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Identification, Characterization and Synthesis of Process Related Unknown Impurity in Cetirizine Dihydrochloride

JAGADEESH NARKEDIMILLI[1,2,*], RAVINDRA KUMAR YALAVARTHI[1], SANDEEP MOHANTY[1], KIRTI KUMAR JAIN[1], SANDEEP REDDY GADDAM[1] and JAYASHREE ANIREDDY[2]

[1]Dr. Reddy's Laboratories Limited, API Plant, Bollaram-III, Plot No 116, IDA Bollaram, Medak District, Hyderabad-502 325, India
[2]Centre for Chemical Sciences and Technology, Institute of Science and Technology, Jawaharlal Nehru Technological University Hyderabad, Kukatpally, Hyderabad-500 085, India

*Corresponding author: E-mail: jagadeeshn@drreddys.com; nark_012@yahoo.com

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Identification, isolation, structural characterization and synthesis of process generated unknown impurity in cetirizine dihydrochloride is described in this work. Process-related unknown impurity was detected in the range of 0.1-0.15 % in cetirizine dihydrochloride by a gradient HPLC and LC-MS methods. Based on LC-ESI/MS^n study, the chemical structure of impurity was presumed as 2-(2-(4-(2-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)ethyl)piperazin-1-yl)ethoxy)acetic acid. The impurity was isolated by preparative HPLC, the complete spectral analysis, MS, 1D NMR (^{1}H and ^{13}C), 2D NMR (COSY, HSQC and HMBC) confirmed the proposed chemical structure of impurity.

Keywords: Synthesis, Liquid chromatography-mass spectrometry, Cetirizine.

INTRODUCTION

Cetirizine dihydrochloride (Fig. 1) is a second-generation antihistamine, well known for its antiallergic, spasmolytic and antihistaminic activity. The molecule has gone off patent in 2001 but still continues to enjoy the commercial success with several generic versions of the active ingredient in the markets across diverse geographies of the world. Though its active enantiomer levocetirizine was introduced later into the market for the treatment of the same health disorder. This molecule still remains popular by considering the increasing number of prescriptions worldwide. Cetirizine dihydrochloride (**1**) is sold as the racemate as Zyrtec in USA, Reactine in Canada and in India, is sold over-the-counter as brand-name "CTZ".

Although each of these drugs commands billions of U.S. dollars of annual sales worldwide the need for the overall assessment of manufacturing processes in terms of the final product quality is of paramount importance to the company. To achieve this, understanding of the overall manufacturing process, quality assessment of raw materials and reagents used in the process and detection, identification, quantification and control of single maximum unknown impurities at each and every step of the process is critical for delivering an active pharmaceutical ingredient (API) of high quality. In addition, to ensure a continuing supply of API for drug product, timely identification of key impurities is essential. A through literature survey [1-20] reveals a variety of synthetic methods, analytical methods, quantitative determination of cetirizine and its related substances, mass spectrophotometric methods and also simultaneous methods for the determination of cetirizine in pharmaceuticals.

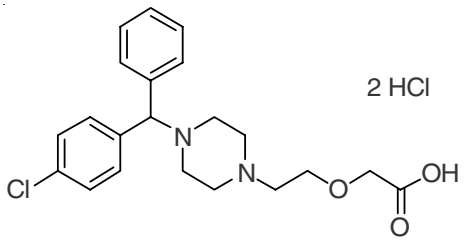

Fig. 1. Structure of cetirizine dihydrochloride (**1**)

In principle, the basic methods of carrying out the synthetic steps described in Fig. 2. Production of (**1**) exploits commercially available key starting material (**5**) and 2-chloroethan-1-ol as starting materials. During the HPLC analysis of laboratory batches of cetirizine dihydrochloride, revealed the presence of an unknown impurity which were between 0.1-0.15 %.

