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Study of β-Cyclodextrin–Ketoconazole–Tartaric Acid Multicomponent Non-covalent Association by Positive and Negative Ionspray Mass Spectrometry

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In continuation of studies of multicomponent non-covalent associations (MCAs) of cyclodextrin (CD) inclusion or host–guest (H–G) complexes of hydrophobic or barely water-soluble drugs with suitable counter ions, which can dramatically increase the hydrosolubility of the guest drug, was the β-CD–KC–tartaric acid (TA) MCA, where KC = ketoconazole, an antifungal drug, investigated by ionspray (IS) mass spectrometry (MS) and MS/MS in both the positive and negative ion modes. In the positive IS mode a protonated 1:1:1 β-CD–KC–TA gaseous species is obtained, which dissociates by the loss of TA upon collisional activation (CA), thus reproducing the same behaviour as observed previously for a β-CD–terfenadine–TA MCA. Unprecedented results were obtained in the negative ion mode. In particular, deprotonated 1:1:1 β-CD–KC–TA MCA was detected, which upon CA yielded mainly deprotonated 1:1 β-CD–TA and tartrate anion. Hence, while a relatively strong interaction binding β-CD to TA within the MCA parent anion emerges, the fair abundance of tartrate anion could suggest the formation of its neutral complementary fragment, 1:1 β-CD–KC, a possibly H–G complex not observed as a negatively charged MS/MS fragmentation product. The role of the KC–TA ionic bonding of the neutral MCA appears very pertinent to the study by positive and negative ISMS of the non-covalent interactions within the gaseous protonated or deprotonated ternary complex thereof. © **1998 John Wiley & Sons, Ltd.**

KEYWORDS: *β*-cyclodextrin; host–guest complexes; ketoconazole; tartaric acid; supramolecular multicomponent associations; ionspray mass spectrometry; tandem mass spectrometry

INTRODUCTION

Inclusion or host–guest (H–G) complexes, formed by molecular encapsulation of guest compounds in the cavities of macrocyclic hosts, are non-covalent associations of current interest for fundamental research in the field of supramolecular systems and for a wealth of technological applications. While the structural characterization and the determination of the association equilibria of H–G complexes classically rely upon diffraction, NMR and other physico-chemical methods, in recent years mass spectrometry (MS) has been employed successfully by exploitation of the so-called 'soft' ionization methods for the study of such weakly bonded associations in the gas phase.[1]

Cyclodextrins (CDs)[2] are water-soluble naturally available cyclic oligosaccharides with a hydrophobic cavity, widely used as hosts for the complexation of hydrophobic or barely water-soluble guest molecules of interest to, e.g., the pharmaceutical, cosmetics, agriculture, food and beverage industries. Most recently it has been found that CD inclusion complexes can yield non-covalent multicomponent associations (MCAs) with suitable counter ions of guest molecules. Such MCAs are of scientific and technological relevance for their physical, chemical and/or biological properties.[3,4] In the field of pharmaceutical preparations, MCAs of CD–drug inclusion complexes can dramatically enhance the solubility in water of hydrophobic or sparingly soluble guest drugs.[5]

In connection with a research programme on CD inclusion complexes by MS,[6] we are currently engaged with the study of MCAs of *β*-CD–drug H–G complexes with specific organic counter ions such as α-hydroxycarboxylic acids (HAs) and diethanolamine (DEA). Recent studies[7,8] showed the possibility of detecting by ionspray (IS) MS the protonated species of 1:1:1 *β*-CD–drug–HA (drug = terfenadine (TFN), CAS No. 50679-08-8; HA = tartaric or citric acid) and of 1:1:1 *β*-CD–drug–DEA (drug = glybenclamide, CAS No. 10238-21-8, or furosemide, CAS No. 54-31-9) from water–acetonitrile solutions of the related MCA samples containing the three components in defined molar ratios. Interestingly, the collisionally activated dissociation (CAD) patterns of these gaseous protonated ternary MCAs showed substantial differences, which provided information on the relative strength of the interactions binding their components. In particular,

