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Differentiation of lisinopril and its RSS diastereomer by liquid chromatography combined with collision-induced dissociation mass spectrometry

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A simple and sensitive liquid chromatography tandem multiple-stage mass spectrometry (HPLC/MS/MS) method suitable for bulk lisinopril analysis was developed, by which lisinopril and its RSS isomer were separated and differentiated. In the collision-induced dissociation (CID) mass spectra of the $[M + H]^+$ ions, the abundance of the fragment ion of *m/z* 246 for lisinopril was about two times higher than the ion of *m/z* 245; however, the former fragment ion was noted to be a little lower than the latter for RSS isomer at all collision energies. In the CID mass spectra of the $[M + Li]^+$ ion, the abundance of the rearrangement ion of *m/z* 315 for the RSS isomer was about three times higher than that for lisinopril. Furthermore, the difference was supported by the results of energy-resolved mass spectrometry (ERMS) in the test range of collision energies. Similar differences were also observed between the CID mass spectra of lisinopril and RSS isomer methylester, which indicated that the RSS isomer could be rapidly characterized by the CID mass spectra of both the protonated and lithium adduct ion. Elemental compositions of all the ions were confirmed by Fourier Transform ion cyclotron resonance ESI mass spectrometry (FT-ICR-ESI/MS). In addition, theoretical computations were carried out to support the experimental results.

Keywords: lisinopril; RSS isomer; differentiate; collision-induced dissociation; theoretical computation

Introduction

Mass spectrometry has been widely applied to the stereochemical analysis of organic compounds. In general, stereochemical information arises from sterically controlled ionic fragmentations;[1,2] therefore, the fragmentation ions in a mass spectrum could be used to characterize stereochemical differences.[3,4] Since the process of metal ion attachment could strongly influence the fragmentation of various organic molecules, the approach of activating cationized species has been applied effectively to structural studies.[5–7] Alkali metal ions interact selectively with the polar functional groups, and the collisional activation leads to charge-remote fragmentation (CRF).[8] Furthermore, due to the bulk factor, compounds adducted with alkali metal ion sometimes give structural information complementary to collisional activation of the $[M + H]^+$ ion.[9,10]

The fragment information can be significantly enhanced by examining changes in the profile with ramping collision energies, the so-called energy-resolved mass spectrometry (ERMS). A plot of relative intensity of precursor and product ions *versus* collision energy leads to the generation of breakdown curves.[11] The evaluation of breakdown curves can provide information on fragmentation mechanisms, such as describing fragmentation pathways and the identification of isomers.[12,13]

Lisinopril, (2S)-1-[(2S)-6-amino-2-[[(1S)-1-carboxy-3-phenylpropyl]amino] hexanoyl]pyrrole-2-carboxylic acid is an angiotensin-converting enzyme (ACE) inhibitor, used for the treatment of hypertension, heart failure and acute myocardial infarction.[14–16] The RSS isomer of lisinopril ((2S)-1-[(2S)-6-amino-2-[[(1R)-1-carboxy-3-phenylpropyl]amino]hexanoyl]pyrrole-2-carboxylic acid) is an impurity (impurity E) resulting from the synthesis of lisinopril.[17,18] The impurity profile of a drug substance is critical to its safety assessment and is important for monitoring the manufacturing process.[17,19] This, in-turn, requires the development of analytical methods that can rapidly characterize potential isomeric side products. Ion trap mass spectrometry/mass spectrometry (MS/MS) can be used for direct structural elucidation based on characteristic fragmentations, especially for minor impurities in drugs, for which standards are not available. In this paper, we described the identification of the RSS isomer from bulk lisinopril by both protonated and lithium adduct ion with collision-induced dissociation (CID) and ERMS. The elemental compositions of the fragmentation ions were confirmed by FT-ICR-ESI/MS/MS. Furthermore, theoretical computations were carried out to support the proposed mechanisms.

Experimental

Reagents and chemicals

The bulk lisinopril, RSS isomer and their methylester isomers were obtained from JianYuan Inc. (Hangzhou, China). Analytical-grade lithium chloride was purchased from Beijing Xudong Chemical Co. (Beijing, China). HPLC-grade methanol and acetonitrile was obtained from Merck Co. (Darmstadt, Germany), and water was

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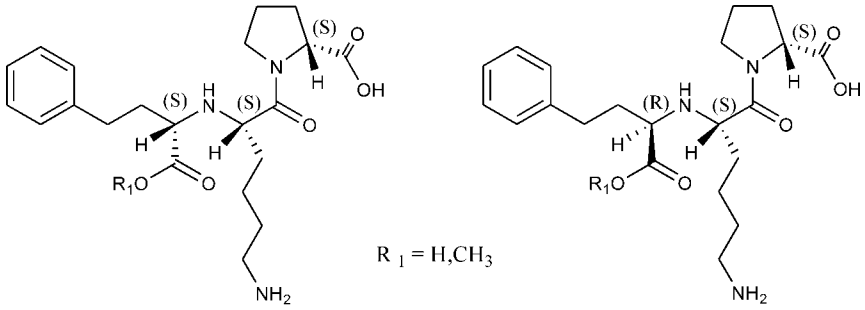


Figure 1. Structure of lisinopril, RSS isomer and methylester diastereomers.

