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RESEARCH PAPER

Isolation and Characterization of Cetirizine Degradation Product: Mechanism of Cetirizine Oxidation

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ABSTRACT

Purpose The goal of the study was to isolate and analyze a cetirizine degradation product, formed within a PEG-containing formulation and to elucidate the mechanism of oxidation of cetirizine.

Methods Cetirizine, formulated in PEG-containing matrix, was subjected to forced degradation conditions in the pH range from 3 to 10, and the product was analyzed by HPLC and LC-MS/MS. Additionally, pure cetirizine was subjected to selective oxidization by hydrogen peroxide and sodium percarbonate. The reaction mixture was purified, and the isolated material was analyzed by ^{1}H NMR.

Results Oxidation process was investigated in order to model the degradation of cetirizine in PEG-containing formulation. Site of oxidation is proposed based on correlation of the results of forced degradation with ionization scheme of cetirizine. The finding was verified by spiking of cetirizine degradation sample with cetirizine N-oxide reference standard.

Conclusions Degradation of cetirizine in polyethylene glycol arose from the reaction between the drug and the reactive peroxide intermediates such as peroxyl radicals formed through oxidation of PEG. Selective oxidation of cetirizine and isolation/characterization of the oxidation product allowed the identification of the oxidation product as cetirizine N-oxide. The mechanism of oxidation is proposed.

KEY WORDS cetirizine · oxidation · PEG

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INTRODUCTION

Cetirizine hydrochloride (cetirizine), the active component of ZYRTEC® tablets and syrup, is an orally active and selective H_1-receptor antagonist used for the treatment of allergy symptoms. Soft gelatin capsules (Softgels) offer the possibility of delivering a liquid in a solid oral dosage form. A softgel can therefore contain the active ingredient in solution, suspension or emulsion, which may inherently lead to better absorption of the active ingredient compared to the delivery in a tablet or powder. Many problems associated with tableting, including poor compaction and lack of content or weight uniformity, can be eliminated if drug is incorporated into this dosage form. Softgel dosage forms are perceived by customers as easier to swallow, help to mask the taste of the medicine and provide faster onset of action (1). In order to develop this highly water soluble and sensitive compound in soft gelatin dosage form, cetirizine was formulated in PEG-containing matrix. However, cetirizine formulated in PEG-containing solution for Softgels was found to degrade during manufacture and storage.

Our preliminary results, supported by the literature reports (2), showed that cetirizine does not degrade under acidic or basic conditions. It is known that cetirizine is susceptible to oxidation under chemical (hydrogen peroxide), biochemical (enzymatic) and electrochemical conditions (3). The metabolism of levocetirizine (R enantiomer of cetirizine) was studied (3) and found to proceed through the metabolic pathways: hydroxylation, O-dealkylation, N-oxidation and N-dealkylation (Fig. 1).

Excipients containing polyoxyethylene chains, for example polyethylene glycol (PEG) 400 and polysorbate, are known to contain some residual peroxides, which accumulate during storage and could potentially affect the stability of oxidation-

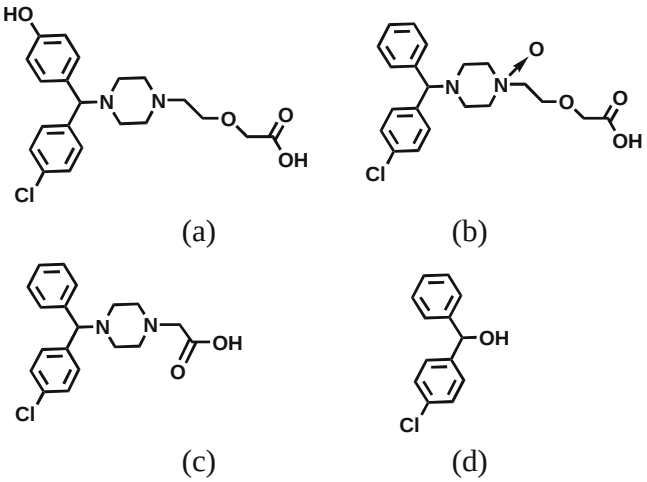


Fig. 1 Metabolic products of levocetirizine oxidation produced by (**a**) Hydroxylation (**b**) N-oxidation (**c**) O-dealkylation (**d**) N-dealkylation (3).

