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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ILAPRAZOLE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, rapid, accurate and precise RP-HPLC method was developed and validated for the estimation of Ilaprazole in pharmaceutical dosage form. Chromatographic separation was carried out by using Hypersil BDS C18 (4.6 x 250 mm) column using a mobile phase consisting of a mixture of Methanol: Water 70:30 pH-3.0 with anhydrous disodium hydrogen phosphate buffer by using an isocratic elution. The following system conditions were maintained throughout development and validation i.e., flow rate 1.0 ml/min, column was maintained at room temperature and the detected by a UV wavelength 237 nm. The Ilaprazole was well resolved and detected at 237nm with retention time 4.4 minutes. Ilaprazole was shown to be linear over a range of 5-25 µgm/ml. The method was validated as per ICH guidelines.

KEYWORDS: Ilaprazole, RP-HPLC, Method validation and C18 Column.

INTRODUCTION

Ilaprazole[-[(4-methoxy-3-methyl-pyridin-2-yl)methylsulfinyl]-6-pyrrol-1-yl-1*H*-benzoimidazole], a substituted benzimidazole, is a new candidate drug that is an H^+/K^+-ATPase inhibitor designed for the treatment of gastric ulcers.[1, 2] Ilaprazole was under development by IIYang Pharmacy Co (Seoul, Korea) and has been proven by a series of animal studies to be a potent and safety antiulcer agent. Seung-Woon Myung *et al* found two metabolites of Ilaprazole using liquid chromatography/tandem mass spectrometry (LC/MS/MS) in rat plasma, the major one being Ilaprazole sulfone.[3] Recently, a new metabolite of Ilaprazole, Ilaprazole thiol ether, was identified.

Quantification of drugs in biological matrices by LC/MS/MS is becoming an increasingly common technology today due to the improved sensitivity and selectivity of these methods. Li *et al* reported that Ilaprazole was metabolized by CYP3A5 and CYP2C9 in Chinese healthy subjects using a LC-MS/MS method to determine Ilaprazole and Ilaprazole sulfone concentrations. [4] B. Satheesh *et al* reported a stability indicating UPLC method was developed and validated for simultaneous determination of Ilaprazole and its impurities in tablets.[5] The proposed RP HPLC method was found to be sensitive, accurate and precise for determination of Ilaprazole in tablets. The method utilizes easily available and cheap solvent for analysis of Ilaprazole hence the method was also economic for estimation of Ilaprazole from tablet dosage form. Hence it can be conveniently adopted for routine quality control analysis of the drugs in pharmaceutical formulation.

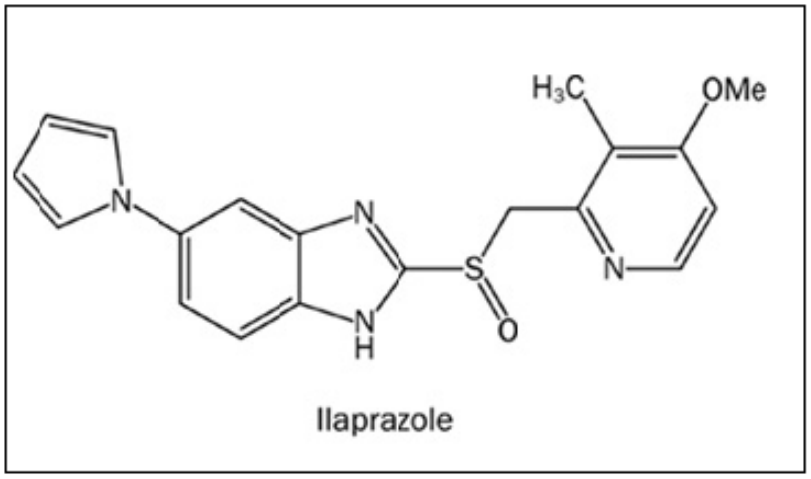

Figure 1 Chemical structure of Ilaprazole

MATERIAL AND METHODS

Reagents and Chemicals

Ilaprazole bulk powder was gifted by Lupin Pharmaceuticals Ltd. Aurangabad, Maharashtra, India. Tablets of Ilaprazole were purchased from local pharmacies. Purified water was obtained from Millipore system. Methanol (HPLC grade) was obtained from E-Merck. All other chemicals used in the analysis were AR grade.

Instrumental and analytical conditions

The HPLC system (Younglin (S.K) isocratic System), consisted of a UV – VIS detector and autochro 3000 software. Sonicator, Branson Ultrasonic's Corporation, USA was used in the study.

EXPERIMENTAL

Preparation of standard stock solution

10.0 mg of Ilaprazole was weighed accurately and transferred into a clean, dry 10ml volumetric flask. 5 ml of Methanol was added and sonicated to dissolve. Volume was adjusted to 10 ml with diluents.

Selection of detection wavelength

The standard solution of Ilaprazole was scanned over the range of 200 nm to 400 nm wavelengths. From overlay spectra, 237nm wavelength was selected for the determination of Ilaprazole.

