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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF ILAPRAZOLE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract

A RP-HPLC method has been developed and validated for the estimation of Ilaprazole in bulk and pharmaceutical dosage form.The isocratic separation was achieved on a Shodex C_{18} column (250×4.6 mm, 5µm).The method was developed using mobilephase comprising of a mixture of 0.1 M phosphate buffer (pH adjusted to 7.6) and Acetonitrile (50:50, v/v) at a flow rate of 1.0mL/min. The analyte was monitored with UV detector at a wavelength of 306 nm. The retention time of Ilaprazole was found to be 5.86 min. The method was validated according to ICH guidelines for various parameters like accuracy, precision, specificity, linearity, robustness, LOD and LOQ. Linearity was observed in the concentration range of 2-16µg/mL with acorrelation coefficient of 0.994. The limit of detection and limit of quantification for Ilaprazolewere found to be0.01µg/mL and 0.1µg/mL respectively. The proposed method is simple, accurate, precise and robust therefore can be used for routine analysis of Ilaprazole in bulk drug and pharmaceutical formulation.

Key Words: ICH guidelines, Ilaprazole,Method Development, RP-HPLC, Validation.

Introduction:: Ilaprazole (IPZ) is a proton pump inhibitor which is chemically {2-[[(4-methoxy-3-methyl)-2-pyridinyl] methylsulfinyl-5-(1Hpyrrol- 1-yl)-1H-benzimidazole.The chemical structure of the drug is shownin Figure 1.

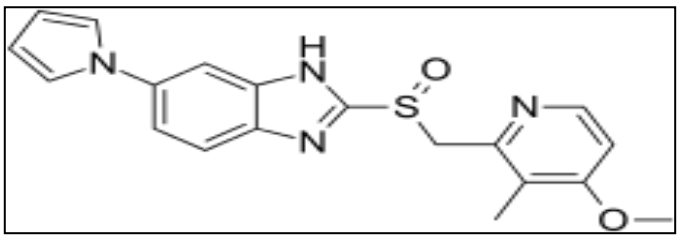

Figure 1: Chemical structure of Ilaprazole (IPZ)

IPZ is used in the treatment of dyspepsia, peptic ulcer disease,gastroesophageal reflux disease and duodenal ulcer[1].Literature survey reveals that there are several analytical methods reported for the determination of IPZfrom biological fluids using hyphenated techniques like HPLC-ESI-MS/MS[2, 3, 4], HPLC-NMR[2]. There is also a method reported for enantiomeric separation of IPZ and other proton pump inhibitors on new generation chiral columns using HPLC and supercritical fluid chromatography.[5] However, there is no method reported for the determination of IPZin bulk and formulation. Hence the present work aims to introduce a novel RP-HPLC method for the determination of IPZ in its bulk and tablet form. This method is very simple in application in comparison with the previously reported methods and at the same time it offers a high degree of accuracy and precision.

MaterialsandMethods

Materials: IPZwas obtained as a gift sample fromAjanthapharma, Aurangabad,Maharashtra HPLC grade methanol;monobasic potassium dihydrogenophosphate and o-phosphoricacid were purchased from Molychem Manufacturers & Importers of Laboratory Reagents & Fine Chemicals. All other chemicals& used were of analytical grade purchased fromMolychemIndia. Double distilled water used in the study was preparedin house using borosil glass distillation unit.

Instrumentation

The analysis was performed using Agilent 1200 seriesquaternary pump HPLC system equipped with variablewavelength programmable UV detector with precision loopinjector (Rheodyne20µl). 50µL Hamilton injection syringewas used for sample injection. The data was processedusing Chemstation (B.02.01) software. UV method analysiswas performed on a double beam Jasco V-630spectrophotometer with Spectramanager software. Allchemicals were weighed using Shimadzu electronicbalance, Measurement of pH of buffer solutions was madeusing Equip-tronics digital pH meter with magnetic stirrer.All solutions used in HPLC analysis were filtered using a0.45µm nylon membrane filtration apparatus with vacuumpump. Oscar Ultrasonics bath sonicator was used for degassing the mobile phase.