sensitive pharmaceuticals (4,5). It was reported (5,6) that auto-oxidation can progress through formation of hydroperoxides and peroxyl radicals in polyoxyethylene linkages containing substances such as polysorbate surfactants and PEG. Since the cetirizine soft gelatin capsule matrix contains PEG, we believe that this may be the main cause for cetirizine oxidation. In order to identify cetirizine degradation products, caused by PEG-containing formulations, we selectively oxidized cetirizine. The resulting degradation product was isolated and analyzed. Hence, the aim of this work was to identify a degradation product, investigate, and establish the mechanism of the degradation.

MATERIALS AND METHODS

Reagents and Chemicals

Cetirizine hydrochloride was supplied by Matrix Laboratories Limited (Lot CTP 0171106). Cetirizine N-oxide was obtained from LGC GmbH (Lot 380.13.07.03). PEG 400 was obtained from BASF. Ethyl acetate (Fisher Scientific), methanol (EMD), ammonia (JT Baker) and acetonitrile (EMD and Burdick & Jackson) were of the HPLC grade. Potassium phosphate monobasic monohydrate, magnesium sulfate, hydrogen peroxide, and sodium percarbonate were of the ACS grade and were purchased from JT Baker. Phosphoric acid (NF FCC) was also purchased from JT Baker. Trifluoroacetic acid and NMR solvent were purchased from Sigma-Aldrich.

HPLC/UV Chromatographic Method

An Agilent 1200 series, calibrated HPLC system equipped with a binary pump and UV detector was used. The chromatograms were acquired using Chemstation LC 3D Rev A 10.02 software (Agilent Technologies). The detector wavelength was set at 230 nm, and the injection volume was 25μL. The HPLC column was Xterra-RP C18, 5 μm, 250× 4.6 mm (Waters Corporation). Column temperature was maintained at 25°C. A binary mixture of potassium phosphate buffer: acetonitrile 70:30 (v/v) (solvent A) and potassium phosphate buffer: acetonitrile 35:65 (v/v) (solvent B), both at pH 2.5, was pumped at the flow rate of 1.0 mL/min.

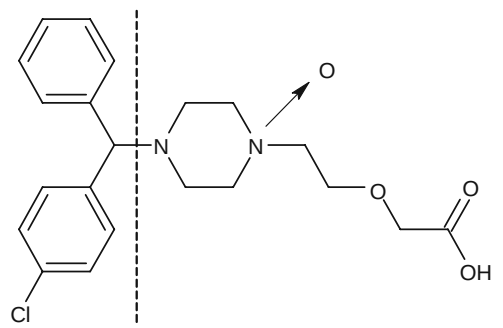
LC-MS/UV and LC-MS/MS/UV Method

LC-MS/UV and LC-MS/MS-UV data were obtained using an Agilent 1200 series HPLC system, equipped with UV detector, HTS PAL autosampler and MDS Sciex/ Applied Biosystems QTRAP 4000 mass spectrometer with Analyst 1.4.2 software. The flow rate was 1.0 mL/min, and the injection volume was 10 μL. The analytical column used was Xterra-RP C18, 5 μm, 250×4.6 mm (Waters Corporation). Column temperature was maintained at 25°C. The mobile phase consisted of 0.1% trifluoroacetic acid and acetonitrile in the ratio of 75:25 (v/v)–0.1% trifluoroacetic acid and acetonitrile in the ratio of 25:75 (v/v).

Synthesis and Purification of Cetirizine Degradation Product

Method 1

Cetirizine was oxidized by aqueous hydrogen peroxide. For this reaction, cetirizine hydrochloride (1 g) was exposed to 30% aqueous solution of hydrogen peroxide for 5 h at 70–80°C, then the reaction mixture was extracted with ethyl acetate. Organic phase was separated and concentrated. The crude material was purified by preparatory TLC, as described by Hamburger *et al.* (5) with some modifications. Spotting was done on precoated silica gel aluminum plate 60 F254. The solvent system consisted of ethyl



m/z 201

Fig. 2 Estimated structure of degradation product (m/z 405/407).

acetate-methanol-ammonia (80:15:5, v/v/v) mixture. Scraped spots were extracted with ethyl acetate, dried over magnesium sulfate, concentrated and analyzed by HPLC/UV and ^{1}H NMR.

