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Short communication

Identification of a new impurity in lisinopril

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Abstract

LC–UV scan of lisinopril revealed the presence of an unknown impurity (∼0.14%) at a relative retention time of 3.26 employing phosphate buffer-acetonitrile as binary gradient system while LC–MS analysis with binary gradient system comprising of a ammonia–ammonium acetate buffer (pH 5.0) and acetonitrile indicated it to be $C_{31}H_{41}N_3O_7$. The impurity was isolated by preparative HPLC utilizing a linear gradient of water and acetonitrile. The structural analysis of the isolated product by ^1H, ^{13}C NMR, mass spectroscopy and FT-IR revealed it to be a 4-phenyl butanoic acid derivative (CL) of lisinopril.

Keywords: Lisinopril; Angiotensin converting enzyme inhibitor; HPLC; LC–MS/MS; NMR; FT-IR

1. Introduction

During the synthesis of the angiotensin converting enzyme (ACE) inhibitor lisinopril (1-[N^2-[(*S*)-1-carboxy-3-phenylpropyl]-L-lysyl]-L-proline dihydrate), seven known impurities are reported that include (i) (2*RS*) 2-amino-4-phenylbutanoic acid, (ii) 4-methylbenzenesulphonic acid, (iii) *S*,*S*,*S*-diketopiperazine, (iv) *R*,*S*,*S*-diketopiperazine, (v) lisinopril *R*,*S*,*S*-isomer, (vi) cyclohexyl analogue, and (vii) 2-(2-oxo-azocan-3-ylamino)-4-phenyl-butyric acid [1–4]. In the regulatory guidelines of the International Conference on Harmonization (ICH-Q3A), it is recommended that the impurities higher than 0.1% should be identified and characterized [5,6]. Employing a binary gradient LC–UV system, we observed the presence of additional impurity (CL) at a relative retention time of 3.26, which is higher than this identification threshold (about 0.14%). It is worth mentioning that this impurity is not detectable by the Ph. Eur. method [2]. This paper describes the isolation and characterization of this impurity using chromatographic and spectroscopic techniques.

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2. Experimental

2.1. Chemicals

LC-grade water (resistivity less than 18.2 MΩ cm at 25 °C and total organic carbon less than 5 μg l^{-1}) was prepared by purifying distilled water with a Milli-Q water purification system from Millipore (Bedford, USA). Acetonitrile (gradient grade for chromatography) was purchased from Qualigens (Glaxo-SmithKline Pharmaceuticals, Mumbai, India) while acetic acid (∼99% p.a.) was obtained from RanKem (New Delhi, India). AR grade ammonia solution (∼25%) and sodium dihydrogen phosphate were purchased from S.D. Fine Chem. Ltd. (Mumbai, India).

2.2. Instrumentation

The LC–UV analysis was performed on Shimadzu LC2010A system. The LC–MS/MS analysis was carried out on Perkin-Elmer liquid chromatograph coupled with a PE SCIEX model API 3000 Triple Quadruple mass spectrometer with a turbo ion spray source (ESI mode). The positive ion spray mode was used. The MS and MS/MS spectra were obtained under following conditions: ionization volt energy (IVE), 4000 V at 400 °C,

declustering potential 6 V, focusing potential 60 V while CAD spectrum was recorded at 6 V with 38 eV collision energy.

The impurity was isolated using preparative HPLC (Shimadzu LC-8A) instrument equipped with a system controller SCL–10 AVP, UV detector SPD-10 AVP and HPLC data were processed using class VP 5.03 software. The ^{1}H and ^{13}C spectra were recorded on Bruker Avance DRX–200 MHz NMR spectrometer using D_2O as the solvent at ambient temperature. FT-IR measurements were performed on Perkin-Elmer FTIR model 1600.

2.3. Preparation of lisinopril substance solution for isolation of impurity

0.225 g of concentrated lisinopril mother liquor (obtained in the final stage of purification) lyophilized sample was dissolved in 4 ml water and the solution was filtered through 0.45 μm syringe filter.

