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RESEARCH ARTICLE

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ILAPRAZOLE AND DOMPERIDONE IN THEIR COMBINED DOSAGE FORM

Prasanna Kumar Pradhan *, Dhaval Mavani, Mayank Patel, Naimish Raiyani, Umesh Upadhyay

ABSTRACT
Ilaprazole is a proton pump inhibitor and Domperidone is a specific blocker of dopamine receptors. This combination of drugs will be used to treat peptic ulcers. In RP-HPLC method, a mobile phase of Phosphate Buffer (pH 3)**:** Methanol (40:60 v/v) was used to resolve Ilaprazole and Domperidone from a mixture. Phenomex C18 column was used and flow rate was selected as 1 ml/min. Detection was carried out at 229 nm which is Isobestic point of Ilaprazole and Domperidone. The linearity range obtained for the RP-HPLC method were 5 – 15 µg/ml and 15 - 45 µg/ml with corresponding correlation coefficient of 0.999 and 0.999 for Ilaprazole and Domperidone respectively. The method was found to be rapid, accurate and precise. This method was validated according to ICH guidelines.
Keywords: Ilaprazole, Domperidone, RP-HPLC, Simultaneous Estimation

1. INTRODUCTION

Ilaprazole **(ILA)**, chemically is known as 2-[(RS)-[(4-methoxy-3-methylpyridin-2-yl)methyl]sulfinyl]-5-(1H-pyrrol-1-yl)-1H-benzimidazole, (trade name Noltec) is a proton pump inhibitor (PPI) used in the treatment of dyspepsia, peptic ulcer disease (PUD), gastroesophageal reflux disease (GORD/GERD) and duodenal ulcer. Domperidone **(DOM),** chemically is known as 5-chloro-1-{1-[3-(2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)propyl]piperidin-4-yl}-2,3-dihydro-1H-1,3-benzodiazol-2-one, a specific blocker of dopamine receptors. It speeds gastrointestinal peristalsis, causes prolactin release, and is used as antiemetic and tool in the study of dopaminergic mechanisms. This combination of drugs will be used to treat peptic ulcers.[1-2]

The literature is enriched with several methods for determination of ILA and DOM in pharmaceutical dosage forms either as a single drug or in combination with some other drugs. The most extensively used technique for estimation of ILA are by UV[3] HPLC[4], UPLC[5] and LC-MS/MS[6] methods and most extensively used technique for estimation of DOM are by UV [7-9] and RP-HPLC[10-17]. The aim of study is Development and Validation of Analytical HPLC method for Simultaneous Estimation of Ilaprazole and Domperidone in their combined Dosage form.

The present study was designed to develop a simple, precise, and rapid analytical RP-HPLC procedure, which can be used for the analysis of assay method for simultaneous estimation of Ilaprazole and Domperidone as there was only individual methods reported for both drugs. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of these two drugs in their combined dosage forms. Literature survey of Ilaprazole and Domperidone revealed several methods for detecting these drugs individually but there is no method for their simultaneous estimation using RP-HPLC.

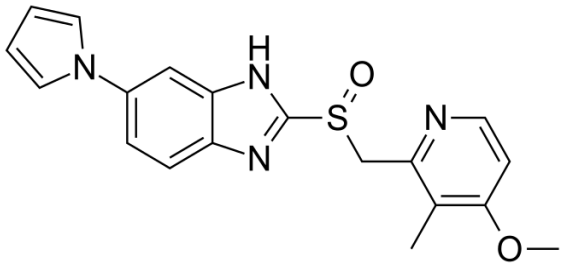

FIGURE 1: Chemical structure of Ilaprazole

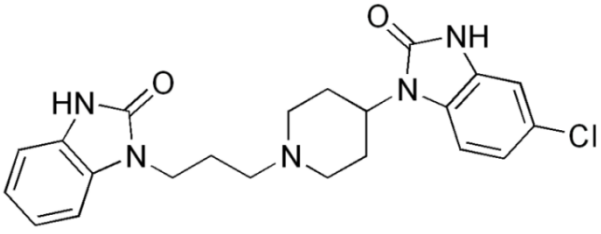

FIGURE 2: Chemical structure of Domperidone

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2. MATERIAL AND METHODS

2.1 Apparatus and Instrument

The analysis was carried out on a HPLC system (Shimadzu LC-20- AT) equipped with UV detector. Other apparatus and instruments used were a micro analytical balance (Shimadzu), Ultrasonic Cleaner (EIE Instruments Pvt. Ltd. Ahmedabad), Nylon Membrane Filters (0.22mcm, 47 mm D). All instruments and glass wares were calibrated.

