monomers and the dimers in a single experiment, the EFG was set wide (from 0 to −165 V).

Fig. 3 Changes in the mobility of small molecular ions after formation of cation adduct complexes. Most of the target analytes followed a trend in which the CCS increases with an increasing mass of the cation adduct. In

the graph, the species marked as black dots represents the first conformation of the ion, and the ones with grey dots represents the second conformation of the ions

Figure 5 shows overlaid EIM traces of the monomers and dimers of THC and of CBD generated with silver and sodium ions. They show that THC dimers with silver and sodium are formed more readily compared to CBD, which could be associated with the molecular geometry and thus flexibility of the

compound. Due to the closed pyran ring THC exhibits greater rigidity in comparison to CBD, which has more freedom to rotate [32]. Again, the more rigid compound shows to be more prone to form dimers with the selected cation. The formation of dimers with the use of the silver cation in THC and CBD

Fig. 4 Dimer formation of the small molecular ions analysed. Extracted ion mobilograms (EIM) of the cation adduct monomer (black trace) and dimer (grey trace). X-axis shows the inverse reduced mobility value $1/K_0$ (Vs/cm^2), and the y-axis represents the signal intensity of the mobility peak. Dimers were formed more readily in the case of compounds with limited number of rotating bonds, which are: **a**) alpha-naphthoflavone with silver adducts, **b**) rutaecarpine with silver adduct, **c**) alpha-

naphthoflavone with sodium adduct, **d**) rutaecarpine with cesium adduct, **e**) alpha-naphthoflavone with lithium adduct, **f**) rutaecarpine with lithium adduct, **g**) ellipticine with silver adduct. The **h**, **i**, **j** and **k** figures show compounds exhibiting more structural flexibility, i.e. tolbutamide, fluvoxamine, amoxicillin and carvedilol respectively. The more flexible compounds showed dimer formation. The dimers were of lower intensity than the monomer, except of fluvoxamine

was further investigated by generating a calibration curve of the two compounds with concentrations ranging from 0.6–20 μM using ESI-LC-MS (data not shown). This analysis

was performed in order to be sure that this difference was not caused by the use of rather elevated concentrations of the compounds and to ensure that the effect was present over

Fig. 5 Dimers of the isomers: cannabidiol (CBD) and tetrahydrocannabinol (THC). Extracted ion mobigrams of monomers (black trace) and dimers (grey trace) of CBD and THC silver and sodium adduct ions. The formation of dimers was observed to be larger in THC than in CBD, when

adducted to silver and to sodium ions. AI: CBD 2 μ M, AII: 0.2 μ M, AIII: 0.02 μ M, BI: THC 2 μ M, BII: 0.2 μ M, BIII: 0.02 μ M, CI: CBD 2 μ M, BII: 0.2 μ M, BIII: 0.02 μ M, DI: THC 2 μ M, BII: 0.2 μ M, BIII: 0.02 μ M. The concentration of AgNO_3 and NaCl was 20 μ M in all experiments

the large concentration range. Silver adduct ions of THC and CBD were formed by post-column infusion of silver nitrate. The calibration curves generated showed THC to form dimers already at 2.5 μ M, whereas CBD does not form a dimer with a silver ion at any of the tested concentrations. Additionally, it was shown that CBD forms more readily a monomer with a silver ion than THC. These results were in agreement with those obtained with TIMS-MS earlier in this study (Fig. 5).

THC dimer was shown to be present at all tested concentrations, while CBD dimer were not formed even at the high concentration of 20 μ M. There was, however, one discrepancy between the two sets of the data: in the LC-MS chromatogram, CBD did not form any dimer even at the highest concentration of the compound tested, whereas in the TIMS data dimer formation (signal intensity of 473 cts) was observed already at a concentration of 0.02 μ M. A possible explanation

for this could be that the dimer of CBD was formed in the drift section of the TIMS tube. The observed absence of dimers during LC-MS analysis could also be due to the lower sensitivity of the mass spectrometer used for generating the calibration curve as compared to TIMS-MS.