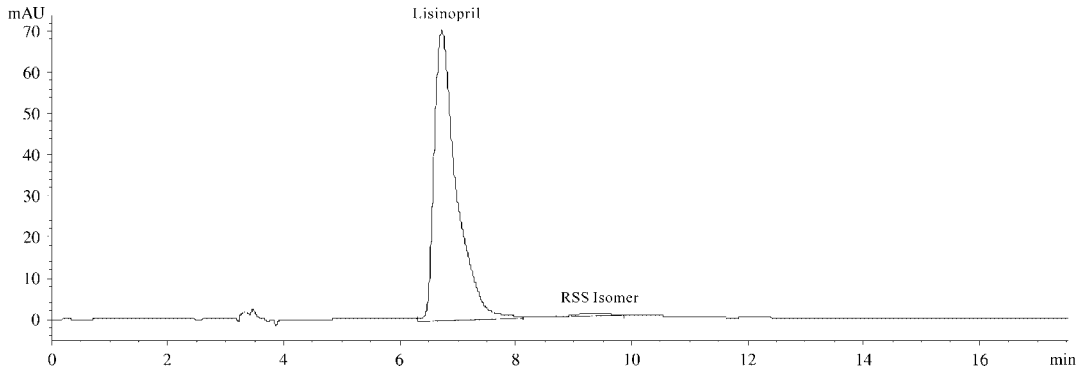


Figure 2. Liquid chromatogram of the bulk lisinopril.

purified by a Milli-Q purification system (Millipore, Bedford, MA, USA). The bulk lisinopril was prepared in methanol and water (50 : 50, v/v) at concentration of 0.5 mg ml^{-1} for LC/MS analysis, and other samples were at concentration of 0.2 mg ml^{-1} for offline analysis.

HPLC/MS/MS and FT-ICR/MS analysis

An Agilent 1100 series HPLC system (Agilent Technologies, Palo Alto, CA, USA) coupled to a Bruker Esquire 3000^{plus} ion trap mass spectrometer (Bruker–Franzen Analytik GmbH, Bremen, Germany) with an ESI source was used for HPLC/MS/MS analysis. A Hypersil ODS2 column (4.6 × 250 mm, 5 µm) was used for the separation. The mobile phase consisted of a mixture of 20 mM ammonium acetate buffer adjusted to pH 4.5 with glacial acetic acid and acetonitrile (90 : 10, v/v) at a flow rate of 1.0 ml min^{-1}. For lithium adduct ion analysis, the buffer solution mixed with lithium chloride at a concentration of 1 × 10^{-9} M. The column temperature was maintained at 30 °C with the detection wavelength of 254 nm. The samples were infused into the mass spectrometer from the HPLC system through a T-junction with a splitting ratio of 2 : 1. The ion source temperature was set at 250 °C, and the ESI needle voltage was always set at 4.0 kV. Nitrogen was used as the nebulization gas at a pressure of 30 psi and the dry gas at a flow rate of 10 l min^{-1}. For tandem mass spectra, they were obtained by CID with helium as collision gas after isolation of precursor ions, and optimization of the collision energy between 0.3 and 0.8 V to maximize the ion current for all samples.

The accurate mass spectrometric experiments were performed on an Apex III (7.0 T) Fourier transformation ion cyclotron resonance (FT-ICR) mass spectrometer (Bruker, Billerica, MA, USA). Precursor ions were generated in the positive ion mode using ESI source. XMASS software version 6.1.1 was used for instrument control, data acquisition and processing. The spray voltage was 4.5 kV. The temperature of the capillary was 250 °C. Nitrogen was used as the nebulizing and drying gas, and argon was used as the collision gas. Loop injections were made into a mobile phase stream at a flow rate of 0.18 ml h^{-1}.

The MS/MS results of electrospray ionization in negative ion mode did not offer much information (data not shown).

Computational procedure

All theoretical calculations were carried out by the semi-empirical PM3 method with the Gaussian 03 program.[20] Semi-empirical molecular orbital methods based on the neglect of diatomic differential overlap (NDDO) approximation continue to find widespread use in variety of applications. The semi-empirical PM3 optimized structures were shown by Gauss View version 3.09 software to give higher quality images of these structures.

Result and Discussion

During routine impurity profiling of bulk lisinopril through analytical HPLC, an impurity level of 1.35% was characterized as the RSS isomer (impurity E) by comparing with the standard. Lisinopril (S,S,S), RSS isomer, a pair of methylester isomers (S,S,S) and (R,S,S) are shown in Fig. 1.

