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Separation，quantification and structural study of (+)-catechin and (-)-epicatechin by ion mobility mass spectrometry combined with theoretical algorithms

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Two catechin epimers and their non-covalent complexes with γ-cyclodextrin were studied by using ion mobility coupled with mass spectrometry (IM-MS). Rapid separation of complexes was achieved with the peak-to-peak resolution reaching 0.86 after optimization of IM condition. Collision cross section (CCS) was measured to explore the structural difference of complexes. A gap of 11.75 Å2 between two complexes was found. Molecular modeling and theoretical CCS calculation were adopted to explain the measurement results. Two binding ways of both complexes were found and the calculated CCS corresponds accurately to the measured CCS. Quantification of catechins in mixtures were performed and the relative error was less than 15%, indicating the effectiveness of quantification by IM-MS.

Background and Originality Content

Catechins are part of the chemical family of flavan-3-Ol and widely found in plants such as tea,[1] cocoa,[2] peaches[3] and so on. Catechins as well as their gallic acid conjugates are seen as a family which is a kind of important functional components in plants. Bioactivity studies showed that catechins have various physiological effects like antibacterial,[4] antioxidant,[5] anticarcinogenic[6] and antiallergic[7] properties.

The non-gallated catechins consist of two phenyl rings A and B with four phenolic hydroxyl groups and a pyran ring C with a hydroxyl group on carbon 3 (Figure 1). Obviously, there are two chiral centers on carbons 2 and 3, bringing four diastereoisomers which are (+)-catechin, (-)-catechin, (+)-epicatechin and (-)-epicatechin. The most common isomers found in plants and food are (+)-catechin (CA) and (-)-epicatechin (EC). Studies indicated differences of the two catechins in bioavailability,[8] antioxidant[9] and other properties. Therefore, it is necessary to distinguish and quantify different catechin isomers in complex samples.

Traditionally, high-performance liquid chromatography mass spectrometry (HPLC-MS) and gas chromatography (GC) were the most frequently used methods for separating and analyzing complicated components including isomers.[10, 11] As for CA and EC, thin-layer chromatography (TLC)[12] and capillary electrophoresis (CE)[13] were also adopted as identification methods. Although these techniques provide high sensitivity or separation resolution for isomer analysis, long period of analysis and tedious pretreatment may reduce the analysis efficiency.

Ion mobility spectrometry (IMS or IM) is another effective way for separating molecules in gas phase based on their size and shape.[14] Unlike differentiating ions analyzed by their mass to charge (m/z) in MS or tandem MS, IM separates ions according to their flight time in the buffer gas. This method is somehow similar to LC or GC but spends much less time (miliseconds) than the two methods (minutes). Combining IM with MS (IM-MS) provides an additional analytic dimension, which makes the conformational information of molecules as an important segment in explaining structural differences or intermolecular interaction. Recently, IM-MS has been used in studying biomolecules such as oligonucleotides,[15] carbohydrates,[16] lipids,[17] proteins[18] and structural description of complexes like protein-inhibitor.[19] Collision cross section (CCS) is usually adopted to describe the structural information by calculating rotationally averaged cross section of a molecule based on the overall size and molecular architecture.[20] CCS can be experimentally measured by IM to provide a quantified result of structure information. In addition, CCS can also be calculated from theoretical models derived by molecular dynamics (MD) simulations or other modelling techniques.[21] Comparing CCS from these two methods makes the cognition of molecule structures more reliable.

In this study, we reported our attempt of separating CA and EC by IM-MS method. In consideration of potential problem of inadequate resolution of IM,[22] several kinds of cyclodextrins were introduced to explore the possibility of separating the two catechins at a higher resolution. Cyclodextrins (CDs) are chiral, cyclic oligosaccharides comprised of D-glucopyaranoside units linked together by α→1, 4 glycosidic linkage. Structures of three typical CDs were shown in Figure 1. These amphiphilic molecules were widely used in the chiral recognition method usually called as host-guest method because of their multiple chiral centers.[23] Our present work carried out a further study on the interaction between CDs and catechins. It was the first attempt to study the conformations of catechin-CD complexes by combining IM-MS measurement with theoretical molecular model construction and CCS calculation. In addition, the quantitative capability of IM-MS method was evaluated by quantifying catechin-CD mixtures.

Results and Discussion

IM-MS analysis of CA/EC-CDs

Enough conformation difference is necessary for IM separation. Some isomers are highly different in structure, causing distinct CCS and easy separation in IM. But some isomers, especially

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epimers with small group at isomerism sites, may have nearly the same CCS. One strategy for chiral separation by IM is using chiral gas as buffer gas.[24] However, as chiral gas molecules have more complex structures than helium or nitrogen, theoretical model of collisions and interactions in gas phase may be much more unpredictable, which makes caculating CCS extremely difficult. Another way, as described above, is making covalent or non-covalent complexes of target analytes with other molecules and measuring the complexes. Amino acids and cyclodextrins are usually used as chiral reagent.[22,25] The selectivity from their chiral center makes it easy to form different conformation of complexes.