Chromatographic Conditions

The chromatographic separation was performed in aShodex C_{18}-4E column (5 µm; 250 × 4.6 mm, Showa DenkoAmerica Inc., USA). The mobile phase comprised of amixture of 0.1 M phosphate buffer (pH adjusted to 7.6) and

acetonitrile (50:50, *v/v*) at a flow rate of 1.0 mL/min withiscocratic elution. The injection volume was 20 µL and the run time was 10 min. Detection was carried out at 306 nm.

Preparation of Standard Stock Solution

Accurately about 10 mg of IPZ wasweighed and transferred to a 10 ml volumetric flask. Thevolume was then made up to the mark with methanol toget a standard solution of ilaprazole at a concentration of1000µg/mL.

Preparation of Working Standard Solutions

Working standard solutions for HPLC injections wereprepared on a daily basis Aliquots of the standard stocksolution were taken and diluted with the mobile phase toget solutions in a concentration range of 2 -16µg/mL.

Assay of Tablet Formulation[6]

Twenty marketed tablets of IPZ(ILAPRO tab® 10mg) were accurately weighed andtriturated. The average weight per tablet was calculatedand tablet powder equivalent to 10mg of IPZ wasweighed and transferred into 50ml volumetric flask. 70mlof methanol was added to dissolve the contents of the flaskwith the aid ofultrasonication for 10mins and volume wasmade up to100ml with methanol to get a stock solutionwith concentration of 100µg/mL. 1ml of the stock solutionwas further diluted up to 10ml with the mobile phase to get a final concentration of 10µg.ml. The resulting solution wassubjected to chromatographic analysis. From the resultsobtained the percentage assay of the drug was calculated.

Method Validation

The method was validated as per ICH guidelines[7] todemonstrate that it is suitable for the intended purpose.The method was validated for system suitability, linearity,accuracy, precision, limit of detection, limit ofquantification and robustness.[8]

a. System Suitability: System suitability parameters were studied to ensure thatthe instrument is suitable for the intended purpose.Retention time, tailing factor and theoretical plates wereevaluated. The drug solution was injected five times into chromatographic system under the optimized conditionsand the parameters were evaluated.

b. Linearity: Series of dilutions were prepared from the standard stocksolution of IPZ in the concentration range of 2–16 µg/mL. 20µL of each of these solutions was then injectedinto the column and the chromatographic characteristicswere studied under the optimized conditions.

c. Specificity:

Marketed tablets of IPZ wereanalyzed to determine the specificity of the optimizedmethod in the presence of excipients. The chromatogramswere observed for the interfering peaks at the retentiontime of IPZ.

d. Accuracy:

The recovery studies for the method were carried out bystandard addition method. It was evaluated at threeconcentration levels (80, 100 and 120%) and thepercentage recoveries were calculated.

e. **Precision:**

The precision of the method was determined by intra andinter day precision studies. This was evaluated by injectingthree different sample preparations of IPZ from asingle formulation at three different concentration levelson the same day (Intra day) and on three different days(Inter day). From the resulting data the % Relative standarddeviation was calculated.

f. Limit of Detection and Limit of Quantification:

The Limit of Detection (LOD) and Limit of Quantification(LOQ) were determined based on the standard deviation ofthe response and the slope of the calibration curve. Thesensitivity of the method was established by the LOD andthe LOQ values.

g. **Robustness:**

Robustness was established by introducing small deliberatechanges in the HPLC optimized conditions which include thechange in wavelength, flow rate and percentage ofmethanol in mobile phase. This was studied using threereplicates at a concentration level of 10µg/mL.

Results and Discussion

A simple RP-HPLC method has been developed for determination of IPZ. The method wasoptimized to provide a good separation of the component (acceptable theoretical plates) with a sufficient sensitivityand suitable peak symmetry in a short run.[9]For this purpose, the analytical column, solvent selection, mobilephase composition, flow rate, and detector wavelength were studied.[10] The chromatographic separation wasachieved using an RP C_{18} column. Our experiments using acetonitrile along with low pH phosphate buffer as mobilephase gave a good peak shape (peak symmetry) and

resolution for IPZ. The optimizedchromatographic conditions are given in Table 1. The representative chromatogram of standard IPZ is shown in Figure 2.

