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Identification and Synthesis of Potential Impurities of Losartan Potassium - A Non-peptide Angiotensinogen II Receptor Antagonist†

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In the process for the preparation of **1**, identified four potential impurities ranging from 0.05-0.15 % were detected in HPLC. Based on the mass spectral data obtained by LC-MS analysis structure of these impurities were characterized as potassium salt of 2-*n*-butyl-5-chloro-4-hydroxymethyl-1-[(2'-(2H-tetrazole-5-yl)-1,1'-biphenyl-4-yl)methyl]-1H-imidazol (**Imp-A**, Isolosartan potassium), potassium salt of 5-(4'-methyl-1,1'-biphenyl-2yl)-2H-tetrazole (**Imp-B**, biphenyl tetrazole analogue of **1**), 2-butyl-4-chloro-1-[[2'-(1H-tetrazol-5-yl)-1,1'-biphenyl)-4-yl]methyl]-1H-imidazole-5-ethanoate (**Imp-C**, ester analogue of **1**) and 2-*n*-butyl-4-chloro-1-[[2'-(1H-tetrazol-5-yl)-1,1'-biphenyl)-4-yl]-5-triphenyl methoxy methyl-1H-imidazole (**Imp-D**, *o*-trityl losartan). These impurities were synthesized from an unambiguous route, confirmed the structure by collecting various spectral data and co-injected with **1**, retention time is matching with expected impurities. To our knowledge the impurities **A-D** were not reported as process impurities elsewhere.

Key Words: Synthesis, Impurities, Identification, Losartan.

INTRODUCTION

Losartan potassium (**1**, HYZAAR, COZAAR®), the first of a new class of antihypertensives, is non-peptide angiotensin II receptor (type AT_1) antagonist. Losartan had been launched for hypertension in several major markets, alone or in combination with agents such as hydrochlorothiazide (HCTZ)[1]. Losartan was also being investigated as a potential treatment for diabetic nephropathy in Japan and for anxiety disorder and glaucoma in the US. Losartan potassium chemically known as 2-butyl-4-chloro-1[*p*-(*o*-1H-tetrazol-5-ylphenyl) benzyl] imidazole-5-methanol monopotassium salt (**1**).

The reported literature studies reveal the preparation of **1** in different methods[2,3]. It has less of an effect in black patients, similar to ACE inhibitors, such as captopril (Capoten), enalapril (Vasotec) and lisinopril (Zestril).

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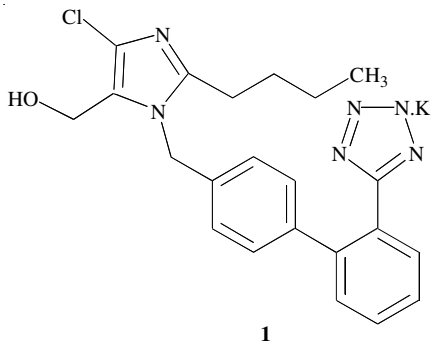


1

In this paper, the synthesis and characterization of impurities of **1** have been reported.

EXPERIMENTAL

The investigated samples of **1** bulk material (B.No. LOS-Pharma) and crude samples (B.No LOS-Crude) were obtained from Dr. Reddy's Lab. Ltd., Bulk Actives-III, Hyderabad, India.

High performance liquid chromatography: The synthesized impurities were used to validate the liquid chromatography method below. The validation performed was carried out in line with the International Conference on Harmonization (ICH) requirements.

A Waters 717 plus auto sampler equipped with a water 2996 photo diode array UV detector was used. An in-house LC method was developed for the analysis of Losartan and its intermediates, were a C18 column (Nucleosil C18 250 × 4 × 5 mm i.d., Flexit Jour Laboratories Pvt Ltd.) with a mobile phase consisting of a mixture of 0.02 M KH_2PO_4 and acetonitrile in the ratio of 750:250 (v/v) (pH 2.5) was used with UV detection 220 nm at flow rate of 1.0 mL/min for the resolution of all impurities. The data was recorded using Waters Empower software. This LC method was able to detect these impurities, which ranged from 0.05 to 0.15 % in parent compound.

Mass spectrometry: Mass spectra were obtained using an AB 4000 Trap LCMS/MS mass spectrometer with energy set to 4500 V. The samples were introduced *via* HPLC pump Agilent 1100 Series Auto sampler. The source temperature maintained at 250 and 400°C, respectively.

