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DEVELOPMENT AND VALIDATION OF NEW RP-HPLC METHOD FOR ESTIMATION OF ILAPRAZOLE IN BULK AND TABLET DOSAGE FORMS

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ABSTRACT

This paper describes a validated high-performance liquid chromatographic (HPLC) method for estimation of Ilaprazole in tablet dosage form. The HPLC separation was achieved on a stainless steel column 15cm x 4.6 mm, packed with octylsilane bonded to porous silica (5µm) using a mobile phase of 60 volumes of buffer, 40 volumes of acetonitrile in the ratio of 60:40 and pH adjusted to 5.5 with dilute orthophosphoric acid, with a flow rate of 1.0 ml per minute, with an injection volume of 10µl and elution measured at 305nm spectrophotometer. The calibration curve showed good linear relationship with r^2 = 0.9953 for Ilaprazole standard. The method was validated in terms of linearity, accuracy, precision (repeatability), precision (intermediate), specificity, solution stability, and robustness based on ICH guidelines (Q_2 R_1). The method was successfully applied for routine analysis of Ilaprazole in Pharmaceutical Tablet dosage forms.

KEYWORDS: Ilaprazole, Tablet Dosage form, HPLC, validation, ICH guidelines.

1. INTRODUCTION

Ilaprazole, a proton pump inhibitor (PPI), is a newly developed medicine in the management of acid-related disorders. Several studies have shown that Ilaprazole is a highly effective and safe PPI compared with other PPIs in the treatment of duodenal ulcer. Ilaprazole can be recommended as a therapy for acid related disorders, especially in Asian populations.

Duodenal ulcer is a very common digestive disease with a high incidence all over the world.[1,2,3]

As the first proton pump inhibitor (PPI), Omeprazole has been used therapeutically for many years, and shown great efficacy in treating peptic ulcers.[4,5,6] Currently, research is focused on more effective PPIs with a lower dose and comparative safety.[7,8]

Ilaprazole (also known as IY-81149), the latest proton pump inhibitor (PPI) has been less well reported in clinical practice, as a newly developed medicine in the management of acid related disorders.[9,10]

Ilaprazole (IPZ) is used in the treatment of dyspepsia, peptic ulcer disease, gastroesophageal reflux disease and duodenal ulcer. Literature survey reveals that there are several analytical methods reported for the determination of IPZ from biological fluids using hyphenated techniques like HPLC-ESI-MS/MS[11, 12, 13] HPLC-NMR.[11] 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.[14] However, there is no method reported for the determination of IPZ in 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.

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 shown in Figure 1.

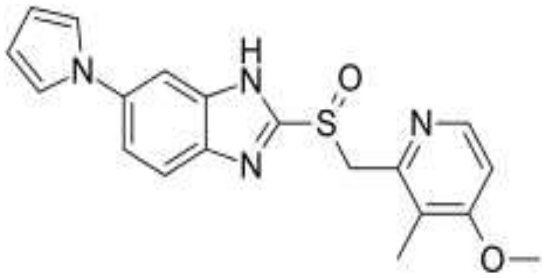

Figure 1: Chemical structure of Ilaprazole (IPZ).

2. MATERIALS AND METHODS

2.1 MATERIALS

The pure Ilaprazole raw material was received from Metrochem API Pvt. Ltd, Telangana, India. The working standard of Ilaprazole had Potency of 101.83% on dry basis. Similarly, Acetonitrile (Gradient Grade), Disodium hydrogen phosphate, and Orthophosphoric acid were received from Hi-media, India.

2.2 APPARATUS

The method was developed using a Shimadzu LC-2030 (Prominence-I).

A stainless steel column 15cm x 4.6mm, packed with octylsilane (C8) bonded to porous silica (5µm) was used with a flow rate of 1.0ml per minute. The elution was monitored at 305 nm and injection loop volume was 10µl.

Standard and sample solutions ware filtered through a 0.45µm nylon membrane prior to HPLC injection.

2.3 PREPARATION OF REFERENCE SOLUTION

50 mg of Ilaprazole Reference standard was weighed accurately and transferred to 50 ml volumetric flask. Then, it was diluted and dissolved with acetonitrile up to the mark. 5ml of this solution was taken and further diluted to 50 ml with this solvent.

2.4 PREPARATION OF TEST SOLUTION

Powder equivalent to 10mg of Ilaprazole Reference Standard was weighed from finely powdered tablets and transferred to 100 ml volumetric flask which was then dissolved and diluted up to the mark. Then, the final solution was filtered through a 0.45µm nylon membrane prior to HPLC injection.

