Validated Stability-indicating assay method for determination of Ilaprazole in bulk drug and tablets by high performance liquid chromatography

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Abstract

A validated stability-indicating HPLC method has been reported for the determination of Ilaprazole in bulk drug and tablet. The drug was subjected to the various stress conditions as per the ICH guidelines. The degradation behavior of Ilaprazole was studied under hydrolytic, oxidative, photolytic and thermal conditions and was found to be unstable in almost all conditions except under alkaline and photolytic conditions. The separation of drug and its degraded products was carried out on Kinetex C-18 100A (5μ, 250×4.6 mm) column. The initial mobile phase composition used was Acetonitrile and water in the ratio 50:70v/v for 1 min then changed to 70:30v/v in next 6 min and finally equilibrated back to initial composition in 14min. The method was applied for the determination of Ilaprazole in marketed tablet formulation. The detection was carried at 305 nm using PDA detector with a flow rate of 1.0ml/min and injection volume 20μl. The validation of developed method was performed for linearity, accuracy, precision, selectivity and specificity and robustness.

1. Introduction

For the development of stability-indicating assay method, the drug is subjected to various ICH (International Conference on Harmonization) stress conditions such as photolytic, hydrolytic, thermal and oxidative [1]. As per the ICH drug stability test guidelines Q1A (R2), validated stability-indicating assay method should be developed for the analysis of drug substance and drug product [2,3]. The ICH guidelines Q6A explains about specifications [4] and tests criteria for drug substance and product in order to perform stability-indicating assays. Ilaprazole (Fig. 1); 2-[[(4-Methoxy-3-methyl-2-pyridinyl)methyl]sulfinyl]-6-(1H-pyrrol-1-yl)-1H-benzimidazole is a new proton pump inhibitor used in the treatment of peptic ulcer disease, dyspepsia, gastro esophageal reflux disease and duodenal ulcer which reduces acid secretion by inhibiting the parietal cell H^+/K^+ ATP pump. In literatures, few analytical methods have been reported of analysis of Ilaprazole such as UPLC [5], LC-MS/MS [6,7], HPLC-ESI-MS/MS [8], however no stability-indicating HPLC method have been reported for the analysis of Ilaprazole in presence of its degradation products as per ICH recommended approach. Hence the aim of the present study was to develop and validate stability-indicating HPLC method for determination of Ilaprazole (IPZ) bulk drug as per the ICH stress

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conditions. The developed method is stability-indicating and was successfully utilized for the determination of Ilaprazole in tablet formulation.
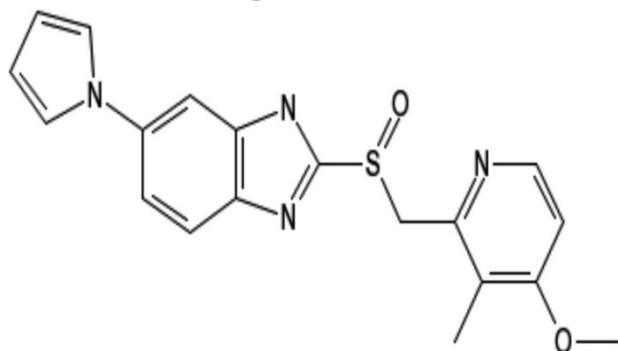

Fig. 1: Structure of Ilaprazole

2. Experimental

2.1 Materials:

Ilaprazole API (Active Pharmaceutical Ingredient) was received as gift sample from Precise Chemipharma Pvt. Ltd. HPLC grade Acetonitrile (Spectrochem) and deionized water was used for the study. All other chemicals used in the study were of Analytical reagent (AR) grade.

2.2 Instrumentation:

The development and validation of Ilaprazole was carried out using WATERS (Milford, USA) HPLC system having pump (600E), autosampler, controller (Waters^{TM} 600), in-line degasser (Waters AF module) and Photodiode array (PDA, 996) detector. The data collection and processing was done using EMPOWER build (1154) software. The analysis of Ilaprazole and its degraded products was performed using Kinetex C-18 100A (5μ, 250×4.6 mm) column.