Since the mobile phase employed for HPLC/UV analysis of lisinopril consisted of nonvolatile sodium dihydrogen phosphate,[17] it was necessary to modify the mobile phase to make it suitable for LC/MS analysis. The obtained LC of bulk lisinopril using the modified method described above is shown in Fig. 2. The LC/MS/MS

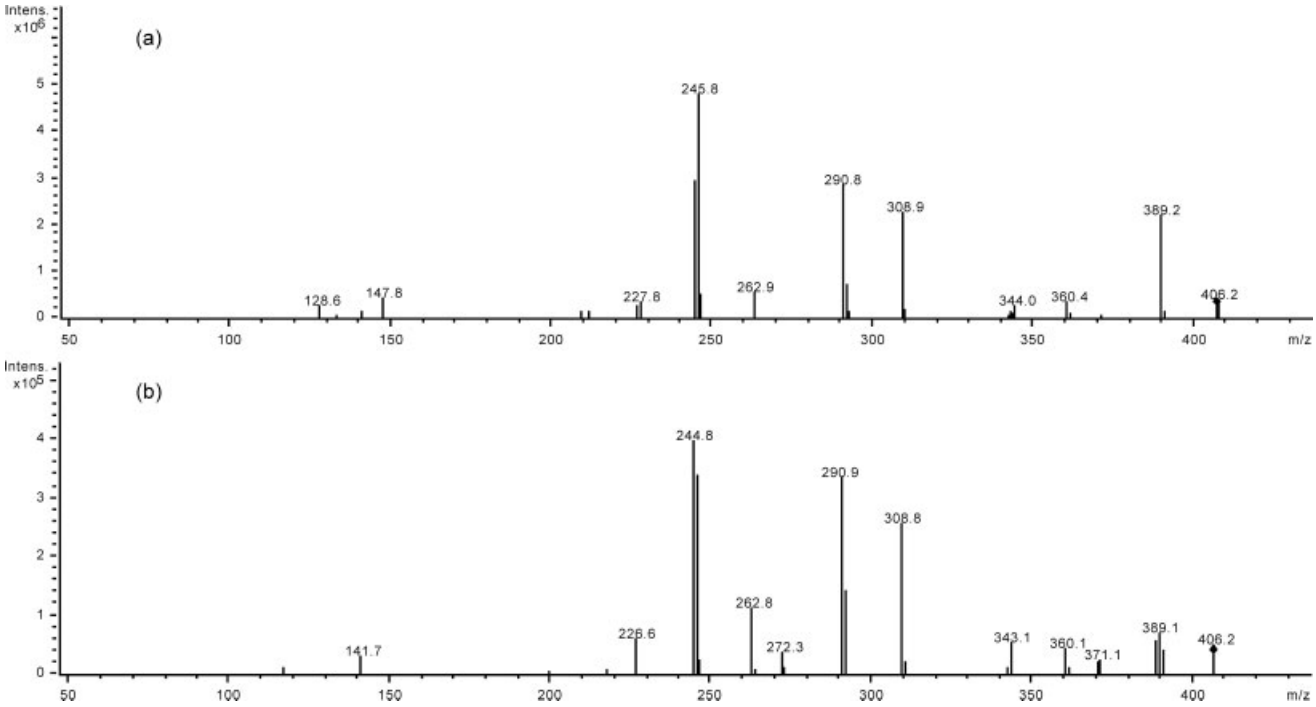


Figure 3. CID spectra of protonated precursor ions. (a) lisinopril; (b) RSS isomer.

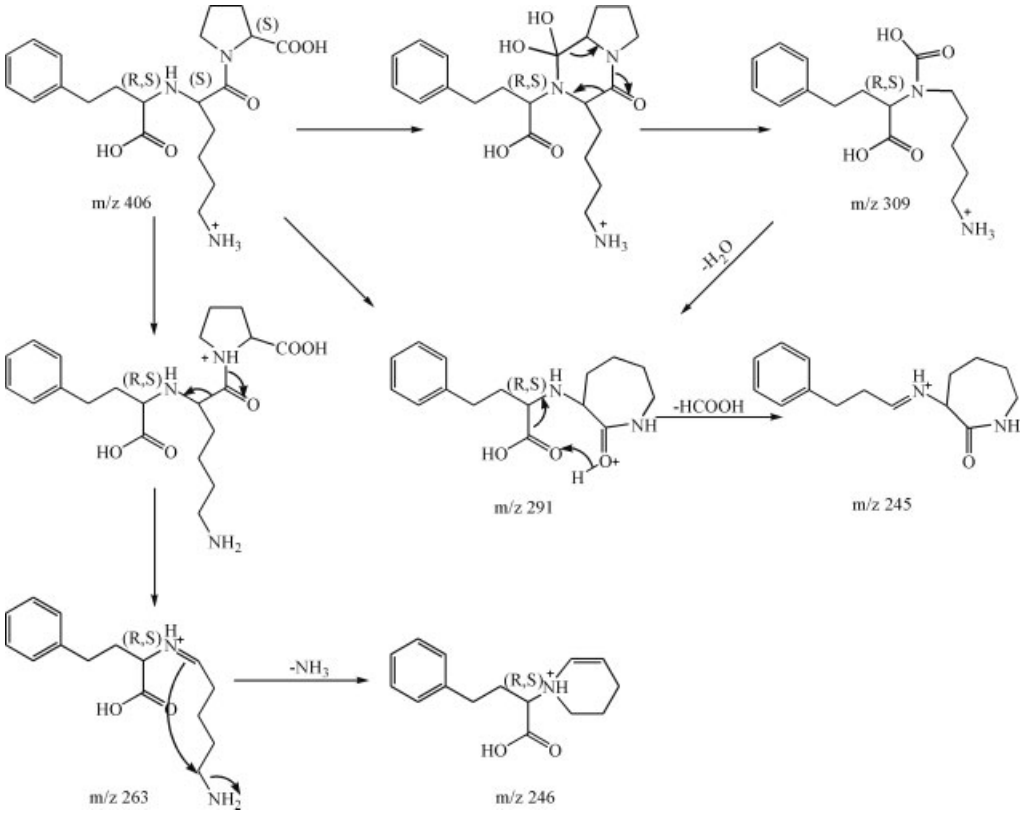
analysis was performed and the RSS isomer was detected with molecular mass of 405 by both positive and negative ion mass spectrum, respectively.