In this work, five kinds of CDs were chosen to bind with CA/EC. Individual epimeric catechin-cyclodextrin mixture was firstly analyzed. MS spectra of 10 samples were shown in Supporting Information (Fig. S1). It was found that all cyclodextrins could bind with two catechins by 1:1, but the charge states of the complexes were different. To determine the possibility of CA/EC separation by IM, the IM spectra of CA/EC and their complexes with CDs were extracted from MS peaks. The drift time shown in IM spectra in Figure 2 indicated that there was nearly no resolving possibility for negtive charged CA/EC. For complexes of catechins and β-CD (m/z 711.2285), HP-β-CD (m/z distributed from 800 to 950), DM-β-CD (m/z distributed from 1500 to 1600), HP-γ-CD (m/z distributed from 900 to 1050), the results were similar. However, the IM spectra of CA/EC-γ-CD complexes (m/z 792.3511) complexes showed an inspiring result. The drift time of the two base peaks had a difference of 0.61ms, which indicated the possibility of seperation. Thus, further experiments were all based on catechin-γ-cyclodextrin complexes.

Interestingly, there appeared two additional weak peaks in both spectra of CA/EC-γ-CD complexes, and the drift time of one's peak was just nearly the same as the other's base peak. This phenomenon was not found in any other complexes or CA and EC. A study about the structure of CA/EC-β-CD pointed out that inclusion ways of CA/EC and β-CD were completely different.[26] Considering that different cyclodextrins contains different hydrophobic cavity area and polar groups, we speculated that CA/EC-γ-CD complexes may also take different binding ways which could cause CCS diversity. In addition, a larger hydrophobic cavity in γ-CD may provide more freedom and flexibility than β-CD while including catechins, leading to CCS difference of one single complex. Therefore, a computing simulation was done to search the possible conformations of catechin-γ-CD complexes. The results are shown and discussed in later section.

Catechin separation in mixture and optimizing IM condition

A mixture of CA, EC and γ-CD was prepared at a concentration ratio of 10μM:10μM:50μM to optimize the IM condition and evaluate the resolution. As ralated work reported,[22] the separation efficiency of T-wave IM was mostly decided by wave height (WH) and wave velocity (WV). The resolution of IM can be determined by peak-to-peak resolution (R) as Equation (2) shown, where t_1 and t_2 represent the drift time of two peaks and w_1 and w_2 represent their baseline width.

$$R=2(t_2-t_1)/(w_1+w_2) \quad (2)$$

Figure 3 shows a comparison of IM spectra of CA-γ-CD, EC-γ-CD and their mixtures at the same IM condition. Obviously, the two peaks of mixture corresponded to the two base peaks of individual complex. Although the two weak peaks were overlapped by the two strong ones because of similar drift time, qualitatively judging that the two catechins were successfully separated was not influenced.

The mixture was tested at six IM conditions to find a best one of separation effective. Resolution of each spectrum was calculated and listed in Table 1. The six IM spectra were shown in Supporting Information, Figure S2. The two analytes could be separated at all six conditions. Although the resolution differed in a small range, the best condition was determined as WH 28V and WV 450m/s.

CCS measurements

Polyalanine was measured at eight IM conditions to build calibration curves. As the charge state of components in mixture were different (one negative charge for catechins and two for γ-CD and CA/EC-γ-CD complexes), two calibration curve clusters of these charge states were individually built. The calibration curves were shown in Supporting Information Figure S3, S4 and Table S1, S2.

CA-γ-CD and EC-γ-CD samples were also measured at the same IM conditions. In all cases, two peaks of complex ions were found. Thus two CCS values were calcuted for both complexes. Table 2 shows the results of CCS measurement. Relative standard deviation (RSD) of each group was <1%, illustrating a high precision of measurement. There was only a disparity of 0.32$Å^2$ between CA and EC. It was an apparent result because of the structural similarity and symmetry of buffer gas molecule. However, the disparity came to 11.75 $Å^2$ when comparing the main peaks of two complexes. Each complex itself was divided into two groups by CCS disparity of 12.33$Å^2$ for CA-γ-CD and 13.62$Å^2$ for EC-γ-CD. Table 3 shows the relative difference (RD) between two complexes as well as two catechins. Such distribution of CCS indicated that catechin-γ-CD complexes may adopt a variety of conformations. Interestingly, the CCS only increased about 15~30 $Å^2$ from γ-CD to the complexes, which was extremely different from the CCS of catechins. As reported,[27] CDs with sufficiently large hydrophobic cavity mostly bind with small molecules taking the form of "inclusion", which means that the hydrophobic part of small molecule enters into the cavity while the polar groups bind with the hydroxyl groups at CDs' cavity rims. Thus, the CCS measurement in this work suggested that catechins may be highly included into the cavity of γ-CD while forming complexes. The interaction between the polar groups may cause shrinkage of the hydrophobic cavity.