NMR spectroscopy: Characterization of impurities was achieved by using NMR Varian unity plus 200/400 MHz instrument. The samples were dissolved in DMSO-d_6 or $CDCI_3$, containing 0.03 % v/v TMS in 5-mm NMR tube. TMS was used as an internal reference standard. All the spectra were recorded at 25°C and no shift relaxation agents were employed. The ^{1}H NMR chemical shift values were reported on the δ scale in ppm, relative to TMS (δ = 0.00), respectively.

FT-IR spectroscopy: The IR spectrum of the impurities **A, B, C** and **D** were detected in the solid state as KBr dispersion using Perkin Elmer 1650 FT IR spectrophotometer and reported in cm^{-1}.

Melting point determination: Melting points of impurities **A** to **D** were determined on a LABINDIA visual melting range apparatus (MR-VIS).

RESULTS AND DISCUSSION

Detection of impurities A, B, C and D: A typical analytical LC chromatogram of a production batch of Losartan potassium **1** bulk drug recorded using the LC method is shown in Fig. 1. The target impurities under study were marked as **Imp-A, Imp-B, Imp-C** and **Imp-D**. These impurities were synthesized by synthetic methods.

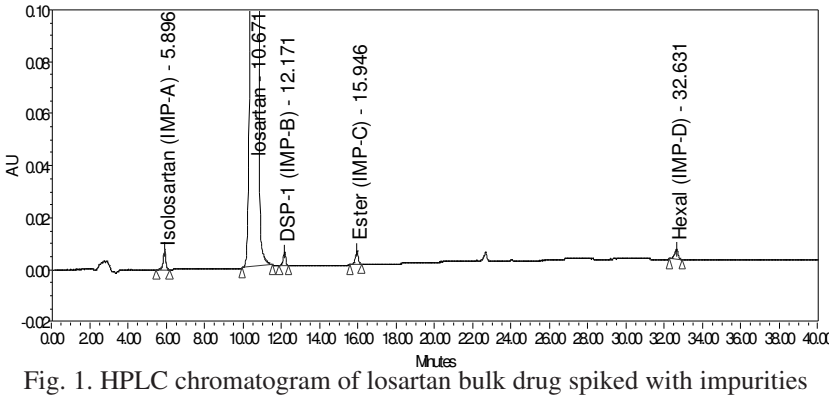
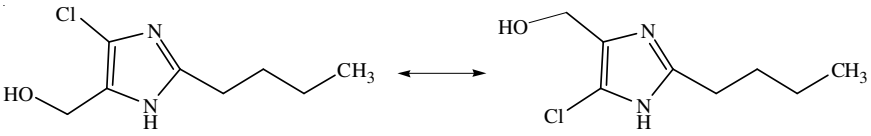


Fig. 1. HPLC chromatogram of losartan bulk drug spiked with impurities

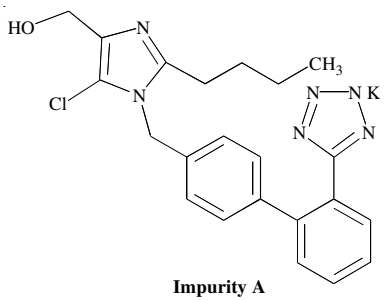
Identification of Impurities A, B, C and D

Potassium salt of 2-*n*-butyl-5-chloro-4-hydroxymethyl-1-[(2'-(2H-tetrazole-5-yl)-1,1'-biphenyl-4-yl)methyl]-1H-imidazole (5): **Isolosartan potassium (Imp-A):** In the preparation of trityl losartan (**5**), imidazole derivative was reacted with N-(triphenylmethyl)-5-[4-(bromomethyl)-1,1'-biphenyl-2'-yl]tetrazole in presence of alkali solution in biphasic system. Due to delocalization of lone pair of electrons it exists in two regio isomeric forms.



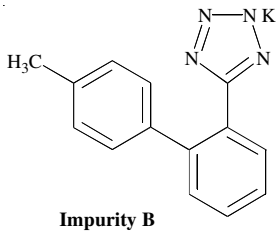
2 **3**

The compound **3** react with 4'-bromomethyl-2-(triphenyl methyl-2H-tetrazole-5yl)-1,1'-biphenyl (**4**) at higher temperatures and carried up to the final stage, with levels of less than 0.15 % in the drug substance.