CID of protonated molecule

Lisinopril and its RSS isomer exhibited strong protonated molecule $[M + H]^+$ allowing online HPLC/MS/MS analysis. The CID mass spectrum of lisinopril is shown in Fig. 3(a). As reported, the most thermodynamically favored protonated site would be the ε-amino group for lisinopril, then the proton can migrate to other sites in the molecule where leading to fragmentation.[21] One site is the nitrogen atom of the proline moiety, this will result in the simultaneous loss of proline and carbon monoxide to give *m/z* 263 (*a*-ion), followed by the subsequent loss of ammonia to yield *m/z* 246. The other site to which the proton can migrate is the amide carbonyl moiety, which will result in the loss of dihydropyrrole and carbon monoxide to give *m/z* 309. The product ion of *m/z* 309 might also form through a CRF mechanism as presented in Scheme 1, and the mechanism does not associate with the location of the ionizing proton and its mobility, just depend on the interaction of one basic functional group with another.[22] The product ion at *m/z* 291 results from several dissociation processes involving an acyclic *b*-ion by cleavage of the amide bond, and by loss of H_2O from *m/z* 309.[22,23] A stable seven-membered ring *b*-ion for *m/z* 291 formed by cyclization with the side-chain amino group has been confirmed by the previous work in our group.[24] When the ion is isolated and collisionally activated, an exclusive fragmentation pathway to form *m/z* 245 can be observed without obvious ion *m/z* 263 (*a*-ion) being observed. This suggests that product ion *m/z* 263 results mainly from direct cleavage of the peptide backbone between the *a*-carbon and the amide carbonyl carbon. To confirm the molecular formulae of proposed ions, the accurate masses were detected using FT-ICR/MS/MS. The most probable elemental compositions of these ions were obtained with a high degree of confidence. The relative errors between accurate masses and exact masses are within $\pm$1.30 ppm (Table 1).

The RSS isomer of lisinopril showed similar fragmentation routes to lisinopril (Fig. 3(b)). However, a significant difference was found in the CID spectra between the two diastereomers. The ion of *m/z* 245 and 291 from the RSS isomer were much higher than that from lisinopril. The abundance of ion *m/z* 246 was about two times higher than the ion of *m/z* 245 for lisinopril, while the former showed a little lower than the latter for RSS isomer. The breakdown curves (Fig. 4(a) and (b)) which measure the relative abundances of precursor and product ions for both lisinopril and RSS isomer indicate that the difference exists in all the way in the range of 0.38–0.54 V excitation voltage. In fact, the chiral carbon under investigation does not directly take part in the fragmentation process. However, for the ions of *m/z* 291, the carbonyl unit at the chiral carbon for the RSS isomer may be nearer to the positive charge than lisinopril (S,S,S) and even form a hydrogen bond with the proton. The ion of *m/z* 245 and 291 may be more energetically favored for the RSS isomer. Therefore, the ion of *m/z* 291 for the RSS isomer will be more stable and easier to lose formic acid then yield the ion of *m/z* 245. Such a result implicates different yields of product ions, relating to the different structural conformation.

Simple theoretical computations of these two compounds were carried out to investigate the structures of the fragment ions. A schematic potential configuration for the proposed ions of *m/z* 291 is given in Fig. 5. An H-bond with the bond length of 1.76 Å formed between two carbonyls group in the RSS isomer favors the proton immigrating to another carboxyl group and thus easy losses of formic acid or water and carbon monoxide. No additional energy is required for bond rotation to facilitate the proton transfer process. For the same fragment ion of lisinopril, the distance from one carboxyl group to the other is too great and therefore cannot form a hydrogen bond. Furthermore, an H-bond form ion of *m/z* 291 from RSS isomer had the lower



Scheme 1. Proposed fragmentation pathway of $[M + H]^+$ ion for lisinopril and RSS isomer.

