

Research Article

Stress Degradation Studies on Cetirizine Dihydrochloride and Development of Degradation Pathways

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ABSTRACT

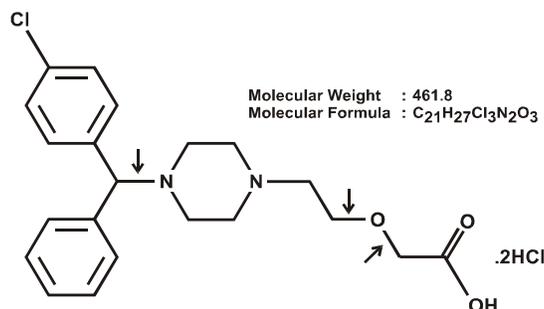
Cetirizine dihydrochloride, a non-sedative antihistaminic is subjected to different stress conditions like hydrolytic conditions (neutral, acidic, alkaline) oxidative and photolytic conditions according to ICH guidelines. The separation study has been carried out using HPLC and an analytical method has been developed to optimally resolve the drug peak and degradation methods. Drug undergoes extensive degradation (around 99%) in oxidative, neutral and hydrolytic conditions over 48 hrs. In photolytic conditions, degradation is around 30-50%, whereas in alkaline conditions, it undergoes insignificant degradation. The resulting degradation products DCZI-DCZ3 are isolated, purified and characterised by spectral analysis. In neutral and acidic hydrolytic conditions same degradation product is formed which is α -(4-chlorophenyl) benzyl alcohol, whereas in oxidative conditions it is characterised as 4-chlorobenzophenone. Degradation pathways leading to these degradation products have also been proposed.

Keywords: Cetirizine dihydrochloride; Stress conditions; Degradation products.

1. INTRODUCTION

The International Conference on Harmonization (ICH) guidelines¹ for stability testing require conduct of forced decomposition studies under a variety of conditions, like light, oxidation, dry heat, susceptibility to hydrolysis across a wide range of pH values etc. and separation of drug from degradation products. It requires that stress testing be carried out to elucidate the inherent stability characteristics² of the active substance. It also suggests that degradation products, formed under a variety of conditions, should be identified and degradation pathways established, to support suitability of proposed analytical procedures³. The ICH guidelines[1] (Q1A) on *Stability testing of new drug substances and products* emphasizes that the testing of those features which are susceptible to change during storage and are likely to influence quality, safety and/or efficacy, must be done by validated stability-indicating testing methods⁴.

Cetirizine (CZ) is chemically 2-[2-[4-[(4-chlorophenyl) phenylmethyl] piperazin-1-yl] ethoxy]acetic acid and is used as non-sedating antihistaminic. Cetirizine is a piperazine-derived second-generation antihistaminic drug, and its nontoxic topical formulation is recommended for treatment of pruritus associated with atopic dermatitis⁵. Non-sedating antihistamines, like cetirizine are the first-choice treatment for all forms of urticaria, especially in non-responsive patients⁶. Several other uses of cetirizine have also been reported such as in acute and chronic symptoms of allergic rhinitis in children⁷.



Several methods have been reported for determination of Cetirizine including titrimetric⁸, spectrophotometric⁹, chromatographic¹⁰. A High-Performance Liquid Chromatography (HPLC) method showed the degradation of cetirizine dihydrochloride in acidic and oxidative conditions¹¹. The results showed that cetirizine dihydrochloride was unstable in 2 M HCl and 0.5% H₂O₂. The kinetics of the acidic degradation showed a pseudo-first-order reaction in the temperature range of 70-90°C. In addition, the kinetics of hydrogen peroxide mediated degradation was pseudo-first-order in the temperature range of 50-80°C.

The main aim of this work is to establish the inherent stability of the drug substance under various stress conditions, along with establishment of degradation pathways which can be further used to develop and validate stability indicating method for Cetirizine.

2. EXPERIMENTAL

2.1 Materials

Cetirizine was procured from Panacea Biotec Ltd. Lalru, India and was used without further purification. All AR grade reagents like potassium dihydrogen orthophosphate, phosphoric acid, potassium hydroxide pellets and all HPLC grade solvents including acetonitrile, methanol, isopropyl alcohol, tetrahydrofuran were procured from S.D. Fine Chem Ltd. Triple distilled water produced in the lab was used for preparation of all solutions.

2.2 Instrumentation

Heating mantle (Perfit, India) was used for generation of stress samples. Photo stability studies were done in a stability chamber (KBWF 240, WTB Binder, Germany) capable of controlling tolerances in temperatures (2°C) and humidity (5% RH) as specified in ICH guideline Q1A. The chamber was equipped with illumination bank made of light sources defined under Option 2 in the ICH guideline Q1B [16]. The light bank consisted of a combination of two black light lamps OSRAM L73 and eight lamps OSRAM L20. The blacklight lamps (L73) had a spectral distribution between 345 and 410 nm with maximum at around 365 nm. The output of the white fluorescent lamps (L20) was similar to that specified in ISO 10977. Both UV and VIS lamps were put on simultaneously. The samples were placed at a distance of 9 inches from the light bank. The overall illumination at the point of placement was 7000 lux.