Selection of mobile phase

The mobile phase was selected on the basis of best separation, peak purity index, peak symmetry, theoretical plate etc. So no. of trials was taken for the selection of mobile phase. So also for this Ilaprazole, the same mobile phase in different ratio was used to optimize best result. Also different solvent mixtures were used to optimize best result.

Assay

Weigh 20 tablets and determine average net content of blend. Accurately weigh and transfer quantity of tablet contents equivalent to about 10.0 mg of Ilaprazole into 10 ml amber colored volumetric flask. Add 10ml of Methanol and sonicated for about 20 minutes. Dilute to volume with Methanol and mix. Filter resulting solution with what man's filter paper discarding first few ml of filtrate. Further dilute 0.25 ml of this solution to 10.0 ml with mobile phase and mix.

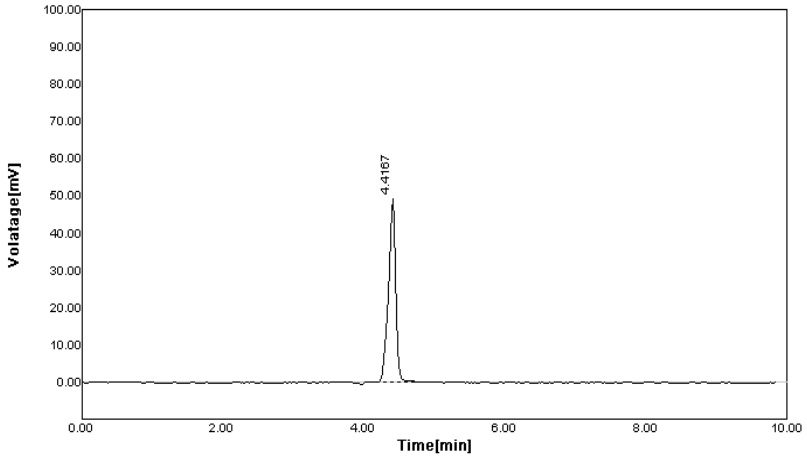

Fig. 2: Chromatogram of Ilaprazole using studies Methanol: Water (70:30 v/v) as a mobile phase.

METHOD VALIDATION

The proposed method has been developed and validated for the determination of Ilaprazole in pharmaceutical dosage forms. According to International Conference on Harmonization (ICH) guidelines, validation of the method was carried out by using accuracy, linearity, LOD, LOQ, precision, ruggedness and robustness, and system suitability parameters.

System suitability

A standard solution was prepared by using Ilaprazole working standards as per test method and was injected 5 times into the HPLC system. The system suitability parameters were evaluated from the USP tailing and USP plate count values obtained from standard chromatograms.

System Suitability Component Parameter	Ilaprazole
Retention times (RT) min	4.4
Theoretical plates (N)	8007.5
Tailing factor (AS)	0.9375
Slope	14.1
Intercept	1.3
Coefficient of variance	0.999
Linear range	5-25 mg/ml

Linearity of test solution

A series of solutions were prepared from standard stock solution at concentration levels from 5-25µg/ml for Ilaprazole. The linearity of the method was determined for the formulation at five concentration levels ranging from 5-25 µgm/ml Ilaprazole. The equation for regression line was y = 14.14x-1.3 R^2 = 0.999 for Ilaprazole.

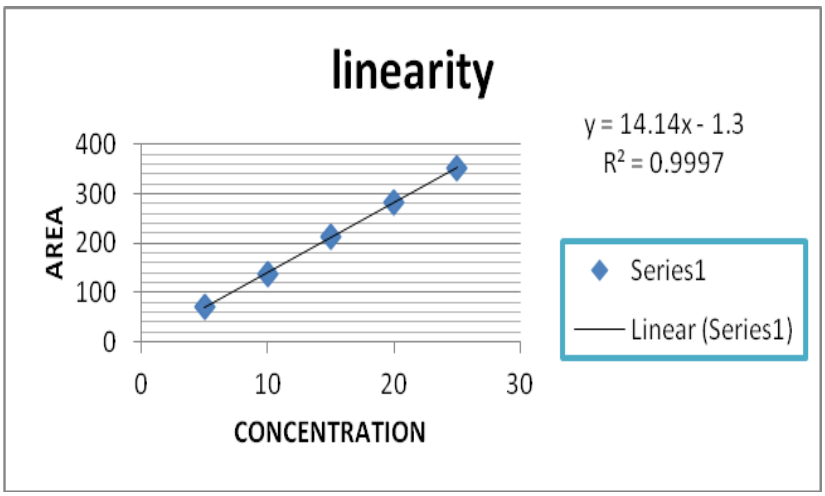

Fig. 3 Calibration curve for Ilaprazole

Table 1 Linearity of Ilaprazole by RP-HPLC method

Sr No.	Concentration (µgm/ml)	Area I	II	Mean area
1	5	71.94	71.71	71.825
2	10	137	138	137.5
3	15	212	213	212.5
4	20	282	283	282.5
5	25	348	356	352

Recovery study

The recovery studies were carried out at three levels of 80, 100 and 120% and the percentage recovery was calculated and presented in Table 2.