Separation of isomers employing cation adduct formation

As mentioned in the previous section, despite their small structural difference, THC and CBD exhibit quite different pharmacological activity. The most important difference is that THC has psychoactive properties, and CBD does not [31, 33, 34]. This makes THC a drug of abuse and correct identification of this compound is thus important from a forensic point of view. The two cannabinoids, can be separated using chromatographic or electrophoretic methods, such as planar chromatography [35], LC-UV [36], LC-MS(MS) [37, 38], CE-LED-induced fluorescence detection [39], which require a considerable analysis time (5–30 min). In the present study, we studied the potential of TIMS for the fast separation of THC and CBD. The two compounds were diluted to a concentration of 2 μM (for experiments with Na^+ , Li^+ and Cs^+) and 0.2 μM (for experiments with Ag^+) and mixed with acetic acid or three different salt solutions, i.e. LiCl , CsCl and AgNO_3 . A lower concentration of the compounds in the case of adduct formation with silver ions was used as silver was shown to increase the peak intensity especially in the case of CBD. Silver ions, known as dopant for compounds exhibiting double bonds, is expected to coordinate with double bond at C4 position of CBD [40]. The mixtures were analysed by direct infusion ESI-TIMS-MS. The addition of the salts to THC and CBD aimed at generating cation-adducts complexes. Gas phase orientation of cation-adduct complexes formed with analytes was shown to influence the analytes shape and CCS. As a consequence, changing the cation (e.g. from H^+ to Ag^+) turns out to be an important feature to adjust / improve the separation of isomers [8, 9, 11]. Mobilograms showing the TIMS analysis of THC (grey trace) and CBD (black trace) as their adduct ions are depicted in Fig. 6. A clear shift in the mobilities between the two cannabinoids was observed after the formation of cation adduct complexes with lithium, silver and sodium ions. The difference was sufficient to see in the mobilograms the two isomers separated from each other, however a baseline separation was not achieved (R of ~ 50). In all cases, apart from the protonated and cesiated forms for which the CCS of THC and CBD was found not to differ, a tendency for CBD to acquire a more compact structure (smaller CCS and higher mobility) than THC was observed. As a consequence of the dimer formation of CBD with a silver ion

Fig. 6 Separation of tetrahydrocannabinol (THC) and cannabidiol (CBD) using cation adduct formation. Extracted ion mobilograms (EIM) of CBD (black trace) and THC (grey trace), measured separately. The dashed line represents the two compounds measured in a mixture (only with lithium, silver and sodium adducts). X-axis shows the inverse reduced mobility value $1/K_0$ ($\text{V}\cdot\text{s}/\text{cm}^2$), and in the y-axis represents the signal intensity of the mobility peak. **a:** EIM of the protonated THC (2 μM) and CBD (2 μM); **b:** EIM of CBD (2 μM) and THC (2 μM) upon lithium adduction; **c:** EIM of CBD (0.2 μM) and THC (0.2 μM) upon silver adduction; **d:** EIM of CBD and THC upon sodium adduction; **e:** CBD (2 μM) and THC (2 μM) upon cesium adduction

(see Fig. 5), the complex is supposed to still show flexibility and the bond between the aromatic ring and cyclohexenyl should freely rotate. As discussed, silver ions typically bind with the π electrons of a double bond.

If the silver ion complexes to the double bond on the carbon 8 (Fig. 1), the rotation of the bond between carbon 4 and carbon 8 can make the CBD more compact than THC. Sodium ion adduction also shows a shift in the mobility between the two compounds ($\Delta K_0 = 0.042 \text{ cm}^2/\text{Vs}$; $\Delta \text{CCS} = 7.08 \text{ \AA}^2$). The most probable localization of the sodium ion is near the oxygen atom by ion-dipole attractions [41]. Therefore, the mechanism by which the conformation in the two structures changes most probably differs from the one proposed for silver cation adducts. When looking at the structure of CBD and THC, the oxygen in THC is present in the pyran ring while in CBD the oxygen comes from a hydroxyl group attached to the phenol ring. This means that the conformational changes happen in this particular region of the two compounds. Lithium also separates CBD and THC in gas phase. Lithium ions, similarly to sodium ions, are known to interact with oxygen and nitrogen atoms by electrostatic forces [42, 43]. The increase in the CCS of $[\text{THC} + \text{Li}]^+$ is substantial considering the size of lithium's ionic radius ($0.76 \text{ \AA}^2$) and is larger compared to the sodium ion adduct $[\text{THC} + \text{Na}]^+$ and the cesium ion adduct $[\text{THC} + \text{Cs}]^+$ for which the ionic radius is $1.02 \text{ \AA}^2$ and $1.67 \text{ \AA}^2$, respectively. Although this result is surprising, similar examples in which the conformation of the compounds and their mobilities are influenced by coordination rather than the size of the metal cation adduct, have been reported [9]. The cesiated CBD and THC ions were observed to have very close mobility values and thus CCSs (191.562 and $192.801 \text{ \AA}^2$), which mean that the cesium ion does not improve the separation of the two isomers. There are, however, examples in literature where cesium ions improved separation and helped in compound identification [11]. In the protonated species, a similar result was obtained and no improvements in the mobility and CCS were achieved.