The analysis of stress samples was performed on Waters HPLC system having binary waters 515 pumps, with software of millennium 2.1, Waters 2487 dual wavelength absorbance detector, with Rheodyne injector. The analytical column used for chromatographic separation of Cetirizine and its degradation products was of Waters spherisorb[®] S5ODS2 (4.6mm×150 mm i.d.). The C18 guard column with dimensions of 10mm×4.6 mm i.d. of nucleosil was placed before the analytical column. The data was acquired and processed by millennium software ver. 2.1 (all equipment from waters). The mobile phase was filtered using Millipore filter through nylon membrane (0.45 µm) and was degassed using sonicator bath (ELMA 507/H).

LC-MS spectra were recorded on Finnegan Mat LCQ ion trap equipment. IR spectra were recorded on Nicolet impact 410 FT-IR and Perkin Elmer, using KBr disc. NMR spectra were determined on Bruker 300 MHz system. GC-MS spectra were performed on GC-MS QP 5000, (Shimadzu, Japan).

2.3 Degradation studies

For hydrolytic degradation studies, 1%w/v solution of Cetirizine dihydrochloride was prepared in three different media (H₂O, 0.1N HCl, 0.1N NaOH) and 100ml of each solution was refluxed for 48 hrs. Simultaneously samples were withdrawn at 0, 1, 2, 4, 6, 8, 10, 12, 24, 36, 48 hrs. In alkaline conditions, the samples were clear, so they were injected as such on HPLC. In neutral and acidic conditions, turbid samples were diluted quantitatively with tetrahydrofuran and injected on HPLC. For oxidative degradation studies, 1%w/v solution of Cetirizine dihydrochloride in 0.3% H₂O₂ was refluxed for 48 hrs. Simultaneously samples were withdrawn at 0, 1, 2, 4, 6, 8, 10, 12, 24, 36, 48 hrs. Turbid samples were diluted quantitatively with isopropyl alcohol and injected on HPLC. For photolytic studies 1%w/v solution of Cetirizine dissolved in 0.01N HCl, 0.1N NaOH and water respectively was divided into two sets. One set was kept in light chamber for 8 days to allow exposure to UV-visible radiations. Other set covered with aluminium foil was also kept with the first set which served as control for photostability samples. Samples were withdrawn starting from 1, 2, 4, 8 days from light and dark solutions, and were analysed by HPLC. All the samples were clear, so they were injected on HPLC without any dilution.

2.3 Separation studies

Initially methanol and water mixture in different proportions was used as mobile phase with a wavelength of 254nm, at a flow rate of 1ml/min, with a C8 column (OS-4.6×150mm, spherisorb®) but with no retention. Then acetonitrile was used in combination with (0.02M) KH_2PO_4 solution in different proportions (ranging from 10:90 to 50:50). The optimal resolution between drug peak and degradation products peak was attained with acetonitrile : (0.01M) KH_2PO_4 (35:65) with a wavelength of 230nm, at a flow rate of 2ml/min, with a C18 column (ODS2-4.6×150mm). This method was chosen as the analytical method for stress samples of hydrolytic and photolytic studies. For oxidative studies, method was modified with the same mobile phase but in the ratio of KH_2PO_4 (0.01M): acetonitrile (60:40) for optimal resolution. The mobile phase was filtered through 47mm/0.45 μm nylon membrane. The injection volume was 20 μl .

The analysis of samples collected during stress testing of Cetirizine in oxidative, photolytic and hydrolytic conditions showed two peaks around 15 min and 8 min. By overlaying the chromatograms obtained for different samples, the peak of Cetirizine was observed around 8 min and peak of degradation product was observed around 15 min in all stress conditions. The purity of the degradation product was verified by HPLC where a single peak was obtained.

2.4 Isolation of degradation product

The degradation product in neutral and acidic conditions was insoluble. So that was filtered, washed with equal volume of water and dried in air. In case of oxidative conditions, the degradation product was separated as oil which was converted to crystals upon addition of water. However, by keeping the sample overnight in refrigerator more quantity was isolated. The product was filtered washed and dried in air.