2.4. Method development for impurity isolation and identification

Inertsil C-8 column (GL Sciences Inc., Japan), 250 mm × 4.6 mm, and 5 μm particle size was employed for LC–UV. Mobile phase (A) was prepared by dissolving 3.026 g of sodium dihydrogen phosphate in 970 ml distilled water and 30 ml acetonitrile while mobile phase (B) contained 1.248 g of sodium dihydrogen phosphate in 400 ml distilled water and 600 ml acetonitrile. The pH of both solutions was adjusted to 5.0 with sodium hydroxide. The binary gradient conditions were as follows: (i) linear gradient of 100–70% of A for 0–35 min; (ii) 70% A for 35–45 min; (iii) 100% of A for 45–50 min; (iv) reconditioning column for 10 min. The flow rate was 1.8 ml min^{-1}. The column oven temperature was set at 50 °C. Twenty microliters of 1 mg ml^{-1} solution was injected. After the peak of lisinopril, the additional peak corresponding to CL impurity was observed at 26.82 min (relative retention time 3.26) (Fig. 1). Spiking experiments have shown that this impurity is not identical with any of the known impurities listed above. LC–MS study was necessary for the determination of the structure of CL. Due to the incompatibility of the phosphate buffer with HPLC–MS another gradient HPLC system employing ammonium acetate buffer was adopted which was found to be suitable for similar purposes [7,8].

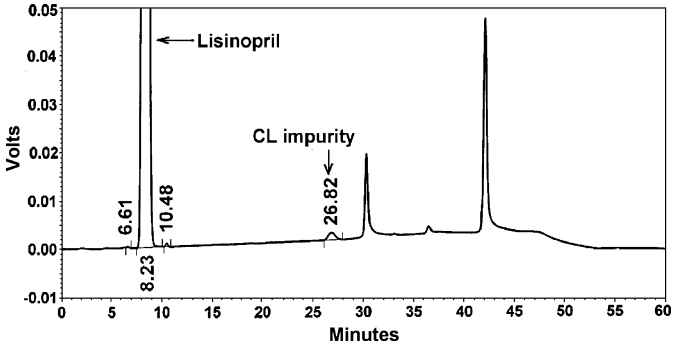


Fig. 1. LC–UV chromatogram of lisinopril containing CL impurity.

LC–MS/MS analysis was carried out using Inertsil ODS C-18 column (150 mm × 4.6 mm, particle size 5 μm; GL Sciences Inc., Japan). The mobile phase (C) contained 0.05% glacial acetic acid in distilled water adjusted to pH 5 with ammonia, while mobile phase (D) consisted of mobile phase (C) and acetonitrile in 20:80 proportions. The gradient conditions were: (1) 100% of C for 0–5 min, (2) linear gradient from 100 to 85% of C for 5–15 min and continued till 20 min, (3) linear gradient from 85 to 80% of C for 20–25 min, (4) linear gradient from 80 to 75% of C for 25–30 min, (5) linear gradient from 75 to 70% of C for 30–35 min, (6) linear gradient from 70 to 50% of C for 35–40 min, (7) 100% C for 41–42 min, and (8) reconditioning of the column for 4 min. The flow rate was 1.5 ml min^{-1}. The column oven temperature was set at 45 °C. The lisinopril sample concentration was 1 mg ml^{-1} dissolved in mobile phase (D). The injected volume was 20 μl. The LC–MS scan revealed the existence of a compound with molecular mass of 568 at relative retention time of 2.08, which was further confirmed by MS fragmentation pattern.

For the isolation of this impurity, X-Terra RP18e column (300 mm × 19 mm, 7 μm, Waters, USA) was used with distilled water (E) and acetonitrile (F) as mobile phases. The gradient conditions for the analysis were as follows: (a) for 0–7 min, 100% of E, (b) linear gradient from 100 to 75% of E for 7–12 min and continued till 15 min, (c) linear gradient from 75 to 70% of E for 15–20 min, (d) linear gradient from 70 to 65% of E for 20–25 min, (e) linear gradient from 65 to 60% of E for 25–30 min, (f) linear gradient from 60 to 0% of E for 30–35 min, (g) 100% of E for 35–40 min, and (h) reconditioning of the column for 10 min. The flow rate was 15 ml min^{-1}.

Approximately, 0.225 g of sample was injected onto the column and this loading was continued until 200 mg of CL was isolated (∼9 injections). The LC–UV analysis indicated that the impurity CL was eluted around 22 min on preparative column. The fractions containing >90% of CL were collected, concentrated using rotary evaporator at 35 °C under vacuum and finally lyophilized to obtain solid product.