Conformational simulations and theoretical CCS calculations

Computational modeling was performed to generate the theoretical structures of catechins-γ-CD complexes to explain the separation of two complexes and heterogeneity of their conformation. Docking and MC/MD simulation provided about 500 conformations totally. 31 of them were preliminary selected according to energy, including 14 for CA-γ-CD and 17 for EC-γ-CD. Two general kinds of binding ways were found from docking results for both complexes. One was that the A-ring of catechins was included into the cavity and the other one was that the B-ring was included. Therefore, the selected conformations were further divided into four groups for CCS/energy calculation and comparison. Calculated CCS and energy of selected structures were listed in Supporting Information Table S3. An energy-CCS map of the conformations was ploted in Figure 4 to show their distribution. The conformations at minimum energy from four groups were

shown in Figure 5.

According to the energy results, the most stable conformation was the "A-ring included" conformation for both complexes (Figure 5 A and C). In this situation, the A-ring was included while B-ring "lies" on the rim of γ-CD because of the possible H-bond interaction. However, when it came to the situation of "B-ring included" (Figure 5 B and D), the B-ring was included deeper while the A-ring "stands" outside the cavity. The energy of "B-ring included" conformations was just slightly higher than the other one. Specifically, the energy gap between lowest-energy "A-ring included" conformation and "B-ring included" conformation was 20.715kJ/mol for CA-γ-CD and 23.905kJ/mol for EC-γ-CD. Such a gap allows the four lowest-energy conformation to be formed and detected by IM. Other studies also provided envidences for discovery of multiple conformations with similar energy.[21] In combination with the relative intensity of measured peaks, it is valid to believe that the stronger peaks corresponded to the "A-ring included" comformation and the other peak corresponded to the "B-ring included" comformation for both complexes.

Table 4 showed comparison of theoretical CCS (Ω_{cal}) and experimental CCS (Ω_{mes}). A difference of about 5~10 Å^2 between theoretical and experimental CCS values of complexes was found while the gap decreased a lot when it came to catechins and γ-CD. Considering the calculating theory,[28] this slightly difference was acceptable. The distribution width of theoretical CCS was nearly the same as measured (about 20 Å^2) and the theoretical CCS gap between two complexes was 9.3 Å^2, which verified the reliability of conformation searching results.

Quantitative analysis

IM-MS have been proved as effective methods for quantitative analysis.[22, 29] The way using IM to quantify compounds in complex samples is similar as HPLC. In the present work, catechins-γ-CD complexes were considered as substance to build standard curves to indirectly quantify catechins. However, there remained a problem that the two complexes were not completely separated. The overlapped peaks may influence the quantitative accuracy. Thus the peak intensity provided by the overlapped weak peaks should be removed while quantifying the complexes in mixture. In particular, mixtures of individual catechin at different concentration and γ-CD at fixed concentration were firstly measured to build calibration curves as Equation set (3) shows.

$$y_{CA}=a_1x_{CA}+b_1$$
$$y_{EC}=a_2x_{EC}+b_2 \quad (3)$$

Then the proportion (k) of the stronger peak to total peak area (γ'/γ) was calculated as Equation set (4) shows.

$$y_{CA}'=ky_{CA}$$
$$y_{EC}'= ky_{EC} \quad (4)$$

The peak intensity of mixture of two complexes (A) was seen as a superposition of stronger peak (γ') of one complex and weak peak of the other complex (γ-γ') as Equation set (5) shows.

$$A_1=y_{EC}'+y_{CA}-y_{CA}'$$
$$A_2=y_{EC}-y_{EC}'+y_{CA}' \quad (5)$$

The quantitative process is shown in Figure 6 and the concentration of catechins (x) can be calculated by solving the equation set (3), (4) and (5). The solution of was shown in equation set (6).

$$x_{CA} = \left(\frac{(1-k_{EC})A_1-k_{EC}A_2}{1-k_{CA}-k_{EC}} - b_1\right)/a_1$$

$$x_{EC} = \left(\frac{(1-k_{CA})A_2-k_{CA}A_1}{1-k_{CA}-k_{EC}} - b_2\right)/a_2 \quad (6)$$

The measurements were performed under optimized conditions mentioned above. Calibration curves were shown in Supporting Information Figure S5. Both curves possessed good linearity (R^2>0.99). The peak area of all stronger peaks was extracted to calculate the proportion (k). Results were listed in Supporting Information Table S4. Mixtures of complexes at different CA/EC ratios were then measured to verify the accuracy of calibration curves. Table 5 shows measured concentrations of catechins and the relative error (RE) between calculated concentrations and actual concentrations. All the measured concentrations of seven validation groups were at a believable level (RE<15%). Such a result proved that IM-MS was comparable to HPLC-MS in the ability of quantifying concentrations of compounds in complex mixture.