2.5 METHODS

2.5.1 Analytical Method Validation for Adapalene

2.5.1.1 Linearity

A series of standard solution of five concentrations: 70%, 80%, 90%, 100%, 120% and 130% of target concentration were prepared; five replicates at each concentration were analyzed. Regression was plotted against graph and found to be significant**.**

2.5.1.2 Accuracy

Samples were prepared at three concentrations over the range of 80%, 100%, and 120% of the target concentration.

2.5.1.3 Precision

Repeatability: Five replicate injections of standard solution were performed at 100% of expected concentration.

Method Precision: Samples were prepared at three concentrations covering 80,100 and 120% of target concentration with triplicate sample of each concentration so as at least nine determinations are covered. For each concentration recovery percentage was determined.

Intermediate Precision: Samples were prepared in triplicate of target concentration (100%) by two different analysts on two different days.

Solution Stability

Sample was prepared and stored at 2-8°C for 24hours to demonstrate the solution stability.

2.5.1.4 Specificity

Specificity was investigated by injecting the blank solution and placebo solution to demonstrate the absence of interference with the elution of analyte.

2.5.1.5 Robustness

The investigation of robustness was done by altering the mobile phase ratio from Buffer: Acetonitrile (60:40) to (58:42), changing the flow rate from 1.0ml to 1.2 ml/minute, changing column from CLM8-2 to CLM8-3**.**

2.5.1.6 System Suitability parameters

During the whole analysis, the column efficiency should be not less than (NLT) 1200 theoretical plates, the tailing factor should be less than 2.0, and the relative standard deviation of replicate injections should not be more than 2.0% to comply with the system suitability test.

3. RESULTS AND DISCUSSION

The representative chromatograms for Ilaprazole in standard and test solutions are shown in figures 2 and 3.

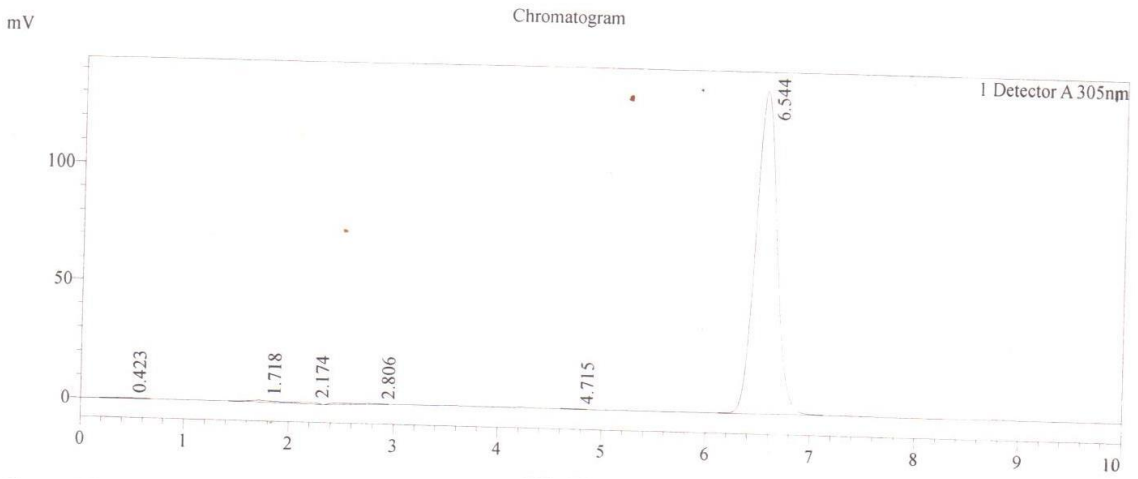

Figure 2: A representative chromatogram for Ilaprazole in standard solution.

