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Research Article

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Isolation and Characterization of Forced Degradative Products of a Proton Pump Inhibitor Drug Ilaprazole by LC/MS Technique

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ABSTRACT

The liquid Chromatography-Mass spectroscopy (LC-MS) method was developed for isolation and characterization of degradation products of a proton pump inhibitor drug Ilaprazole. The method for the same was developed on Shimadzu LC-8030 with PDA System and Zodiac CN C18, (250mm x 4.6mm, 5µm) column using isocratic elution of mobile phase. The experiment was focused on force degradation studies like (as suggested in ICH guidelines) acid degradation, base hydrolysis, oxidative effects, ultraviolet light effect and thermal stability. The retention time of drug was a minor change in various condition but it was easily characterized by their mass spectra. Various degradants were found in conditions like acidic, basic and oxidative degradation. The chemicals structures of the degradants were identified by using mass spectroscopic data.

Keywords: Ilaprazole; PPI; LC/MS; Degradation; Characterization

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INTRODUCTION

Ilaprazole (ILA), benzimidazole derivative is excellent proton pump inhibitors. Ilaprazoe is very effectively used in the treatment of peptic ulcer disease, dyspepsia, and duodenal ulcer. It is administered orally in enteric coated form to protect them from the muscular transformation in the acidic gastric juice. It reduces the amount of acid in the stomach which helps in relief of acid-related indigestion and heartburn. Ilaprazoe drug is least potent in the class of proton pump inhibitors as per clinical study reports but the various other clinical study shows that Ilaprazole significantly prevented the development of reflux oesophagitis[1-6].

Ilaprazole is not listed officially in any pharmacopeia and is listed in Martindale [7] and Rec. INN (WHO Drug Information Vol. 16, No. 3, 2002) [8]. Ilaprazole irreversibly binding with H+ K+ ATPase and eventually inhibits HCl secretion. The action mediated by disulphide bond formation at binding with cysteine chain of the enzyme. Ilaprazole has a prominent effect on healing of acetic acid-induced chronic ulcers through an hydrogen-potassium adenosine triphosphate inhibition mechanism [6].

Several researchers [9-13] reported a simple and validated RP-HPLC method, HPTLC and UV Methods for estimation of Ilaprazole in single and in bulk drug pharmaceutical dosage forms.

The present work was focused on development and optimization of chromatographic condition for better validation parameter for estimation of Ilaprazole. The work was extended for forced degradation study of Ilaprazole in various condition mild to strong.

EXPERIMENTAL SECTION

Ilazprazole was obtained from Zydus Cadila Pvt. Ltd. as a gift sample. Chemicals used in the present study include Methanol of HPLC Grade was procured from SpectroChem, Acetonitrile of HPLC Grade was procured from SpectroChem, Ammonium Formate of AR Grade was procured from Sigma Aldrich and Mili-Q Water of AR Grade.

A Shimadzu LC 8030 separation module equipped with PDA UV detector having column oven CTO-20AC department. This system is controlled by Lab Solutions Software 5.72B supplied by Shimadzu. The method was developed by using Zodiac CN C18 (250 mm × 4.6 mm, 5 µ m). The analytical wavelength of 254 nm was selected. 10 mM Ammonium Acetate in water with pH 5 was prepared by taking 0.7708 gm of Ammonium Acetate and diluted up to 100 ml of HPLC grade water and pH adjusted to 5.0. The diluent was prepared by mixing 500 ml Methanol and 500 ml Acetonitrile and stored up to 3 days at ambient temperature. The mobile phase was used with the mixture of 10 mM Ammonium Acetate in water with pH 5 and diluent in the ration of 45:55 and this solution was used within 3 days from the date of preparation.