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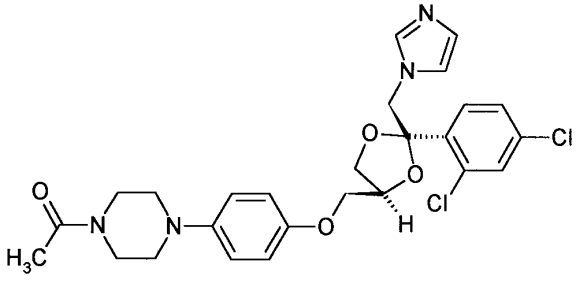

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our MS/MS results appeared consistent with the relative stability of the corresponding 1:1 drug–β-CD H–G complexes in solution.[8]

The present paper reports a study[9] by positive and negative ISMS and MS/MS of an MCA of β-CD–KC–TA, where KC = ketoconazole, namely *cis*-1-acetyl-4-(4-{[2-(2,4-dichlorophenyl)-2- (1*H*-imidazol-1-ylmethyl)-1,3- dioxolan-4-yl]methoxy}phenyl)piperazine, an antifungal drug, CAS No. 65277-42-1, and TA = tartaric acid.

EXPERIMENTAL

Preparation of the multicomponent complex

The multicomponent complex β-CD–KC–TA was prepared at Cyclolab, (Budapest, Hungary) by lyophilizing an aqueous solution containing the components in a 1.5:1:1 molar ratio.

Mass spectrometry

A bench-top Perkin–Elmer Sciex API 365 triple-quadrupole mass spectrometer, equipped with a standard API-IonSpray ionization source, operated in the positive or negative ion mode with a cluster-breaking orifice voltage of 60 V, was employed for the MS and MS/MS experiments, which were performed with a resolution of 0.8 u (measured at half-height) for both the resolving quadrupoles. The acquired spectral data were processed with the Multiview 1.3 software package. CAD MS/MS experiments were performed through the closed-design Q2 collision cell operating with a collision energy (E_{tab}) of 30 eV, using nitrogen as collision gas at a pressure of 8 mTorr (1 Torr = 133.3 Pa).

The multicomponent complex β-CD–KC–TA was dissolved in water–acetonitrile (50:50, v/v), diluted to concentrations of 10 000, 1000 or 100 ppm and then introduced into the ion source by an infusion pump in the continuous flow mode at a flow-rate of 2 μl min^{-1}. The positive ion mode ISMS experiments were also performed with the same sample solutions containing 5 mM ammonium acetate.

RESULTS AND DISCUSSION

Positive ion ISMS allows the detection of a protonated 1:1:1 β-CD–KC–TA ternary gaseous species (*m/z* 1815.5) (Fig. 1) from water–acetonitrile (50:50, v/v) solutions containing 100, 1000 or 10 000 ppm of a β-CD–KC–TA lyophilized MCA preparation (see Experimental). The relative abundance of this ternary ion represents ~2% of the base peak at *m/z* 531.1, which is due to protonated KC. Two other significant peaks at *m/z* 266.0 (~15%), due to diprotonated KC, and *m/z* 1665.8 (~5%), corresponding to protonated 1:1 β-CD–KC, possibly an H–G complex, are also present. In previous studies[6–8,10,11] on β-CD complexes using IS or fast atom bombardment MS, we detected ammonium cationized species, possibly due to residual and/or added ammonium ions. Otherwise, in the present case no significant formation of ammonium adducts was observed, even upon addition of 5 mM ammonium chloride to the sample solutions.