Table 1. Summary of accurate mass measurements for the precursor and product ions of lisinopril

Precursor ions	Product ions	Exact mass	Accurate mass	Formula	Error (ppm)
406 $[M + H]^+$	–	406.2337	406.2335	$C_{21}H_{32}N_3O_5^+$	−0.49
	389	389.2071	389.2066	$C_{21}H_{29}N_2O_5^+$	−1.28
	309	309.1809	309.1811	$C_{16}H_{25}N_2O_4^+$	0.51
	291	291.1703	291.1704	$C_{16}H_{23}N_2O_3^+$	0.34
	263	263.1754	263.1757	$C_{15}H_{23}N_2O_2^+$	1.13
	246	246.1489	246.1489	$C_{15}H_{20}NO_2^+$	0
	245	245.1648	245.1648	$C_{15}H_{21}N_2O^+$	0
412 $[M + Li]^+$	–	412.2421	412.2421	$C_{21}H_{31}N_3O_5Li^+$	0
	315	315.1893	315.1894	$C_{16}H_{24}N_2O_4Li^+$	0.32
	297	297.1787	297.1784	$C_{16}H_{22}N_2O_3Li^+$	−1.00

energy (272 kcal mol^{-1}) than that from lisinopril (287 kcal mol^{-1}). This means that the ion *m/z* 291 from lisinopril is less stable than that from the RSS isomer. The results of theoretical computations are consistent with the experimental data and provide a possible explanation as to the difference in the CID spectra between the two diastereomers.

Similar differences on the abundance of ions *m/z* 245 and 305 were also observed in the CID mass spectra of their methylester isomers. Compared with the ion *m/z* 246, the abundance of ions *m/z* 245 and 305 are higher in the RSS isomer than in lisinopril. Results suggest that lisinopril and the RSS isomers, along with the methylester derivatives, can be discriminated by the CID mass spectrometry. In particular, the differences in the abundance of the ions of *m/z* 245 and 291 from the RSS isomer as well as the ions of *m/z* 245 and 305 from its methylester are notable.

CID of lithium adduct ion

Since alkali metal ion attachment might result in the product ions in a different way, the fragmentation behaviors of lisinopril series compounds coordinated to lithium were investigated in this study.

As the low concentration of lithium chloride in HPLC elution results in deposition approximately equal to 3×10^{-7} μg min^{-1}, it therefore does not have any detrimental effect on mass spectrometry,[25] this allows the MS/MS experiments on the $[M + Li]^+$ precursor ion to be performed online. Analysis of the product ion spectrum of $[M + Li]^+$ of lisinopril clearly indicates that the precursor ion behaves similarly to the $[M + H]^+$ ion, yielding two main ions of *m/z* 297 and 315 (Fig. 6(a)), which correspond to the ion of *m/z* 291 and 309 from the $[M + H]^+$. The CID spectrum of $[M + Li]^+$ ion for the RSS isomer shows the same fragmentation characteristics as lisinopril (Fig. 6(b)); however, the