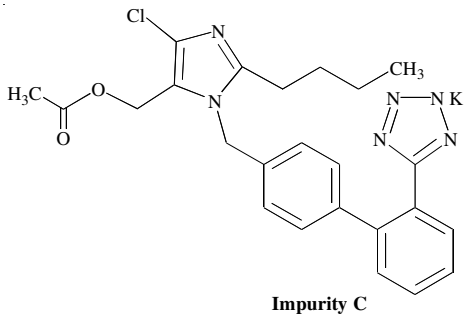
Impurity A

Potassium salt of 5-(4'-methyl-1,1'-biphenyl-2yl)-2H-tetrazole (6) (Imp-B): The said impurity is obtained from one of the unreacted starting material N-(triphenylmethyl)-5-[4 (methyl)1,1'-biphenyl-2'-yl]tetrazole at the initial stages and is carried up to the drug substance as its potassium salt. This impurity was seen at 0.10 % levels by HPLC.



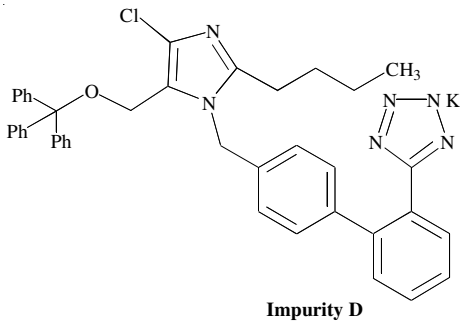
Impurity B

2-Butyl-4-chloro-1-[[2'-(1H-tetrazol-5-yl)-1,1'-biphenyl)-4-yl]methyl]-1H-imidazole-5-ethanoate (Imp-C): The said impurity is formed in the final stage, during the product isolation in acetone. The alcohol group will be converted to an ester derivative and this impurity was seen at 0.05 % levels by HPLC.



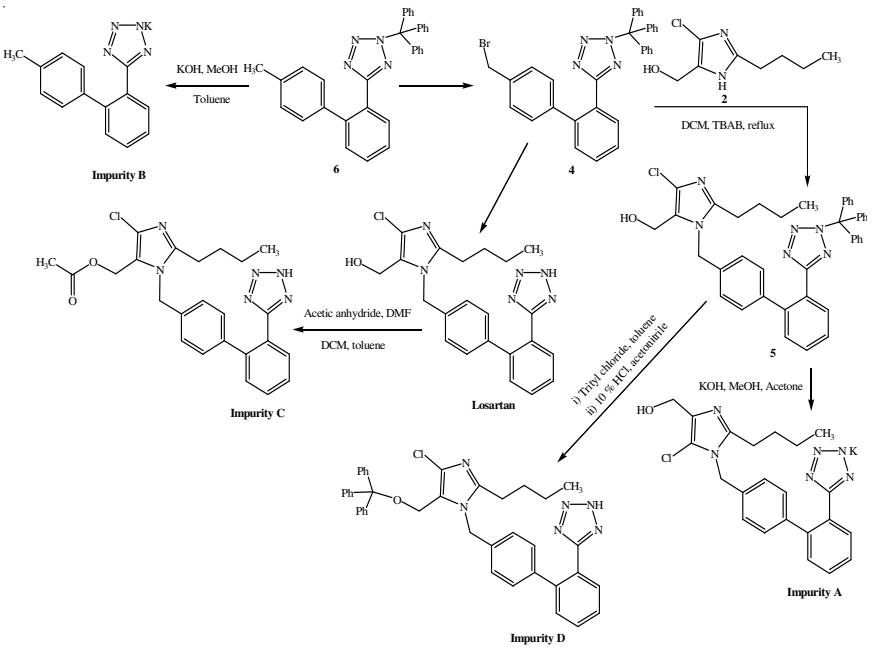
Impurity C

2-*n*-Butyl-4-chloro-1-[[2'-(1H-tetrazol-5-yl)-1,1'-biphenyl)-4-yl]-5-triphenyl methoxy methyl-1H-imidazole (Imp-D): The said impurity is formed during the preparation of losartan potassium, the trityl losartan is deprotected with potassium hydroxide in methanol to give the biproduct triphenyl methyl ether. The biproduct reacts with alcohol group of imidazole to give this impurity and it was seen at levels greater than 0.05 % in the drug substance.



Impurity D

Synthesis of impurities: Losartan potassium and the impurities were synthesized as per the **Scheme-I**. Significant quantities were required for confirmation of structure, validation and use as an analytical standard for further analysis.