The tandem mass spectrum (Fig. 2) of the protonated 1:1:1 β-CD–KC–TA parent species (*m/z* 1815.5) shows the prominent loss of TA, yielding a protonated 1:1 β-CD–KC (*m/z* 1665.8) complex. Such behaviour appears analogous to the dominant loss of HA (HA = tartaric or citric acid) already described[7] for protonated 1:1:1 β-CD–TFN–HA ternary complexes, which had been attributed[8] to a relatively strong non-covalent binding interaction, with a possible hydrophobic H–G contribution, between β-CD and drug within the protonated ternary parent species. For instance, the MS/MS dissociation (Fig. 2) of the *m/z* 1665.6 parent ion, which is present in the ISMS spectrum (Fig. 1) of β-CD–KC–TA MCA, yields only a protonated KC fragment, according to its expected (see above) structure of protonated 1:1 β-CD–KC, as a possible H–G complex.

If we now assume very reasonably that within the ternary parent ion the proton should be most preferentially located at one of KC basic nitrogens, we would expect to obtain protonated KC and/or its associations with β-CD or TA as the only charged MS/MS non-covalent dissociation products. Actually, as shown in Fig. 2 and Scheme 1, only some protonated KC (*m/z* 531.2) and the most abundant protonated β-CD–KC (*m/z* 1665.6) can be observed, although the former could also partially originate from further dissociation of the latter according to the MS/MS dissociation (Fig. 2) of the *m/z* 1665.6 parent ion.

Interestingly, no 1:1 KC–TA association was observed, either as a possible protonated species or, most unlikely for proton affinity reasons, as a neutral MS/MS dissociation product, whereas its charged complementary fragment (i.e. protonated β-CD, *m/z* 1135.4) is absent.

Hence the relative abundance of the charged (protonated) and their complementary neutral MS/MS products allows one to propose the strength rank order outlined in Table 1 for the non-covalent intermolecular bonding of the parent ion, which is selectively dissociated under the present experimental conditions.

Moreover, we are able to provide unprecedented results from negative ISMS and MS/MS experiments. In particular, we detected (Fig. 3) the deprotonated 1:1:1 β-CD–KC–TA MCA species (*m/z* 1813.5), with a relative abundance of about 2% of the base peak at *m/z* 149.1 due to tartrate anion. Other negative ions of nominal mass 299 and 1283 can be attributed to the