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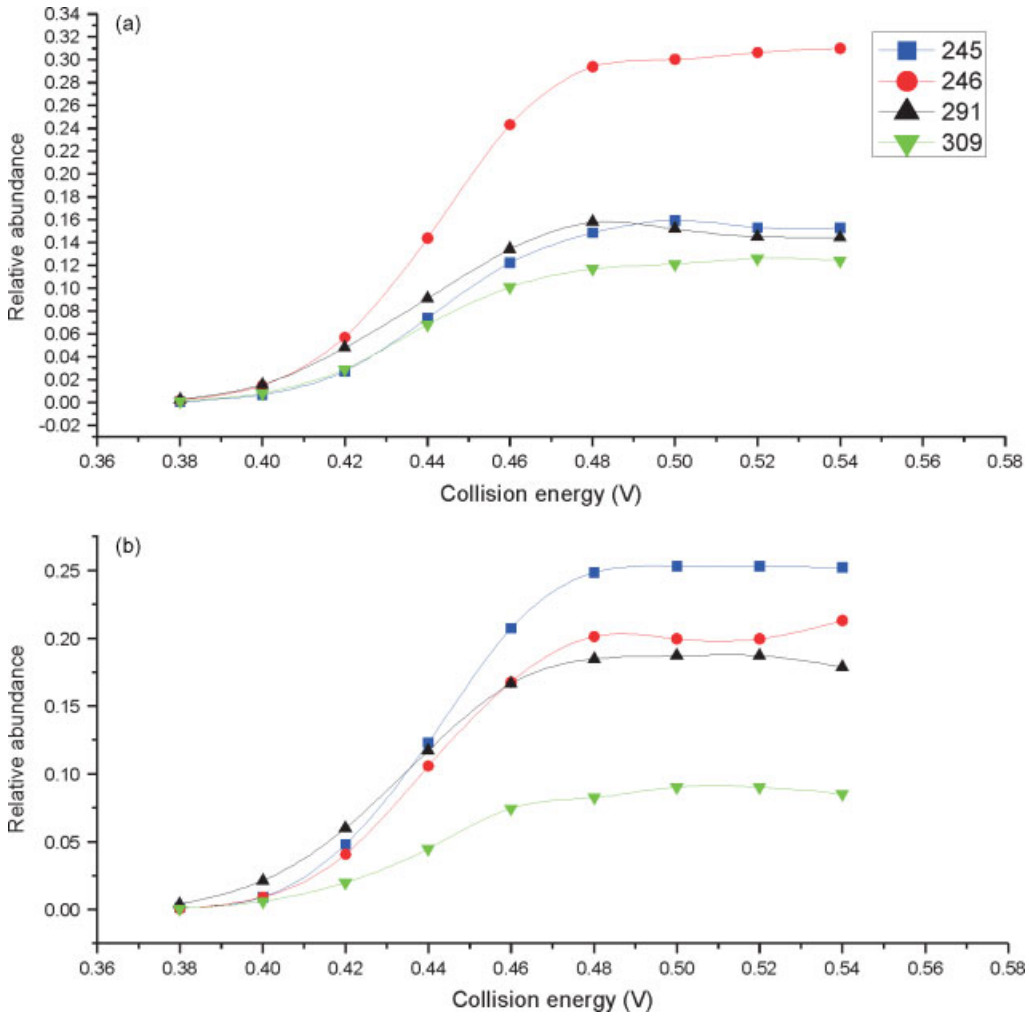


Figure 4. The ERMS of $[M + H]^+$ ion. (a) lisinopril; (b) RSS isomer.

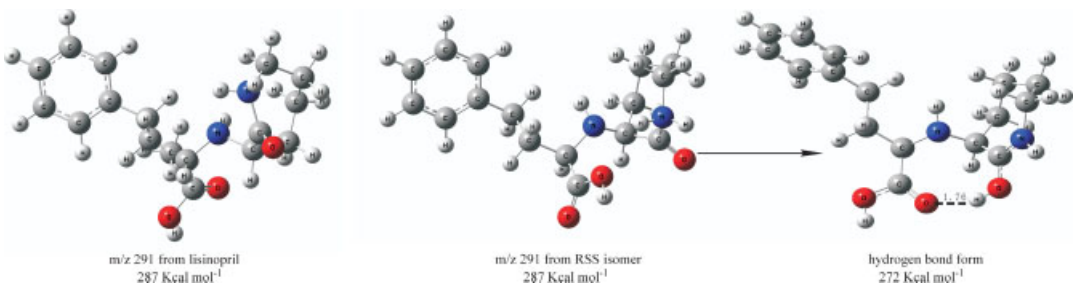


Figure 5. PM3 optimized structures of ion *m/z* 291 for lisinopril and RSS isomer.

abundance of the ion of *m/z* 315 is higher for the RSS isomer than for lisinopril. The breakdown curves of the lithium adduct ion for both lisinopril and its RSS isomer were measured in the range of 0.38–0.52 V collision voltage (Fig. 7(a) and (b)). As for lisinopril, the base peak changed to the ion of *m/z* 297 from the precursor ion of *m/z* 412 at 0.44 V, and the RSS isomer changed to the ion of *m/z* 315 at 0.46 V. The abundance of the ion at *m/z* 297 was about three times higher than that of the ion *m/z* 315 for lisinopril, while the former was almost the same as that of the latter for RSS isomer. However, the abundance of ion *m/z* 309 from the $[M + H]^+$ was almost same in both lisinopril and its diastereomer. Although the difference in the abundance of the ion at *m/z* 315 between these two diastereomers implied the ion at *m/z* 315 from $[M + Li]^+$ underwent a different fragmentation pathway with the ion of *m/z* 309 from $[M + H]^+$. As reported, the fragment ion of *m/z* 315 was a lithium adduct $b_1 + OH$ ion, which was formed through a CRF mechanism.[8,26,27] The lithium ion polarizes the carbonyl, increasing the partial positive charge on the carbon and making it susceptible to nucleophilic attack by hydroxide group, resulting in the loss of the C-terminal amino acid, and then yielding the ion *m/z* 315.[28] The ion *m/z* 297 is proposed as a seven-membered ring *b*-ion just as *m/z* 291 from $[M + H]^+$. For the RSS isomer, the ion of *m/z* 315 may be energetically more favorable and stable. High-resolution mass analysis of the ion of *m/z* 315 indicated an