3. Results and discussion

The isolated impurity CL was subjected to structural analysis using LC–MS/MS, MS, ^{1}H, ^{13}C NMR and FT-IR spectroscopic methods.

CL impurity displays a peak at *m/z* 568 on the LC–MS scan of lisinopril and its presence was confirmed with MS analysis. Moreover, the fragmentation patterns for both are similar indicating that they possess structural resemblance [1]. The fragmentation of isolated CL molecule (Table 1) exhibits a mass difference of 162 units, which probably can be explained on the basis of –CH(COOH)CH_2CH_2Ph moiety attached to the primary amino group of the lisinopril molecule.

^{1}H NMR spectrum of CL displays following peaks: δ 7.36–7.27 (m, 9H, aromatic), 4.40 (m, 1H, $C^{2}H$), 4.22 (t, 1H, $C^{7}H$), 3.58 (m, 2H, $C^{5}H_2$), 3.65 (m, 2H, $C^{13}H$, $C^{23}H$), 3.01 (t, 2H, $C^{11}H_2$), 2.72 (m, 4H, $C^{15}H_2$, $C^{25}H_2$), 2.27–1.98 (m, 10H, $C^{3}H_2$, $C^{4}H_2$, $C^{8}H_2$, $C^{14}H_2$, $C^{24}H_2$), and 1.53–1.17 (m, 4H, $C^{9}H_2$, $C^{10}H_2$). It can be seen from these data that there are

Table 1
MS/MS fragmentation data of isolated CL impurity

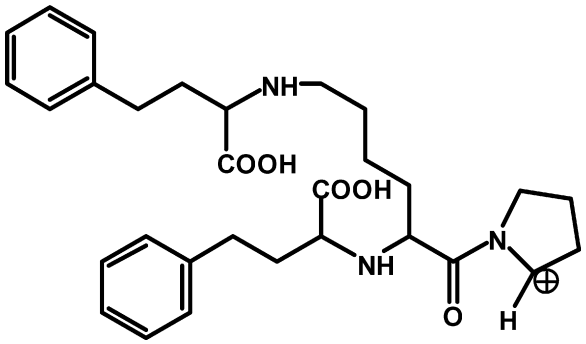
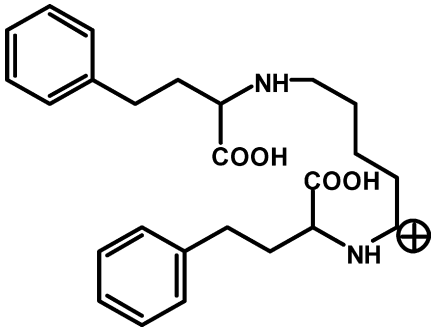
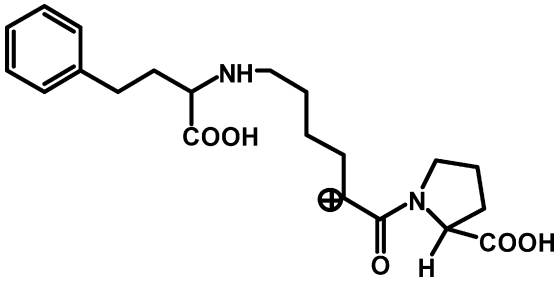
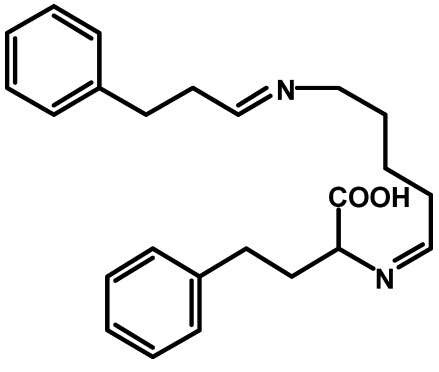
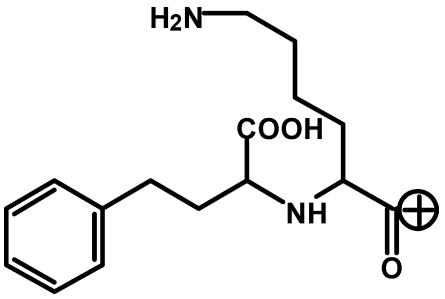
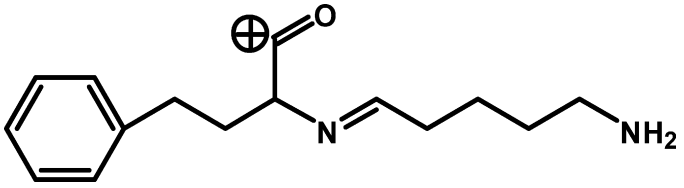
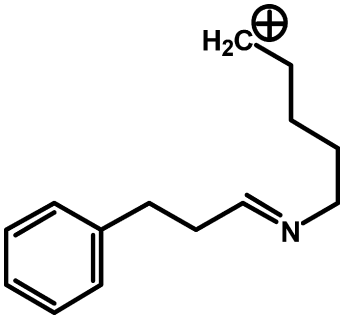
m/z (amu)	Fragment no.	Assignment
522	I	
471	II	*m/z* 568 minus H_2O gives *m/z* 549 which on further loss of phenyl radical gives *m/z* 471
425	III	
406	IV	*m/z* 568 minus —CH(COOH)CH_2CH_2Ph
389/390	V	
378	VI	
342	VII	*m/z* 406 minus H_2O (*m/z* 387), *m/z* 387 minus —COOH