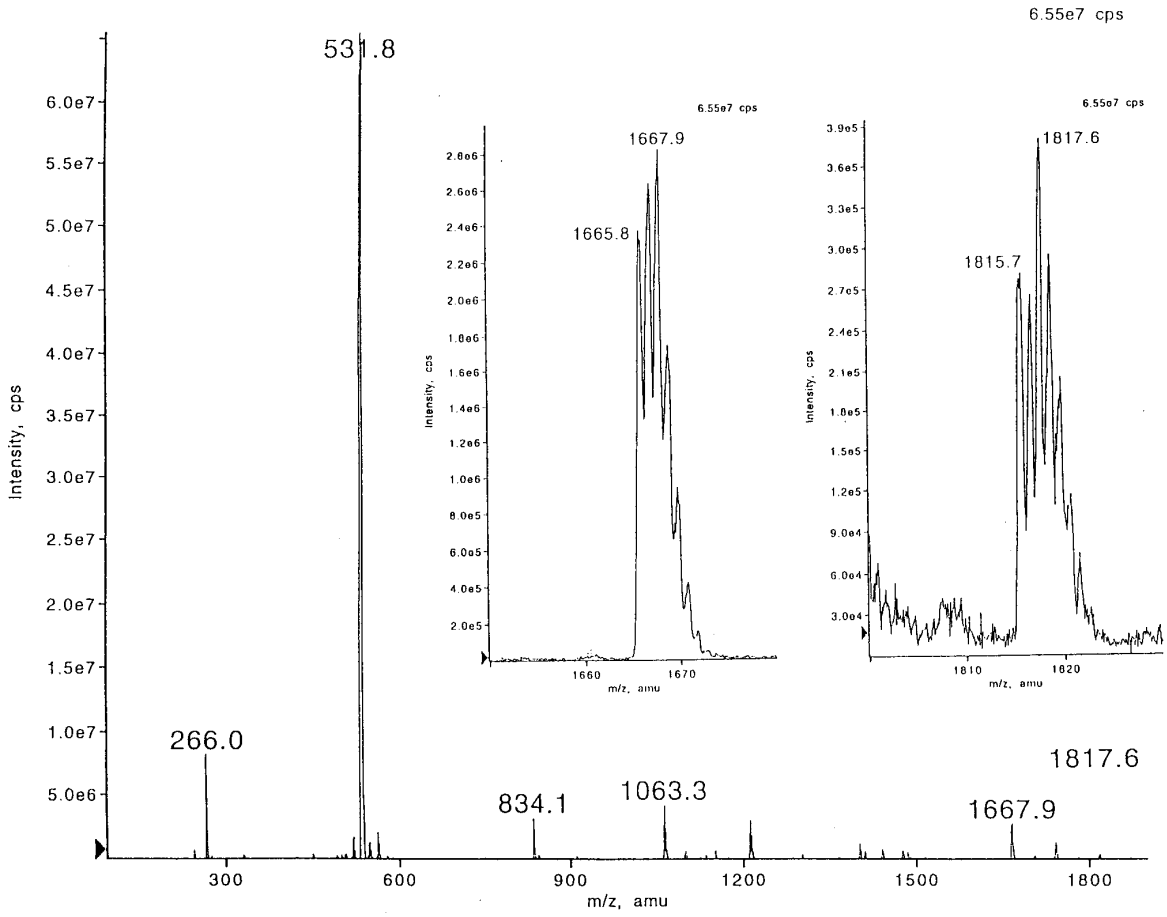


Figure 1. Positive ion IS mass spectrum of a 10 000 ppm water–acetonitrile (1 :1, v/v) solution of the related MCA sample. The two expanded insets show the peak clusters of protonated 1 :1 β-CD–KC (*m/z* 1665.8) and of 1 :1 :1 β-CD–KC–TA (*m/z* 1815.5) complexes.

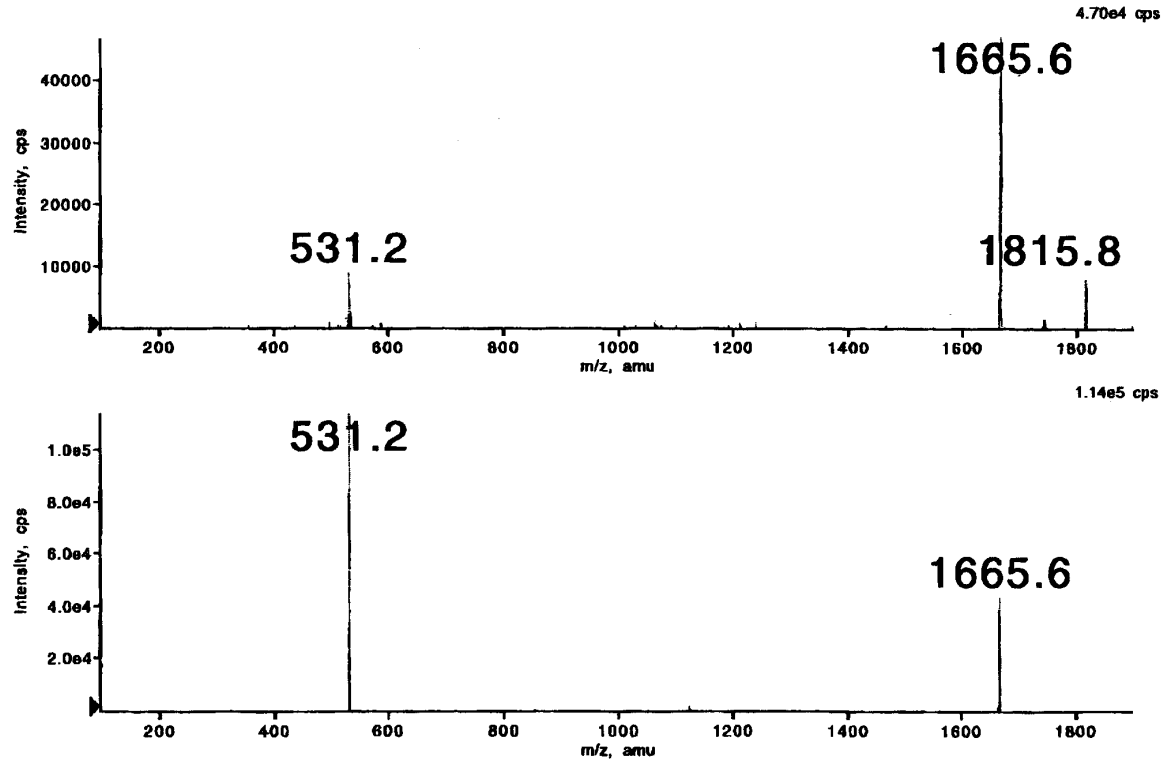
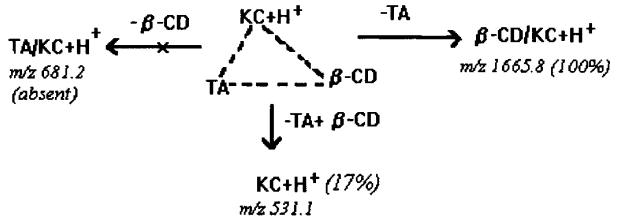