To commercialize an active pharmaceutical ingredient, it is mandatory for the manufacturer to identify and characterize all the unknown impurities. For impurities in new drug substances, according to International Conference on Harmonization

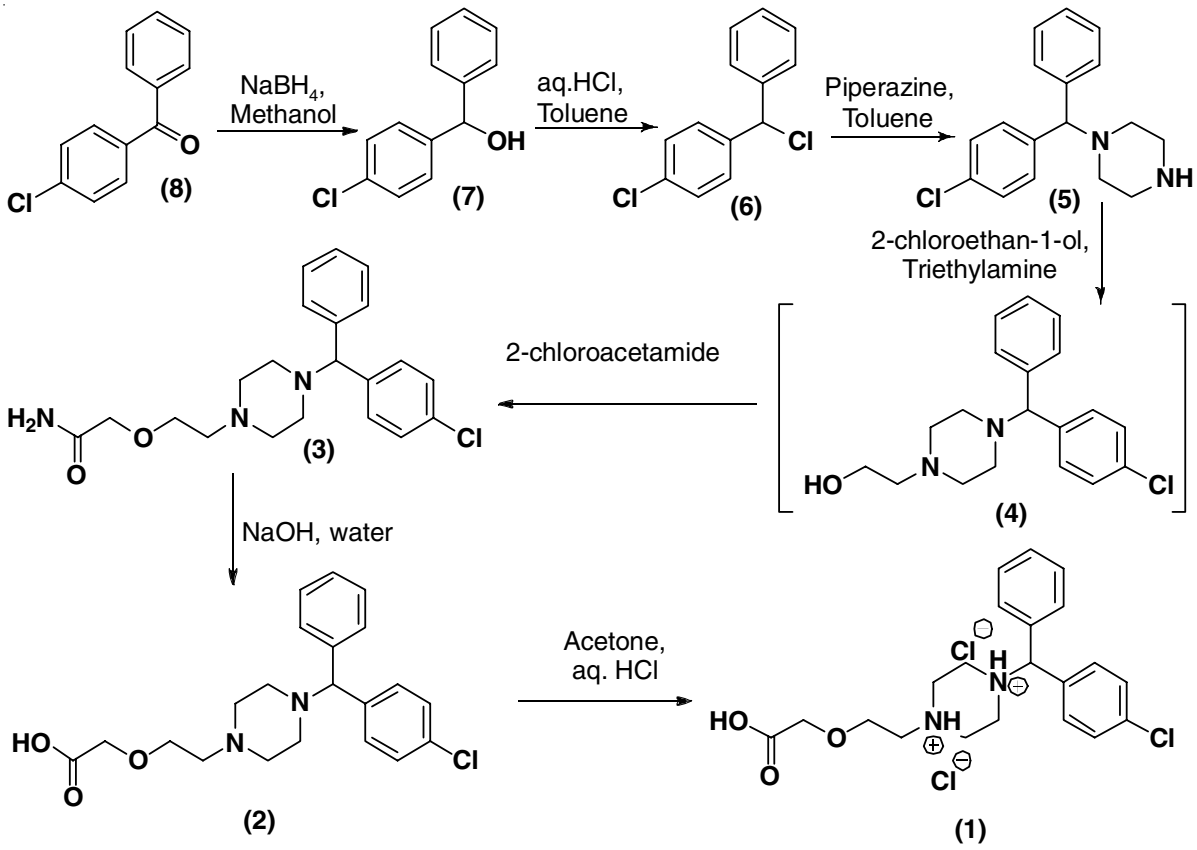

Fig. 2. Manufacturing process of cetirizine dihydrochloride (**1**)

(ICH) guidelines for a maximum daily dose ≤ 2 g/day of a drug substance, the reporting and identification thresholds are 0.05 and 0.10 %, respectively [21]. In this context, a comprehensive study was taken for identification and characterization of this impurity so that minimization at a level of NMT 0.1 % at the final product can be worked out for delivering an API of high quality.

To the best of our knowledge, impurity is novel and unknown. No earlier reports have been discussed on this impurity profile study of cetirizine dihydrochloride (**1**).

EXPERIMENTAL

The samples of cetirizine dihydrochloride were obtained from synthetic R&D laboratory of Dr. Reddy's Laboratories Ltd., CTO-I, Hyderabad, India. HPLC grade methanol and acetonitrile were obtained from Merck, Mumbai, India. AR grade monobasic sodium phosphate salt, tetrabutyl ammonium hydrogen sulfate and sodium hydroxide were obtained from Merck, Mumbai, India. Dimethylsulphoxide-d_6 was purchased from Aldrich Chemical Co., USA. Water used for preparing mobile phase was purified using Millipore (MA 01821, USA) water purification system.