3. RESULTS AND DISCUSSION

3.1 optimisation of method of analysis

Many methods of analysis of Cetirizine dihydrochloride are reported in literature¹²⁻¹⁵. Starting from zero, a C8 column and mobile phase composed of methanol and water (60:40) was used as a first trial. But no elution was obtained till 25 minutes. Replacing water with phosphate buffer (0.02M) (pH 7.5) and changing the ratio to 50:50 resulted in drug peak at 28 min. Further replacing the organic modifier methanol with acetonitrile shifted the RT to 2.5 min. As the RT was very less, so the proportion of aqueous phase was increased upto 75% with corresponding proportional decrease in acetonitrile. As the proportion of acetonitrile was decreased from 50% to 25%, the drug RT was increased from 2.5 min to about 20 min. Finally mobile phase composed of KH_2PO_4 (0.02M) and acetonitrile in 65:35 ratio was selected (RT=8.5min), but significant peak tailing was observed. Addition of 0.5% tetrahydrofuran reduced the tailing to some extent, but shifted the RT to 6 min. This method was not able to resolve the degradation products. At this stage rather than changing the mobile phase, column was changed from C8 to C18 and wavelength was shifted from 254 to 230nm. With the same mobile phase the elution was late and also peak was broad. So, the flow rate was increased to 2ml/min and in mobile phase buffer was replaced by (0.01M) KH_2PO_4 solution. The resulting method eluted the drug at around 7.5 min and also degradation products were resolved significantly in case of neutral, alkaline and hydrolytic conditions and photolytic degradation conditions. However in case of oxidative hydrolysis, peaks of degradation products were slightly superposed over each other. Hence the proportion of aqueous phase was decreased from 65% to 60% with corresponding increase in proportion of acetonitrile to 40% resulting in optimal separation of degradation products.

3.2 Degradation Behaviour

The results of stress testing of Cetirizine are summarized in table 1:

Table 1:

Medium	Condition	Time	%degradation
Hydrolytic conditions			
H ₂ O (Neutral)	Reflux	4 hr	72.46%
		24 hr	87.20%
		48 hr	99.71%
0.1N HCl (Acidic)	Reflux	6 hr	81.71%
		24 hr	99.41%
		48 hr	99.94%
0.1N NaOH (Alkaline)	Reflux	48 hr	insignificant degradation
Oxidative conditions			
0.3% H ₂ O ₂	Reflux	½ hr	23.40%
		8 hr	33.29%
		48 hr	92.58%
Photolytic conditions			
Water (Neutral)	Temperature 40°C, Relative Humidity (70%)	1 day	30.61%
		8 days	54.37%
0.01N HCl (Acidic)	Temperature 40°C, Relative Humidity (70%)	1 day	8.99%
		2 days	13.20%
		8 days	30.11%
0.1N NaOH (Alkaline)	Temperature 40°C, Relative Humidity (70%)	1 day	1.53%
		4 days	7.64%
		8 days	31.07%

The chromatograms for individual stress conditions are shown in figure 1-7, depicting the decrease in concentration of Cetirizine with corresponding increase in degradation product concentration.

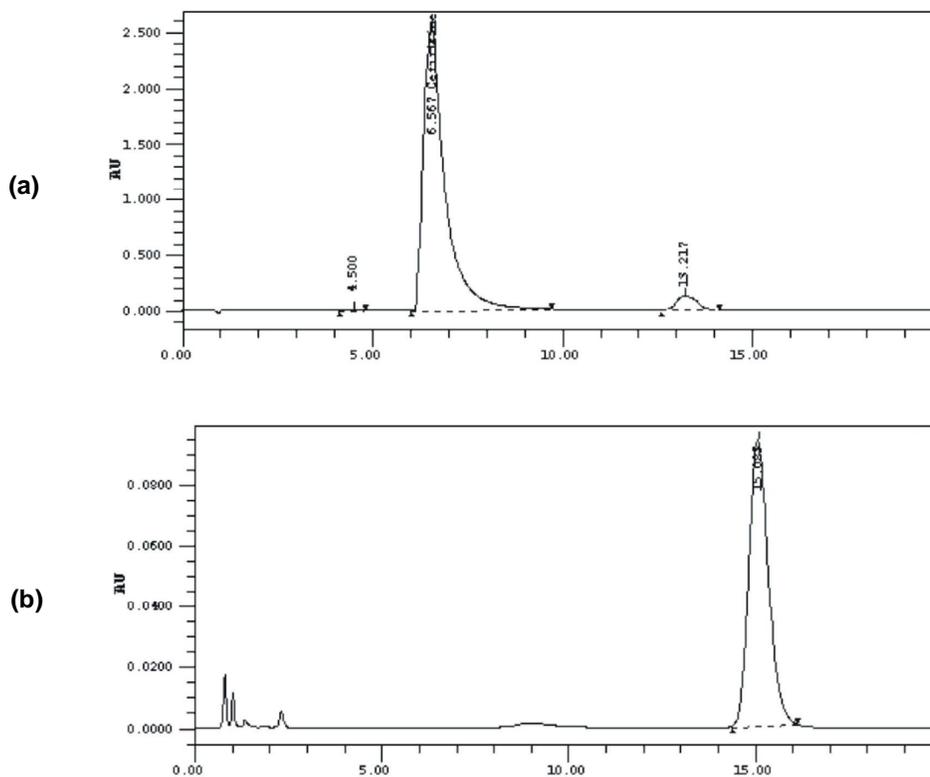


Fig. 1: Progressive degradation of Cetirizine in neutral hydrolytic conditions (a) initial sample, (b) 48 hr sample