2.3 Stock and standard solutions of Ilaprazole

Ilaprazole stock solution (100μg/ml) was prepared by dissolving 100mg in 100ml Acetonitrile and further diluted to prepare standard solution (100μg mL^{-1}).

2.4 Degradation Studies:

All stress studies for Ilaprazole were performed at concentration of 1mg/ml. The neutral (water) degradation study was performed by refluxing the drug solution at 80^0C for 8h. The alkaline degradation study was carried out by refluxing drug solution in 0.1M NaOH at 80^0C for 48h. The drug solution was refluxed with 0.01M HCl at 80^0C for 6h to conduct degradation study under acidic conditions. For degradation study in hydrogen peroxide (H_2O_2) drug solution was refluxed with 3% H_2O_2 at 80^0C for 8h. Photolytic stress degradation study was carried out by exposing the drug powder to UV light for 48h. Thermal degradation behavior of Ilaprazole was studied by exposing the drug powder to dry heat in an oven at 60^0C for 24h. Stressed samples were withdrawn periodically and analyzed by HPLC after suitable dilution.

2.5 Analysis of Tablet formulation:

Twenty tablets (Iladay$^{®}$) were accurately weighed and finely powdered. An accurately weighed amount equivalent to 10mg of Ilaprazole was sonicated with 30ml of acetonitrile for 30 min. The contents after filtration were transferred into a 100ml volumetric flask and

volume was made upto the mark with acetonitrile. The resultant solution was diluted further to obtain a concentration of 10µg/ml. The resultant solution was analyzed by the developed HPLC method.

3. Results and discussion

3.1 Method development

The HPLC gradient programming was utilized to analyze drug and its degradation products. The separation was achieved with mobile phase consisting of Acetonitrile and Water in the ratio of 50:50 (v/v) for 1 min. then changed to 70:30 (v/v) in next 6 min. and brought back to initial composition 50:50 (v/v) from 7min to 14 min. The detection was carried out at 305nm with a flow rate of 1ml/min and injection volume of 20µl. The typical chromatogram of standard solution of Ilaprazole is shown in Fig. 2A.

3.2 Degradation behavior of IPZ

Ilaprazole was subjected to above mentioned stress conditions and showed following degradation behavior.

3.2.1 Hydrolytic studies

IPZ when refluxed with water at 80^0C showed significant degradation after 1h of reflux. The degradation products appeared at RTs 2.063 (NDP-I) and 8.534 (NDP-II) shown in Fig. 2. The drug was also found to be unstable under acidic condition which was seen after refluxing it with 0.1M HCl at 80^0C. The major degradation products appeared at RTs 2.017, 5.491, 8.465 (HDP-I, HDP-II, HDP-III) shown in Fig. 3. The drug was found to be sufficiently stable under alkaline condition. No degradation product was seen after refluxing drug solution in 0.1M NaOH at 80^0C for 48h shown in Fig. 4.
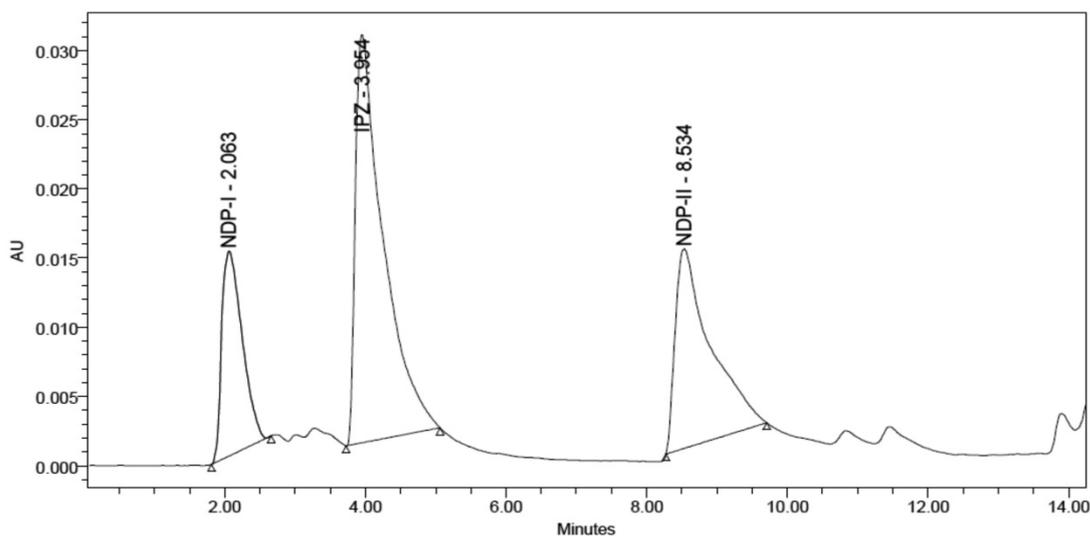