2.2 Reagents and Materials

Ilaprazole and Domperidone were obtained as gratis sample from Accurate Pharmaceuticals, Godhra. Methanol HPLC Grade, Water HPLC Grade, Phosphate Buffer, O-Phosphoric Acid was used which were obtained from Samir Tech-Chem Pvt. Ltd. A stock-standard solution of ILA and DOM was prepared by dissolving accurately weighed amount of pure drug in mobie phase.

2.3 Mobile Phase: Phosphate Buffer (pH 3): Methanol(40:60 v/v). The mobile phase was filtered through Millipore filter paper type HV (0.45 µm) and degassed by sonication.

2.4 Chromatographic conditions

Chromatographic analysis was carried out on an inertsil C-18 column, (5 µm, 250mm x 4.6mm i.d) LC-20 AT. The mobile phase consisted of Phosphate Buffer (pH 3): Methanol (40:60 v/v). The mobile phase was filtered through Millipore filter paper type HV (0.45 µm) and degassed by sonication, was pumped at 1.0 ml/min flow rate. The column was thermostated at room temperature. Under these conditions the runtime was 10 min.

2.4.1 Preparation of standard stock solution of Ilaprazole (100 µg/ml) and Domperidone (300µg/ml)

A 10 mg of standard Ilaprazole and 30 mg of standard Domperidone was weighed and transferred to a 100 ml volumetric flask each and dissolved in 25 ml mobile phase. The flask was shaken and volume was made up to the mark with mobile phase to give a solution containing 100 µg/ml Ilaprazole and 300 µg/ml Domperidone.

2.4.2 Preparation of combined working standard solution containing Ilaprazole and Domperidonein ratio of 1:3

Accurately weighed 10 mg Ilaprazole and 30 mg of Domperidone were transferred to 100 ml volumetric flask, dissolved in sufficient amount of mobile phase and diluted up to mark with mobile phase to get concentration of 100 µg/ml Ilaprazole and 300 µg/ml Domperidone. This solution was diluted further to get the concentrations in range of 5, 7.5, 10, 12.5, 15 µg/ml of Ilaprazole and 15, 22.5, 30, 37.5, 45 µg/ml of Domperidone.

2.5 Method Validation

2.5.1 Precision

Repeatability: Precision of the method was studied by making repeated injections of the mixture of drugs on the same day for intraday precision. The % RSD after six determinations was determined at 10 µg/ml for ILA and 30 µg/ml for DOM.

Intraday and Inter-day Precision: Intraday and Inter-day precision for method were measured in term of %RSD. The experiment was repeated three times in a day for intraday and on three different days of same for inter-day precision by taking lower, middle and higher concentration of ILA(5, 10, 15 µg/ml) and DOM(15, 30, 45 µg/ml).

2.5.2 Linearity: The linearity of measurement was evaluated by analyzing standard solutions of ILA and DOM in the range of 5–15 µg/ml and 15-45 µg/ml for both drugs respectively and calibration plot was constructed.

2.5.3 Limit of Detection (LOD) and Limit of Quantitation (LOQ): LOD and LOQ of ILA and DOM were determined by calibration curve method. Solutions of Ilaprazole and Domperidone were prepared in the range of 5–15 µg/ml and 15-45 µg/ml for both drugs respectively and injected in triplicate.

2.5.4 Accuracy: Accuracy of the method was calculated by recovery studies at three levels by standard addition method i.e. spiking 80%, 100%, 120% of ILA and DOM to the standard solutions containing 5 µg/ml of ILA and 15 µg/ml of DOM.

