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Isolation, Synthesis and Characterization of Novel Process Related Impurities in Cetirizine Dihydrochloride by Mass and NMR Spectrometry

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ABSTRACT

Two new process related impurities were observed in Cetirizine dihydrochloride samples when analyzed by United States Pharmacopeia (USP) described high performance liquid chromatography method (HPLC). These impurities were isolated using Preparative high performance liquid chromatography followed by full characterization using analytical techniques like mass, ^{1}H, ^{13}C-NMR and designated as (±)-2,2-bis[4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid and bis[[1-(4-chlorophenyl)-1-phenyl]methyl]ether. These impurities were also synthesized and reconfirmed by co-injection in HPLC.

Keywords: Cetirizine; Process-related impurities, Identification, Synthesis, HPLC

I. INTRODUCTION

(±)-[2-[4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid also known by the generic name of cetirizine is a non-sedating type histamine H1-receptor antagonist and is used for the treatment of allergic syndromes, such as chronic and acute allergic rhinitis including seasonal and perennial allergic rhinits, allergic conjunctivitis, pruritus, urticaria, and the like [1-3]. Cetirizine is a second-generation antihistamine and is less able to cross the blood-brain barrier and therefore have diminished effects on the central nervous system compared to first-generation drugs [4]. Cetirizine, and more in particular its dihydrochloride is a molecule of great success on the market and hence different methods of its synthesis have been studied [5-8]. Our route of synthesis has shown in Figure 1.

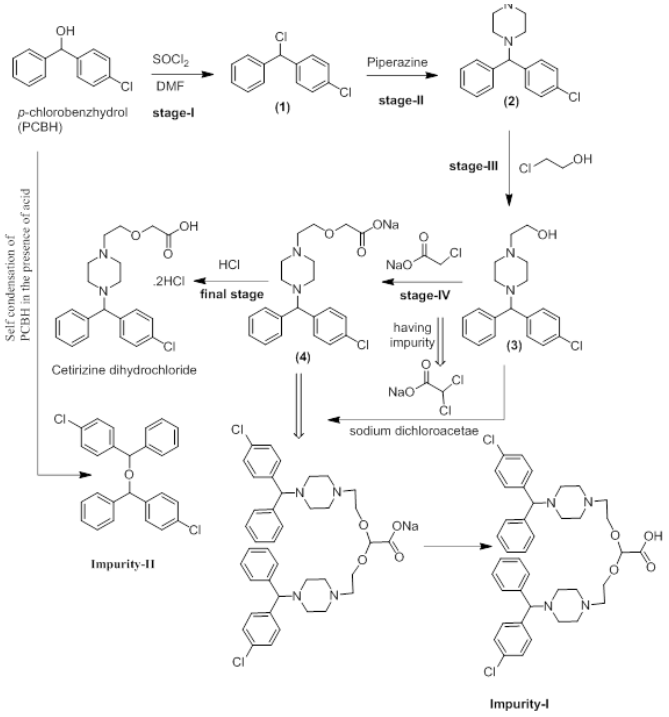


Figure 1. Route of synthesis of cetirizine dihydeochloride showing the plausible formation of impurities 1 and 2

Safety and quality of pharmaceutical products can be affected by the impurities present in the Active

Pharmaceutical Ingredients (APIs); hence impurity profile study of the API to be used in the manufacturing of drug substance is necessary. The impurity profile of API may vary if there are any changes in synthetic route and/or key raw materials. Therefore it is essential to know all the possible impurities that can be generated during the manufacturing process of drug substance. According to International Conference on Harmonization (ICH) guidelines identifying and characterizing all impurities that are present at a level of 0.10% or more are recommended [9].

On HPLC analysis of the batches which were manufactured during the lab development process, it was found that apart from USP listed impurities there were two impurities which were new and unknown [10]. These two impurities were observed at RT 62.886 (impurity-I) and RT 94.482 (impurity-II) at a level > 0.1%. Since these impurities were present at a level more than the identification threshold, therefore identification of these impurities was done by their isolation using Preparative high performance liquid chromatography, followed by full characterization using spectral techniques like MS, ^{1}H and ^{13}C-NMR. After identification these impurities were synthesized and further confirmed by co-injection in HPLC.

Herein we wish to discuss the identification, isolation, synthesis and full characterization of two new process related impurities using sophisticated techniques like Mass and NMR spectrometry.

II. MATERIALS AND METHODS

Samples and reagents

Cetirizine dihydrochloride batches were synthesized at manufacturing site of Ipca Laboratories Ltd. (Mumbai, India). HPLC grade acetonitrile and concentrated sulfuric acid was from Merck. Deuterated chloroform and D2O were purchased from Merck KGaA, Darmstadt, Germany. Millipore MilliQ Plus Water purification system was used to obtain high purity water.

