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Isolation, Identification and Characterization of Process Related Impurities in Losartan Potassium Drug Substance

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Two impurities in losartan drug substance at level *ca.* 0.5 % is detected by RP-HPLC. These impurities are isolated from the impurity enriched sample using reversed phase preparative HPLC. Based on the spectral data (IR, NMR and MS) both the impurities are characterized as Positional Dimer of Losartan having same molecular weight.

Key Words: Impurity, Spectroscopy, Isolation, Identification, Characterization, Separation.

INTRODUCTION

During the analysis of different laboratory batches of losartan, two impurities are detected consistently in almost all batches, whose area percentage ranged from *ca.* 2-4 % by RP-HPLC method. A comprehensive study has been done to isolate and characterize these impurities by spectroscopic techniques[1-4]. The impurity profile study has to be carried out from any final drug substances to identify and characterize the unknown impurities. The requirement of identification and characterization of the impurity in the final drug substances is extremely necessary to meet the stringent regulatory or customer requirements[5].

EXPERIMENTAL

The investigated samples of losartan drug substance and impurity enriched samples were obtained from Research Laboratory of Alembic Research Centre, Alembic limited, Vadodara, Gujarat (India).

High performance liquid chromatography (analytical): A Waters Alliance separation module equipped with UV detector was used. Kromasil 100-5C_{18} column having dimensions 250 × 4.6 mm i.d. and 5 µm particle size was used for analysis. The column was maintained at 35 °C and the eluent was monitored at 220 nm and the data was recorded using Empower-II software. Mobile phase A (0.1 % phosphoric acid in water) and mobile phase B (acetonitrile) is used for the separation. The diluent was mobile phase A:B::50:50.

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High performance liquid chromatography (preparative): A Waters LC-2000 separation module equipped with 2487 UV detector and system controller were used. Luna C-18 column having dimensions 250 × 50 mm i.d and 15 µm particle size was used for the impurity isolation work. A 10 mL injection loop was used and the eluent was monitored at 220 nm and the data was recorded using Millenium software. About 80 mg of the sample was dissolved in a methanol and loaded on preparative column. Mixture of trifluoracetic acid in water and acetonitrile is used as mobile phase. Flow rate was adjusted to 35 mL/min and the eluent was monitored at 220 nm.

Mass spectrometry: Mass spectra were obtained using AB Sciex API-2000 LC/M-MS mass spectrometer in negative ion ionization mode.

NMR spectroscopy: NMR measurement (^{1}H and ^{13}C) were performed on a Bruker Avance 300 MHz instrument at 25 °C in deuterated dimethyl sulfoxide and chloroform and the chemical shift values were reported on the d scale relative to TMS.

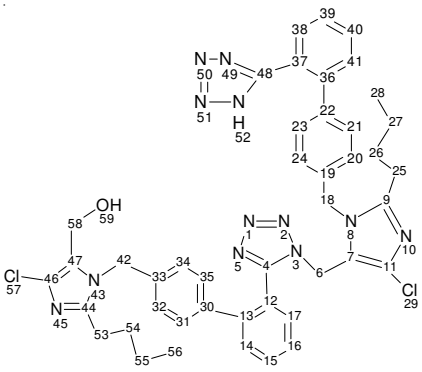
RESULTS AND DISCUSSION

Detection of impurity: A typical analytical LC chromatogram of a laboratory was recorded using LC method. The target impurity under study is eluting at retention time 21.8 and 24.4 min, respectively. Both the impurities are isolated from enriched impurity sample of losartan potassium on preparative LC. Attempts were also made to synthesize the impurity.

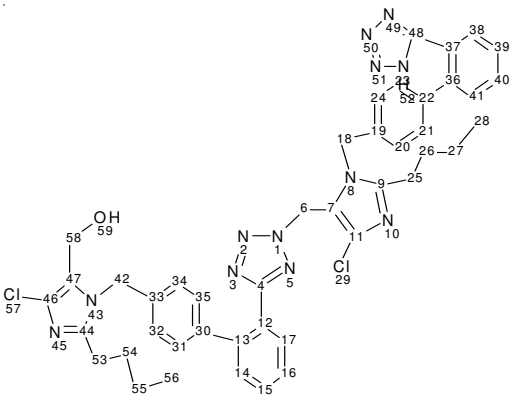
Isolation of impurity by preparative HPLC: A reversed phase solvent system discussed under section 2.3 was used for the isolation of impurities. The enriched impurity sample was loaded on the preparative column and the fraction collected were pooled together and analyzed using analytical HPLC to confirm the RRT and purity of the isolated impurity[6]. The pooled fraction was concentrated under high vacuum Buchi Rotavapour R-124 to distill out the acetonitrile solvent. The remaining aqueous layer is subjected to Lyophilization in Vertis 6L lyophilizer to get a pure compound. The chromatographic purity of the impurity is tested by analytical LC separately before and after concentration[6]. The isolated fluffy solid mass used for spectral studies.

Structure elucidation of dimeric impurity: The EI mass spectrum of both the impurities in negative ionization mode exhibits a molecular ion peak at m/z 825.2 atomic mass unit (amu) which was 403 amu more than losartan which shows both the impurities are dimer. Both the impurities (Structures **1** and **2**) are formed by condensation of two monomers (molecular weight 422) by elimination of water molecule[2].

Based on the above spectral data (Tables 1 and 2) the molecular formula of dimeric impurities was confirmed as and the corresponding structure was characterized as [2-butyl-1-[[2'[1-[[2-butyl-4-chloro-1-[[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-imidazol-5-yl]methyl]-1*H*-tetrazol-5-yl]biphenyl-4-yl]methyl-4-



Structure **1**



Structure **2**

TABLE-1
CHEMICAL SHIFT ASSIGNMENT BY ^{1}H NMR OF IMPURITY-1

Functional group	Number of protons	Chemical shift	Multiplicity
28,56 –CH_3	6	0.72-0.74	Triplet
55,25 –CH_2	4	1.41-1.21	Multiplet
54,26 –CH_2	4	1.37-1.41	Multiplet
53,25 –CH_2	4	2.37-2.42	Triplet
58 –CH_2	2	4.27	Singlet
6 –CH_2	2	4.81	Singlet
42 –CH_2	2	4.94	Singlet
18 –CH_2	2	5.19	Singlet
Ar-CH	16	6.57-7.75	Multiplet

TABLE-2
CHEMICAL SHIFT ASSIGNMENT BY ^{1}H NMR OF IMPURITY-2

Functional group	Number of protons	Chemical shift	Multiplicity
28,56 –CH_3	6	0.79-0.94	Triplet
57,27 –CH_2	4	1.53-1.58	Multiplet
54,26 –CH_2	4	1.70-1.75	Multiplet
53,25 – CH_2	4	2.64-2.70	Triplet
58 –CH_2	2	5.21-5.22	Singlet
6,42 –CH_2	2	5.65	Singlet
18 –CH_2	2	6.37-7.99	Multiplet
Ar-CH	16	6.57-7.75	Multiplet
Ar-CH	16	6.57-7.75	Multiplet

chloro-1*H*-imidazol-5-yl]methanol and [2-butyl-1-[[2'[2-[[2-butyl-4-chloro-1-[[2'-(1*H*-tetrazol-5-yl) biphenyl-4-yl]methyl]-1*H*-imidazol-5-yl]methyl]-2*H*-tetrazol-5-yl]biphenyl-4-yl]methyl-4-chloro-1*H*-imidazol-5-yl]methanol.

The HPLC purity of lyophilized impurity1 is found to be 91.67 % and impurity 2 is 87.50 %, respectively by area normalization.

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