Quantification of catechins in catechu

Components are firstly ionized and then separated in IM-MS measurement. Such a characteristic brings a challenge quantifying target compounds in complex system due to the limited ionization efficiency. In this study, we made a preliminary attempt to quantify catechins in extracts of a kind of herb, catechu, using the method mentioned in previous section.

A mixture of diluted extractive solution with γ-CD was firstly tested to make sure whether catechins had been extracted and binded with γ-CD. The results showed that catechins in catechu extracts formed complexes with γ-CD and the complexes could also be detected by IM-MS dispite possible effects of matrix. Then, quantification was performed according to the procedure introduced before. The calibration curves and calculation results of IM peak ratio were shown in Supporting Information Figure S6 and Table S5. Measurement and calculation results of extracts were shown in Table 6. It was found that (+)-catechin was the main component in the extracts, which was about 150mg/g or 15%, while (-)-epicatechin was only about 4%. Although as introduced before, γ-CD could form complexes with variety kinds of compounds and the detection of complexes may be hard because of the matrix effects, catechins in extracts could still be mesured and quantified. Considering the binding effect, γ-CD was at a high concentration in all samples. But the singal intensity of γ-CD was not at high level due to the strong ionization tendency of catechins. This made the quantification less likely interpreted by γ-CD or other compounds in the complex system。

The same extracts were also measured by HPLC. Experimental details were shown in Supporting Information. The results shown in Table 6 gave similar weight percentages as the method in this work measured, indicating a credible accuracy and higher time efficiency (1 minute for one sample while more than 10 minutes using HPLC) of current method compared with HPLC.

Conclusions

In this study, we attempted to separate (+)-catechin and (-)-epicatechin by using IM-MS. The negative charged epimers as well as complexes of catechins and β-CD, HP-β-CD, DM-β-CD or HP-γ-CD showed almost no separation in IM spectra. But when it came to the complexes of catechins and γ-CD, a remarkable improvement of separation was found. The catechins-γ-CD complexes could also be separated from mixtures with drift time difference >0.5ms and the peak-to-peak resolution up to 0.86 after condition optimization. Two individual peaks of both complexes were detected, indicating a diversity of conformations of complexes. Further efforts provided the experimental CCS of the compounds in complex mixtures. The CCS difference between CA and EC was only 0.32Å^2 while it increased to 11.75 Å^2 between the two complexes. The slight increase of CCS from γ-CD to catechin-γ-CD complexes sugggested that the epimers may highly included into the hydrophobic cavity of γ-CD while forming the non-covalent complexes.

Computational modeling and calculation was performed to provide theoretical support to the experimental results. 31 models were selected from a large number of modeling results according to energy and CCS of these models was calculated. Two binding ways of both complexes were found and the lowest-energy conformations of each way shared small energy gaps, indicating the coexistence possibility of these conformations. CCS calculation revealed that the two separated peaks in IM may correspond to the "A-ring" included conformations of two complexes because of their lower energy and theoretical CCS difference. The two additional peaks of both complexes may correspond to the "B-ring" included conformations with higher energy.

Quantitative analysis by IM-MS proved it an effective method to quantify catechins according to the contents of separated complexes indirectly. Although IM peaks may overlap at a certain degree because of the conformational diversity, mathematical optimization for concentration calculation could reduce the influence from overlapped peaks to ensure the accuracy of quantitative analysis. The method was also intoduced to quantify catechins in complex system and showed similar accuracy and higher analysis speed comparaed with traditional method such as HPLC.

The results obtained from the present study promised a hopeful future of rapid separation and quantification of small molecule isomers. The combination of IM-MS measurement and theoretical calculation provided a clear sight of conformation difference of isomers and their complexes with chiral selectors. This way can not only be applied in isomer separation, but also in other field such as protein-ligand interaction and inhibitor screening. Our further experiment will aim at mechanisms of interaction between flavonoids and proteins based on these separation and theoretical methods.