High performance liquid chromatography

HPLC analysis was performed on Waters Alliance HPLC with, pump-alliance (2695), auto sampler (2695); with empower software ^{R} (Waters USA) using UV detector (2489) as well as 2996 photo diode array (PDA) detector. The output was being monitored with Empower 3.0 Software version. The separation was carried out on Symmetry shield RP-18, 250 × 4.6 mm × 5µ m column. The mobile phase was a mixture of solution A (dissolve2 g of tetrabutylammonium hydrogen sulfate and 3 g of sodium phosphate monobasic in 1litre of water, pH adjusted to 2.7 with 1N sodium hydroxide) and solution B (methanol) with time gradient program as mentioned in USP [10]. The injection volume was 10 µl and chromatographed under prescribed condition at 232 nm.

Preparative high performance liquid chromatography

A Waters Model Alliance 2555 Separation Module (Waters Corporation, Milford, MA, USA) equipped with a Waters 2489 UV Detector and Empower software was used. The column used for separation was Unisphere C18 250 ×50 mm× 10µ m. Mobile phase used for the separation was Buffer: ACN (50: 50). Buffer was the water having pH 2.5 adjusted with TFA. Injection volume was 5ml with 2.5 g of sample. The flow rate was maintained at 30 ml / min with output monitored at 232 nm.

Mass spectrometry

The High Resolution Mass Spectra of isolated impurity was obtained from a Thermo Scientific Q-Exactive-Orbitrap mass spectrometer (Waltham, Massachusetts, United States**)** with 17500 resolutions. Sample prepared in isocratic mixture of acetonitrile and water (50:50, v/v) was introduced by syringe pump and ionization was achieved by HESI ionization source in the positive ion detection mode. Nitrogen was used as both the sheath gas and the auxiliary gas at 30psi and 10 psi respectively. HESI source parameters were set as capillary temperature $320^{0}C$, spray voltage of 4 kV and heater temperature $250^{0}C$. Scanning of sample was done over mass range of 150-1000 m/z. Operating software used was Thermo Xcalibur 3.0.63.

Nuclear magnetic resonance spectroscopy

The ^{1}H, ^{13}C NMR spectra were recorded on AVANCE 400 (Bruker, Fallanden, Switzerland) instrument at 300K. Solvent used for impurities sample run was $CDCl_3$ and for Cetirizine it was D_2O. Distortionless enhancement by polarization transfer (DEPT) spectral editing revealed the presence of methyl and methine groups as positive peaks while the methylenes as negative peaks

III. RESULTS AND DISCUSSIONS

Detection of unknown impurity

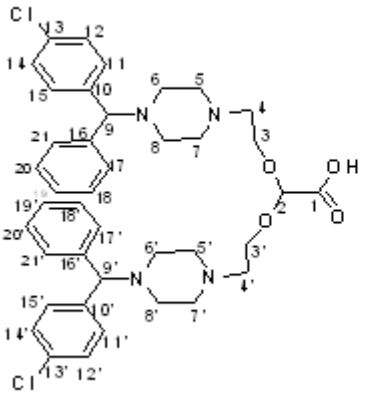
Cetirizine dihydrochloride samples were analyzed by the USP described HPLC method (section 2.2). Two new impurities at RT 62.886 (impurity-I) and 94.482 (impurity-II) were observed along with Cetirizine at RT 32.396. These two new impurities were isolated from the sample by using Preparative high performance liquid chromatography as described in section 2.3 and fully characterized to get the exact structure.

Structure elucidation of impurities

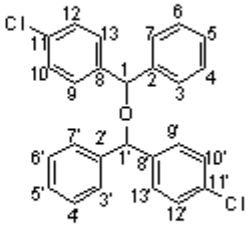
Impurities isolated were found to have HPLC purity ≥ 95%. These impurities were directly used for structure elucidation without any further purification. On the basis of spectral data like mass, ^{1}H and ^{13} C-NMR (Table 1), structure of theses impurities were designated.

Table 1. 1H and 13C NMR data of impurities

Impurity-I



Impurity-II



Position	Integrat	δ(ppm) multiplicit	^{13}C δ(ppm)	Position	Integra	δ(ppm) multiplicity	^{13}C δ(ppm)
1	-	-	172.04	1, 1’	2H	5.43, s	79.6, 79.5
2	1H	4.98, s	99.7				
3, 3’	4H	4.05, t	61.9				
4, 4’		(3.27-3.31)	56.1				
6,6’	12H	bs + m	48.7				
8,8’			48.7				
5, 5’	8H	2.72, bs	53.1				
7, 7’			53.1				
9, 9’	2H	4.31, s	74.5				
10,10’	-	-	140.1	2, 2’	-		140.6, 140.7
11,11’	2H		129.1	3, 3’	2H		127.2-127.9
12,12’	2H	m(7.18-7.34	128.9	4, 4’	2H		128.5-128.7
13,13’	-	-	133.2	5, 5’	2H	m (7.28-7.41)	128.5-128.7
14,14’	2H		128.9	6, 6’	2H		128.5-128.7
15, 15’	2H		129.1	7, 7’	2H		127.2-127.9
16,16’	-	-	140.9	8, 8’	-		141.4, 141.5
17,17’	2H		127.8	9, 9’	2H		127.2-127.9
18,18’	2H		128.9	10, 10’	2H		128.5-128.7
19,19’	2H		128.9	11, 11’	-		133.3, 133.4
20,20’	2H		128.9	12, 12’	-		128.5-128.7
21, 21’	2H		127.5				