Table 1 (*Continued*)

m/z (amu)	Fragment no.	Assignment
292	VIII	
246	IX	
202	X	

no appreciable changes in the spectrum of CL as compared to lisinopril [1].

^{13}C NMR spectrum of CL consists of following peaks: C^1 (177.78), C^2 (61.87), C^3 (24.35), C^4 (20.9), C^5 (46.25), C^6 (166.18), C^7 (61.87), C^8 (30.65), C^9 (25.17), C^{10} (30.47), C^{11} (47.82), C^{12} (172.74), C^{13} (58.50), C^{14} (31.43), C^{15} (29.00), C^{16} (140.54), C^{17} (128.42), C^{18} (128.71), C^{19} (126.45), C^{20} (128.71), C^{21} (128.42), C^{22} (172.74), C^{23} (61.87), C^{24} (31.89), C^{25} (29.28), C^{26} (140.35), C^{27} (128.42), C^{28} (128.71), C^{29} (126.45), C^{30} (128.71), and C^{31} (128.42). CL impurity displays significant changes in the values of certain carbon atoms that are close to C^{11} carbon bearing the –CH(COOH)CH_2CH_2Ph group. The difference is observed for C^{11} resonance, which is downfield by about 8 ppm indicating the presence of the electron withdrawing –CH(COOH)CH_2CH_2Ph group. Moreover, changes are observed for the adjacent carbons (C^{10} and C^9) as a consequence of their interaction with C^{11} thus corroborating the influence of the –CH(COOH)CH_2CH_2Ph group. Furthermore, the analysis of DEPT 135 carbon NMR spectrum indicates the presence of 11 CH_2 groups confirming the presence of additional two CH_2 group as compared to lisinorpil molecule. The ^{1}H, ^{13}C NMR spectra and data from DEPT experiment revealed the presence of 11 methylenes (C^3, C^4, C^5, C^8, C^9, C^{10}, C^{11}, C^{14}, C^{15}, C^{24}, and C^{25}), 10 sp^2-hybridized methines (C^{17}, C^{18}, C^{19}, C^{20}, C^{21}, C^{27}, C^{28}, C^{29}, C^{30}, and C^{31}), 4 sp^3-hybridized methines (C^2, C^7, C^{13}, and C^{23}) and 6 quaternary sp^2-carbons (C^1, C^6, C^{12}, C^{16}, C^{22}, and C^{26}). COSY spectrum of the CL impurity also supported the above assignments. Because of minor peaks were not observed in the NMR spectra, we conclude that the CL impurity was obtained in a stereochemically homogenous form. However, absolute configuration of the impurity could not be established.

The FT-IR spectrum of CL impurity shows a broad signal at 3422 cm^{-1} unlike the splitted pattern for lisinopril owing to –OH stretching vibrations. Peaks corresponding to stretching and bending vibrations of $-NH_2$ in lisinopril are absent in CL impurity suggesting that its amine function is derivatised. However, other peaks of lisinopril remain almost unaffected after the formation of this impurity [1].