Method 2

Cetirizine was selectively oxidized with sodium percarbonate. The kinetics of oxidation was monitored by HPLC/

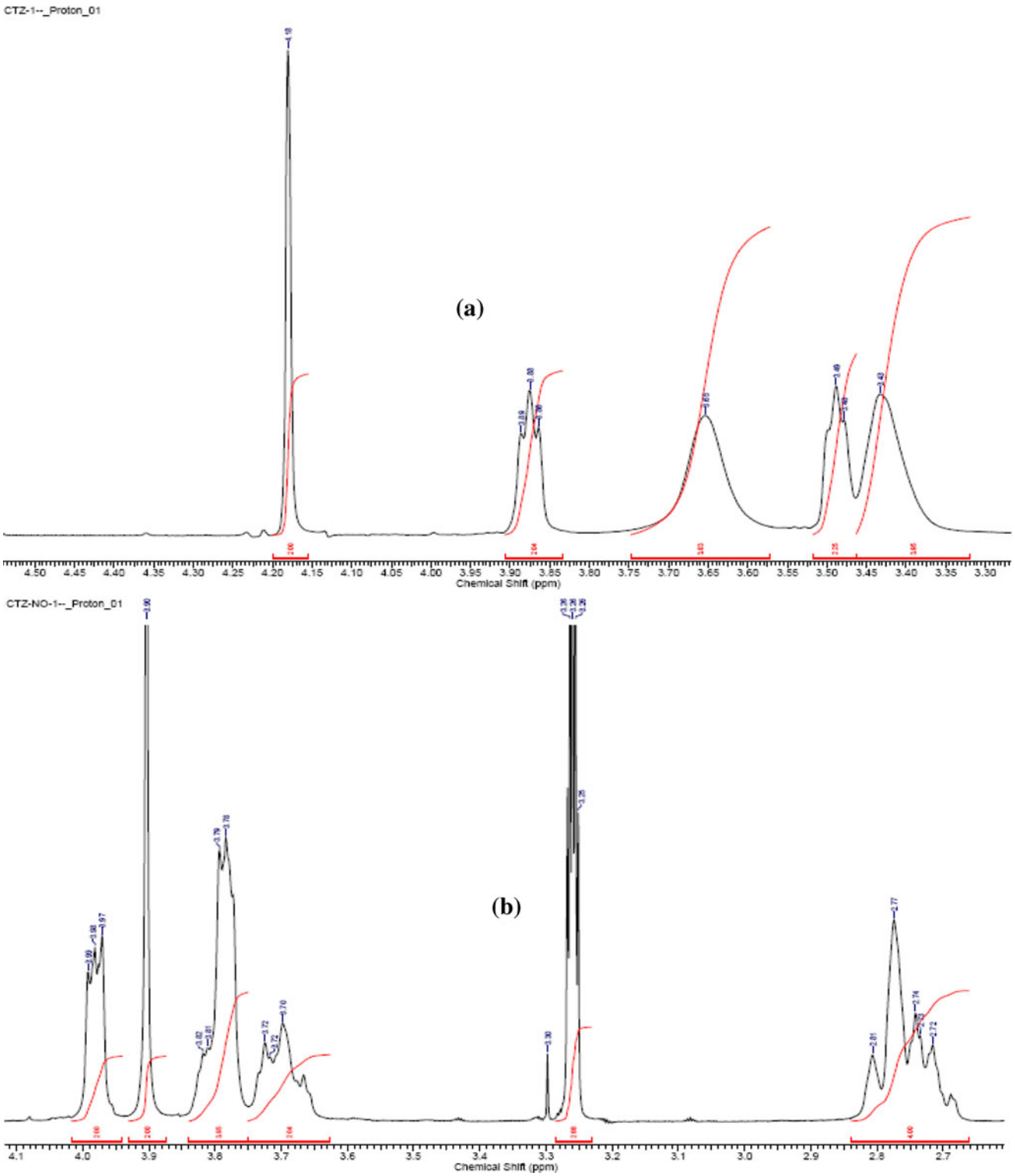


Fig. 3 ^{1}H NMR spectra of cetirizine (**a**) and degradation product (**b**).