Experimental

Materials

β-cyclodextrin (β-CD), γ-cyclodextrin (γ-CD), (2-Hydroxypropyl)-β-cyclodextrin (HP-β-CD), 2,6-Di-O-methyl-β-cyclodextrin (DM-β-CD), (2-Hydroxypropyl)-γ-cyclodextrin (HP-γ-CD) were purchased from Aladdin (China). (+)-Catechin, (-)-epicatechin, ammonium acetate were obtained from Sigma-Aldrich (USA). Catechu was obtained from Tongrentang Changchun Pharmacy (China). Methonal, acetonitrile and acetic acid were supplied by TEDIA Company (USA). The ultrapure water for all experiments was purified using a Milli-Q Water System (USA).

Sample preparation

To prepare stock solutions, standard (+)-catechin and (-)-epicatechin were dissolved in methonal, while each kind of cyclodextrin was dissolved in water. The concentration of all stock solutions was 1mg/ml. For pre-seperation of two catechins, eatablishing quantitative standard curve and CCS measurment, (+)-catechin and (-)-epicatechin were individually mixed with each cyclodextrin at a concentration ratio of 10μM:20μM. All samples were prepared in ammonium acetate buffer (500μM) in methonal/water (50/50, v/v).

For quantification of catechins in complex system, 1g of catechu powder was mixed with 10mL of ethanol/water (70/30, v/v) in a 50mL centrifuge tube. Then, the mixture was treated by ultrasound for 1h at room temperature. The extractive solution was obtained by centrifugation and stocked in -20℃for further use. While preparing samples for quantification, the solution was diluted to a series of concentration and mixed with γ-CD (50μM) and ammonium acetate buffer (500μM) in methonal/water (50/50, v/v).

Instrument parameters

All IM-MS measurements were conducted on a quardupole ion-mobility time-of-flight (Q-IM-Tof) mass spectrometer (Synapt G2-S HDMS, Waters) equipped with an electrospray ionization (ESI) source. The IM instrument had an intrinsic resolution of >40 (Ω/ΔΩ). All the samples were directly infused by a syringe pump at a flow rate of 10μl/min and detected on negative ion mode with a capillary voltage at 2.1kV. The cone voltage was set at 40V. The flow of cone gas was 50L/h and desolvation gas was 450L/h, respectively. The source temperature was 80℃ and the desolvation temperature was 350℃. As for IM gas parameters, the helium cell gas flow was set at 200mL/min and IM gas flow was set at 120mL/min.

CCS mesurement

As described everywhere,[30] since a T-wave IMS applies constantly changing electric field in its mobility cell to transport ions through the buffer gas, the CCS of analytes can't be caculated directly according to drift time. It must be determined by calibration of standard materials with appropriate molecular weight range. In this work, polyalanine was employed to build CCS calibration. The form of calibration should be Equation (1). CCS (Ω) of polyalanine at different polymerization degrees and charge statements was known.[31] The calibration could be built by measuring the drift time (t_D) of polyalanine.

$$\Omega = At_D^B \quad (1)$$

Stock solution of polyalanine (1mg/ml) was diluted to 0.1mg/ml in water/acetonitrile/acetic acid (50/50/1) and acquired at eight different conditions of IM. The conditons of ESI source were the same as those of acquiring analytes.

Computational methods

Autodock (version 4.2) was applied to build initial models of catechin-cyclodextin complex for further simulation.[32] Semiflexible docking method was used with genetic algorithm to search complex models. The grid box was set to 0.3 and contained the whole cyclodextrin. In every simulation, 10 results were output and scored by a built-in function of the software. According to the scores and structures, appropriate models were chosen to peform a second dock or molecular dynamics simulation.

The molecular dynamics simulation and geometry optimizations were performed by Ascalaph Designer with MdynaMix software package.[33] The output structures from Autodock were treated by a short optimization to remove atoms clashes and then simulated by Monte Carlo (MC) method mixed with molecular dynamics (MD). The simulation temperature was 300K, and the MD time was set to be 500ps with 2.0fs of time step. Structures of last 100ps were extracted from every 5ps to be optimized by Hybrid LS and CD method. Molecular mechanics method was used to calculate energy. The OPLS force field was

chosen for all simulation and energy calculation.

Reasonable simulation results were then chosen to calculate theoretical CCS by Collidoscope, an open source program developed by Prell et al.[34] This program uses trajectory method to calculate CCS. For all CCS computation, the net charge was set to 0 and spherical nitrogen model was chosen as simulation gas phase.

Supporting Information

The supporting information for this article is available on the WWW under https://doi.org/10.1002/cjoc.2018xxxxx.

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Figure legends

Figure 1 Suctures of catechins and three typical cyclodextrins.

Figure 2 IM spectra of CA/EC and their 1:1 noncovalent complexes with five kinds of CDs.