Development of Method by HPLC

Stock solution

Ilaprazole was weighed accurately equivalent to 50 mg of Ilaprazole and the appropriate volume of Methanol was added to make a final concentration of Ilaprazole equivalent to 5 mg/Ml. The prepared solution was kept in a cool condition at 5 ± 3°C. The same solution was further used within 7 days from the date of preparation.
The following forced degradation conditions were carried out as stipulated in ICH Q1A (R2) and Q1B [14-16] guidelines.

Acidic degradation

To 1.0 ml of stock solution of Ilaprazole, 1.0 ml of 0.1 N Hydrochloric Acid was added to a 10 ml volumetric flask. 6 such flasks were prepared. It was kept for 1 hour and then 1 ml of 0.1 N NaOH was added to neutralize the solution. The resultant solution was diluted with mobile phase up to 10 ml to obtain required concentration. Then 20 µl solution was injected into the chromatographic system and the chromatogram was recorded to access the stability of the sample. Likewise was done after 3, 6 and 48 hours. The chromatograms for 1, 3, 6 and 48 hours were recorded.

Alkali degradation

To 1.0 ml of stock solution of Ilaprazole, 1.0 ml of 0.1 N Sodium Hydroxide was added to a 10 ml volumetric flask. 6 such flasks were prepared. It was kept for 1 hour and then 1 ml of 0.1 N HCl was added to neutralize the solution. The resultant solution was diluted with mobile phase up to 10 ml to obtain required concentration. Then 20 µl solution was injected into the chromatographic system and the chromatogram was recorded to access the stability of the sample. Likewise was done after 3, 6 and 48 hours. The chromatograms for 1, 3, 6 and 48 hours were recorded.

Oxidative degradation

To 1.0 ml of stock solution of Ilaprazole, 1.0 ml of 3% Hydrogen Peroxide (H_2O_2). The resultant solution was diluted with mobile phase in a 10 ml volumetric flask to obtain required concentration. Then 20 µl solutions were injected into the chromatographic system and the chromatograms were recorded after 1, 3, 6 and 48 Hours to access the stability of the sample.
Photolytic Degradation: It was carried out by exposing 1 gm Ilaprazole to UV light (254 nm), in UV chamber for 7 days. The resultant solution was diluted with mobile phase in a 10 ml volumetric flask to obtain required concentration. Then 20 µl solutions were injected into the chromatographic system and the chromatograms were recorded for 12, 24 and 48 hours to access the stability of the sample.

Thermal degradation

For dry heat degradation, 1 gm of the Ilaprazole drug was placed in an oven at 70°C for 12, 24 and 48 hrs. Appropriate dilutions were prepared in mobile phase up to 10 ml in a volumetric flask. Then 20 µl solutions were injected into the chromatographic system and the chromatograms were recorded for 12, 24 and 48 hours to access the stability of the sample.

RESULTS AND DISCUSSION

Force degradation study was performed on taking pure API Form of Ilaprazole, In the present study, the high-performance liquid chromatography method was developed for estimation and separation of Ilaprazole and its generated degradants. The major degradants were characterized by using their mass spectra. The Ilaprazole was degraded in acidic, Basic and oxidative conditions and was stable in photolytic and thermal degradation (Figures 1-11).

Analysis of Acidic Degradation Sample by HPLC

Ilaprazole undergoes 7.15% degradation by 0.1 N HCl within 1 hour and 27.28% degradation by 0.1 N HCl within 24 hours.

Analysis of Alkali Degradation Sample by HPLC

Ilaprazole undergoes 6.58% degradation by 0.1 N NaOH within 1 hour and 23.28% degradation by 0.1 N NaOH within 24 hours.

Analysis of Oxidative Degradation Sample by HPLC

Ilaprazole undergoes 5.12% degradation by 3% H_2O_2 within 1 hour and 22.57% degradation by 3% H_2O_2 within 24 hours.

Structural Identification of degradation products

Liquid chromatography-mass Spectroscopy was performed for identification of structural property of our Ilaprazoe pure API and their degradants. The molecular ion and fragment ion peaks were identified using mass spectra of individual degradants [17].