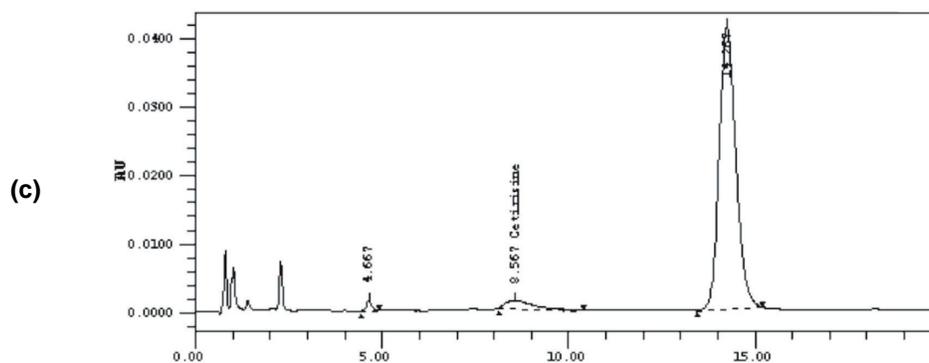


Fig. 2: Progressive degradation of Cetirizine in acidic hydrolytic conditions (c) 48 hr sample

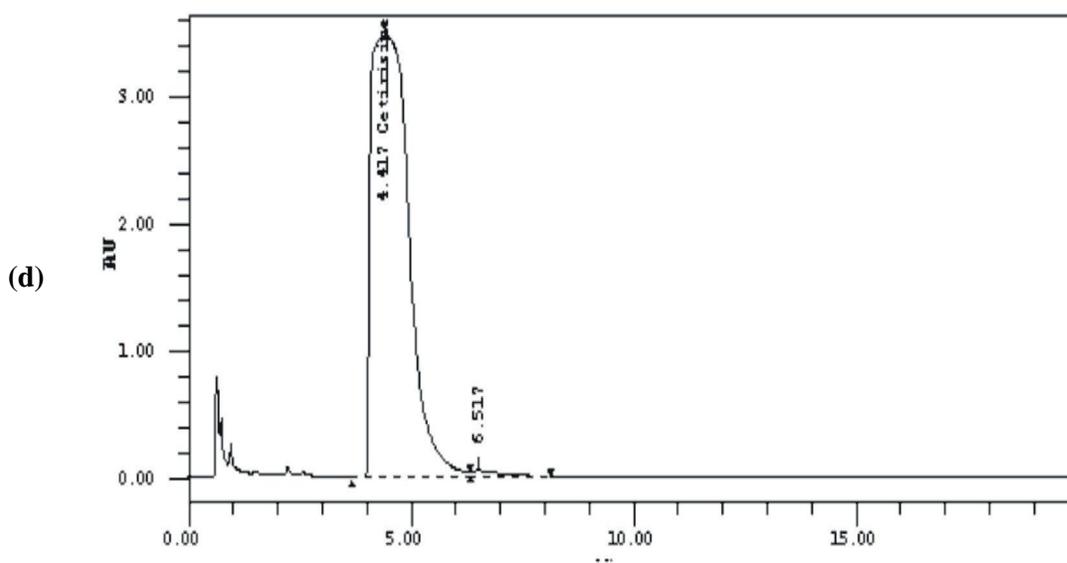


Fig. 3: Progressive degradation of Cetirizine in alkaline hydrolytic conditions (d) 48 hr sample

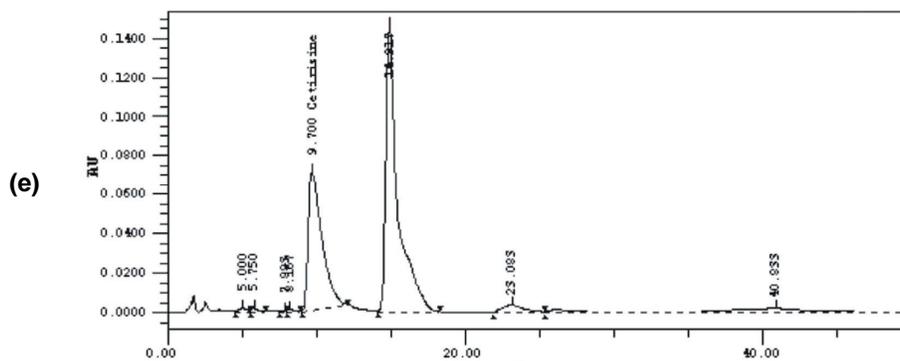


Fig. 4: Progressive degradation of Cetirizine in oxidative conditions (e) 48 hr sample