Figure 3 Overlapped IM spectra of CA-γ-CD, EC-γ-CD and their mixture. The intensity was normalized by the strongest peak of all spectra.

Figure 4 Energy-CCS distribution of the selected conformations.

Figure 5 Side view (upper) and top view (below) of theoretical conformations of catechins-γ-CDcomplexes at lowest energy states including: A) A-ring of CA included (-111.686kJ/mol, Ω_{cal}=405.43Å2), B) B-ring of CA included (-90.971kJ/mol, Ω_{cal}=392.54Å2), C) A-ring of EC included (-112.212kJ/mol, Ω_{cal}=396.13Å2), D) B-ring of EC included (-88.738kJ/mol, Ω_{cal}=405.10Å2). Possible H-bonds were also labeled.

Figure 6 Quantification process of CA and EC by catechins-γ-CD complexes in mixture.

Table 1 Resolution of IM spectra of CA/EC-γ-CD mixture at different wave height (WH) and wave velocity (WV)

WH(V)/WV(m/s)	t1(ms)	t2(ms)	w1+w2(ms)	R
28/400	6.06	6.61	1.44	0.76
28/450	7.27	7.81	1.25	0.86
28/500	8.05	8.71	1.98	0.67
20/270	7.94	8.71	2.09	0.74
25/400	7.83	8.60	2.09	0.74
27/400	6.62	7.28	1.54	0.85

Table 2 Measured CCS of catechins, γ-CD and their non-covalent complexes under eight conditions. Ω_{str} and Ω_{weak} mean CCS calculated from the drift time of stronger peak and weaker peak of complex ions. Ω_{mes} represents average CCS of each group.

WH(V)/WV(m/s)	$\Omega_{CA\text{-}\gamma\text{-}CD}$(Å^2)		$\Omega_{EC\text{-}\gamma\text{-}CD}$(Å^2)		Ω_{CA}(Å^2)	Ω_{EC}(Å^2)	$\Omega_{\gamma\text{-}CD}$(Å^2)
	Ω_{str}	Ω_{weak}	Ω_{str}	Ω_{weak}			
30/400	399.77	388.16	386.60	401.26	153.47	153.47	372.48
35/400	400.28	387.66	390.41	402.48	153.00	154.31	372.55
28/450	400.03	386.47	388.20	401.68	154.80	154.80	373.61
30/450	401.78	389.24	387.78	401.78	154.49	154.49	371.64
35/450	394.78	381.97	386.30	398.93	152.99	153.84	366.51
40/450	392.06	380.82	383.93	397.52	152.18	153.75	369.65
35/500	399.52	386.71	384.64	399.52	153.88	152.66	371.20

40/500	397.97	386.52	384.34	397.97	154.09	154.09	372.30
Ω_{mes}(Å^2)	398.27	385.94	386.52	400.14	153.61	153.93	371.24
RSD	0.82%	0.77%	0.58%	0.47%	0.57%	0.43%	0.60%

Table 3 Comparison of measured CCS between CA and EC as well as their complexes. For CA-γ-CD and EC-γ-CD, the relative difference of CCS calculated from strong and weak peak was given out. The relative difference of two complexes were calculated from their strong peaks.

	CA-γ-CD	EC-γ-CD	CA-γ-CD/EC-γ-CD	CA/EC
RD	3.19%	3.52%	3.04%	0.21%

Table 4 Theoretical CCS (Ω_{cal}) and experimental CCS (Ω_{mes}). Difference between two values were also shown ($\Delta\Omega=\Omega_{cal}-\Omega_{mes}$).

	CA-γ-CD		EC-γ-CD		CA	EC	γ-CD
	A-ring	B-ring	A-ring	B-ring			
Ω_{mes}(Å^2)	398.27	385.94	386.52	400.14	153.61	153.93	371.24
Ω_{cal}(Å^2)	405.43	392.54	396.13	405.10	152.81	150.50	373.47
ΔΩ(Å^2)	7.16	6.60	9.61	4.96	-0.80	-3.43	1.23

Table 5 Results of complex mixtures measurements. A_1 and A_2 are area of two peaks. Measured concentrations (x_{CA} and x_{EC}) were calculated from Equation set (6). Relative errors (RE) were calculated by x_{CA}/c_{CA} and x_{EC}/c_{EC}.

c_{CA}(μM)	c_{EC}(μM)	A_1	A_2	x_{CA}(μM)	x_{EC}(μM)	RE_{CA}	RE_{EC}
10	10	1.93E+05	1.82E+05	9.22	8.84	-7.85%	-11.55%
10	20	3.79E+05	1.99E+05	9.34	17.62	-6.56%	-11.90%
10	30	5.66E+05	2.25E+05	9.92	26.39	-0.78%	-12.05%
10	40	7.81E+05	2.41E+05	9.89	36.55	-1.12%	-8.62%
20	10	2.43E+05	3.65E+05	18.23	10.05	-8.83%	0.46%
30	10	2.71E+05	5.54E+05	27.64	10.16	-7.86%	1.58%
40	10	2.92E+05	7.66E+05	38.23	9.79	-4.41%	-2.14%

Table 6 Results of extracts measurements (Crude drug concentration is 10 μg/mL) acquired by MS and HPLC methods.