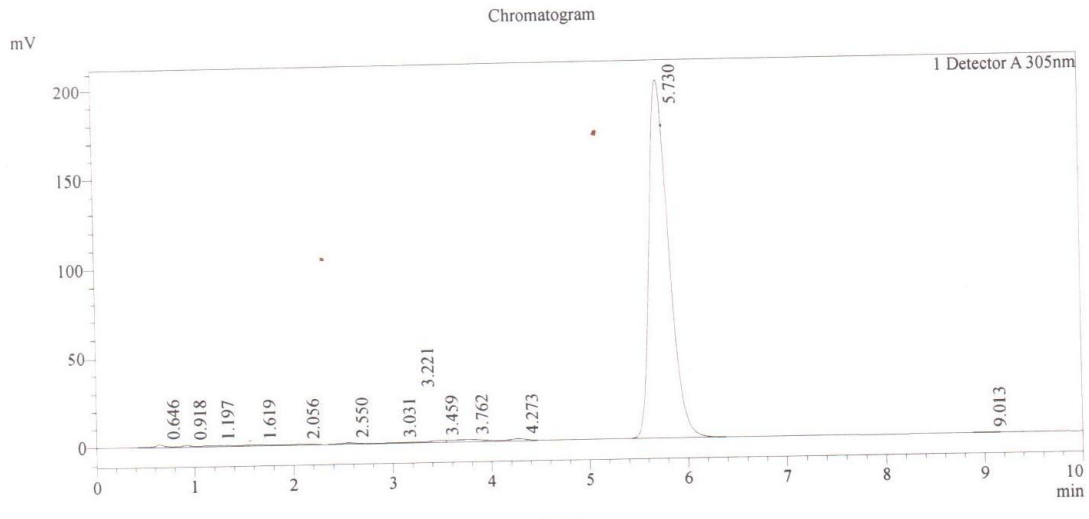

Figure 3: A representative chromatogram for Ilaprazole in test solution.

Calibration Curve

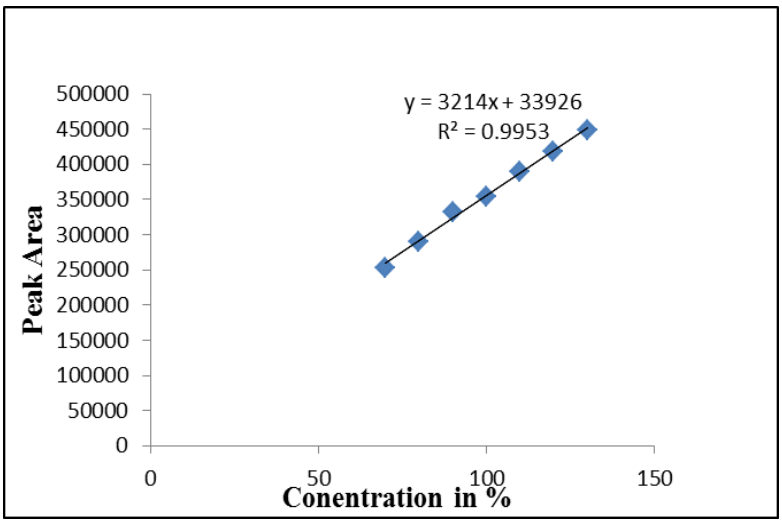

Fig. 4: Linearity curve for %concentration vs. peak area for Ilaprazole (Standard).

The correlation coefficient observed was 0.995, which meets the requirement ($r^2 \geq 0.98$). So the analytical method fit properly for calibration curve.

ACCURACY

Table 1: Accuracy datasheet for standard.

Replicate	Area	Mean	Std. Dev.	RSD %
Std 1	2826148	2834103.8	4573.58	0.16138
Std 2	2836258			
Std 3	2837551			
Std 4	2834546			
Std 5	2836016			

Table 2: Accuracy datasheet for test.

Parameters	Replicate	Level		
		80%	100%	120%
Area	Test 1	2218247	2830739	3147514
	Test 2	2092925	2808337	3118747
	Test 3	2214072	2763118	3123724
Assay %	Test1	78.77	99.97	118.31
	Test2	78.37	100.01	118.02
	Test3	78.47	99.61	118.44
Recovery %	Test1	98.46	99.97	98.59
	Test2	97.96	100.01	98.35
	Test3	98.09	99.61	98.7
Mean of recovery		98.17	99.86	98.47

The accuracy was in the range of 98.17% to 99.86%, which is within the limit specified (98%-102%).

PRECISION

Repeatability

Table 3: Datasheet for Precision.

Inj. No.	Retention time	Peak Area	Peak Height
1	6.7	2717601	201083
2	6.699	2731617	201903
3	6.692	2762982	202718
4	6.706	2753828	203911
5	6.686	2739163	203269
Mean	6.6966	2741038.2	202576.8
Std. Dev.	0.0077	17935.2103	1114.0706
RSD (%)	0.1155	0.6543	0.5499

Table 4: Interpretation of Results.

Parameter	Result	Specification
RSD (%) Retention time	0.11548	Not more than 2.0%
RSD (%) Peak Area	0.6543	Not more than 2.0%
RSD (%) Peak Height	0.5499	Not more than 2.0%

The relative standard deviation for retention time, peak area and peak height was not more than 2%. Hence the method complies for repeatability.

Method Precision

Table 5: Datasheet for standard solution.

