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Research Article

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Ilaprazole and Domperidone in Pharmaceutical Dosage Form

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ABSTRACT

A specific, accurate, precise and reproducible RP-HPLC method has been developed and subsequently validated for the simultaneous determination of Ilaprazole and Domperidone in pharmaceutical dosage form. The proposed HPLC method utilizes hypersil (Thermo scientific) C18 column (250 mm × 4.6 mm id, 5 µm particle size), and mobile phase consisting of methanol:phosphate buffer (40:60) and pH adjusted to 4.0 with 0.1M glacial acetic acid at a flow rate of 1.0 mL/min. Quantitation was achieved with UV detection at 230 nm based on peak area with linear calibration curves at concentration ranges 5-15 µg/ml for Ilaprazole and 15-45 µg/ml for Domperidone. The retention time of Ilaprazole and Domperidone were found to be 3.433 min and 5.860 min respectively. The method was validated in terms of accuracy, precision, linearity, limits of detection, limits of quantitation and robustness. This method has been successively applied to marketed formulation and no interference from the formulation excipients was found.

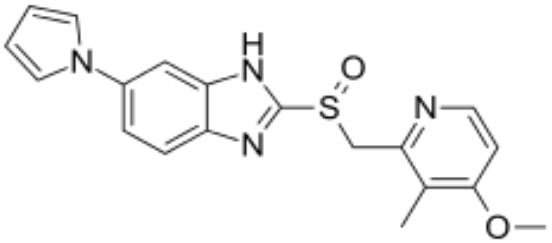
Keywords: RP-HPLC, Ilaprazole, Domperidone, Pharmaceutical Dosage Form

INTRODUCTION

Ilaprazole is a proton pump inhibitor(PPI) used in the treatment of dyspepsia, Peptic ulcer disease (PUD), gastroesophageal reflux disease (GORD/GERD) and duodenal ulcer. It is available in strengths of 5, 10, and 20 mg.Clinical studies show that Ilaprazole is at least as potent a PPI as omeprazole when taken in equivalent doses. Studies also showed that Ilaprazole significantly prevented the development of reflux oesophagitis.[1-3].

Ilaprazole is chemically 2-[(RS)-[(4-methoxy-3-methylpyridin-2yl)methyl]sulfinyl]-5-(1H-pyrrol-1-yl)-1H-benzimidazole[4-6].

Fig.1.Chemical structure of Ilaprazole[2]



Domperidone chemically is 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[7]. Fig.2

Domperidone acts as a gastrointestinal emptying (delayed) adjunct and peristaltic stimulant. The gastroprokinetic properties of domperidone are related to its peripheral dopamine receptor blocking properties.

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Domperidone facilitates gastric emptying and decreases small bowel transit time by increasing esophageal and gastric peristalsis and by lowering esophageal sphincter pressure. Antiemetic: The antiemetic properties of domperidone are related to its dopamine receptor blocking activity at both the chemoreceptor trigger zone and at the gastric level. It has strong affinities for the D2 and D3 dopamine receptors, which are found in the chemoreceptor trigger zone, located just outside the blood brain barrier, which - among others - regulates nausea and vomiting.

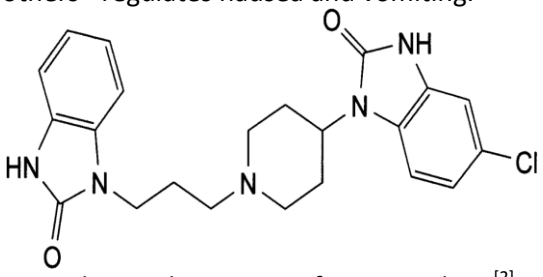

Fig.2.Chemical structure of Domperidone[2]

Combination of Ilaprazole and Domperidone are used in treatment of peptic ulcer and gastroesophageal reflux disease (GORD/GERD) and duodenal ulcer. In the literature survey it was found that Ilaprazole and Domperidone were estimated individualy or in combination with other drugs by UV, HPLC, HPTLC Spectrofluori methods [7-21]. But no method has been found for simultaneous estimation of Ilaprazole and Domperidone by chromatographic method. In the view of the need in the industry for routine analysis of Ilaprazole and Domperidone in formulation, attempts are being made to develop simple and accurate RP-HPLC method for simultaneous estimation of Ilaprazole and Domperidone and extend it for their determination in formulation.