Conclusions

In this study, we used TIMS to investigate the behavior of twelve pharmaceutically relevant compounds upon cation adduct formation. The selected compounds varied in regard to their molecular shape and flexibility, which resulted in differences in their behavior in the gas phase upon cation adduct formation. In this study four interesting observations of gas phase ion behavior were made: 1) cation adduct can generate different conformation of molecules; 2) for most compounds the correlation between the cross section and the radius of the cation was observed; 3) more planar and rigid molecules tend to form dimers more readily than molecules exhibiting greater flexibility due to the presence of rotating bonds; 4) the

separation of structural isomers, THC and CBD, was facilitated by the formation of lithium, cesium and silver adducts. The study presented here confirms previous findings on the cations adduct formation with target analytes and enhances our understanding of the behavior of ions in the gas phase, which is related to the molecular structure of the analytes. The knowledge gained from our observations should prove to be particularly valuable in characterization of unknown molecular structures and in analyzing complex mixtures in which the additional selectivity provided by TIMS is required. Considering the impact that the cations have on the small molecular ions, this study provides a base for future studies on structural isomers, for example, drug metabolites present in complex mixtures.

Acknowledgements The authors acknowledge ARIADME, a European FP7 ITN Community's Seventh Framework Programme under Grant Agreement No. 607517. We are grateful names, (Bruker) for providing the opportunity to work with a prototype TIMS instrument, and their useful support. Thanks for Prof. Michel Nielsen from the Wageningen University for providing us with THC and CBD.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Kanu AB, Dwivedi P, Tam M et al (2008) Ion mobility-mass spectrometry. *J Mass Spectrom.* <https://doi.org/10.1002/jms.1383>
- Lanucara F, Holman SW, Gray CJ, Eyers CE (2014) The power of ion mobility-mass spectrometry for structural characterization and the study of conformational dynamics. *Nat Chem* 6:281–294. <https://doi.org/10.1038/nchem.1889>
- Creaser CS, Griffiths JR, Bramwell CJ et al (2004) Ion mobility spectrometry: a review. Part I. Structural analysis by mobility measurement. *Analyst* 129:984. <https://doi.org/10.1039/b404531a>
- Adams KJ, Montero D, Aga D, Fernandez-Lima F (2016) Isomer separation of polybrominated diphenyl ether metabolites using nanoESI-TIMS-MS. *Int J Ion Mobil Spectrom* 1–8. doi:<https://doi.org/10.1007/s12127-016-0198-z>
- Silveira JA, Ridgeway ME, Park MA (2014) High resolution trapped ion mobility spectrometry of peptides. *Anal Chem* 86: 5624–5627. <https://doi.org/10.1021/ac501261h>
- Ujma J, Giles K, Morris M, Barran PE (2016) New high resolution ion mobility mass spectrometer capable of measurements of collision cross sections from 150 to 520 K. *Anal Chem* 88:9469–9478. <https://doi.org/10.1021/acs.analchem.6b01812>
- Kemper PR, Dupuis NF, Bowers MT (2009) A new, higher resolution, ion mobility mass spectrometer. *Int J Mass Spectrom* 287:46–57. <https://doi.org/10.1016/j.jms.2009.01.012>
- Huang Y, Dodds ED (2015) Ion-neutral collisional cross sections of carbohydrate isomers as divalent cation adducts and their electron transfer products. *Analyst* 140:6912–6921. <https://doi.org/10.1039/c5an01093d>
- Huang Y, Dodds ED (2013) Ion mobility studies of carbohydrates as group i adducts: isomer specific collisional cross section