Scheme-I Synthesis of losartan and impurities

Impurity A: Potassium salt of 2-*n*-butyl-5-chloro-4-hydroxymethyl-1-[(2'-(2H-tetrazole-5-yl)-1,1'-biphenyl-4-yl) methyl]-1H-imidazole prepared by condensation of N-(triphenylmethyl)-5-[4 (bromo methyl)-1,1'-biphenyl-2'-yl]tetrazole (**4**) with 2-butyl-4-chloro-5-hydroxymethyl imidazole (**2**) in the presence of phase transfer catalyst in dichloromethane at reflux temperature.

Potassium salt of 2-*n*-butyl-5-chloro-4-hydroxymethyl-1-[(2'-(2H-tetrazole-5-yl)-1,1'-biphenyl-4-yl) methyl]-1H-imidazole prepared by reacting 2-*n*-butyl-5-chloro-4-hydroxymethyl-1-[(2'-(2-triphenylmethyl tetrazol-5-yl)-1,1'-biphenyl-4-l)methyl]-1H-imidazole with potassium hydroxide in methanol and isolating the required compound from acetone.

A mixture of N-(triphenylmethyl)-5-[4 (bromomethyl)1,1^{1}-biphenyl-2^{1}-yl]tetrazole (**4**, 4.5 g, 0.109 mol), sodium hydroxide (50.0 g, 0.089 mol), tetra butyl ammonium bromide (3.5 g, 0.01 mol) and water (100.0 mL) were stirred at 25-30°C for 15 min. 2-Butyl-4-chloro-5-hydroxymethyl imidazole (**2**, 17.0 g, 0.09 mol) and dichloromethane (300 mL) were added to the reaction mass and the contents were heated to 35-40°C, stirred for 35 h. The separated organic layer was washed with water (3 × 50.0 mL) and concentrated under reduced pressure. The resulted compound **5** was isolated and recrystallized from acetonitrile and ether (40 g, 67 %).

A mixture of 2-*n*-butyl-5-chloro-4-hydroxymethyl-1-[(2'-(2-triphenyl-methyl tetrazol-5-yl)-1,1'-biphenyl-4-yl) methyl]-1H- imidazole (**5**, 40 g), potassium hydroxide (3.4 g) and methanol (250 mL) were stirred at 60-65°C for 4-5 h, the same contents were stirred for 2 h at 0-5°C, the separated bi product was filtered and washed with methanol (25 mL). The filtrate was concentrated under reduced pressure, acetone (40 mL) was added to the residue and distilled completely under reduced pressure. The residue was dissolved in acetone (180 mL) and stirred at 40-45°C for 1 h followed by stirred at 0-5°C for 1-1.5 h. The reaction mass was concentrated, isolated from the residue and characterized as **impurity-A** (25 g, 90 % yield, 93.83 % purity).

^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 0.82 (t, 3H, CH_3), 1.33 (m, 2H, CH_2), 1.50 (m, 2H, CH_2), 2.51 (t, 2H, CH_2), 4.32 (s, 2H, CH_2), 4.91 (s, 1H, CH), 5.20 (t, 2H, CH_2), 7.05 (d, 2H, CH_2), 7.11 (d, 2H, CH_2), 7.56 (m, 3H, CH_3) and 7.72 (t, 1H, CH); IR (KBr, cm^{-1}): 2961, 2931 and 2863; MS (m/z): 423 M^+ (with out potassium) corresponding to the molecular formula $C_{22}H_{23}ClN_{26}O$, m.p. 185-188°C.

Impurity B: Potassium salt of 5-(4'-methyl-1,1'-biphenyl-2yl)-2H-tetrazole prepared by reacting N-(triphenylmethyl)-5-[4 (methyl)1,1'-biphenyl-2′-yl]tetrazole with potassium hydroxide in methanol and isolating the required compound from acetone.

A solution of potassium hydroxide (2.6 g, 0.043 mol) in water (20 mL) was added to the mixture of 4'-methyl-2-(triphenyl methyl-2H-tetrazole-5-yl)-1,1'-biphenyl (**6**, 0.041 mol) and methanol (200 mL) were heated to reflux temperature till to the completion of the reaction. The methanol was distilled from the reaction mass under reduced pressure, water (60 mL) was added to the crude, the filtered biproduct was washed with water (24

mL). The filtrate was washed with toluene (2 × 20 mL), the separated aqueous layer was distilled completely under reduced pressure. Finally water traces were removed by azeotropic distillation with toluene. The crude was dissolved in methanol (20 mL) and carbon (0.88 g) was added stirred for 0.5 h. The reaction mass was filtered through high-flow bed and washed with methanol (16.0 mL), filtrate was concentrated under reduced pressure. The obtained solid was dried at 80-90°C and characterized as **impurity-B** (5.6 g, yield 48.8 %, purity 99.86 %).