Figure 2. CAD tandem mass spectra of protonated complexes 1 :1 :1 β-CD–KC–TA (*m/z* 1815.7) (top) and of 1 :1 β-CD–KC (*m/z* 1665.8) (bottom) generated by ISMS.



Scheme 1

association of tartrate anion with TA or β-CD, respectively, while doubly charged species of 1:1 β-CD–TA and 1:1:1 β-CD–KC–TA with *m/z* 641.3 and 907.5, respectively, are also revealed (Fig. 3).

The tandem mass spectrum of deprotonated 1:1:1 β-CD–KC–TA (Fig. 4) shows deprotonated 1:1 β-CD–TA (*m/z* 1283.4) as the most abundant product anion, formed by the loss of a neutral KC molecule and fairly abundant (70%) tartrate anion (*m/z* 149.1), originating from possible loss of neutral β-CD–KC H–G association, whose existence as a stable entity is not definitely demonstrated by the present data. However, tartrate anion could also form by further dissociation of the most abundant deprotonated 1:1 β-CD–TA (*m/z* 1283.4) fragment.

As sketched in Scheme 2, the negative charge due to a missing proton within the ternary parent anion should most preferentially reside on TA and the MS/MS non-covalent dissociation could yield tartrate anion and/or its 1:1 associations with β-CD or KC as the only possible negatively charged products. Actually, tartrate anion and its association with β-CD are the only observed MS/MS products, whose relative abundances indicate (see Table 2) that the weakest binding is between KC and TA, as for the corresponding protonated ternary association, whereas, in contrast, β-CD appears able to establish a relatively stronger interaction with tartrate than with KC. However, the relative strength of the β-CD–KC interaction of possible H–G type can be only indirectly presumed on the grounds of the fair abundance of tartrate anion, which is the charged complementary fragment of a possible 1:1 β-CD–KC neutral MS/MS product.

Such a strength hierarchy of the non-covalent interactions emerging from the MS/MS data presented suggest the following:

1. The relatively stronger ($\sim 10^2$ kcal mol^{-1}) non-covalent intermolecular interaction within a hypothetical neutral 1:1:1 β-CD–KC–TA gaseous ternary

Table 1. Strength rank order, assigned from MS/MS data, and type of the non-covalent intermolecular binding within the gaseous protonated 1:1:1 β-CD–KC–TA association generated by positive ISMS

Intermolecular binding	Strength rank order	Interaction type
β-CD–KC	Strongest	H–G and/or polar
β-CD–TA	Medium[a]	Polar
KC–TA	Weakest	Polar

[a] Rank order attributed from indirect evidence of the relative abundance of the complementary protonated KC MS/MS product.