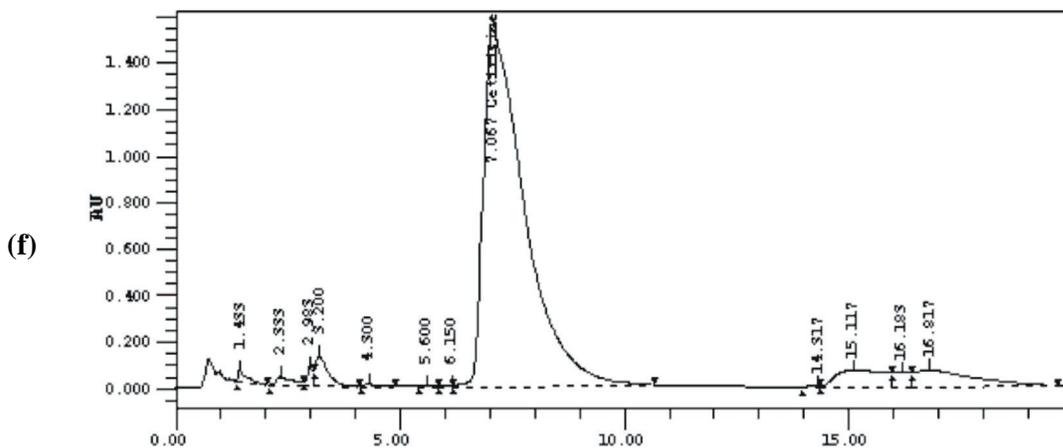


Fig. 5: Progressive degradation of Cetirizine in neutral photolytic conditions (f) 8 day sample

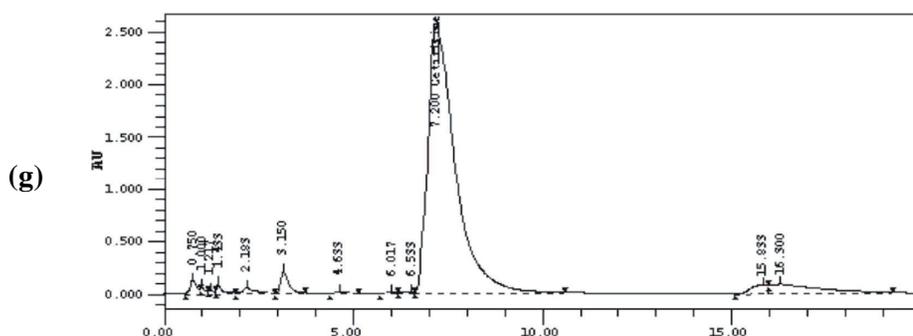


Fig. 6: Progressive degradation of Cetirizine in acidic photolytic conditions (g) 8 days sample

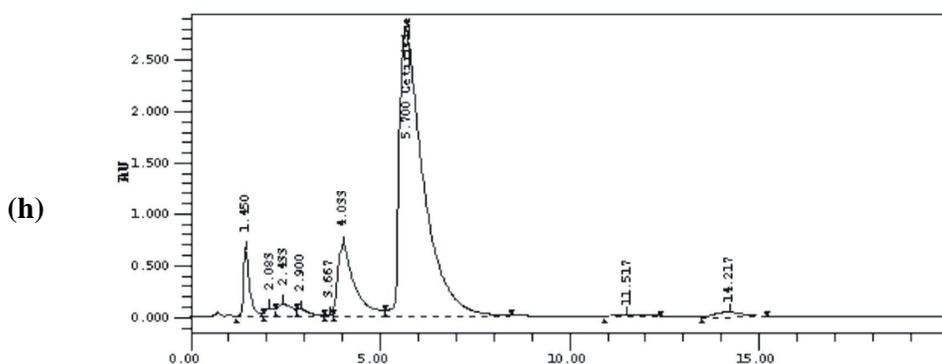


Fig. 7: Progressive degradation of Cetirizine in alkaline photolytic conditions (h) 8 days sample

3.3 Characterisation of degradation products

IR spectra of degradation products (DCZ_1 and DCZ_2) formed by neutral and acidic hydrolysis exhibited exactly the same pattern of bands. A broad band stretching from 3361 cm^{-1} revealed presence of O–H group. A medium intensity band at 1401 cm^{-1} was assigned to O–H bending, whereas a strong intensity band at 1016 cm^{-1} was due to C–O stretching. Another strong band at 1086 cm^{-1} corresponding to aromatic C–Cl stretching revealed the presence of chloroaryl moiety in the product. Aromatic and aliphatic C–H stretches were present at usual positions. Presence of aromatic C–H stretching revealed the aromatic nucleus, whereas aliphatic C–H stretches postulated

the presence of aliphatic region also. NMR spectra of both degradation products also exhibited the similar pattern of signals. A 1 proton broad singlet at 2.50δ which exchanged with D_2O confirmed the presence of hydroxyl proton. A 1 proton singlet at $\delta 5.7$ might be due to an aliphatic proton, present in a strongly deshielded region.

In case of acidic degradation product, a complex multiplet at $\delta 7.39-7.19$ accounted for nine protons, whereas in neutral degradation product, nine aromatic proton regions is divided into two singlets, the one at $\delta 7.29$ accounted for four protons, whereas the other at $\delta 7.25$ accounted for five protons. This revealed that there are two phenyl groups, the one with 5 protons and the other with 4 protons. In both neutral and acidic conditions the molecular ion peak was observed at 218 (relative intensity 3.96%). The mass spectrum of both compounds exhibited similar mass fragmentation pattern. Loss of 17 mass units confirmed the presence of OH group whereas peak at m/z 77 revealed the presence of a phenyl ring. A peak at m/z 165 obtained by the loss of HCl confirmed the presence of a Cl group. The presence of Cl group on a phenyl ring is further supported by a peak at m/z 111. A signal at m/z 77 obtained by loss of CO from m/z 105 revealed that OH group is attached to aliphatic carbon.