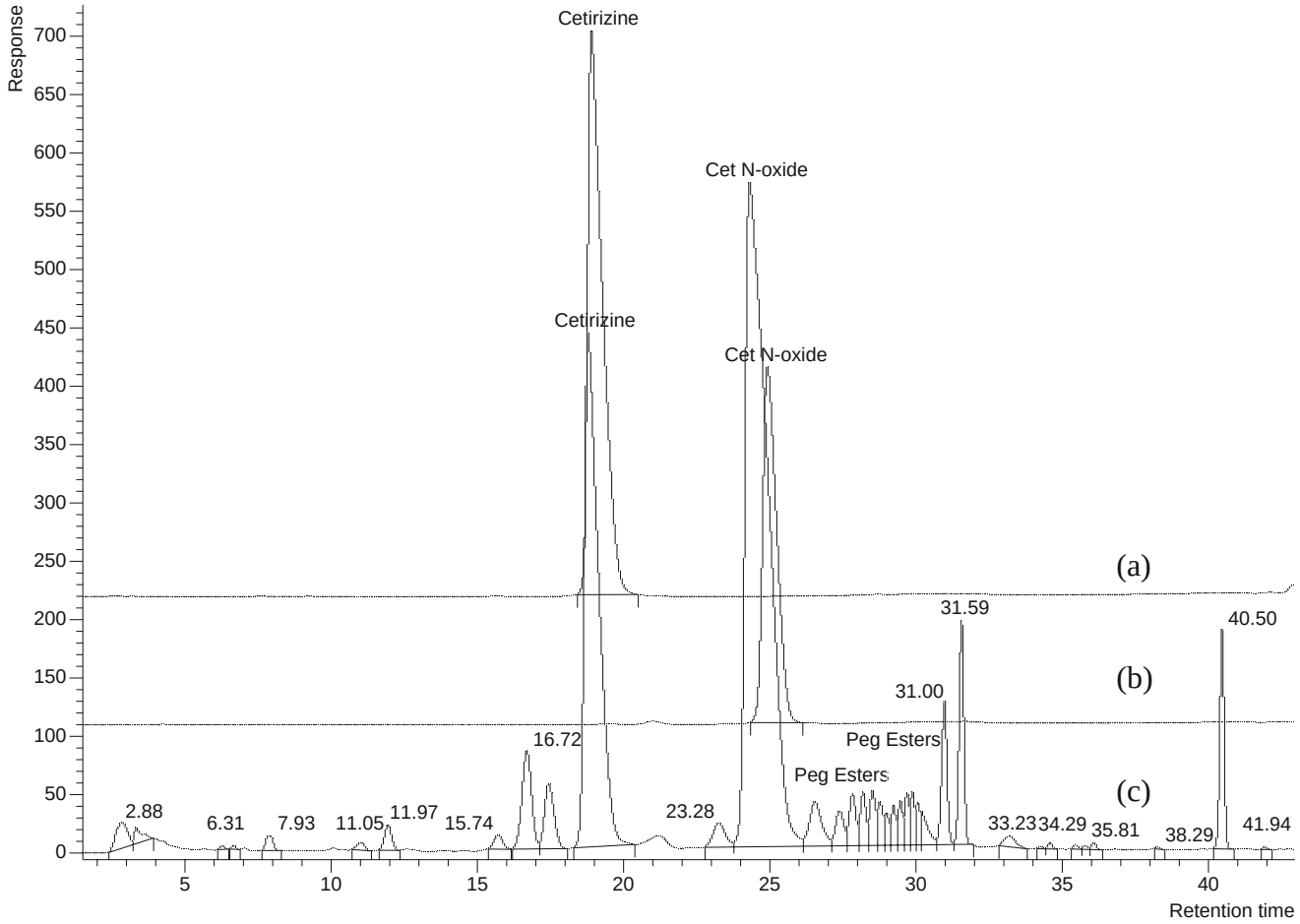


Fig. 4 Chromatograms of cetirizine (**a**), cetirizine N-oxide standard (**b**), and stressed cetirizine solution, spiked with cetirizine N-oxide (**c**).