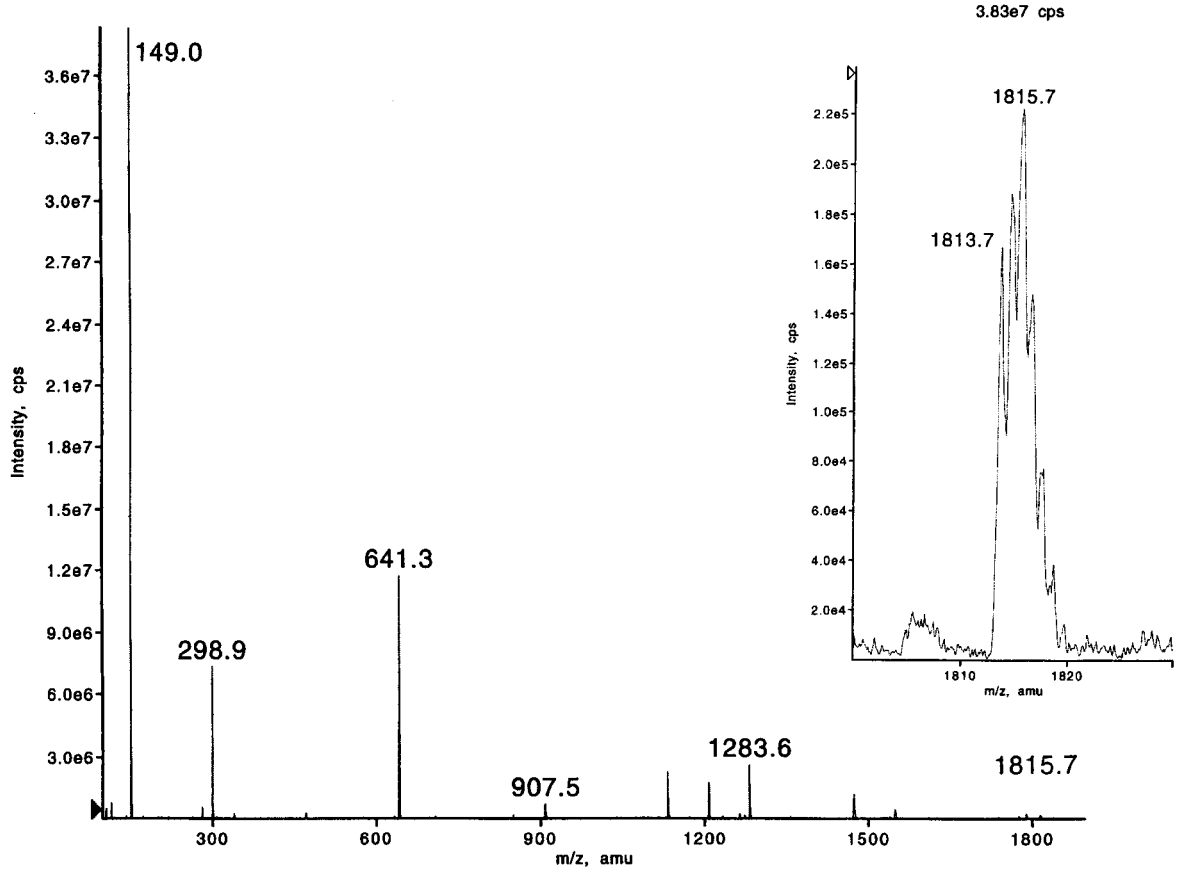


Figure 3. Negative ion IS mass spectrum of a 10 000 ppm water–acetonitrile (1:1, v/v) solution of the related MCA sample. The expanded inset shows the peak cluster of 1:1:1 β-CD–KC–TA deprotonated species (*m/z* 1813.7).