- dependence on metal ion radius. *Anal Chem* 85:9728–9735. <https://doi.org/10.1021/ac402133f>
10. Fenn LS, McLean JA (2011) Structural resolution of carbohydrate positional and structural isomers based on gas-phase ion mobility-mass spectrometry. *Phys Chem Chem Phys* 13:2196–2205. <https://doi.org/10.1039/C0CP01414A>
 11. Jackson SN, Ugarov M, Post JD et al (2008) A study of phospholipids by ion mobility TOFMS. *J Am Soc Mass Spectrom* 19:1655–1662. <https://doi.org/10.1016/j.jasms.2008.07.005>
 12. Chen L, Gao YQ, Russell DH (2012) How alkali metal ion binding alters the conformation preferences of gramicidin a: a molecular dynamics and ion mobility study. *J Phys Chem A* 116:689–696. <https://doi.org/10.1021/jp209430q>
 13. Clowers BH, Hill HH (2006) Influence of cation adduction on the separation characteristics of flavonoid diglycoside isomers using dual gate-ion mobility-quadrupole ion trap mass spectrometry. *J Mass Spectrom* 41:339–351. <https://doi.org/10.1002/jms.994>
 14. Barnett DA, Purves RW, Ellis B, Guevremont R (2000) Separation of o-, m- and p-phthalic acids by high-field asymmetric waveform ion mobility spectrometry (FAIMS) using mixed carrier gases. *J Mass Spectrom* 35:976–980. [https://doi.org/10.1002/1096-9888\(200008\)35:8<976::AID-JMS25>3.0.CO;2-Q](https://doi.org/10.1002/1096-9888(200008)35:8<976::AID-JMS25>3.0.CO;2-Q)
 15. Fernández-Maestre R, Meza-Morelos D, Wu C (2016) Shift reagents in ion mobility spectrometry: the effect of the number of interaction sites, size and interaction energies on the mobilities of valinol and ethanolamine. *J Mass Spectrom* 51:378–383. <https://doi.org/10.1002/jms.3771>
 16. Howdle MD, Eckers C, Laures AMF, Creaser CS (2009) The use of shift reagents in ion mobility-mass spectrometry: studies on the complexation of an active pharmaceutical ingredient with polyethylene glycol excipients. *J Am Soc Mass Spectrom* 20:1–9. <https://doi.org/10.1016/j.jasms.2008.10.002>
 17. Fernandez-Maestre R, Meza-Morelos D, Wu C (2016) Mobility shifts when buffer gas temperature increases in ion mobility spectrometry are affected by intramolecular bonds. *Int J Mass Spectrom* 407:113–117. <https://doi.org/10.1016/j.ijms.2016.06.012>
 18. Morrison KA, Bendiak BK, Clowers BH (2017) Enhanced mixture separations of metal adducted Tetrasaccharides using frequency encoded ion mobility separations and tandem mass spectrometry. *J Am Soc Mass Spectrom* 28:664–677. <https://doi.org/10.1007/s13361-016-1505-y>
 19. Morsa D, Gabelica V, De Pauw E (2011) Effective temperature of ions in traveling wave ion mobility spectrometry. *Anal Chem* 83: 5775–5782. <https://doi.org/10.1021/ac201509p>
 20. Merenbloom SI, Flick TG, Williams ER (2012) How hot are your ions in TWAVE ion mobility spectrometry? *J Am Soc Mass Spectrom* 23:553–562. <https://doi.org/10.1007/s13361-011-0313-7>
 21. Fernandez-Lima F, Kaplan DA, Suetering J, Park MA (2011) Gas-phase separation using a trapped ion mobility spectrometer. *Int J Ion Mobil Spectrom* 14:93–98. <https://doi.org/10.1007/s12127-011-0067-8>
 22. Castellanos A, Benigni P, Hernandez DR et al (2014) Fast screening of polycyclic aromatic hydrocarbons using trapped ion mobility spectrometry – mass spectrometry. *Anal Methods* 6:9328–9332. <https://doi.org/10.1039/C4AY01655F>
 23. Michelmann K, Silveira JA, Ridgeway ME, Park MA (2015) Fundamentals of trapped ion mobility spectrometry. *J Am Soc Mass Spectrom* 26:14–24. <https://doi.org/10.1007/s13361-014-0999-4>
 24. Hernandez DR, DeBord JD, Ridgeway ME et al (2014) Ion dynamics in a trapped ion mobility spectrometer. *Analyst* 139:1913–1921. <https://doi.org/10.1039/c3an02174b>
 25. Lalli PM, Iglesias BA, Toma HE et al (2012) Protomers: formation, separation and characterization via travelling wave ion mobility mass spectrometry. *J Mass Spectrom* 47:712–719. <https://doi.org/10.1002/jms.2999>
 26. Seo Y, Schenauer MR, Leary JA (2011) Biologically Relevant metal-cation binding induces conformational changes in heparin oligosaccharides as measured by ion mobility mass spectrometry. *Int J Mass Spectrom* 303:191–198. <https://doi.org/10.1038/jid.2014.371>