High performance liquid chromatography (Analytical): A Waters Model Alliance 2695 separation module (Waters Corporation, Milford, MA, USA) equipped with a waters 2998 photo diode array detector was used. Data was processed through Waters empower software. A gradient analytical method with Symmetry shield RP18 column, 250 × 4.6 mm i.d., 5 µm particle size (Waters corporation, Milford, MA, USA) with a mobile phase consisting of solvent A: dissolved 2 g of tetrabutyl ammonium hydrogen sulfate and 3 g of sodium phosphate monobasic in 1 L of water, adjust with 1 N sodium hydroxide to a pH of 2.8 ± 0.1 and solvent B: methanol. The gradient program employed a time gradient program of T (min)/%B (v/v): 0/42, 40/42, 68/80, 108/80, 110/42, 120/42 for the separations. Column temperature was maintained at 40 °C. Flow rate was kept at 1.2 mL min^{-1} and the column eluent was monitored at 232 nm.

Preparative liquid chromatography: An Agilent 1200 preparative chromatography system equipped with Agilent G1315D photo diode array UV detector, fraction collector model Agilent G1346B and Rheodyne Injector Model 2260A with 1.8 mL loop was used. A 250 × 20 mm i.d column packed with 10 µm YMC actus triart C18 (YMC Co. Ltd., Japan) was employed for separation with a mobile phase consisting of 0.1 % HCOOH (aq) as solvent A, Acetonitrile as solvent B. The gradient program employed a time gradient program of T (min)/% B (v/v) = 0/30, 12/60, 15/90, 20/90, 21/30, 25/30) was used with UV detection at 232 nm at a flow rate of 16.0 mL min^{-1}. The data was recorded using Agilent Chemstation software.

Liquid chromatography-mass spectrometry: An Agilent 1100 series LC system coupled to a triple quadrupole mass spectrometer (Agilent LC/MS/MS model 6410, Agilent Technologies Inc., Santa Clara, CA, USA) with electrospray ionization (ESI) source was used and ES ionization was done in positive mode. The ion source temperature was set at 300 °C and the ESI needle voltage was set at 3.0 kV. Nitrogen was used as the drying gas at a flow rate of 10 L min^{-1} and as the nebulizer gas at a pressure of 60 psi. For MS/MS studies,

nitrogen was used as the collision gas with collision energy of 50 eV. Symmetry shield RP18 column, 250 × 4.6mm i.d., 5 µm particle size (Waters Corporation, Milford, MA, USA) with a mobile phase consisting of 0.1 % HCOOH (Aq) as solvent A, methanol as solvent B. The gradient program employed a time gradient program of T (min)/% B (v/v): 0/42, 40/42, 68/80, 108/80, 110/42, 120/42 was used with UV detection at 232 nm at a flow rate of 1.0 mL min^{-1}. The column temperature was maintained at 40 °C. The cetirizine sample was prepared in diluent consisting of solvent A and solvent B (50: 50. v/v) at 1 mg mL^{-1} concentration and 10 µL of sample solution was injected in LC/MS/MS system.

The high resolution mass spectra were obtained from a Waters LCT Premier time of flight (TOF) mass spectrometer (Milford, USA) with ESI source. Resolution of the LC-TOF/MS was more than 5000. Leucine enkephalin ($C_{28}H_{37}N_5O_7$) was used as external lock-mass. The source block and desolvation temperatures were 90 and 180 °C, respectively. The nebulizer and desolvation gas (nitrogen) flows were 20 L h^{-1} and 450 L h^{-1}, respectively. The instrument parameters were used as capillary 3000 V, cone 25 V, extractor 2 V and MCP 2700 V. The acquisitions were done in scan mode. MS/TOF studies were performed in ESI positive mode for cetirizine and its isolated impurity. Data acquisition and processing were done using Mass Lynx V.4.0 software.