MATERIAL AND METHOD

Equipment

RP–HPLC instrument equipped with SPD-20 AT UV-Visible detector, (LC-20AT, Shimadzu), Rheodyne injector (20 µl Capacity), BDS hypersil (Thermo scientific) C18 column (250 mm × 4.6 mm, 5 µ particle size) and Spinchrom software was used.

Chemicals and reagents

Reference standard of ILA and DOM were obtained from Montage laboratories PVT. LTD., Himatnagar. Methanol and used was of HPLC grade and Phophate buffer(pH 4.0) and all other reagent were of AR grade.

Preparation of standard and test solutions

Preparation of mobile phase

Mobile phase were prepared by mixing of 400 ml of methanol with 600 ml of phosphate buffer, whose pH was adjusted to pH 4.0 by addition of glacial acetic acid. The mobile phase prepared was degassed by ultrasonication for 20 min, so as to avoid the disturbances caused by dissolved gases. The degassed mobile phase was filtered through 0.45 µ filters to avoid the column clogging due to smaller particles.

Preparation of standard stock solutions

An accurately weighed quantity of ILA(10 mg) and DOM(30 mg) were transferred to a 100 ml volumetric flask and dissolved and diluted to the mark with mobile phase to obtain standard solution having concentration of ILA (100 µg/ml) and DOM (300µg/ml).

Preparation of solutions for calibration curve

The calibration curves were plotted over the concentration range 5-15 µg/ml for ILA and 15-45µg/ml for DOM. From the stock solution 100 µg/ml of ILA and 300 µg/ml of DOM prepared. From these working solutions of ILA and DOM (0.5 ml, 0.75 ml, 1.0 ml, 1.25 ml, 1.5 ml and 0.5 ml, 0.75 ml, 1.0 ml, 1.25 ml, 1.5 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with mobile phase. Aliquots (20 µL) of each solution were injected under the operating chromatographic conditions described above.

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Preparation of sample solution

Take quantity equivalent to 10 mg ILA and 30 mg DOM was transferred to 100 ml volumetric flask in Mobile Phase .The solution was filtered through whatman filter paper No. 41 and the volume was adjusted up to the mark with Mobile Phase. From this 1 ml of solution is diluted to 10 ml with the help of mobile phase to get final concentration of 10 µg/ml ILA and 30 µg/ml DOM.

METHOD VALIDATION[8-24]

The developed method was validated according to ICH guidelines. To check the system performance, the system suitability parameters were measured. System precision was determined on six replicate injections of standard preparations. Number of theoretical plates and asymmetry were measured.

Linearity

Linearity was performed with five concentrations ranging from 5-15µg/ml and 15-45 µg/ml for ILA and DOM respectively. The peak areas versus concentration of drug were plotted and a linear least-square regression analysis was conducted to determine the slope, intercept and correlation coefficient (r) to demonstrate the linearity of the method.

The limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ of ILA and DOM were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where σ = the standard deviation of the response

S = Slope of calibration curve.

Precision

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days 3 different concentrations of sample solutions of ILA (5µg/ml, 10 µg/ml and 15 µg/ml) and DOM (15 µg/ml, 30 µg/ml and 45 µg/ml). Percentage relative standard deviation (RSD) was calculated

Accuracy

Accuracy was performed by adding known amounts of ILA and DOM to the pre-analysed marketed formulation and then comparing the added concentration with the found concentration. Three levels of solutions were made which correspond to 80, 100 and 120% of the nominal analytical concentration (5 µg/ml for ILA and 15µg/ml for DOM). Each level was prepared in triplicate. The percentage recoveries of ILA and DOM at each level were determined. The mean recoveries and the relative standard deviation were then calculated.

Robustness

The robustness of the method was evaluated by assaying the test solutions after slight but deliberate changes in the analytical conditions i.e. flow rate (± 0.2 ml/ min), proportion of buffer and methanol (62:38 and 58:42 v/v), and pH of buffer (± 0.2).

RESULT AND DISCUSSION

System Suitability

The chromatogram of ILA and DOM show retention time 3.473 min and 5.920 min respectively. Mobile phase used for separation was Phosphate buffer(pH 4.0) : Methanol (60:40) pH of buffer adjusted with glacial acetic acid. Standard chromatogram was given in Figure 3.System suitability parameters were shown in Table 1.