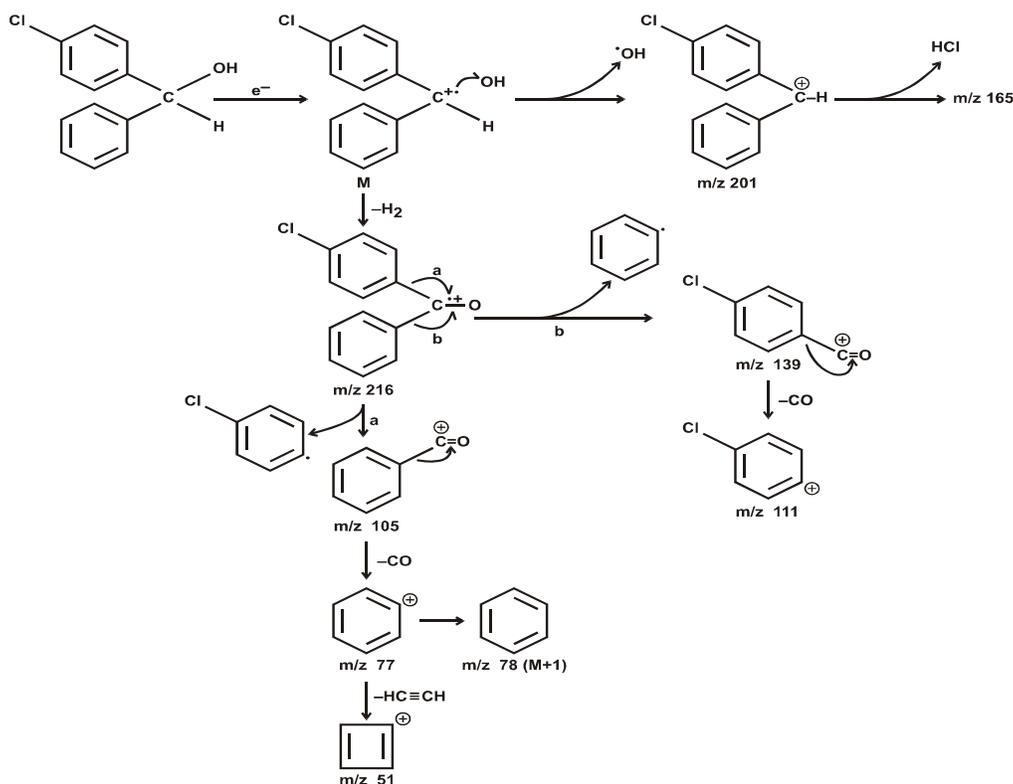
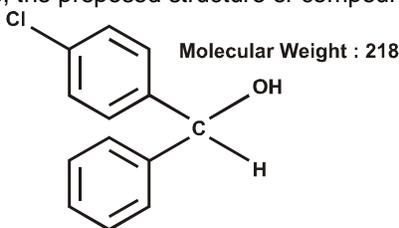


Fig. 8: Mass fragmentation pattern of neutral and acidic degradation products (DCZ₁ and DCZ₂)

On the basis of IR, NMR and MS, the proposed structure of compound is



α -(4-chlorophenyl) benzyl alcohol (DCZ₁ and DCZ₂)

Degradation product formed by hydrolytic oxidative condition (DCZ₃) exhibited different spectral characteristics. In IR spectra, absence of broad stretch around 3360–3390 cm⁻¹ indicated the absence of NH and OH groups. But a strong band at 1650 cm⁻¹ corresponding to Carbonyl vibrations indicated biaryl ketone functional group. A weak intensity stretching and bending at 1147 cm⁻¹ indicated the presence of C–(C=O)–C group. Another strong band at 1086 cm⁻¹ corresponding to aromatic C–Cl stretching revealed the presence of chloroaryl moiety in the product. Presence of aromatic C–H stretches revealed the presence of aromatic nucleus, whereas absence of aliphatic C–H stretches postulated the absence of aliphatic region. So on the basis of IR, the structural components can be summarized as an C=O group, Cl group, aromatic region.

NMR spectrum of the compound showed only one multiplet in aromatic region at δ 7.55–7.77 which accounted for nine protons.