NMR spectroscopy: ^1H, ^{13}C and two dimensional (2D) NMR experiments were performed on 500 MHz Varian Unity Inova FT-NMR instrument in DMSO-d_6. The ^1H chemical shift values were reported on the δ scale in ppm, relative to TMS (δ = 0.00 ppm) and in the ^{13}C chemical shift values were reported on the δ scale in ppm, relative to DMSO-d_6 (δ = 39.5 ppm).

Synthesis of impurity: To a mixture alcohol compound 20 g and chloroform added thionyl chloride and refluxed for 6 h, after completion of the reaction mass evaporated to dryness to get 12 g of crude. The crude was refluxed for 4 h with 50 mL DMF, 10 g potassium carbonate and 10 g BOC piperazine. Reaction mass was poured into 100 mL water and added 10 mL of HCl. The mixture was maintained for 2 h at ambient temperature, pH was adjusted with NaOH solution to basic and extracted with 100 mL toluene. The mixture of above toluene layer, triethylamine and 2-chloro ethanol refluxed for 8 h. Reaction was monitored by TLC. After completion of the reaction organic layer was evaporated to get crude material 5 g. To the above material with 25 mL DMF added 2 g sodium hydride slowly at below 10 °C. Reaction was maintained up to completion and quenched with 30 mL water. To the reaction mixture added NaOH and refluxed for 6 h for completion, pH was adjusted to 3 with acetic acid, extracted with dichloro methane and evaporated to get desired compound (Fig. 3). The synthesized impurity was analyzed by spiking using HPLC to check their retention times. Also the purity of the compound was analyzed by HPLC and found as 86 %.

RESULTS AND DISCUSSION

Detection of impurity: A typical HPLC chromatogram of a laboratory batch of cetirizine dihydrochloride (**1**) was recorded. One unknown peak was identified in the chromatogram at a relative retention time of about 0.55 with respect to the cetirizine peak is shown in Fig. 4. The LC/ESI-MS compatible method was developed and used to detect the impurity. The knowledge about fragmentation pattern of impurity as compared with drug molecule could acquire structural information, the most probable molecular formula for cetirizine and its impurity, calculated with the help of elemental composition calculator, along with errors in ppm and are summarized in Table-1 obtained from MS/TOF.