The data related to retention time and number of prominent degradants from forced degradation by HPLC is presented in Table 1.
The probable Structure, Molecular formula, and mass data are presented in Table 2.

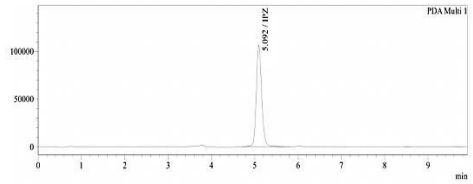


Figure 1: HPLC chromatogram of Ilaprazole (RT-5.092 min)

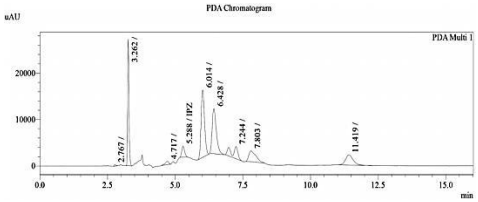


Figure 2: HPLC chromatogram of Acidic Degradation (1 hour)

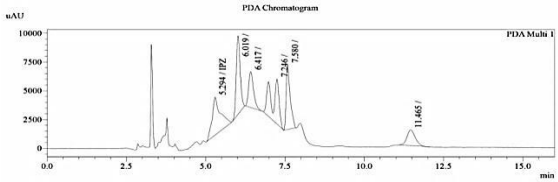


Figure 3: HPLC chromatogram of alkali degradation (1 hour)

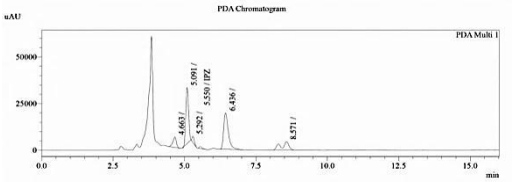


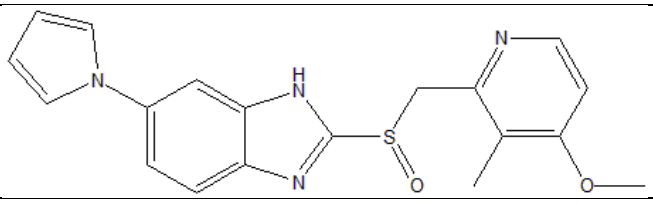
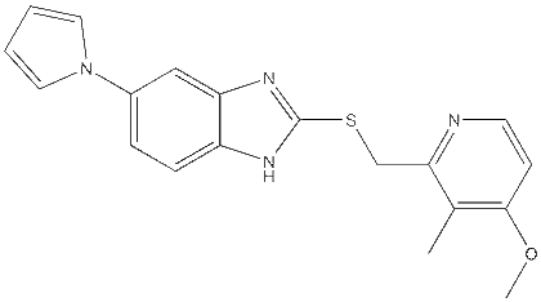
Figure 4: HPLC chromatogram of oxidative degradation (1 hour)