It is evident from the spectroscopic data that the CL impurity is formed during the synthesis of lisinopril and this can be explained on the basis of the incomplete protection (trifluoroacetylation) of the starting material 2,6-diamino-hexanoic acid during the first step of the synthesis. The unprotected 2,6-

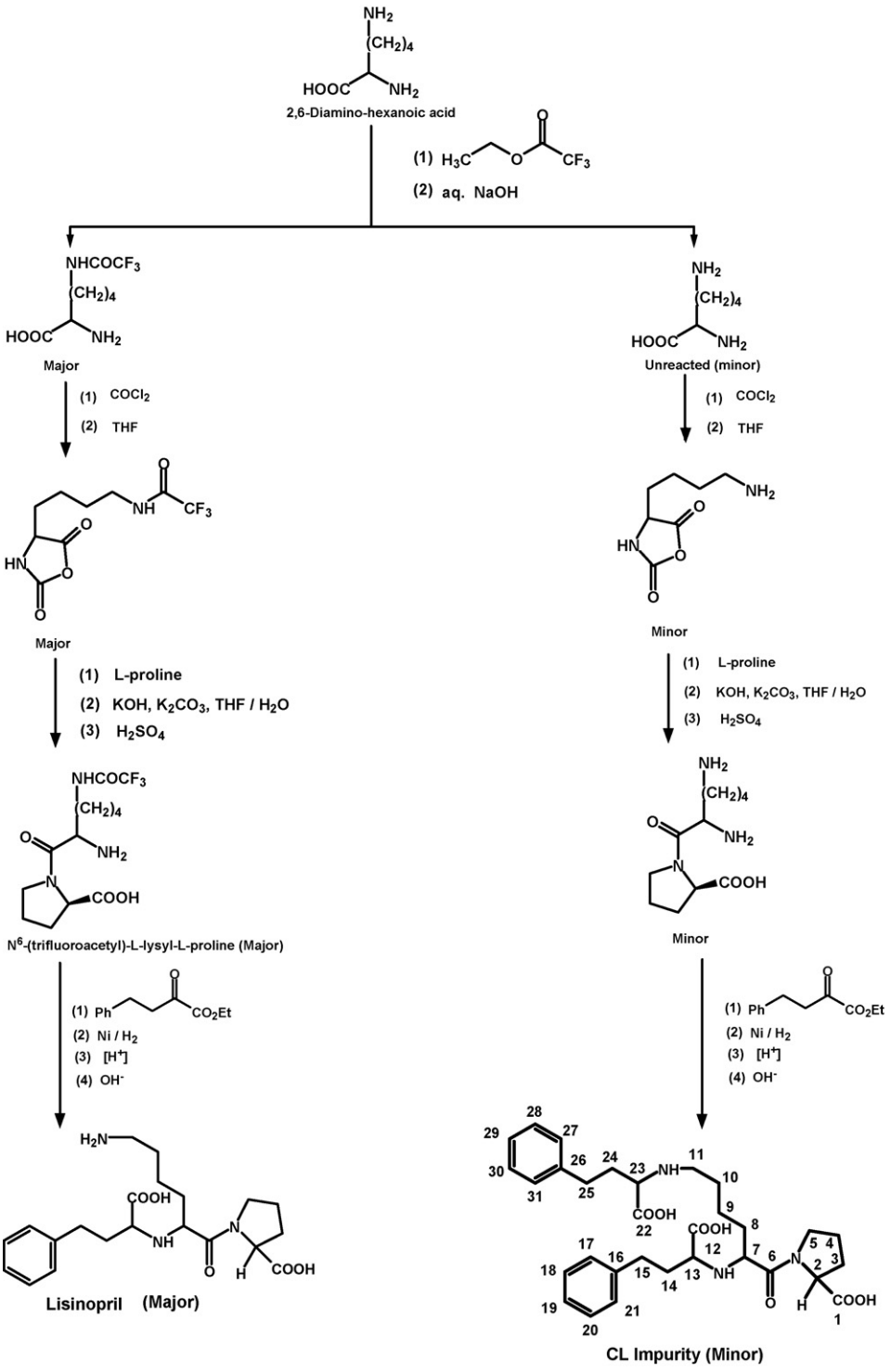


Fig. 2. Proposed formation pathway for lisinopril CL impurity.

diamino-hexanoic acid then undergoes subsequent steps of the synthetic pathway and thus results in the formation of this impurity as a minor product (Fig. 2).

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