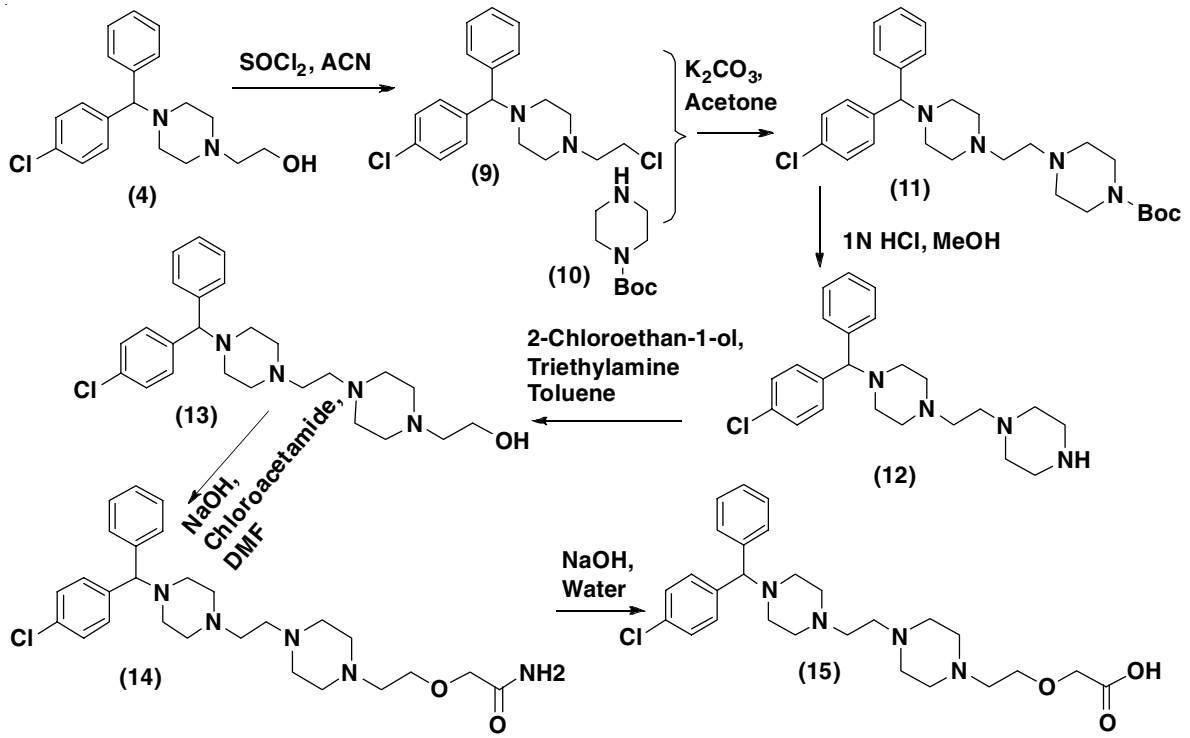

Fig. 3. Synthetic route to prepare impurity

TABLE-1
MS/TOF AND FRAGMENTATION DATA OF CETIRIZINE AND IMPURITY

Name	Accurate mass	Mass of the compound in ESI +ve mode (mass; error in ppm; DBE)	Accurate mass of major fragments in ESI +ve mode
Cetirizine	388.1554 ($C_{21}H_{25}N_2O_3Cl$)	389.1650 ($C_{21}H_{26}N_2O_3Cl$; 4.6; 9.5)	201
Impurity	500.2554 ($C_{27}H_{37}N_4O_3Cl$)	501.2606 ($C_{27}H_{38}N_4O_3Cl$; -5.2; 10.5)	201, 215, 299, 313

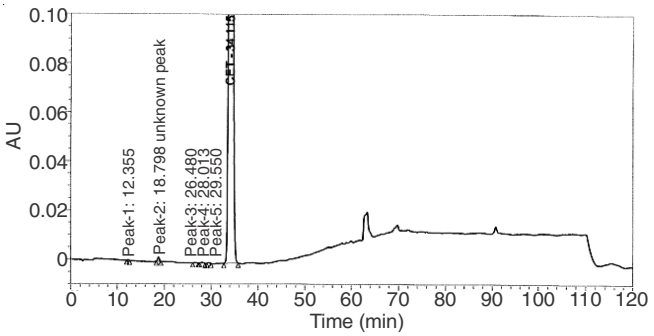

Fig. 4. Typical HPLC chromatogram of cetirizine dihydrochloride

Structural elucidation of impurity: The +ve ESI–MS spectrum of the impurity exhibits protonated molecular ion peak at *m/z* 501.2, the odd *m/z* number of $[M+H]^+$ ions suggest that impurity contains even number of nitrogen atoms (nitrogen rule). From these results the molecular ion of impurity was found to be at *m/z* 500. The mass difference between cetirizine and impurity was found to be 112 amu more. The ESI-MS-MS spectrum displayed daughter ions *m/z* at 313, 299, 215 and 201. The positive HR-MS spectrum showed protonated molecular ion at *m/z* 501.2606 corresponding to molecular formula $C_{27}H_{38}N_4O_3Cl$. When compared with the molecular formula of cetirizine, there was a difference of $C_6H_{12}N_2$. From the complete mass spectral data and plausible mass fragmentation pathway for cetirizine and impurity the difference can be rationalized in terms of the possibility of ethyl piperazine incorporation was observed. Based on this data, the chemical structure of impurity presumed as 2-(2-(4-(2-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)ethyl)piperazin-1-yl)ethoxy)acetic acid (Fig. 5). The proposed structure is further supported by the ^{1}H NMR, ^{13}C NMR and 2D NMR spectra.