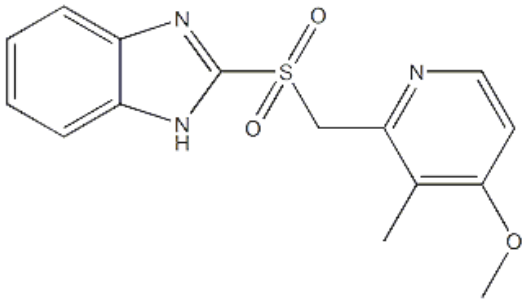
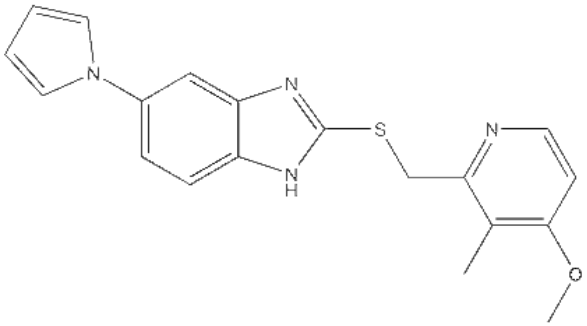
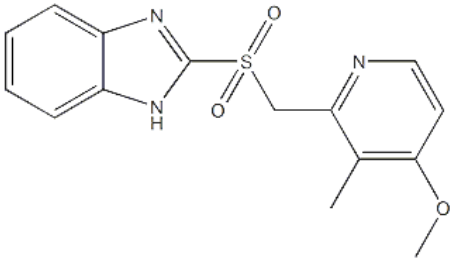
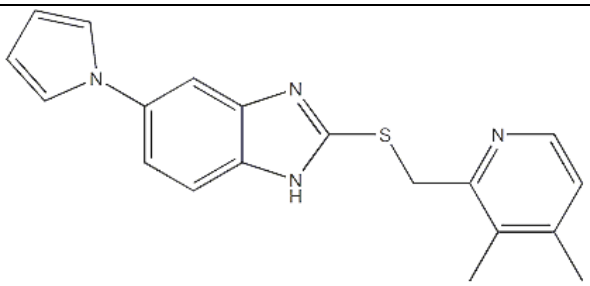
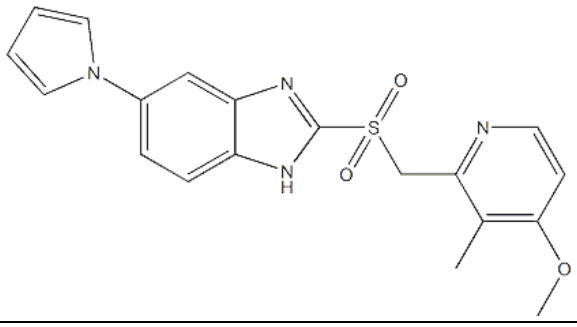
Table 1: Summary of force degradation study

Sr No.	Degradation Condition	RT	Remarks
1	Ilaprazole Pure API	5.092	Drug Substance
2	Acidic	5.288	One Prominent degradant was identified
3	Alkali	5.294	Two Prominent degradants were identified
4	Oxidative	5.55	Three Prominent degradants were identified
5	Photolytic	5.702	No Prominent degradant was identified
6	Thermal	5.731	No Prominent degradant was identified

Degradants Structures obtained from Mass Spectra are summarized in Table 2.

Table 2: Degradants structures obtained from mass spectra

Condition	Probable Structures	Molecular Formula	Molecular Weight (g/mol)
Ilaprazole		$C_{19}H_{18}N_4O_2S$	367
Acidic Degradation Product		$C_{19}H_{18}N_4OS$	351

Alkali Degradation Product 1		$C_{15}H_{15}N_3O_3S$	318
Alkali Degradation Product 2		$C_{19}H_{18}N_4OS$	351
Oxidative Degradation Product 1		$C_{15}H_{15}N_3O_3S$	318
Oxidative Degradation Product 2		$C_{19}H_{18}N_4S$	334
Oxidative Degradation Product 3		$C_{19}H_{18}N_4O_3S$	382