Replicate	Area	Mean	Std. Dev.	RSD %
Std 1	2717601	2741038.2	17935.2	0.65432
Std 2	2731617			
Std 3	2762982			
Std 4	2753828			
Std 5	2739163			

Table 6: Datasheet for test solution.

% Conc. of target analyte	Peak Area			Mean Recovery %	Average of mean of recovery	Std. Dev.	RSD
	I	II	III				
80	2328869	2483930	2493185	100.42	99.21	1.0942	1.103
100	2594704	2553254	2591635	98.92			
120	3005844	3280326	3158343	98.29			

The relative standard deviation between three concentrations is not more than 2%. Hence the method complies with method precision.

Intermediate Precision

Table 7: Intermediate Precision datasheet.

Parameters	Replicate	Analyst 1(Day1)		Analyst 2(Day2)	
		Std	Spl	Std	Spl
Area	Test 1	2864543	2787404	2829525.6	2567435
	Test 2		2872686		2874029
	Test 3		2885729		2895950
% Assay	Test1		101.45		98.73
	Test 2		101.79		100.42
	Test2		101.25		101.13
%Mean Assay			101.50		99.57

Table 8: RSD data across two analysts.

	% Assay	Mean	Std. Dev.	RSD
Analyst 1	101.50	100.53	1.365	1.357
Analyst2	99.57			

The relative percent purity data across two systems have statistical RSD 1.357 which is within the limit (less than 3.0%). Hence the method complies the intermediate precision.

SOLUTION STABILITY

Table 9: Datasheet for solution stability.

	Assay %	Solution Stability %
Fresh Sample	99.18	101.35
Stored sample	100.52	

The solution stability is 101.35% in comparison to freshly prepared solution which is within the limit (97.5% to 102.5%). Hence the sample is stable.

SPECIFICITY

Table 10: Datasheet for specificity.

Chromatograph Results			
S. No.	**Sample**	**Retention Time**	**Area**
1	Blank Solvent	7.034	32332
2	Standard	6.700	2750598
3	Sample	6.698	2603107

As blank solution does not show peak response at same time as sample and standard solution, the method is considered to be specific for the given sample.

ROBUSTNESS

Table 11: Change in mobile phase ratio.

Parameters	**Replicate**	**Buffer: ACN(60:40)**		**Buffer: ACN(58:42)**	
		Std (Avg)	**Spl**	**Std (Avg)**	**Spl**
Area	Test 1	2825213.8	2780014	2848672.6	2703786
	Test 2		2752761		2818523
	Test 3		2872852		2765031
%Assay	Test1		98.3		99.73
	Test 2		100.24		100.43
	Test 3		100.97		99.75
Mean (%Assay)			99.84		99.97

Table 12: Change in flow rate.

Parameters	Replicate	Flow rate(1.0ml/min)		Flow rate(1.2 ml/min)	
		Std(Avg)	Spl	Std(Avg)	Spl
Area	Test 1	2825213.8	2780014	2350453.2	2382444
	Test 2		2752761		2264012
	Test 3		2872852		2290095
%Assay	Test1		98.3		99.01
	Test 2		100.24		99.73
	Test 3		100.97		99.38
Mean (%Assay)			99.84		99.37

Table 13: Change in column.

Parameters	Replicate	Column (Shim pack GIST)CLM8-2		Column (Shim pack GIST)CLM8-3	
		Std(Avg)	Spl	Std(Avg)	Spl
Area	Test 1	2825213.8	2780014	2768011.6	2666217
	Test 2		2752761		2735290
	Test 3		2872852		2735781
%Assay	Test1		98.3		98.44
	Test 2		100.24		99.06
	Test 3		100.97		98.59
Mean (%Assay)			99.84		98.70

The results as shown in the tables after changing several parameters like solvent ratio, change in flow rate, and change in column itself demonstrate that the method is robust enough for the routine analysis. While performing whole analysis the column efficiency was found to be more than 1200 theoretical plates, the tailing factor was less than 2.0, and the relative standard deviation of replicate injections was found to be below 2.0%. So, the analytical method followed complies with the system suitability test.

4. CONCLUSION

This study is a typical example of the development of an assay method following ICH guidelines. A new HPLC method has been developed and validated for determination of Ilaprazole in the tablet formulation. The results of the validated studies showed that HPLC method possesses significant linearity, precision, accuracy, specificity, sensitivity, high efficiency and resolution, and no interference from the excipients, as were demonstrated. The proposed method was successfully applied and is suggested for the quantitative analysis of Ilaprazole in solid pharmaceutical formulations for QC, where economy and time are essential and to assure therapeutic efficacy.

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6. Conflict of Interest

The author declares no conflict of interest and no third party funding in this study.

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