Methods	**ω_{CA}**	**ω_{EC}**
MS	15.50%	4.64%
HPLC	15.71%	4.62%

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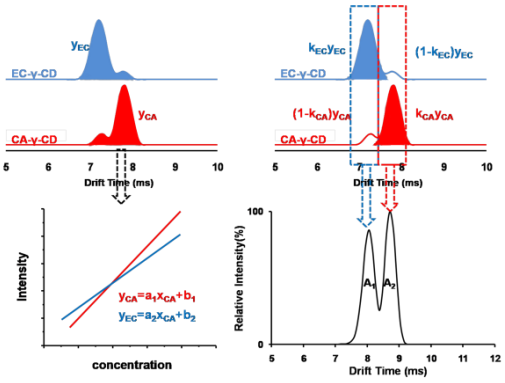
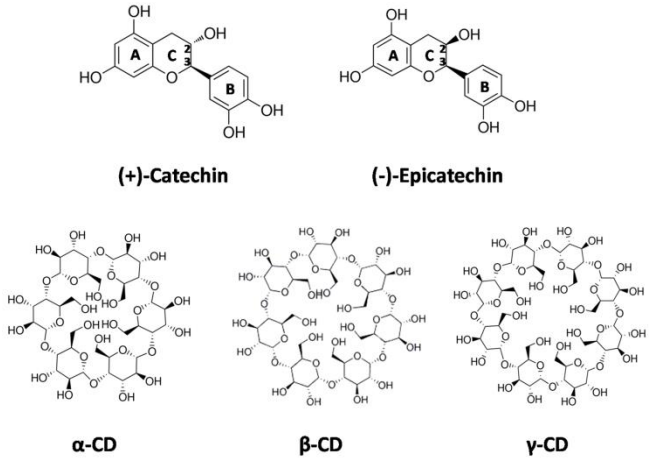
Separation，quantification and structural study of (+)-catechin and (-)-epicatechin by ion mobility mass spectrometry combined with theoretical algorithms

Xinyu Bian, Bing Zhao, Bo Pang, Zhong Zheng, Shu Liu,* Zhiqiang Liu and Fengrui Song*



Quantitative process of CA and EC by catechins-γ-CD complexes in mixture.

Fig 1



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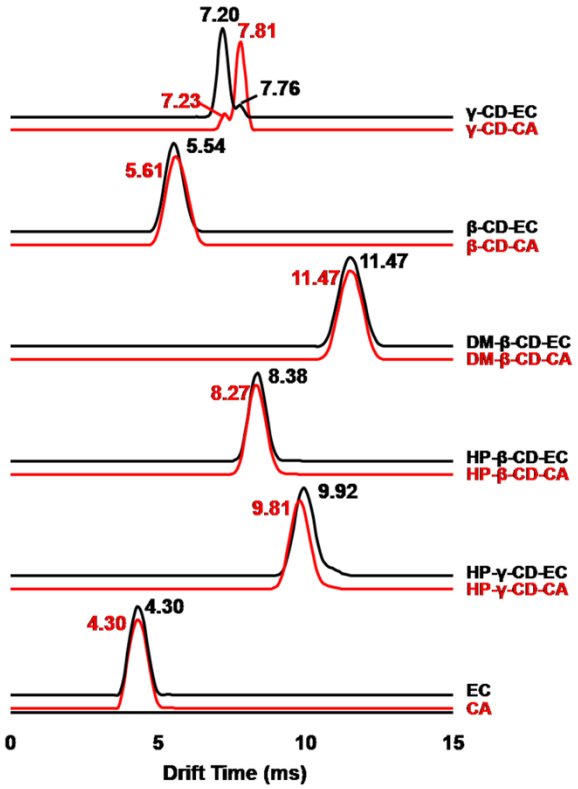

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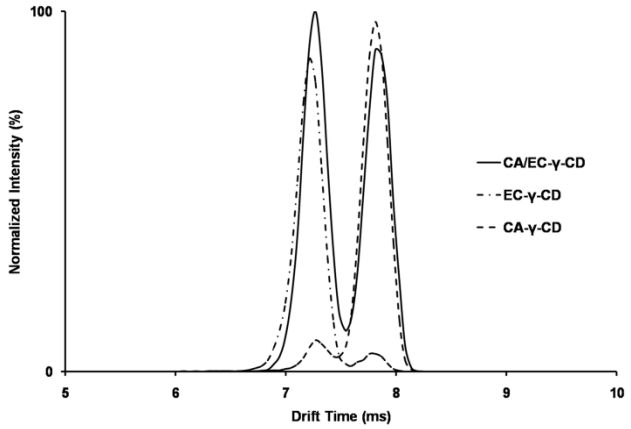