UV. The reaction mixture was purified by preparatory TLC, as described in the Method 1.

^{1}H NMR

The NMR measurements were carried out on a 400 MHZ Varian spectrometer. The NMR spectra were measured in methanol-d_4 and D_2O. Chemical shifts are reported on δ scale (ppm) by assigning the residual solvent peak to 3.30 for methanol.

pH–Stability Profile

A cetirizine solution was prepared by dissolving 8 g of the drug in 8.6 g of DI water and 260 g of PEG 400. The solution was stirred for 1 h at room temperature then transferred into 10 separate vials. The pH of the solution in each vial was adjusted individually to 3.0, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 10.0 using NaOH. Triplicate aliquots of each solution were transferred into separate scintillation vials and purged with nitrogen; the samples were then incubated for 48 days at 30, 60 and 80°C. Aliquots of the solutions were also stored at room temperature as controls. Samples were withdrawn periodically and analyzed by HPLC for cetirizine and related impurities. All solutions were purged with nitrogen each time the vials were opened.

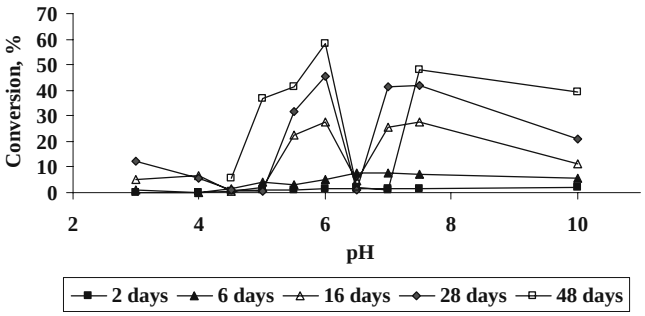


Fig. 5 The effect of pH on the oxidation of Cetirizine in PEG solution under forced degradation conditions.

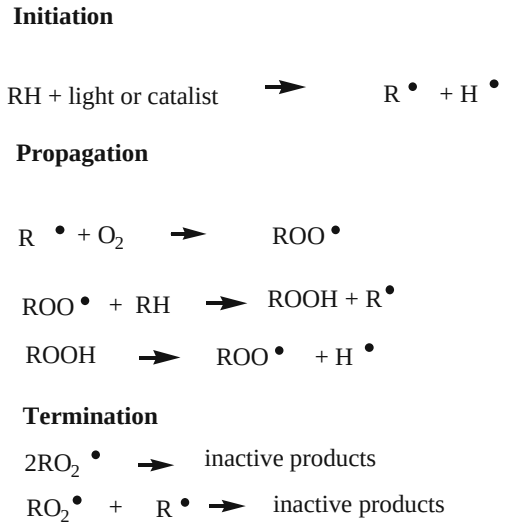


Fig. 6 Auto-oxidation process.

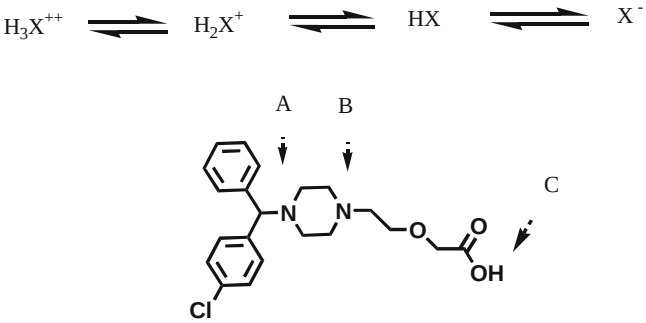


Fig. 8 Cetirizine zwitterionic form HX (predominant specie between pH 3 to 8).