Fig. 2: HPLC chromatograms of Neutral hydrolysis degraded IPZ
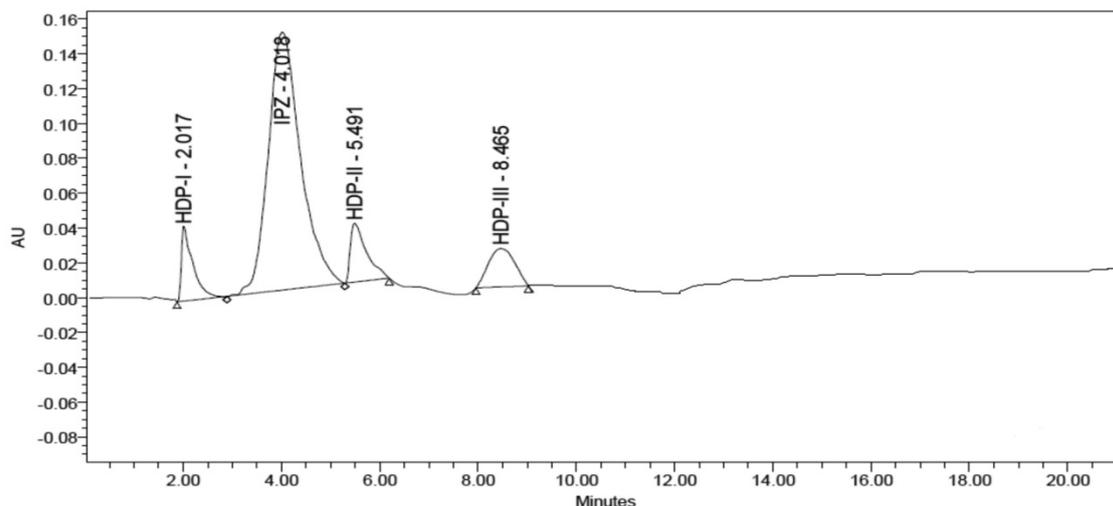
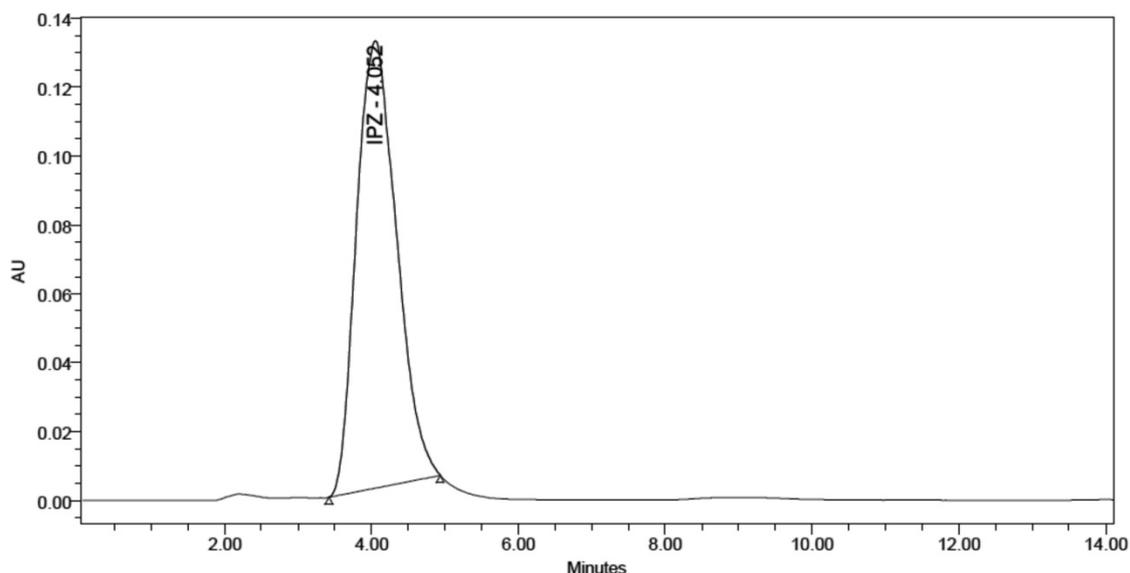**Fig. 3:** HPLC chromatograms of Acid hydrolysis degraded IPZ