Mass Spectrum is supported by appearance of molecular ion peak at 216 (relative intensity 45.13%) which is further supported by M+2 peak at 218 (32.37% of molecular ion). Relative intensity of M+ 2 peaks matched to relative abundance of ³⁷Cl. This confirmed the presence of –Cl group in molecule. The appearance of a peak at m/z 77 due to loss of –CO from m/z 105 revealed the presence of ketonic (C=O) functional group. The mass fragmentation of the degradation product is as shown in figure 9:

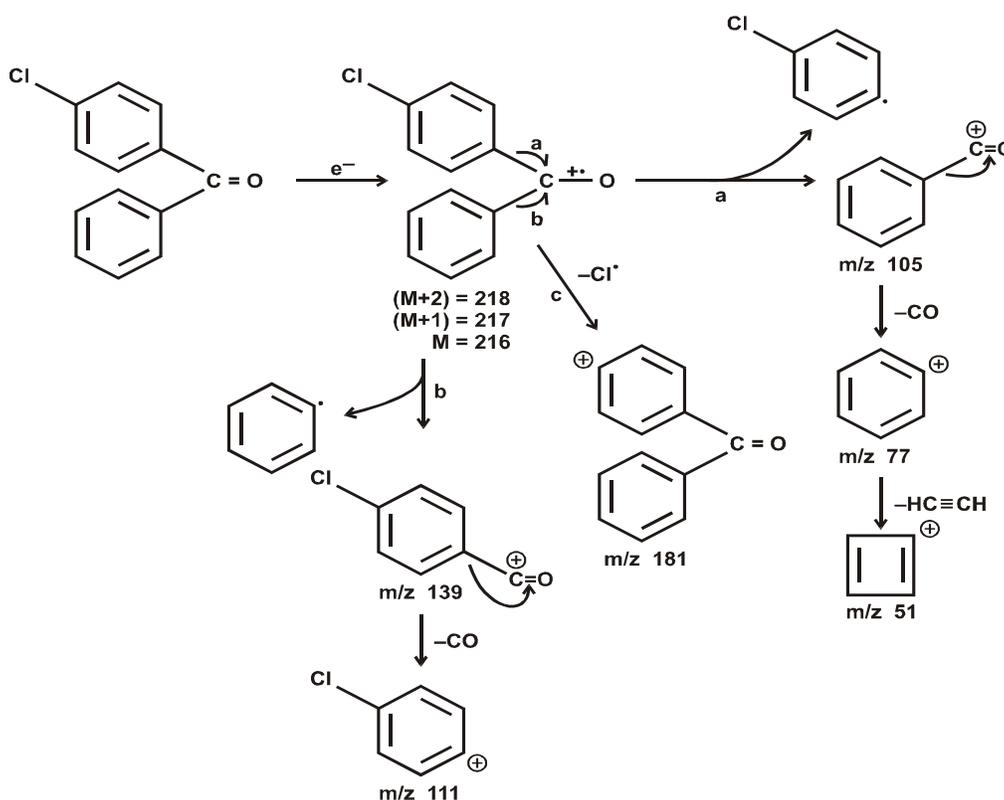
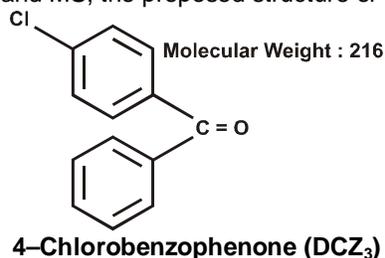


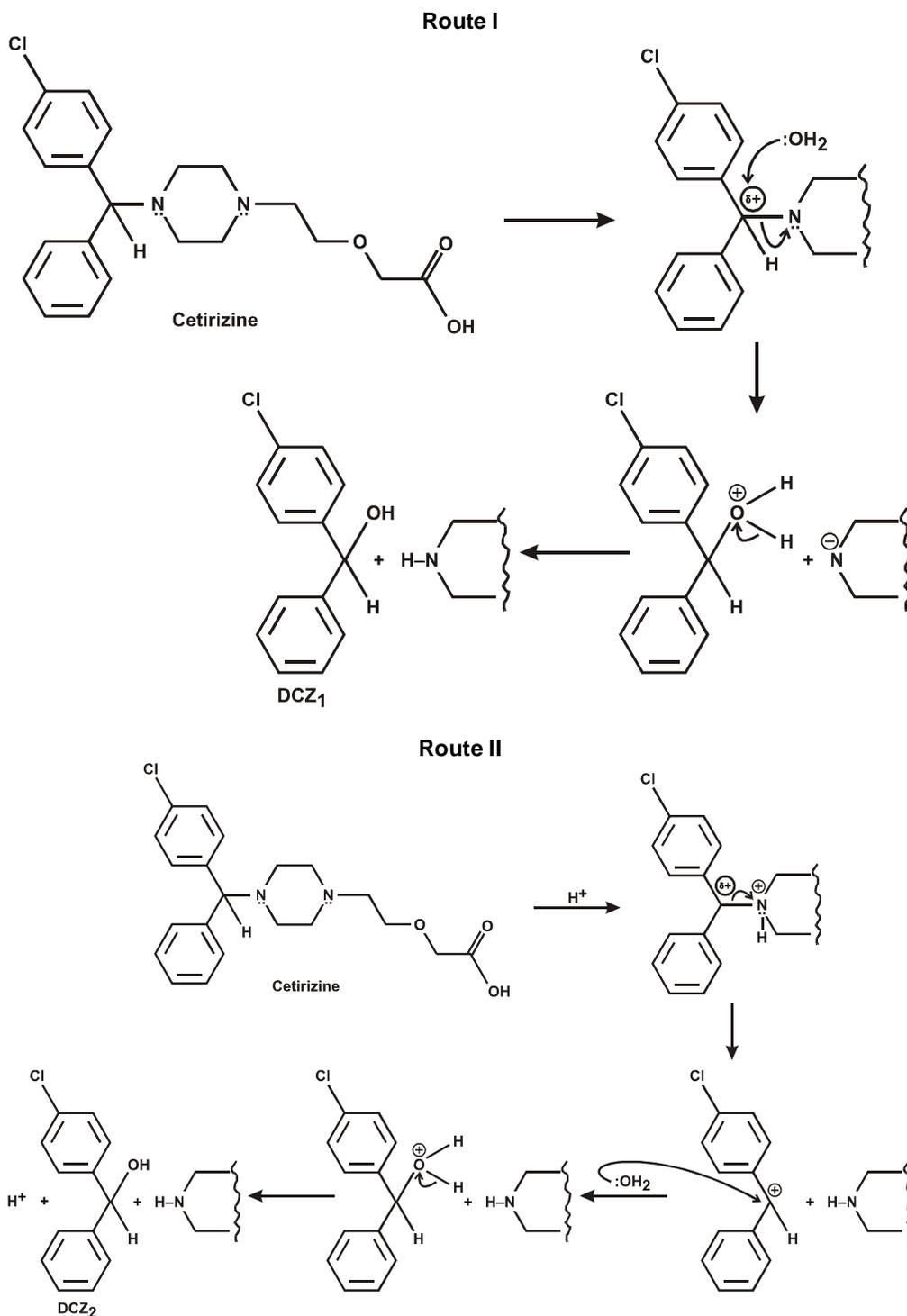
Fig. 9: Mass fragmentation pattern of oxidative degradation product (DCZ₃)