RESULTS

Preliminary LC/MS Results

The preliminary LC/MS and LC/MS/MS results from the study of the stressed solution of cetirizine (MW 388.89) in PEG-containing formulation showed the monochlorinated cluster of peaks at m/z 405/407 (M+16) and a base peak at m/z 201. The intense ion at m/z 201 was assumed to be formed due to the fission of >C-N< bond between aromatic and aliphatic fragment of molecular ion. The value of m/z 405 for piperazine containing aliphatic fragment can be attributed to the monohydroxylated product or N-oxide, suggesting that oxidation occurs in the aliphatic portion of cetirizine. There was no evidence of a m/z fragment of 217 (oxidation on the aromatic fragment) leading to a possible structure as shown in Fig. 2.

Selective Oxidation of Cetirizine and Identification of Oxidation Product

In order to model the degradation process of cetirizine in a PEG-containing formulation, several oxidative methods for preparing the N-oxide of tertiary amines were considered. All methods used hydrogen peroxide in different media (acetic acid, water) and different catalysts (sodium tungstrate, molybdenum trioxide) (7). As a first attempt, cetirizine hydrochloride was successfully oxidized with 30% aqueous hydrogen peroxide. Reaction mixture was purified by preparative TLC as described in the literature (2) and analyzed by HPLC/UV. The retention time of the compound isolated from TLC plates corresponded to the retention time of oxidative peak of interest (R_t=24 min by HPLC) formed during forced degradation.

Additionally, cetirizine was selectively oxidized with sodium percarbonate with higher selectivity and yield of oxidation product than with conventional hydrogen peroxide. The kinetics of oxidation were monitored by HPLC. Results showed that in the presence of sodium percarbonate, conversion of cetirizine approached 50% during 2 h at 80°C. The ^{1}H NMR spectrum of isolated cetirizine oxidation product was compared to NMR of cetirizine hydrochloride (Fig. 3).

The number of protons in the aromatic region of the ^{1}H NMR of the degradation product was similar to that of cetirizine, indicating formation of N-oxide, rather than monohydroxylation. The N-oxide effect can be observed by the difference in the chemical shifts of the proximal protons adjacent to the N-oxide carbons (Fig. 3). The deshielding influence of the N-oxide function, generated

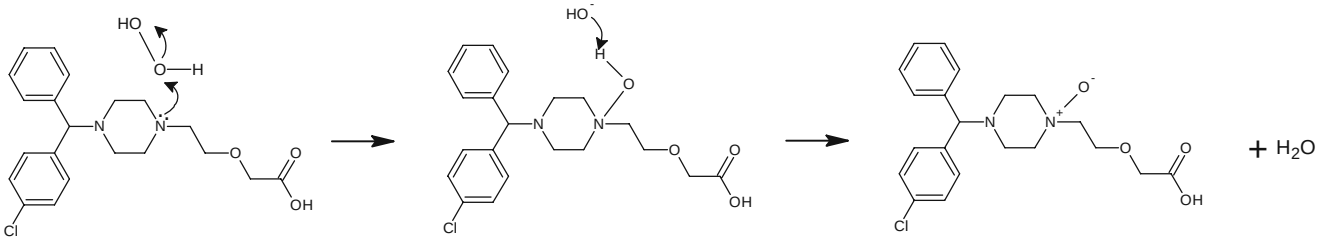


Fig. 7 Proposed mechanism for the oxidation of cetirizine to form N-oxide.

on the nitrogen bearing the ethoxyacetic acid chain, causes a down-field shift of the piperazine ($\Delta\delta = 0.2$) and ethoxy ($\Delta\delta = 0.5$) protons.

This result agreed with the LC/MS data and was verified by spiking the cetirizine degradation sample with cetirizine N-oxide standard. The chromatogram of stressed solution of cetirizine spiked with cetirizine N-oxide (Fig. 4c) showed retention times 19 and 25 min for cetirizine and cetirizine-oxidation products, respectively. Chromatograms of two standards—cetirizine (a) and cetirizine N-oxide (b)—are shown in Fig. 4. Based on these results, the cetirizine oxidation process could be explained by N-oxidation of piperazine nitrogen by hydroperoxides formed from oxidative decomposition of polyoxyethylene chains.