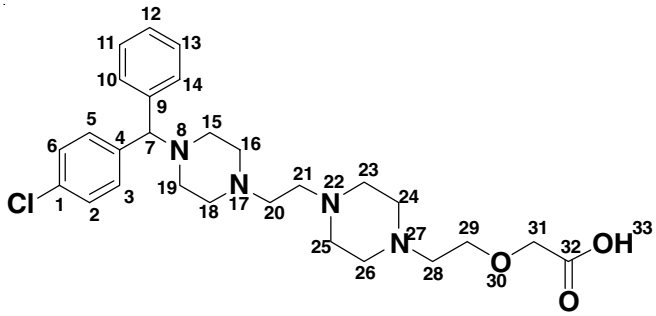

Fig. 5. Structure of unknown impurity, atom numbering used for NMR assignments

^{1}H NMR spectrum collected in DMSO-d_6 showed broad signals, addition of a few drops of deuterated water gave neat spectrum. The 2D data was collected by using D_2O added sample. The proton NMR spectrum showed signals at, δ: 2.9-3.6 (22H), 3.7-3.8 (2H), 4.1-4.2 (2H), 5.1-5.2 (1H), 7.3-7.5 (5H) and 7.5-7.7 (4H) ppm corresponding to thirty six protons.

The ^{1}H and ^{13}C NMR spectra of impurity and cetirizine [22] were compared and significant changes were observed. In ^{1}H NMR spectrum of impurity additional 12 protons in aliphatic region were observed. While in ^{13}C NMR, additional carbon signals can be seen, 6 additional methylene carbons were observed between 48.5 and 50.86 ppm. These signals confirmed the presence of ethyl piperazine moiety in the cetirizine impurity structure. The detailed information for the ^{1}H NMR and ^{13}C NMR spectra can be seen in Table-2. Further to confirm the exact structure of impurity, the 2D NMR HSQC has also been investigated. It was observed that seven methylene signals corresponding to twenty six protons and six methine signal corresponding to ten protons. The proton in aliphatic region showing signal at 4.14 ppm corresponds to C31 which appeared at 67 ppm, signal at 3.86 ppm corresponds to C29 which appeared at 64 ppm, signal at 3.447 ppm corresponds to C28 which appeared at 55 ppm. The 2D NMR HMBC has also been investigated. The methine proton at δ 5.4 ppm showed correlations for carbon C3 and C5 positions at 130 ppm, C10 and C14 positions at 128 ppm, C15 and C19 positions at 48-50 ppm and C4 and C9 positions at 136 ppm, respectively, the methylene proton at δ 3.86 ppm showed correlations for carbon C31 position at 64 ppm and C28 position at 55 ppm, respectively, the methylene proton at δ 4.14 ppm showed correlations for carbon C29 and C32 positions at 64 and 172 ppm, respectively. The methylene proton at δ 3.447 ppm showed correlations for carbon C24 and C26 positions at 48 to 51

TABLE-2
^{1}H NMR AND ^{13}C NMR ASSIGNMENTS FOR IMPURITY

Position[a]	δ_H (ppm)	δ_C (ppm)
1	–	133.76
2, 6	7.79-7.81	129.78
3, 5	7.49-7.51	130.43
4	–	136.36
7	5.45	73.12
8	–	–
9	–	136.92
10, 14	7.74-7.76	128.43
11, 13	7.43-7.46	129.66
12	7.37-7.39	129.21
15, 16, 18, 19	3.447-3.556	48.562-50.70
17	–	–
20	3.17-3.20	48.562-50.7
21	3.4	
22	–	–
23, 24, 25, 26	3.17-3.31	48.562-50.70
27	–	–
28	3.447	55
29	3.86	64.61
30	–	–
31	4.14	67.57
32	–	172.049

[a]The position numbering has given according to impurity (Fig. 5).

ppm. The methylene proton at δ 3.4 ppm showed correlations for carbon C23 and C25 positions at 48 to 51 ppm while the methylene proton at δ 3.2 ppm showed correlations for carbon C16 and C18 positions at 48 to 51 ppm. Based on this data, the structure of impurity was confirmed as 2-(2-(4-(2-(4-((4-chlorophenyl) (phenyl)methyl)piperazin-1-yl)ethyl)piperazin-1-yl)-ethoxy)acetic acid.

Conclusion

A new process related impurity observed in cetirizine dihydrochloride was isolated and characterized by HPLC, LC-MS/MS, MS/TOF and NMR techniques as 2-(2-(4-(2-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)ethyl)piperazin-1-yl)ethoxy)acetic acid and the unknown impurity prepared synthetically.

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