Fig. 4: HPLC chromatograms of Alkali Hydrolysis degraded IPZ

3.2.2 Oxidative studies

The drug degraded rapidly in 3% H_2O_2 upon refluxing it at 80^0C for 8h. The major degradation products appeared at RTs 3.505 (ODP-I) and 8.025 (ODP-II) within 1h (Fig. 5).

3.2.3 Photostability

Ilaprazole drug powder was subjected to UV light for 48h but drug was found to be sufficiently stable under these conditions indicated by the absence any degradation peak (Fig. 6)

3.2.4 Thermal studies

The drug was found to be thermally unstable after exposing it to dry heat in an oven at 60^0C for 24h. The degradation peak appeared at RT 2.677 (TDP-I) shown in Fig. 7.
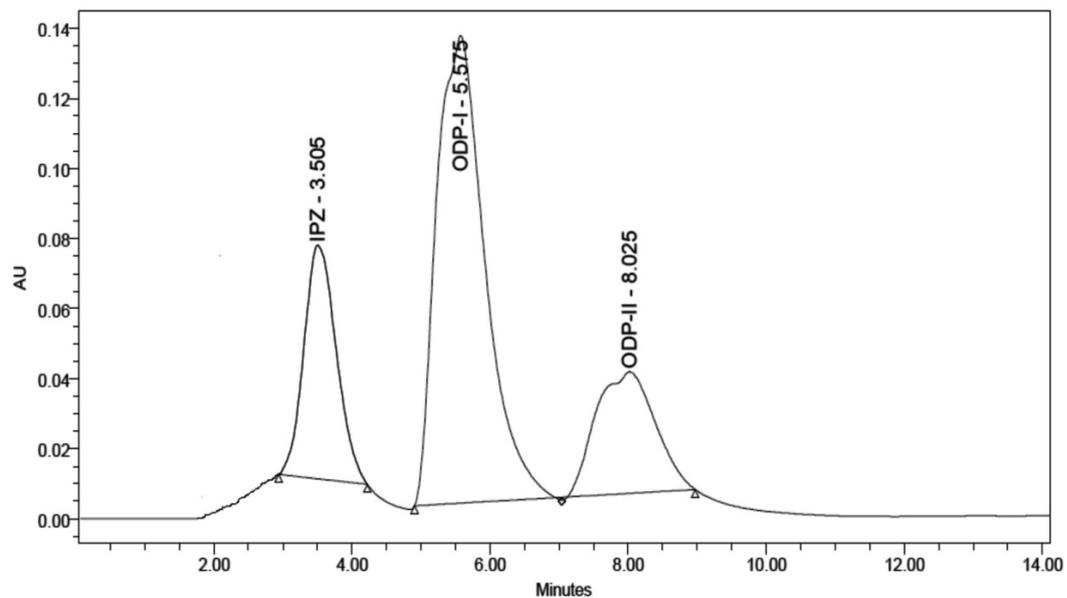
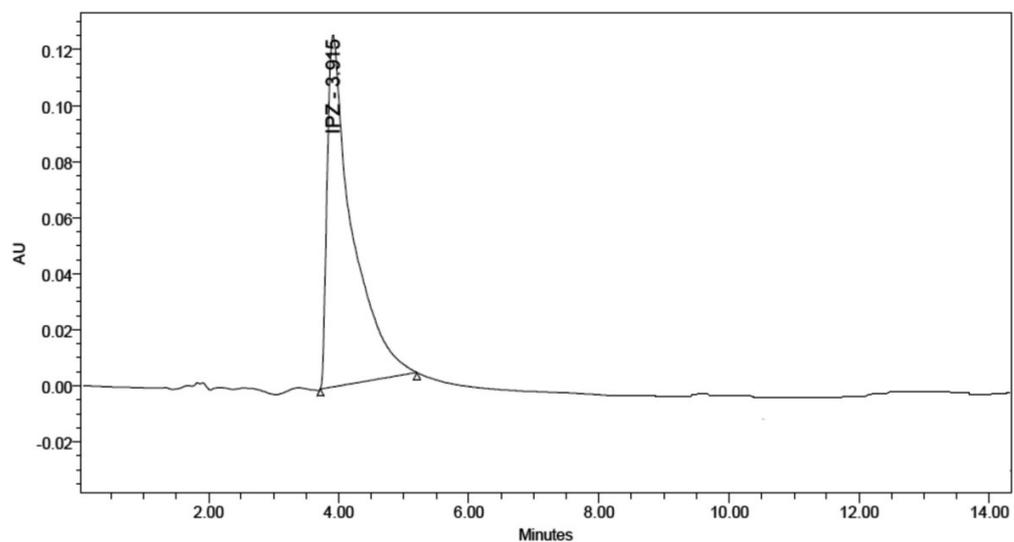**Fig. 5:** HPLC chromatograms of Oxidative degradation of IPZ