2.5.5 Robustness: Influence of small changes in chromatographic conditions such as change in flow rate, that is, ± 0.2 ml/min, mobile phase composition ±2 ml and pH ± 0.2 was studied to determine the robustness of the method for the development of RP-HPLC method for the simultaneous estimation of ILA and DOM and their %RSD was determined.

2.5.6 System Suitability: The stock solution containing 10 µg/ml of ILA and 30 µg/ml of DOM was injected and repeated five times and the chromatograms were recorded. The resolution, number of theoretical plates, and peak asymmetry were calculated to determine whether the result complies with the recommended limit.

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3. RESULTS AND DISCUSSION

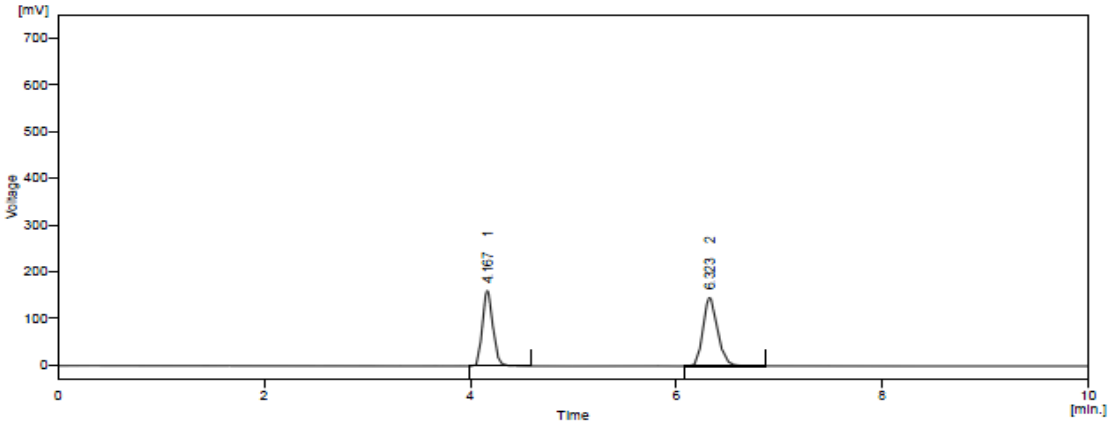

FIGURE 3: Chromatogram of ILA and DOM respectively

3.1 Optimization of Chromatographic conditions

To optimize the chromatographic conditions for separation of ILA and DOM, mobile phase composition, the effect of temperature and wavelength of detection investigated.

During the method development work, enable C18 column (25 cm× 4.6 mm i.d.) with particle size of 5 µm was used and gave the suitable resolution. The mobile phase composition was prepared with appropriate proportion of phosphate buffer (pH 3) : Methanol (40:60 v/v).

It was shown that the most efficient resolution and peak symmetry for Ilaprazole and Domperidone with a mobile phase composed of phosphate buffer (pH 3) : Methanol (40:60 v/v) and a flow rate of 1 ml/min. The retention time for Ilaprazole and Domperidone were found to be 4.21 and 6.4 min respectively.

The wavelength selected was the Isobestic point from the overlay of Ilaprazole and Domperidone. Both the drugs showed typical peak nature and peaks were symmetrical at 229 nm which is the Isobestic point. Hence the wavelength has been selected as the detection wavelength.

3.2 Validation

3.2.1 Linearity and Range

The linearity of measurement was evaluated by analyzing standard solutions of ILA and DOM in the range of 5–15 µg/ml and 15-45 µg/ml respectively for both drugs and calibration plot was constructed.