^{1}H NMR ($CDCl_3$ + DMSO-d_6, 200 MHz, δ ppm): 2.32 (s, 3H, CH_3), 7.05 (d, 2H, Ar-H), 7.11 (d, 2H, Ar-H), 7.30 (m, 3H, Ar-H), 7.73 (d, 1H, Ar-H); IR (KBr, cm^{-1}) : 3027, 3055, 2921, 1506 and 1458; MS (m/z) : 237 M^+ (with out potassium) corresponding to the molecular formula $C_{14}H_{12}N_4$, m.p. 300°C (dec).

Impurity C: 2-Butyl-4-chloro-1-[[2'-(1H-tetrazol-5-yl)-1,1'-biphenyl)-4-yl]methyl]-1H-imidazole-5-ethanoate prepared by reacting the alcohol group on imidazole ring of losartan with acetic anhydride in presence of dimethyl formamide in dichloromethane and isolating the required compound from toluene.

A mixture of 2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2'-(2-H tetrazol-5-yl) 1,1'-biphenyl-4-yl)methyl]-1H-imidazole (Losartan, 40 g, 0.094 mol), dichloromethane (400 mL), acetic anhydride (25 mL) and dimethylformamide (2.5 mL) were heated to reflux till to the completion of the reaction. Water (200 mL) was added to the reaction mass at 25-35°C and pH was adjusted to 8.0-8.5 by using caustic lye (20 mL). The separated aqueous layer was extracted with dichloromethane (100 mL), organic layer was separated, concentrated, the compound was isolated from toluene and characterized as **impurity-C** (27 g, yield 61.4 %, purity 98.52 %).

^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 0.82 (t, 3H, CH_3), 1.33 (m, 2H, CH_2), 1.52 (m, 2H, CH_2), 1.85 (s, 3H, CH_3), 2.50 (t, 2H, CH_2), 4.85 (s, 2H, CH_2), 5.22 (s, 2H, CH_2), 6.85 (d, 2H, Ar-H), 7.19 (d, 2H, Ar-H), 7.43 (d, 1H,Ar-H), 7.62 (m, 2H, Ar-H), 8.06 (d, 1H, Ar-H); IR (KBr, cm^{-1}): 3441,1737; MS (m/z): 465.6 M^+, m.p. 160-162°C.

Impurity D: 2-*n*-Butyl-4-chloro-1-[[2'-(1H-tetrazol-5-yl)-1,1'-biphenyl)-4-yl]-5-triphenyl methoxy methyl-1H-imidazole prepared by reacting N-trityl losartan (**5**) with trityl chloride in toluene followed by reacting with 10 % hydrochloric acid in acetonitrile.

2-*n*-Butyl-4-chloro-5-hydroxymethyl-1-[(2'-(2-triphenylmethyl tetrazol-5-yl) 1,1'-biphenyl-4-yl) methyl]-1H-imidazole (**5**, 25 g, 0.037 mol), triethylamine (25 g, 0.247 mol) and toluene (500.0 mL) were heated to 85°C. A solution of tritylchloride (15 g, 0.053 mol) in toluene (50 mL) was added slowly for 0.5 h and the contents were stirred for 5 h at 80-85°C. Solvent was distilled off from the reaction mass added acetonitrile (100

mL), stirred for 20 min and the separated solid was filtered. The obtained solid was taken in acetonitrile (400 mL), 10 % hydrochloric acid was added and stirred for 2 h at 25-35°C. The separated solid was filtered from the reaction mass and washed with acetonitrile (20.0 mL), dried the solid at 55-60°C and characterized as **impurity-D**.

^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 0.82 (t, 3H, CH_3), 1.27 (m, 2H, CH_2), 1.52 (m, 2H, CH_2), 2.56 (t, 2H, CH_2), 3.88 (s, 2H, CH_2), 5.02 (s, 2H, CH_2), 6.79 (d, 1H, CH), 6.79 (d, 1H, CH), 6.99 (d, 1H, CH), 6.99 (d, 1H, CH), 6.90-7.80 (m, 4H, Ar-H); IR (KBr, cm^{-1}): 3055, 3033, 2960, 2874, 1619, 1492, 1448, 1345, 1228, 1057, 753 and 705; MS (m/z): 665 M^+, m.p. 170-2°C.

Conclusion

The results from various physio-chemical techniques confirm the molecular structures of four impurities of losartan potassium, based on the analytical and the sequence of the preparations, the structures of the four impurities were established.

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