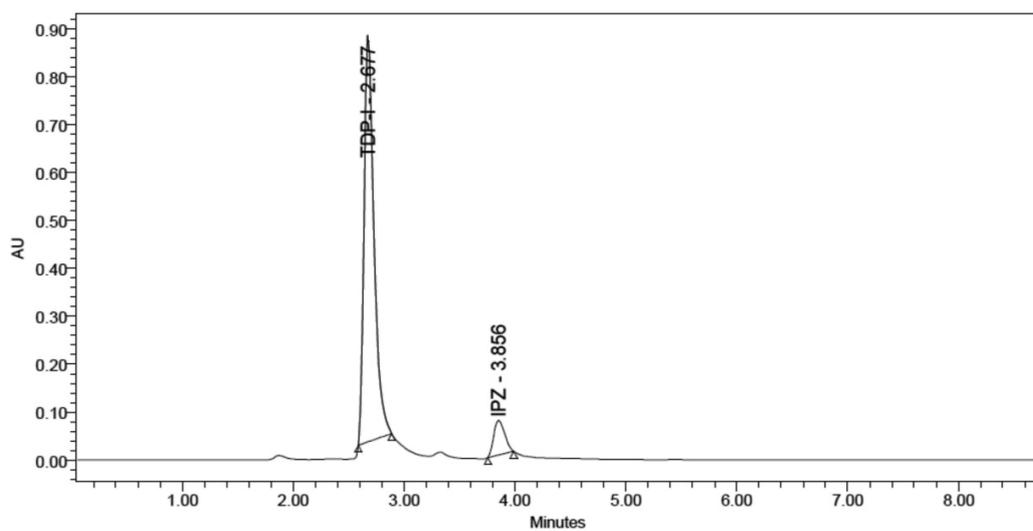**Fig. 6:** HPLC chromatograms of Photolytic degradation of IPZ

Fig. 7: HPLC chromatograms of Thermal degradation of IPZ

3.3 Validation

3.3.1 Linearity

The method was found to be linear in the range from 5-15 µg mL^{-1}. The study was carried out at five different concentration levels and results were recorded in triplicate at each level. The correlation coefficient was found to be 0.998. The result of Linearity is shown in Table 1.