TABLE 1: Statistical parameters for Ilaprazole and Domperidone

Statistical Parameter	Ilaprazole	Domperidone
Average peak area* ± SD	560.67 ± 1.62	718.99 ± 1.66
	827.88 ± 1.53	1061.78 ± 1.73
	1131.98 ± 1.34	1451.96 ± 1.55
	1393.80 ± 1.50	1787.99 ± 1.49
	1695.92 ± 1.69	2175.68 ± 1.64
Concentration Range (µg/ml)	5-15	15-45
Straight line equation	y = 113.46x - 12.517	y = 48.528x - 16.557
Correlation coefficient (R^2)	0.999	0.999
LOD (µg/ml)	0.347	1.04
LOQ (µg/ml)	1.05	3.16

n=5

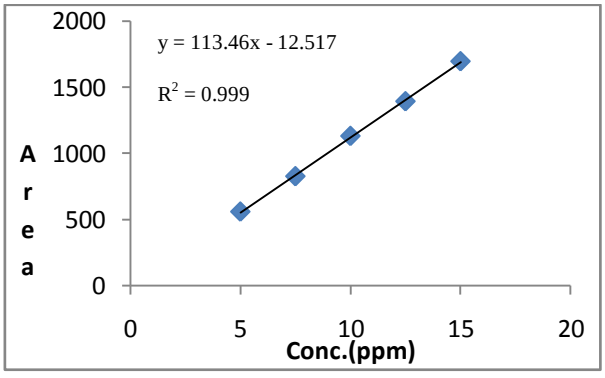

FIGURE 4: Calibration curve of Ilaprazole

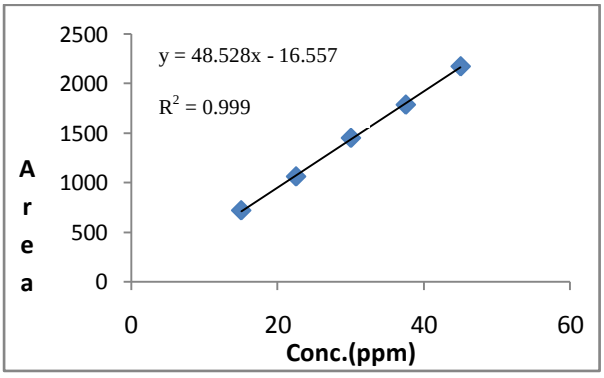

FIGURE 5: Calibration curve of Domperidone

3.2.2 Precision

Precision of the method was studied by making repeated injections of the mixture of drugs. The Relative Standard Deviation (%RSD) after six determinations was 0.7% at 10 µg/ml for ILA and 0.81% at 30 µg/ml for DOM (see Table 2).

TABLE 2: Precision data for ILA and DOM

Precision	Concentration found* (µg/ml)		%RSD	
	ILA	DOM	ILA	DOM
Repeatability	9.97	29.93	0.69	0.54
Intraday Precision	5	15	0.88	1.19
	10	30.01	1.07	1.15
	14.99	44.99	0.61	0.86
Interday Precision	5	15	0.63	0.72
	10	30.01	0.56	0.62
	14.99	44.99	0.46	0.56

n=6

3.2.3 LOD and LOQ

LOD and LOQ of ILA and DOM were determined by calibration curve method. Solutions of ILA and DOM were prepared in the range of 5–15 µg/ml and 15-45 µg/ml respectively and injected in triplicate (see Table 1).

3.2.4 Accuracy

Accuracy of the method was calculated by recovery studies at three levels by standard addition method. The mean percentage recoveries obtained for ILA and DOM were 99.61% and 99.64%, respectively (see Table 3).

TABLE 3: Recovery data for ILA and DOM

Lupila-D® Capsule	Conc. in capsule (µg/ml)	Conc. added (µg/ml)	Total conc. found (µg/ml)	Amount recovered (µg/ml)	Mean Recovery* ± SD (%)
Ilaprazole	5	4	8.94	3.94	99.52 ± 0.65
	5	5	9.95	4.95	99.66 ± 0.65
	5	6	11	6	99.66 ± 0.48
Domperidone	15	12	26.85	11.85	99.77 ± 0.83
	15	15	29.95	14.95	99.60 ± 0.43
	15	18	32.94	17.94	99.56 ± 0.27

n=3

3.2.5 Robustness

The method for the development of RP-HPLC method for the simultaneous estimation of ILA and DOM was found to be robust as the % RSD was found to be less than 2 (see Table 4)