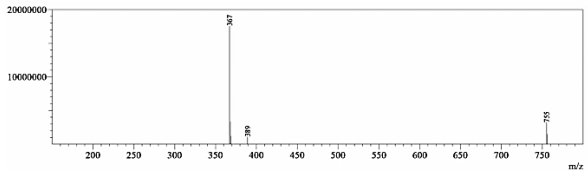


Figure 5: LC/MS-spectrum of Ilaprazole

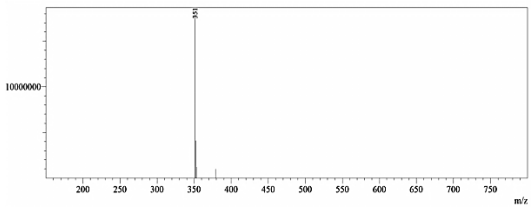


Figure 6: LC/MS spectrum of acidic degradant

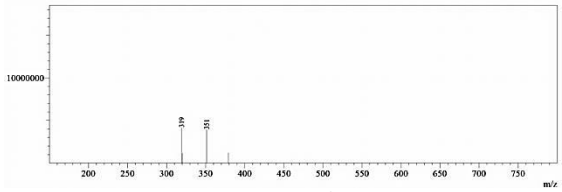


Figure 7: LC/MS spectrum of alkali degradant 1

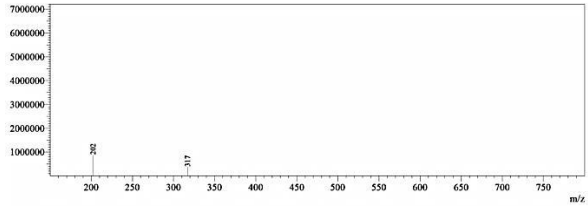


Figure 8: LC/MS spectrum of alkali degradant 2

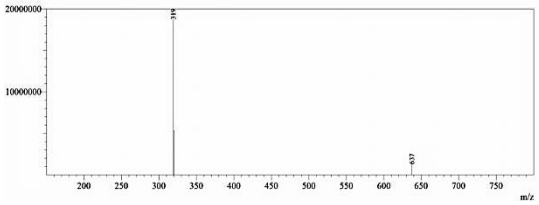


Figure 9: LC/MS spectrum of oxidative degradant 1 (m+1)

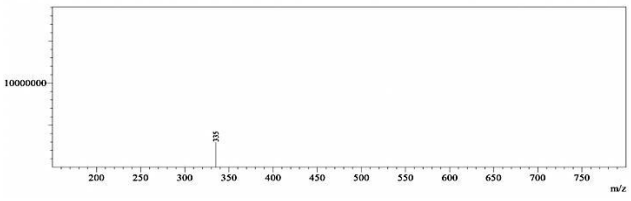


Figure 10: LC/MS spectrum of oxidative degradant 2

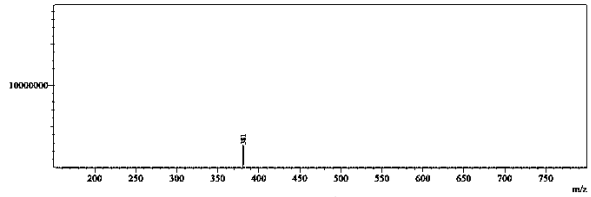


Figure 11: LC/MS spectrum of oxidative degradant 3

CONCLUSION

Ilaprazole degraded in three of five degradation condition like acidic, alkali and oxidative while it remain stable in two of five degradation condition like ultra violet and thermal. The generated prominent degradant were identify by LC/MS the product as 2-((4-methoxy-3-methylpyridin-2-yl)methylthio)-5-(1H-pyrrol-1-yl)-1H-benzo[d]imidazole by Acidic Degradation, 2-((4-methoxy-3-methylpyridin-2-yl)methylsulfonyl)-1H-benzo[d]imidazole and 2-((4-methoxy-3-methylpyridin-2-yl)methylthio)-5-(1H-pyrrol-1-yl)-1H-benzo[d]imidazole by Alkali Degradation and 2-((4-methoxy-3-methylpyridin-2-yl)methylsulfonyl)-1H-benzo[d]imidazole, 2-((3,4-dimethylpyridin-2-yl)methylthio)-5-(1H-pyrrol-1-yl)-1H-benzo[d]imidazole and 2-((4-methoxy-3-methylpyridin-2-yl)methylsulfonyl)-5-(1H-pyrrol-1-yl)-1H-benzo[d]imidazole by Oxidative Degradation.

The highest degradation was found in acidic condition (0.1 N HCl) at 24 hours and there was no any significant degradation was observed in UV and thermal condition. This study was highly useful for a pharmaceutical industry who is making the formulation of Ilaprazole.

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