Table 1: Results of Linearity study (n=3)

Sr. No.	Concentration (µg/mL)	Mean Peak area ± S.D.	R.S.D. (%)
1	5	104265.7±11.16	0.11
2	8	151966±255.37	0.17
3	10	187660±318.63	0.17
4	12	227706.7±480.38	0.21
5	15	273058.3±889.60	0.33

3.3.2 Recovery

The recovery experiments were performed to study the accuracy of method. The standard addition method was utilized for recovery experiments. The result of study showed good recoveries for each added concentration. The data of recovery study is shown in Table 2.

Table 2: Recovery study of IPZ (n=3)

Sr. No.	Actual Concentration (µg/ml)	Concentration Found (µg/ml), ±S.D.	R.S.D (%)	Recovery (%)	Mean % Recovery
01	8	7.92±0.102	1.287	99.00	
02	10	10.11±0.075	0.741	101.10	99.78±1.14%
03	12	11.91±0.199	1.670	99.25	

3.3.3 Precision

The method was found to be precise which was proved through Intra-day and Inter-day precision study data as shown in Table 3. Intra-day and Inter-day precision was carried out by analyzing three different concentration of drug within same day and different days respectively.

Table 3: Intra-day and inter-day precision studies (n=3)

Sr. No.	Spiked Concentration (µg/ml)	Intra-day		Inter-day	
		Concentration found (µg/ml)±S.D.	RSD (%)	Concentration found (µg/ml)±S.D.	RSD (%)
01	8	8.07±0.144	1.784	8.11±0.134	1.652
02	10	9.92±0.137	1.381	10.09±0.053	0.525
03	12	12.10±0.102	0.842	11.72±0.168	1.433

3.3.4 Specificity and Selectivity

The degraded samples of IPZ were analyzed by proposed HPLC method. The method was specific as drug and its degraded products showed adequate resolution thought the analysis. The PDA peak purity study proved that method is selective which can be seen in purity plot of IPZ in presence of degradation products (Fig. 8).
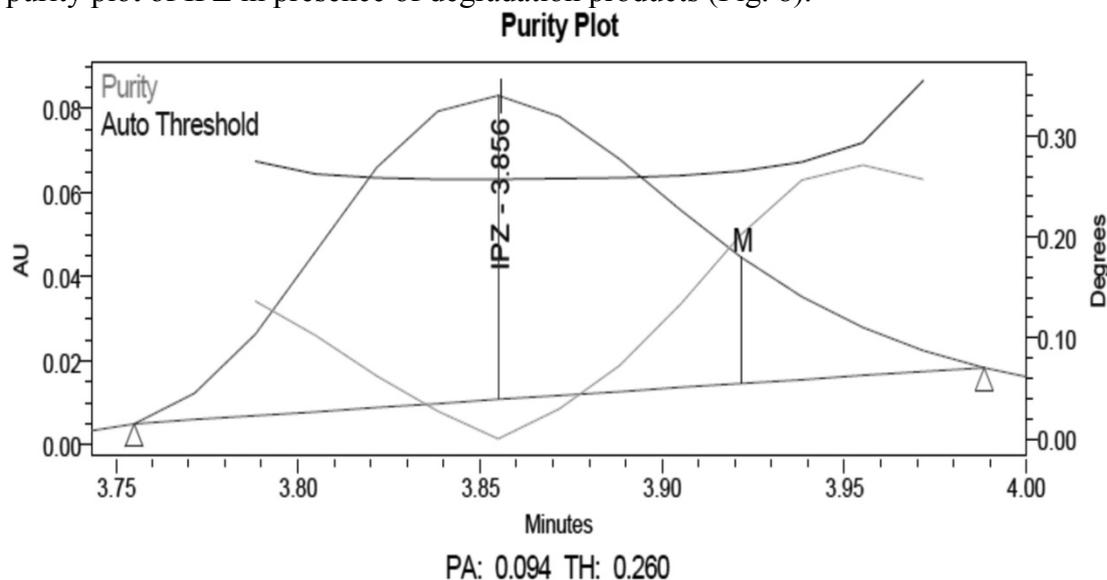

Fig. 8: Purity Plot of Ilaprazole

3.3.5 Robustness

The analysis of Ilaprazole in presence of its degradation products was studied on different HPLC system for three different days, the resolution pattern was found to be similar and thus method was found to be robust.