Accuracy

The accuracy of the method was determined by recovery experiments. Drug assay was performed in triplicate as per test method with equivalent amount of drugs into each volumetric flask for each spike level to get the concentration of drugs equivalent to 80%, 100% and 120% of the labelled amount as the test method.

Table 2 Accuracy and Recovery

Concentration level	Amount added	Area	Amount Recovered	Amount found
80	8	256.01	8.01	18.01
	8 8	255.58	7.99	17.98
		254.47	6.98	16.94
		Mean	7.66	17.98
		SD	0.59	0.04
		% RSD	0.44	0.20
100	10	281.23	9.80	19.80
	10 10	282.38	9.88	19.88
		281.28	9.81	19.81
		Mean	9.83	19.83
		SD	0.04	0.04
		% RSD	0.44	0.22
120	12	310.07	11.83	21.83
	12 12	311.15	11.91	21.91
		310.09	11.86	21.87
		Mean	11.87	21.87
		SD	0.04	0.04
		% RSD	0.19	0.34

Precision

As per the guideline it is given that a minimum of 9 determinations covering the specified range for the procedure (e.g., 3 concentrations/3 replicates each). A 50 µg/ml concentration

solution used, which was prepared from working standard solution for six replicate injections. This was analyzed for intraday precision and inter-day precision. Observation is shown in the [Table 3] which reveals that %RSD is well within the given criteria of 2%.

Table 3 Repeatability of Ilaprazole by RP-HPLC method

Sr No.	Conc.	Area	II	III	Mean	SD	%RSD
1	10	138.58	137.13	136.98	137.56	1.03	0.75
2	15	215.41	213.87	215.37	214.88	0.88	0.41
3	20	285.52	282.95	284.63	284.37	1.31	0.46

Limit of Detection and Limit of Quantitation

The parameters LOD and LOQ were determined on the basis of standard deviation and slope of the regression equation as per International Conference on Harmonization (ICH) guidelines.

Limit of detection (LOD)

As per guideline detection limit can be calculated by using the formula:

LOD = 3.3xσ/S

Where, 'σ' is the standard deviation of response, and 'S' is the Slope of the calibration curve. LOD of the method is given in the [Table 4].

Limit of Quantitation (LOQ)

As per guideline Quantitaion limit can be calculated by using the formula:

LOQ = 10 xσ /S

Where, 'σ' is the standard deviation of response, and 'S' is the Slope of the calibration curve. LOQ of the method is given in the [Table 4]

Table 4 LOD and LOQ of Ilaprazole

Sr No.	Conc.	Area I	II	III	%RSD
1	6	67	67.5	68	0.74
2	8	99	99.5	100	0.5
3	10	131	130.5	130	0.38
4	12	163.2	163.6	164	0.24
5	14	190.4	190.7	191	0.16

Robustness

The robustness study was carried out by deliberate minor variations in the flow rate, mobile phase volume, and wavelength, and system suitability parameters were checked. Results indicate that the selected factors remained unaffected by small variations of these parameters.

Table 5 Robustness of Ilaprazole by RP-HPLC method

Parameters	Variations	Area	T.P.	T.F.
Mobile phase	69:31	210.2	9256	0.8500
	71:29	211.78	9321.5	1.0000
	70:30	213.26	8007.3	0.9375
	MEAN	211.75		
	SD	1.53		
	%RSD	0.72		
Flow rate change	0.9	222.77	11016.2	0.9375
	1.0	223.77	11086.9	0.8889
	1.1	221.37	11037.4	0.9476
	MEAN	222.63		
	SD	1.22		
	%RSD	0.55		
Wavelength	235	222	9397.4	0.9286
	237	223	8007.3	0.9375
	239	225	8937.6	0.9189
	MEAN	223.33		
	SD	1.53		
	%RSD	0.68		

RESULTS AND DISCUSSION

Table 6 Result and discussion

Sr. No.	Validation parameters	Result
1	UV detection wavelength (nm)	237
2	Linearity range (µg/ml)	5-25
3	Standard regression equation	y=14.14x+1.3
4	Correlation coefficient (R^2)	R^2= 0.999
5	Precision (% RSD) Repeatability (n=9)	 0.94
6	% Recovery (Accuracy, n=9)	98.40
7	LOD (µg/ml)	0.13
8	LOQ (µg/ml)	0.39
9	Robustness Mobile phase change Flow rate change Wavelength change	 0.72 0.55 0.64
10	% Assay (% Label claim)	99.42

CONCLUSION

The method describes the quantification of ilaprazole in tablet dosage form. The validation data demonstrate good precision and accuracy which proves the reliability of proposed method. Hence this method can be used routinely for quantitative estimation of Ilaprazole in solid dosage forms**.**

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