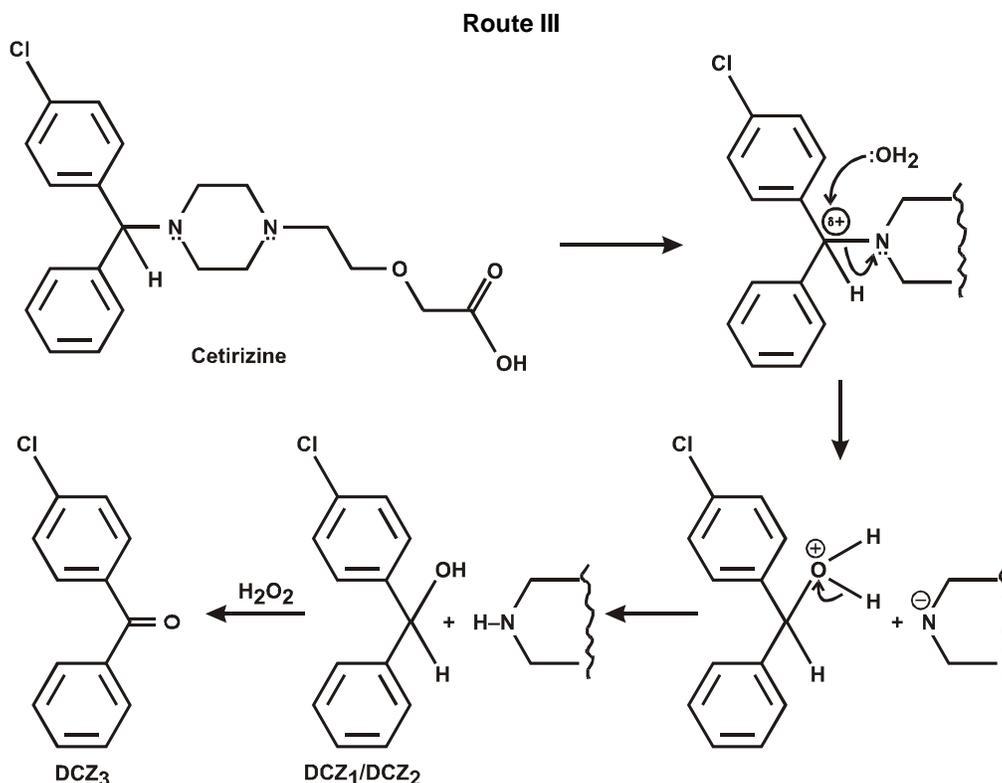
On the basis of IR, NMR and MS, the proposed structure of degradation product is



3.3 Degradation pathways

The proposed mechanism of degradation of Cetirizine leading to DCZ₁ and DCZ₂ (neutral and acidic), and DCZ₃ (oxidative hydrolysis) is outlined in following routes





4. CONCLUSION

The stress testing of Cetirizine, an H₁-antihistaminic, has been performed according to the recent ICH guidelines. The inherent stability of the drug substance, its degradation products under the various stress conditions and the pathway of degradation have been established. This can be used to develop and validate stability-indicating method for Cetirizine.

Cetirizine has been found to be unstable and thus undergo photolytic, hydrolytic and oxidative degradation but it is stable in alkaline media.

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