3.3.6 LOD and LOQ

The sensitivity of developed method was found out by means of limit of detection (LOD) and limit of quantification (LOQ). Both LOD and LOQ were measured as per the methods prescribed by the ICH (International Conference on Harmonization). The LOD and LOQ of Ilaprazole were found to be 0.05μg/ml and 0.14 μg mL^{-1}, respectively. These values are adequate for the detection and quantification of Ilaprazole.

3.4 Assay

The proposed method was applied for the determination of Ilaprazole in Tablet dosage form. The result of assay was 99.27%, % RSD = 1.65 of label claim for Iladay® tablets. The method was found to be selective for Ilaprazole assay as there was no interference from the excipients of tablets. A typical chromatogram obtained after the analysis of Iladay® tablets is shown in Fig. 9.
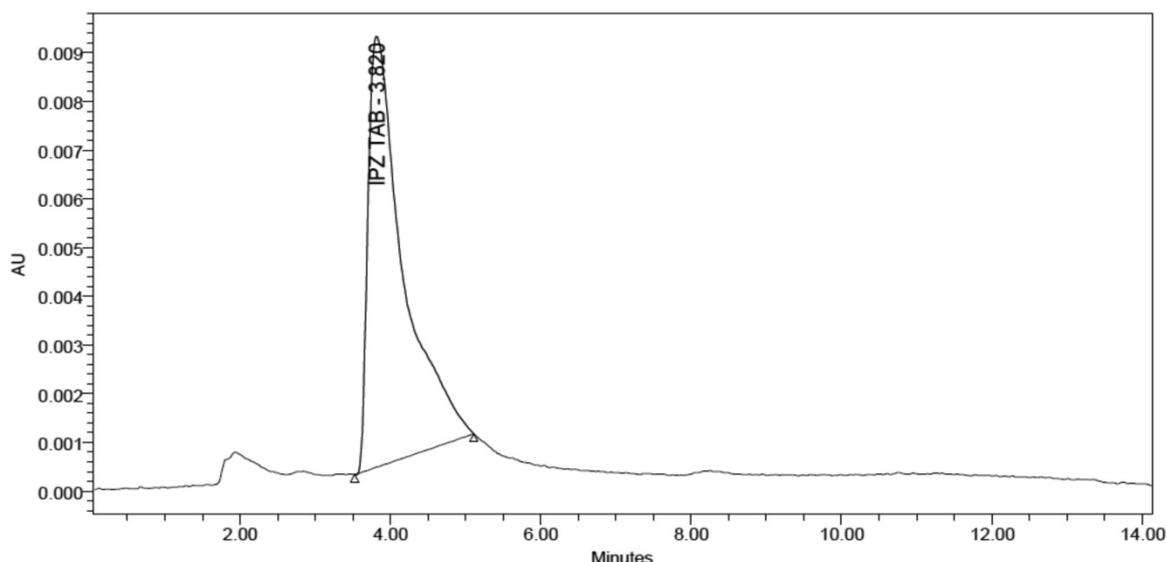
Fig. 9: HPLC chromatograms of Assay of Ilaprazole Tablets.

4. Conclusion

The stability-indicating has been developed and validated for the determination of Ilaprazole in bulk drug and tablet dosage form. The degradation behavior of Ilaprazole was studied as per ICH recommended conditions. The proposed method is simple, precise, accurate, specific, and is able to separate drug from its degradation products. The developed method could also be extended to the analysis of stressed marketed formulation of Ilaprazole, as there is no interference from excipients or other components was observed.

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