TABLE 4: Robustness data for ILA and DOM

Parameters	Variation	% RSD	
		ILA	DOM
Flow rate	1.2	1.31	1.06
	(1 ml/min)	0.69	0.54
	0.8	0.75	0.97
Mobile phase	(42:58)	1.16	1.08
	Phosphate buffer pH 3 : Methanol (40:60)	0.69	0.54
	(38:62)	1.08	0.77
pH (3)	(3.2)	1.10	1.33
	pH (3)	0.69	0.54
	(2.8)	1.19	1.27

3.2.6 System Suitability

The resolution, number of theoretical plates, and peak asymmetry were calculated for the standard solutions. The stock solution containing 10 µg/ml of ILA and 30 µg/ml of DOM was injected and repeated five times and the chromatograms were recorded. The resolution, number of theoretical plates, and peak asymmetry were calculated to determine whether the result complies with the recommended limit (see Table 5)

. **TABLE 5: System Suitability Parameters**

Parameters	Drugs	
	Ilaprazole	**Domperidone**
Retention Time (min)	4.21	6.4
Resolution (Rs)	9.636	
Tailing Factor (t)	0.91	1.38
No. of theoretical plates	7488	9845

TABLE 6: Summary of Validation Parameter:

Sr. No.	Parameters	Results	
		Ilaprazole	**Domperidone**
1.	Linearity Range (n=5) (µg/ml)	5-15	15-45
2.	Regression equation	y = 113.46x - 12.517	y = 48.528x - 16.557
3.	Correlation coefficient (R^2)	0.999	0.999
4.	Limit of detection (n=5) (µg/ml)	0.347	1.04
5.	Limit of quantification (n=5) (µg/ml)	1.05	3.16
6.	Precision		
	Repeatability (%RSD) (n=6)	0.69 %	0.54 %
	Intraday (%RSD)(n=3)	0.86 %	1.07 %
	Interday (%RSD)(n=3)	0.55 %	0.68 %
7.	Robustness (%RSD) (n=3)	1.104 %	1.085 %
8.	Accuracy (Mean ± SD) (%, n=3)	99.61 ± 0.59	99.64 ± 0.51

3.3 Procedure for the analysis of Capsule:

Sample: Ilaprazole and Domperidone

Brand name: LUPILA-D (Ilaprazole 10 mg, Domperidone30 mg)

Manufacturer: Lupin Pharma, Mumbai, Maharashtra, India.

3.3.1 Preperation of sample solution:

LUPILA-D capsule containing 10 mg of ILA and 30 mg of DOM is available in local market which is marketed by Lupin Pharma(Mumbai). For estimation of Ilaprazole and Domperidone mixture was prepared. Mixture equivalent to 10 mg of Ilaprazole and 30 mg of Domperidone was accurately weighed and transferred to volumetric flask of 1000ml capacity. 25 ml of mobile phase was transferred to volumetric flask and Sonicate for 15 mins. The flask was shaken and volume was made up to the mark with mobile phase. The above solution was filtered through Whatman filter paper (0.75µ). From this solution 10 ml was transferred to volumetric flask of 100 ml capacity. Volume was made up to the mark using mobile phase to give a solution containing 100 µg/ml Ilaprazole and 300 µg/ml Domperidone (solution A). From the solution A, 1 ml was transferred to volumetric flask of 10 ml capacity and volume was made up to the mark using mobile phase to give a solution containing 10 µg/ml Ilaprazole and 30 µg/ml Domperidone. This solution was used for the estimation of Ilaprazole and Domperidone.

TABLE 7: Analysis of market formulation

Drug	Label claim (mg)	Conc. taken (µg/ml)	Average Peak Area*	Conc. found from Mixture as per label claim (mg)	Assay* ± SD (%)
Ilaprazole	10	10	1107.373	9.79	97.92 ± 0.56
Domperidone	30	30	1430.931	29.59	98.65 ± 0.04

n=3

CONCLUSION

The proposed RP-HPLC method was used for the simultaneous estimation of Ilaprazole and Domperidone was found to be sensitive, accurate, precise, simple, and rapid. Hence the present RP-HPLC method may be used for routine analysis of the raw materials as well as combined dosage formulations containing Ilaprazole and Domperidone.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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