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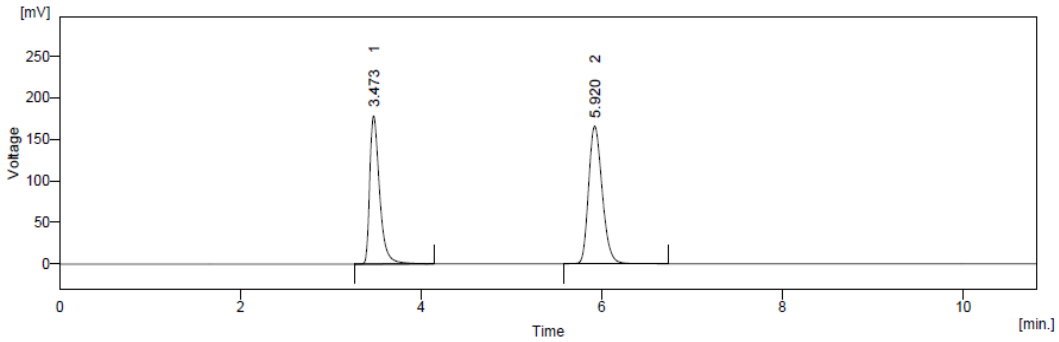

Figure 3: Standard Chromatogram of Ilaprazole and Domperidone

Table 1: System Suitability Parameters Of Chromatogram For ILA & DOM

PARAMETERS	ILA ± SD (n = 6)	DOM ± SD (n = 6)
Retention time (min)	3.473±0.022	5.920±0.016
Tailing factor	1.798±0.018	1.362±0.025
Theoretical plates	4910±2.62	6990±2.14
Resolution	10.163±0.98	

Method validation

The calibration curves were plotted over the concentration range 5-15 µg/ml for ILA and 15-45 µg/ml for DOM are shown in figure 2 and figure 3 respectively. The data for intraday and interday precision for ILA and DOM are shown in Table 2 and Table 3 respectively. Statistical analysis of recovery data is shown in Table 4. Results of robustness study of ILA and DOM are recorded in Table 5. It suggests that the developed method is robust. Summary of validation parameter is shown in Table 6.

Table 2: Linearity Data For ILA and DOM

ILA		DOM	
Conc.µg/ml	**Area* ± SD**	**Conc.µg/ml**	**Area* ± SD**
5	665.892±13.62	15	850.283±10.56
7.5	983.147±13.92	22.5	1258.034±18.26
10	1344.146±15.26	30	1720.292±5.62
12.5	1554.965±18.2	37.5	2018.418±12.65
15	2013.587±30.25	45	2577.797±17.8

*Average of three determination

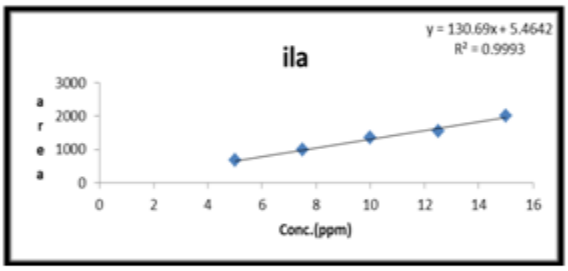

Figure 4: Caliberation curve of ILA

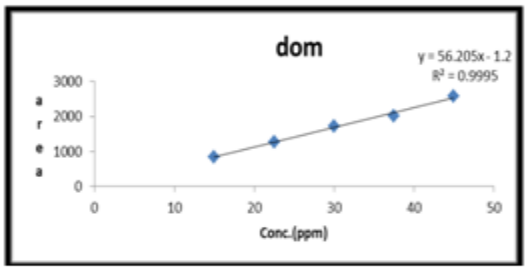

Figure 5: Calibaration curve of DOM

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Table 3: Intraday precision data for estimation of ILA and DOM

ILA			DOM		
Conc.µg/ml	**Peak Area* ± SD**	**%RSD**	**Conc.µg/ml**	**Peak Area* ± SD**	**%RSD**
5	657.391±10.718	1.6304	15	841.19±10.931	1.2997
10	1328.349±19.298	1.4528	30	1706.80±10.914	0.3195
15	1991.963±21.761	1.0924	45	2555.517±12.098	0.6749

Interday precision data for estimation of ILA and DOM

ILA			DOM		
Conc. µg/ml	**Peak Area* ± SD**	**%RSD**	**Conc. µg/ml**	**Peak Area* ± SD**	**%RSD**
5	655.2037±13.31553	2.032273	15	839.377±10.80127	1.28682
10	1330.001±15.17277	1.140809	30	1708.712±5.46059	0.319573
15	1989.005±30.49116	1.532986	45	2556.956±17.2571	0.674908