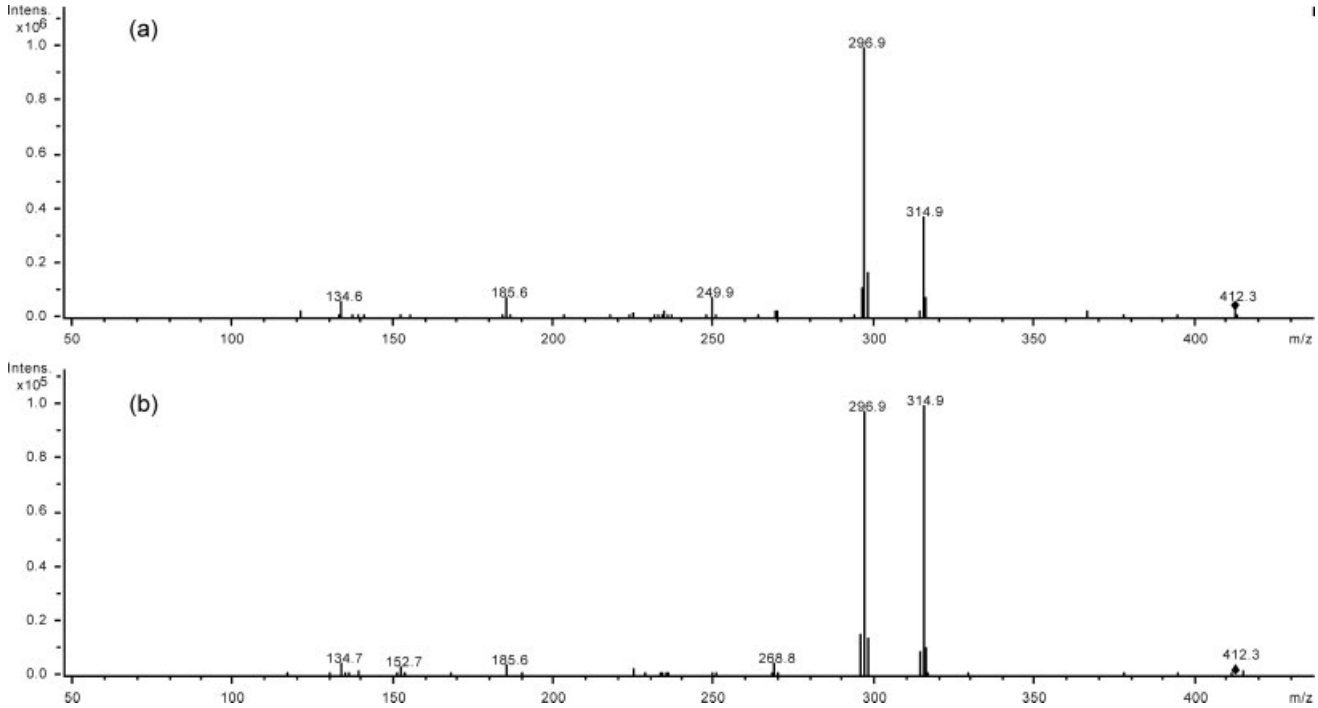


Figure 6. CID spectra of $[M + Li]^+$. (a) lisinopril; (b) RSS isomer.

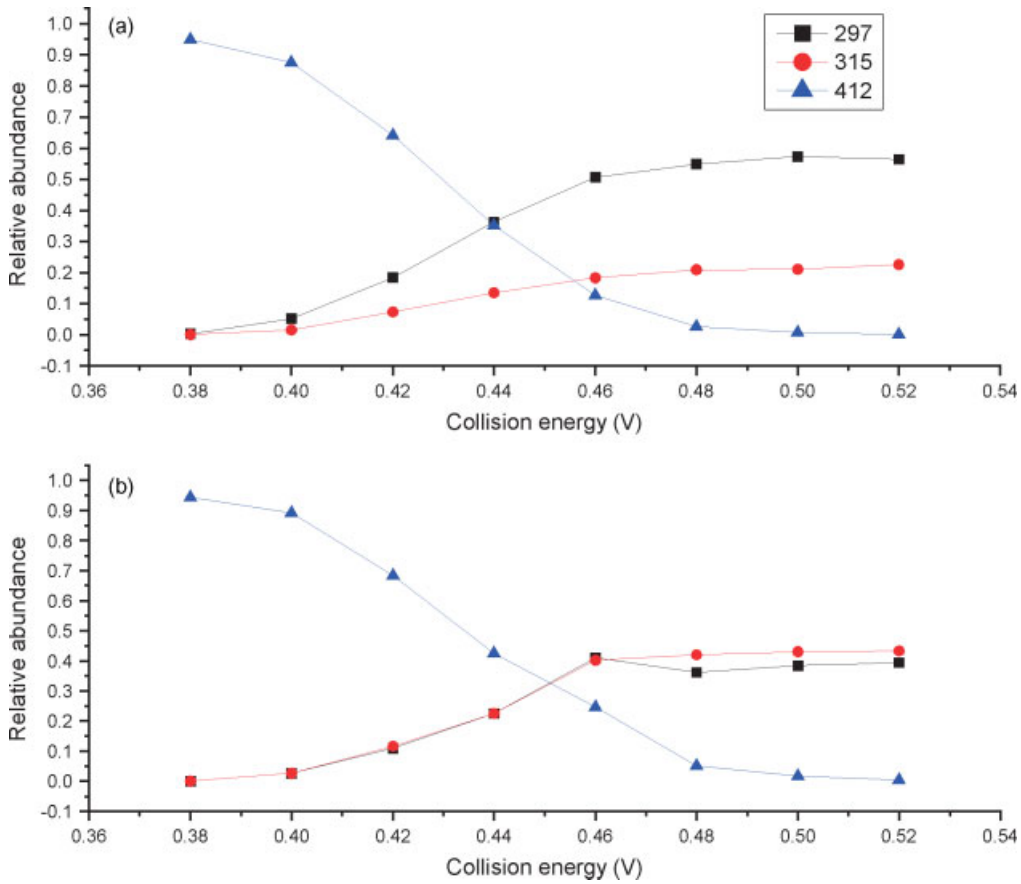
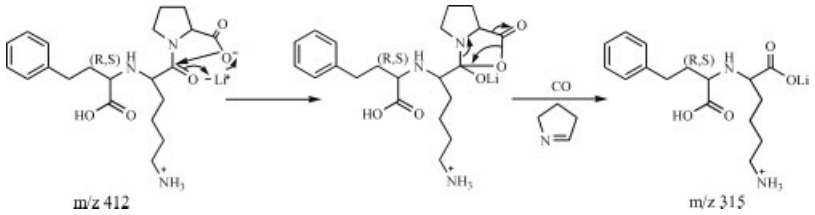