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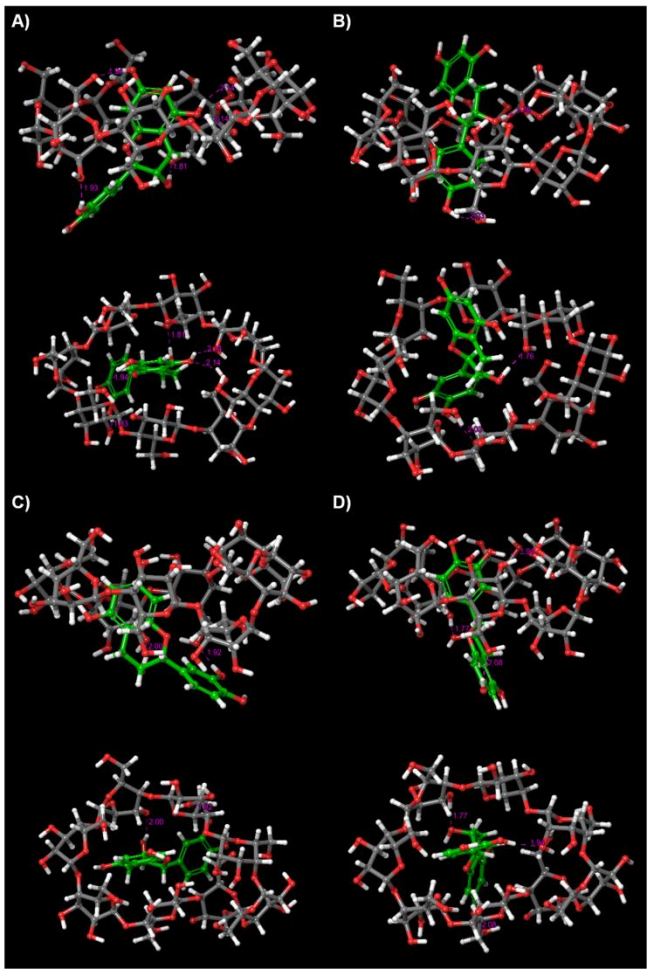

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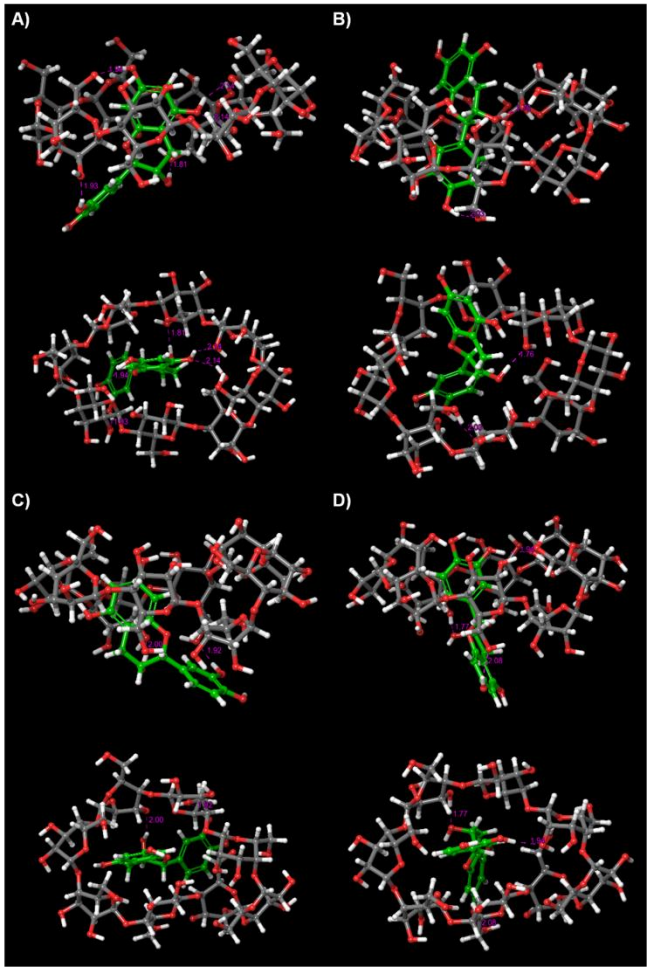

Fig 6