 27. Nikolova-Damyanova B (2009) Retention of lipids in silver ion high-performance liquid chromatography: facts and assumptions. *J Chromatogr A* 1216:1815–1824. <https://doi.org/10.1016/j.chroma.2008.10.097>
 28. Lévêque NL, Héron S, Tchaplai A (2010) Regioisomer characterization of triacylglycerols by non-aqueous reversed-phase liquid chromatography/electrospray ionization mass spectrometry using silver nitrate as a postcolumn reagent. *J Mass Spectrom* 45:284–296. <https://doi.org/10.1002/jms.1713>
 29. Reek JN, Sijbesma R, Nolte RJ (1994) Silver(I) induced conformational changes in a molecular clip. *Tetrahedron Lett* 35:2801–2804. [https://doi.org/10.1016/S0040-4039\(00\)77037-7](https://doi.org/10.1016/S0040-4039(00)77037-7)
 30. Shannon RD, Prewitt CT (1969) Effective ionic radii in oxides and fluorides. *Acta Crystallogr Sect B Struct Crystallogr Cryst Chem* 25:925–946. <https://doi.org/10.1107/S0567740869003220>
 31. Fusar-Poli P, Crippa JA, Bhattacharyya S et al (2009) Distinct effects of Δ^9 -tetrahydrocannabinol and Cannabidiol on neural activation during emotional processing. *Arch Gen Psychiatry* 66:95–105. <https://doi.org/10.1001/archgenpsychiatry.2008.519>
 32. Bow EW, Rimoldi JM (2016) The structure – function relationships of classical cannabinoids: CB1 / CB2 modulation. *Perspect Medicin Chem* 8:17–39. <https://doi.org/10.4137/PMC.S32171.TYPE>
 33. Martin-Santos R, Crippa JA, Batalla A et al (2012) Acute effects of a single, oral dose of d9- tetrahydrocannabinol (THC) and Cannabidiol (CBD) Administration in Healthy Volunteers. *Curr Pharm Des* 18:4966–4979. <https://doi.org/10.2174/138161212802884780>
 34. Jackson AU, Shum T, Sokol E et al (2011) Enhanced detection of olefins using ambient ionization mass spectrometry: ag+ adducts of biologically relevant alkenes. *Anal Bioanal Chem* 399:367–376. <https://doi.org/10.1007/s00216-010-4349-5>
 35. Galand N, Ernouf D, Montigny F et al (2004) Separation and identification of cannabis components by different planar chromatography techniques (TLC, AMD, OPLC). *J Chromatogr Sci* 42:130–134. <https://doi.org/10.1093/chromsci/42.3.130>
 36. Zgair A, Wong JCM, Sabri A et al (2015) Development of a simple and sensitive HPLC–UV method for the simultaneous determination of cannabidiol and 9 -tetrahydrocannabinol in rat plasma. *J Pharm Biomed Anal* 114:145–151. <https://doi.org/10.1016/j.jpba.2015.05.019>
 37. Fabritius M, Staub C, Mangin P, Giroud C (2013) Analysis of cannabinoids in oral fluid by liquid chromatography-tandem mass spectrometry. *Forensic Toxicol* 31:151–163. <https://doi.org/10.1007/s11419-012-0168-z>
 38. Salomone A, Gerace E, D’Urso F et al (2012) Simultaneous analysis of several synthetic cannabinoids, THC, CBD and CBN, in hair by ultra-high performance liquid chromatography tandem mass spectrometry. Method validation and application to real samples. *J Mass Spectrom* 47:604–610. <https://doi.org/10.1002/jms.2988>
 39. Mazina J, Špiljova A, Vaher M et al (2015) Rapid capillary electrophoresis method with LED-induced native fluorescence detection for the analysis of cannabinoids in oral fluid. *Anal Methods* 7: 7741–7747. <https://doi.org/10.1039/C5AY01595B>
 40. Meier F, Garrard KP, Muddiman DC (2014) Silver dopants for targeted and untargeted direct analysis of unsaturated lipids via infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI). *Rapid Commun Mass Spectrom* 28:2461–2470. <https://doi.org/10.1002/rcm.7041>

41. Guo BC, Conklin BJ, Castleman AWJ (1989) Thermochemical properties of ion complexes $\text{Na}^+(\text{M})$ in the gas phase. *J Am Chem Soc* 111:6506–6510. <https://doi.org/10.1021/ja00199a005>
42. Chen R, Li L (2001) Lithium and transition metal ions enable low energy collision-induced dissociation of polyglycols in electrospray ionization mass spectrometry. *J Am Soc Mass Spectrom* 12:832–839. [https://doi.org/10.1016/S1044-0305\(01\)00261-6](https://doi.org/10.1016/S1044-0305(01)00261-6)
43. Fujii T, Tokiwa H, Ichikawa H, Hiroyuki S (1992) An ab initio characterization of Li^+ complexes of benzene, phenol, pyridine and aniline. *J Mol Struct* 277:251–262. [https://doi.org/10.1016/0166-1280\(92\)87143-N](https://doi.org/10.1016/0166-1280(92)87143-N)