Table 4: Recovery

Level of recovery %	**Amount of pure drug added (µg/ml)**		**HPLC Method % recovery**	
	ILAPRAZOLE	**DOMPERIDONE**	**ILAPRAZOLE**	**DOMPERIDONE**
80	4	12	99.6689	100.3718
100	5	15	99.1231	99.5917
120	6	18	99.1576	100.0590
Mean % recovery			99.3165	100.0075
Standard Deviation			0.7059	0.6716
Relative Standard Deviation			0.7107	0.6706

Table 5: Robustness

Condition		**Peak Area**	
		ILA	**DOM**
Flow Rate	1.2 ml/ min	1303.544	1674.575
	0.8 ml/ min	1383.888	1776.575
Mobile phase ratio	62:38	1302.376	1669.903
	58:42	1370.602	1756.954
Ph	4.2	1275.916	1639.688
	4.0	1369.845	1760.354
Average		1334.364	1713.008
S.D		23.082	15.469
% RSD		1.731	0.903

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Assay of the tablet formulation

The proposed validated method was successfully applied to determine ILA and DOM in their capsule formulation shown in figure 6. The result obtained for ILA and DOM were comparable with the corresponding labelled amounts Table 4. No interference of the excipients with the peak of interest appeared.

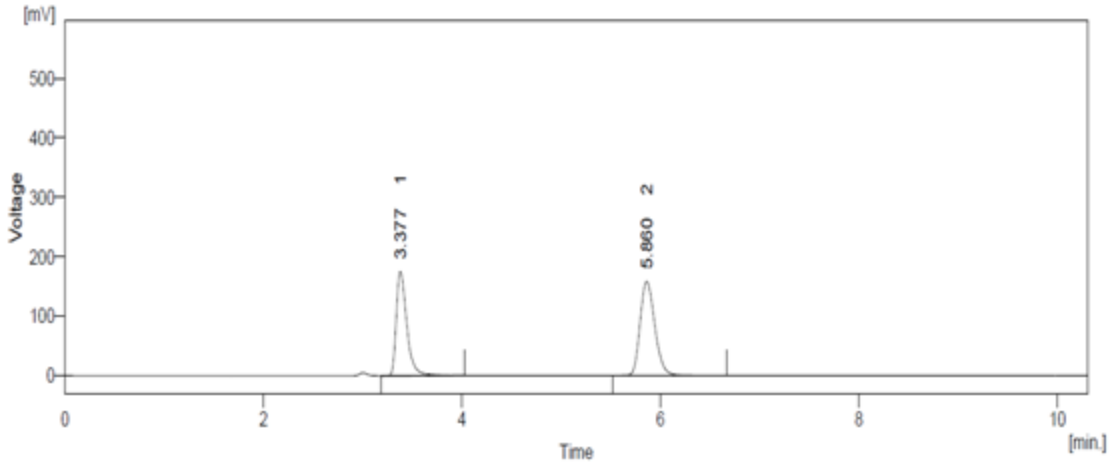

Figure 6: Chromatogram of sample solution of ILA and DOM at 230 nm

Table 6: Assay of formulation

Capsule	mg/capsule		Assay(content in mg)*		(% of labelclaim*) ± % RSD	
LUPILA-D	ILA	10	ILA	9.92	ILA	99.2± 0.80
	DOM	30	DOM	30.42	DOM	101.4± 0.67

Table 7: Summary of Validation Parametere

PARAMETERS	RP-HPLC method	
	ILA	DOM
Concentration range (µg/ml)	5-15	15-45
Slope	130.69	56.205
Intercept	5.4642	-1.2
Correlation coefficient	0.9993	0.9995
LOD(µg/ml)	0.4826	0.9689
LOQ(µg/ml)	0.8659	1.1658
Repeatability (% RSD, n= 6)	1.4858	0.5872
Precision (%RSD) Interday (*n* = 3) Intraday (*n* = 3)	 1.5686 1.3956	 0.7604 0.7647
Accuracy (% recovery), (n=3)	99.3165±0.7059	100.0075±0.6716
% Assay	99.2± 0.80	101.4± 0.67

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CONCLUSION

The RP-HPLC method developed for analysis of Ilaprazole and Domperidonein their capsule dosage form is precise, accurate and with short run time. The method was fully validated showing satisfactory data for all the method validation parameters tested. The developed method is suitable for the quality control of the raw material, formulation and dissolution studies.

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