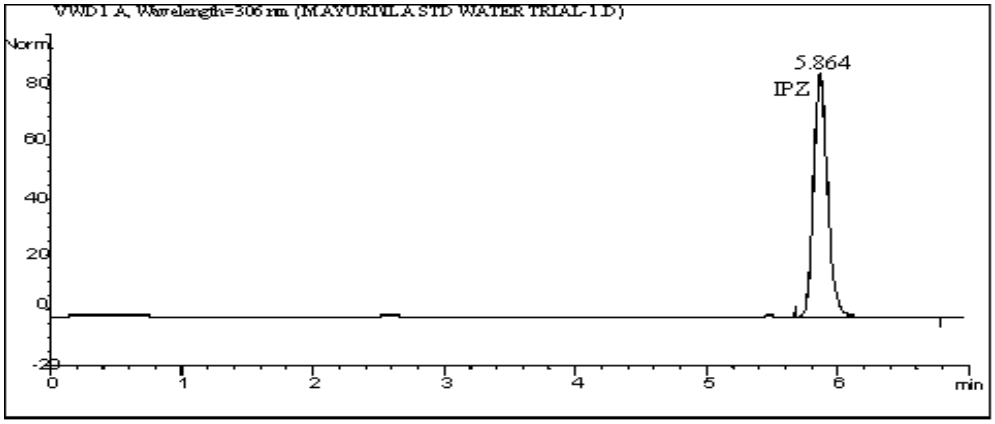

Figure 2: Representative chromatogram of standard IPZ

The chromatogram for the assay of marketed tablets showed a single peak for IPZ at a retention time of 5.86 min. The representative chromatogram is depicted in Figure 3.

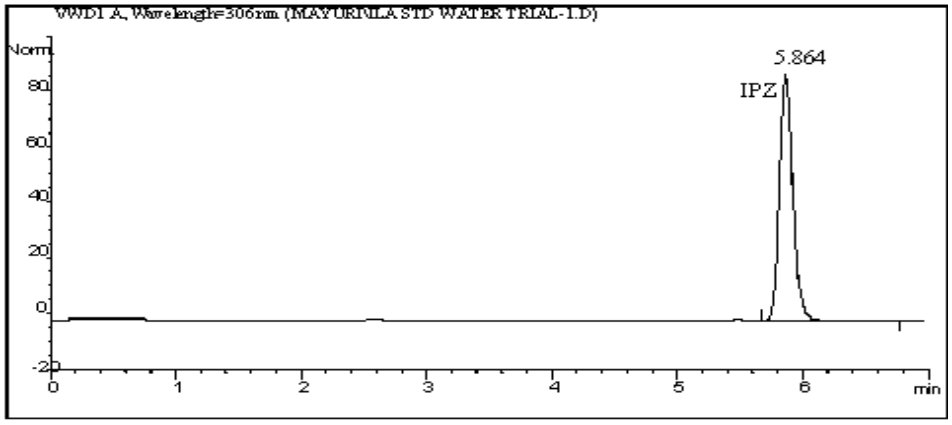

Figure 3: Representative chromatogram of IPZ in tablet formulation

Table 1: Optimized chromatographic conditions for determination of IPZ.

Parameters	**Optimized condition**
HPLC system	Agilent 1200 series quaternary pump system with Chemstation software
Column	Shodex C_{18}-4E (5 µm; 250× 4.6 mm)
Mobile phase	Acetonitrile: Phosphate buffer (pH 7.6; 0.02M) (50:50 V/V)
Flow rate	1mL/min
Detection wavelength	306
Injection volume	20µL
Concentration of Standard IPZ	10 µg/mL

The report for the assay of IPZ tablets is presented in Table 2.

Table 2: Report for assay of IPZ tablets.

Drug	Amount present **(mg/Tablet)**	**Amount found (mg/Tablet)**	**% Label Claim**
IPZ	10	9.86	98.6

The proposed method was found to be simple. The linearity data is tabulated in Table 3. Calibration curve of peak area against concentration was found to be linear in the concentration range of 2 - 16µg/mL as shown in Figure 4 with the regression equation y= 70.86x -10.71 and the correlation coefficient of 0.994.[11]