Figure 7. The ERMS of $[M + Li]^+$ ion. (a) lisinopril; (b) RSS isomer.



Scheme 2. Proposed fragmentation pathway of $[M + Li]^+$ ion for lisinopril and RSS isomer.

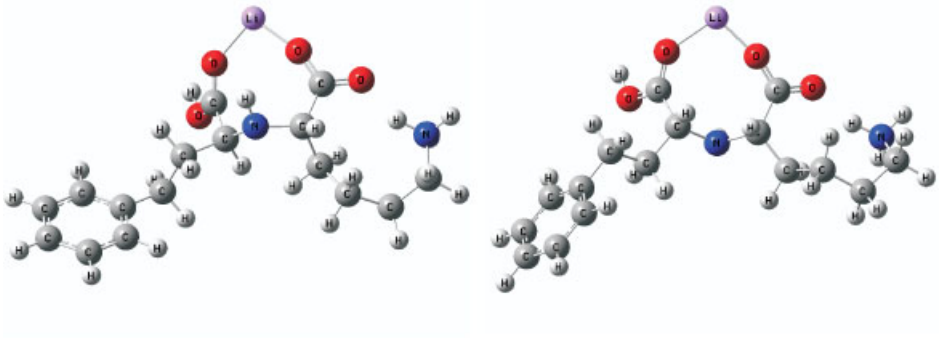


Figure 8. PM3 optimized structures of ion *m/z* 315 for lisinopril and RSS isomer.

element composition of $C_{16}H_{24}N_2O_4Li$, which corresponds to the structure of $[b_1 + Li + OH]^+$ as shown in Scheme 2.

Similar differences are also observed between the CID mass spectra of $[M + Li]^+$ for methylester isomers (*m/z* 420), particularly the ion of *m/z* 329 corresponding to the $[b_1 + Li + OH]^+$ ion, which is greater in the case of the RSS isomer than that of lisinopril. Therefore, the RSS isomer and its methylester could be rapidly differentiated from lisinopril and its methylester by the CID of $[M + Li]^+$.

As shown in Fig. 8, lithium can be stabilized by another carbonyl and form an eight-membered ring in both lisinopril and RSS isomers. However, the conformation difference between the two diastereomers can be observed. For RSS isomer, 1-aminobutyl exists as an e-bond form, whereas 1-aminobutyl is a-bond in lisinopril. This will results in the difference of the stability of ion *m/z* 315.

Conclusions

In this work, HPLC combined with the CID mass spectrometry was used as a rapid and sensitive method for the differentiation of the RSS isomer from bulk lisinopril by the CID of both the protonated and lithium adduct ions. The abundance of the ions at *m/z* 245 and 291 from $[M + H]^+$ and the ion at *m/z* 315 from $[M + Li]^+$ for RSS isomer were much higher than that for lisinopril. Similar differences were also observed for their methylesters. Results indicated that the MS/MS spectra of the lithium adduct ions showed higher isomeric selectivity than that of the protonated ion. Theoretical calculations were also used to shed light on the fragment ions by the semi-empirical PM3 method. Results showed that the variations in the CID spectra arose from the difference in stabilities of fragmentation ions from lisinopril and RSS isomer.

Acknowledgement

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