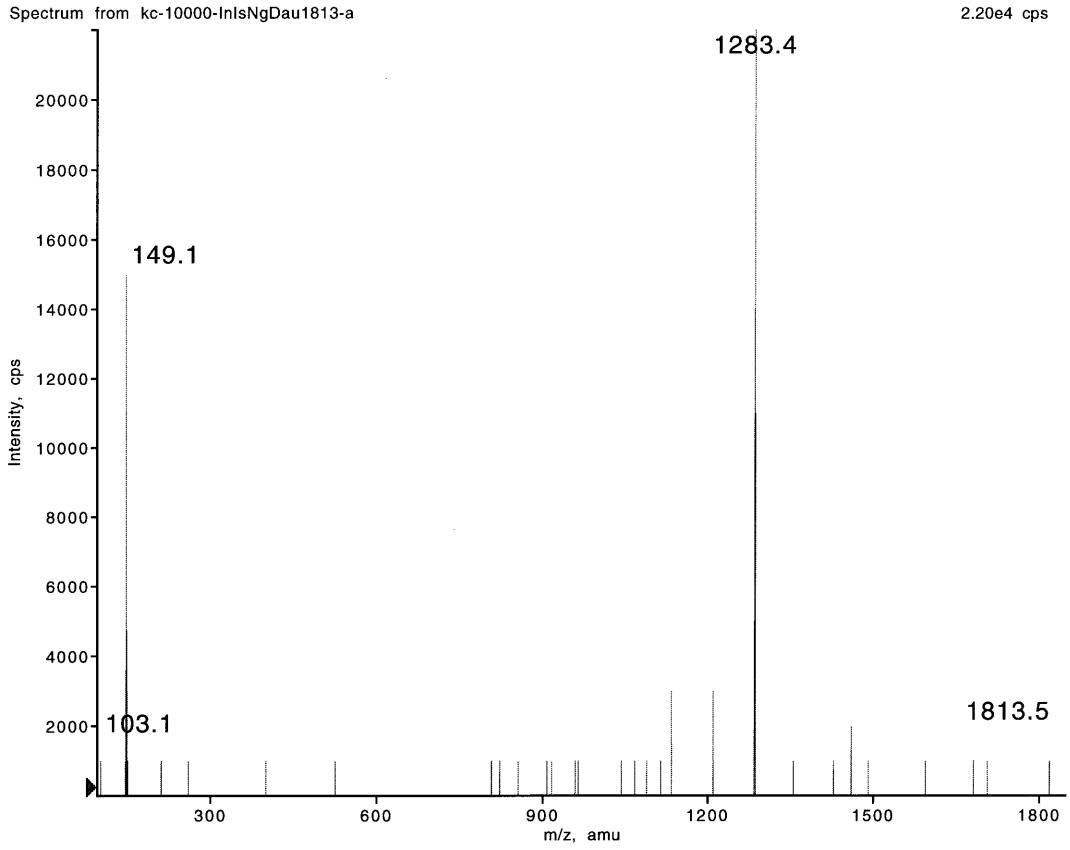
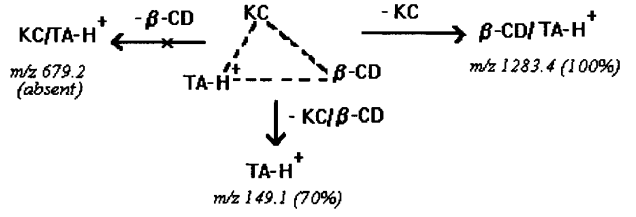


Figure 4. CAD tandem mass spectrum of deprotonated 1 :1 :1 β-CD–KC–TA complex (*m/z* 1813.7) generated by ISMS.

association should be that of essential electrostatic nature between protonated KC and tartrate counter ion. Much weaker ($\leqslant$10 kcal mol^{-1}) should be both the β-CD–KC and β-CD–TA non-covalent binding, whereas (i) the former should rely on the inclusion of the lipophilic dichlorophenyl drug moiety into the β-CD hydrophobic cavity establishing a weak H–G interaction of van der Waals type, as observed by NMR in aqueous solution,[12] which could even be reinforced by polar type contributions due to possible proton bridge and/or hydrogen bonding formation among the not-included polar part of the guest molecule and the external hydrophilic surface of the host; and (ii) the latter could just rely on polar type interactions due to dipole and hydrogen bonding, provided that no evidence of an inclusion complex formation between hydrophilic tartrate anion and β-CD was found by NMR experiments in aqueous solution.[12]