Effect of pH on Oxidation of Cetirizine in PEG formulation

The formation of cetirizine N-oxide in PEG was investigated in a pH range from 3 to 10. Cetirizine, formulated in PEG, was subjected to the forced degradation at different temperatures (30, 60 and 80°C). The aliquots of the product were withdrawn at different times and analyzed by HPLC/UV.

The results obtained at 80°C are presented in Fig. 5. Cetirizine proved to be significantly more stable at pH 4.5 and 6.5 during 48 days exposure at 80°C. Only 0.34–5.7 and 1.5–7.6% of cetirizine N-oxide was formed at pH 4.5 and 6.5, respectively. N-oxide formation increased at pH > 7 and higher N-oxide formation was observed between pH 4.5 and 6.5.

DISCUSSION

Mechanism of Cetirizine Oxidation

The polyoxyethylene-based excipients contain some residual peroxides and could potentially affect the stability of oxidation-sensitive pharmaceuticals. The presence of hydroperoxides and peroxyl radical formed in cetirizine soft gelatin capsule matrix could be the main reason for cetirizine oxidation.

The radical-driven process of cetirizine auto-oxidation predominantly consists of the reactions of initiation, chain propagation and termination. A well-established auto-oxidation process is shown in Fig. 6, where R in our case is $-(OCH_2CH)O-$. The autoxidation starts with decomposition of alkyl polyoxyethylene chain (RH) and subsequent peroxides formation.

The formation of N-oxide progresses according to Fig. 7, producing cetirizine N-oxide with the oxygen atom located on the nitrogen bearing the ethoxyacetic acid chain.

This site of oxidation in the molecule was proposed based on the correlation of results of degradation in pH range from 3 to 10 with ionization scheme of cetirizine and ^{1}H NMR data. The ionization scheme of cetirizine is shown in Fig. 8. The previous studies (8) reported three macro-pKa values for cetirizine (1.52, 2.92 and 8.27), the lowest of which was attributed to the nitrogen nearest to the aromatic rings and pKa 2.9—to the carboxylic acid. The minimum of the oxidation rate at pH of 6.5 could be explained by the protection effect of protons on tertiary amine (9). According to the literature (8,10), zwitterionic form HX is predominant between pH 3 to 8 (Fig. 8).

We assume that between these values of pH the protonation of superoxide radical $\left(O_2^{-\cdot}\right)$ around pH~6 (11) $\left(O_2^{-\cdot} + H^+ \rightarrow HOO^{\cdot}\right)$ may result in the elimination of the charged $O_2^{-\cdot}$ species and therefore decrease the oxidation rate of the protonated tertiary amine. This can happen because the neutral $HOO^{\cdot}$ as an oxidizer would not have the Coloumb attraction as a driving force. As a result, one could obtain another minimum in the oxidation rate at pH below 6.

The peroxide formation in PEG-containing formulations can be inhibited by including an anti-oxidant, such as butylated hydroxytoluene (BHT) and hydroxyl anisole (BHA). The effect of anti-oxidant (BHA) on the rate of cetirizine oxidation under stressed conditions was evaluated in our previous work (12). The data indicated that the addition of BHA reduced the rate of oxidation of cetirizine. The results suggested that the degradation of cetirizine in polyethylene glycol arose from the reaction between the drug and reactive peroxide intermediates, formed through oxidation of PEG.

CONCLUSION

The results of degradation of cetirizine in PEG-containing formulation indicated that cetirizine is susceptible to oxidation. Degradation of cetirizine in polyethylene glycol arose from the reaction between the drug and reactive peroxide intermediates, formed through oxidation of PEG. Cetirizine degradation product was synthesized by the selective oxidation of the sterically less hindered piperazine nitrogen. The qualitative analyses by HPLC, LC/MS/MS along with ^{1}H NMR were used to identify the oxidation product as cetirizine N-oxide. The mechanism of oxidation was proposed. N- oxidation was found to be the major pathway for cetirizine transformation.

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