Impurity I

ESI mass spectrum of impurity-I displayed protonated molecule peak at m/z 717.29 $[M+H]^+$ in positive ion mode, indicating the mass of to be 716.29 which is 61.49 amu less than that of double of Cetirizine. On subjecting to elemental composition calculator this impurity found to have molecular formula $C_{40}H_{46}Cl_2N_4O_4$. This indicates that the impurity can be a molecule which would be structurally close to dimer of Cetirizine molecule. ^{1}H NMR spectrum of this impurity has shown the presence of 18 aromatic protons which are double of Cetirizine aromatic protons. 2 protons showed singlet at δ 4.316 ppm, which could be same as that of CH proton of Cetirizine molecule. Also the singlet of 2 protons (alpha to carboxylic acid) of Cetirizine that appeared at δ 4.06 ppm has been replaced by singlet of 1 proton at δ 4.98 ppm in this impurity. Same conclusion can be withdrawn from ^{13}C NMR spectrum. It showed 1 CH peak at 99.7 ppm instead of Cetirizine CH_2 (alpha to carboxylic) peak. Based on the above collective spectral analysis the structure of impurity was proposed as (±)-2,2-bis[4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]aceticacid. For further confirmation the impurity was synthesized as described in section 3.3 and confirmed by co-injection with the API sample.

Impurity II

ESI mass spectrum of this impurity was done both in positive ion mode and negative ion mode. But due to inconsistency in mass value it was not possible to predict any possible structure. Then its proton NMR and Carbon NMR was taken. In ^{1}H NMR there were only 2 aliphatic protons found to shown singlet at δ 5.431 ppm rest 18 aromatic protons were in aromatic region. This pattern was same as of its starting material p-chlorobenzhydrol (PCBH). Only difference was in the number of protons which were only 2 protons less (20 protons) than the double of PCBH protons (11× 2= 22 protons). This made us think on the possibility of the self condensation of PCBH to give ether linkage dimer type molecule with loss of one water molecule. Looking at ^{13}C spectra of the impurity same conclusion can be drawn as the ^{13}C spectra pattern was same as that of PCBH with double number of carbon atoms. For further confirmation this possible impurity was synthesized as discussed in section 3.3 and confirmed after co-injection with the API sample.

IV. SYNTHESIS OF IMPURITIES

Preparation of impurity I

3.28 g (0.01 mole) of Cetirizine ethanol (Stage –III intermediate stage) and 0.03 moles of potassium t-butoxide was dissolved in 15 ml of dimethylformamide. Reaction mass was heated to 55-60 °C for 1 h. then 0.5 g (0.003 mole) of sodium dichloroacetate was added to this reaction mixture and heated to 55-60 °C for 6h (monitored by TLC). Reaction mass was cooled to room temperature and 30 ml of water was added to it. Wash the aqueous layer thrice with ethyl acetate (20 ml ×3). pH of aqueous layer was adjusted to 4 by adding dilute hydrochloric acid. Extract this aqueous layer with methylene dichloride (MDC). MDC was distilled out to obtain the crude mass which was purified using MDC: methanol (95.5:0.5) as an eluent with HPLC purity ≥ 95%.

Preparation of impurity II

5 g (0.0228 mole) of p-chlorobenzhydrol and 0.5 g (0.003 mole) were taken in 50 ml toluene. Reaction mass was heated to 110-115 °C for 2 h (monitored by TLC). Water was removed azeotropically and reaction mass was cooled to room temperature. 50 ml water was added to reaction mixture followed by layer separation. Toluene layer was washed with 8% sodium bicarbonate solution and dried over sodium sulfate. Solvent was removed under reduced pressure to get the crude mass which was purified by passing it through a column of silica gel using ethyl acetate: hexane (1:9) as an eluent. HPLC purity of impurity-II obtained was more than 95%.

Pathway of the formation of impurities

The route of synthesis of Cetirizine dihydrochloride along with the formation of these identified impurities has shown in fig. 1. Possibility of the formation of impurity-I seems from the presence of sodium dichloroacetate impurity in key starting material sodium monochloroacetate. During the formation of intermediate **(4),** impurity sodium dichloroacetate reacts with intermediate **(3)** to give the impurity-I. While PCBH (one of the USP-listed impurity), which might be present as an impurity in intermediate **(4)** will undergo self condensation via removal of water molecule in the presence of hydrochloric acid at final stage to give impurity-II.

V. CONCLUSIONS

To the best of our knowledge this is for the first time two new process related impurities named as (±)-2,2-bis[4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid and bis[[1-(4-chlorophenyl)-1-phenyl]methyl]ether have been identified in Cetirizine dihydrochloride drug substance when analyzed by USP described chromatographic method.

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