Scheme 2

Table 2. Strength rank order, assigned from MS/MS data, and type of the non-covalent intermolecular binding within the gaseous deprotonated 1:1:1 β-CD–KC–TA association generated by negative ionspray MS

Intermolecular binding	Strength rank order	Interaction type
β-CD–TA	Strongest	Polar
β-CD–KC	Medium[a]	H–G and/or polar
KC–TA	Weakest	Polar

[a] Rank order attributed from indirect evidence of the relative abundance of the complementary tartrate anion MS/MS product.

2. Protonation or deprotonation of such neutral 1:1:1 β-CD–KC–TA gaseous ternary non-covalent association should occur by neutralization of tartrate anion or of protonated KC, respectively, with the consequent effect of suppressing the originally strongest KC–TA ionic interaction. This is in complete agreement with the experimental MS/MS evidence (Schemes 1 and 2 and Tables 1 and 2).

3. The effects of protonation or deprotonation appear less dramatic for both the β-CD–KC and β-CD–TA interactions within the ternary complex. In particular, protonation by neutralization of tartrate anion should (i) increase the strength of β-CD–KC binding by leaving almost unaffected the H–G interaction and, owing to the lack of the counter ion, by enhancing the polar binding contributions of proton bridge and/or hydrogen bonding formation; and (ii) reduce the polar β-CD–TA interaction due to the loss of the ion–molecule dipole contribution. These statements also appear perfectly consistent with the hierarchy established by MS/MS for the strength of the non-covalent interactions within the protonated ternary parent species (Table 1).

4. Deprotonation of the hypothetical neutral 1:1:1 β-CD–KC–TA gaseous ternary non-covalent association, involving the loss of the proton at KC, should (i) not

significantly support the H–G interaction between β-CD and KC, while the polar binding contribution to such interaction would be greatly reduced, since any possibility of proton bridge and ion–molecule dipole formation has been lost; and (ii) increase the polar interaction of tartrate anion with β-CD, principally for ion–molecule dipole formation, owing to the lack of the protonated KC counter ion within the ternary anion.

Accordingly, the strength rank order of the β-CD–KC and β-CD–TA non-covalent interactions, determined by MS/MS for the ternary anion (Table 2), appears reversed with respect to the corresponding protonated complex (Table 1), although, as indicated above, the relative abundance of β-CD–KC association as a possible neutral fragment of the ternary anion can be only assumed indirectly from the abundance of its complementary tartrate anion.

5. The strongest ionic drug–counter ion bonding of the neutral MCA plays a strategic role in the study by MS of the non-covalent interactions among the gaseous protonated or deprotonated ternary species thereof, whereas (i) it vanishes upon protonation or deprotonation, so that the remaining intrinsically weak non-covalent interactions between β-CD and KC or TA are not overwhelmed and become the strongest within the resulting charged complex; and (ii) it is able to address both protonation and deprotonation well outside the β-CD cavity, so that the charge of the resulting ions, which is essential to any MS experiments, should not produce any important perturbation to the weak β-CD–drug H–G interaction.

CONCLUSIONS

This study has shown that not only, as observed previously,[7,8] protonated gaseous 1:1:1 β-CD–drug–counter ion complexes, but also analogous deprotonated species from an MCA of interest to the pharmaceutical industry, can be obtained by positive or negative ISMS, respectively. Most interestingly, the combined results of the MS/MS experiments on both protonated and deprotonated ternary complexes show selective non-covalent bonding dissociations, which provide valuable information on the relative strength of the intermolecular binding interactions. In particular, the presence of the drug/counter ion electrostatic bonding in the neutral MCA fulfils a strategic role whereas, while its strength vanishes upon ISMS protonation or deprotonation, it is able to address the charge sites of the resulting ternary ions well outside the host cavity and so it does not strongly influence the β-CD–drug H–G interaction within the gaseous ternary complex.

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