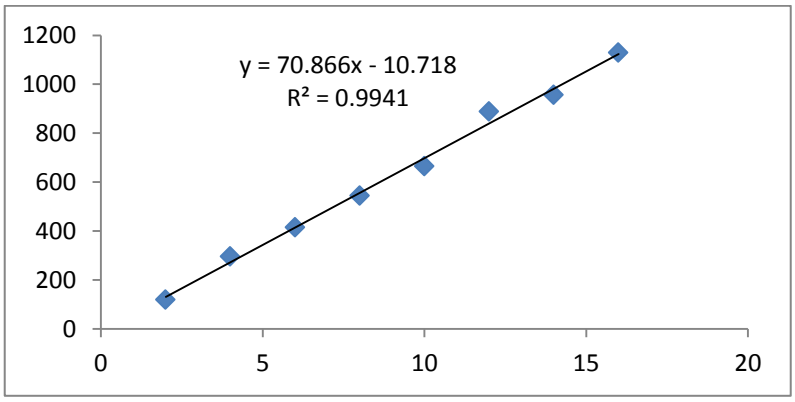

Figure 4: Calibration curve of IPZ.

Table 3: Linearity data.

Concentration (µg/mL)	**Peak Area (mAU*s)**
2	120.11
4	296.56
6	414.84
8	545.24
10	665.19
12	889.09
14	956.37
16	1129.22

System suitability parameters indicate high columnefficiency with large number of theoretical plates (.>2000).The tailing factor was found to be 0.80 which does notexceed the critical value of 2. The average retention time was found to

be 5.86 min. No interference was seen fromany of the excipients of the marketed tablet of IPZ indicating the specificity of the method. Theresults of recovery studies are tabulated in Table 4. Goodrecovery of the spiked drug was obtained at each addedconcentration, and the %RSD was found to be in the rangeof 0.24-0.80.

Table-4: Recovery data of IPZ.

% Spike Level	Amount added (mg)	Amount found (mg)	% Recovery	Mean % Recovery ± SD (n=3)	%RSD
80%	8.00	8.18	101.15	101.50 ± 0.748	0.812
	8.00	8.15	100.91		
	8.00	8.39	102.44		
100%	10.00	10.18	100.88	101.98 ± 0.979	0.961
	10.00	10.46	102.32		
	10.00	10.55	102.75		
120%	12.00	12.11	100.46	100.49 ±0.224	0.224
	12.00	12.07	100.28		
	12.00	12.18	100.73		

SD: Standard deviation, %RSD: % Relative standard deviation

The data for precision is represented in Table 5. The %RSDwas found to be 0.28-0.97 for intraday and 0.49 – 1.24 for inter day precision studies. Thus the developed methodwas found to be accurate and precise as the % RSD valuewas less than 2.[12]

Table-5: Precision data for IPZ.

Sr. No.	Conc. (µg/mL)	Intraday precision		Inter day precision	
		Mean*± SD	%RSD	Mean*± SD	%RSD
1	8	8.46 ± 0.11	0.89	12.14±0.05	0.41
2	10	10.83 ±0.08	0.37	14.4±0.04	0.25
2	12	12.28±0.06	0.37	16.79±0.06	0.36

SD: Standard deviation, %RSD: % Relative standard deviation

The limit of detection and limit of quantification for Ilaprazole were found to be 0.01µg/mL and 0.1µg/mL respectively. The results of robustness study are given in Table 6. It was found that there was no drastic change in the resolution of IPZ when deliberate changes were introduced in the optimized chromatographic conditions thus confirming robustness of the developed method.

Table-6: Results of Robustness Study.

Parameter	Optimized	Variation	Mean peak area (mAU*s)	Mean Retention time (min)	Mean No. of theoretical plates	Mean Tailing factor
Detection wavelength	306 nm	304 nm	664.10	5.75	15234	0.81
		-	665.19	5.86	15282	0.80
		308 nm	664.45	5.81	15267	0.79
Flow rate	1 ml/min	0.8ml/min	664.17	5.74	15290	0.77
		-	665.19	5.86	15282	0.80
		1.2ml/min	664.78	5.82	15251	0.80
% of acetonitrial in mobile phase 50	50%	48%	666.89	5.46	15278	0.78
		-	665.19	5.86	15282	0.80
		52%	667.23	5.99	15245	0.80

Conclusion

A validated RP-HPLC analytical method has been developed for the determination of IPZ in bulk and tablet dosage form. The proposed method is accurate, precise, specific and suitable to use for the routine analysis of IPZ. The simplicityof the method allows for application in laboratories thatlack sophisticated analytical instruments such as LC–MSand GC–MS.

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