Research Article

Development and Validation of Stability Indicating HPTLC method for Simultaneous Estimation of Ilaprazole and Domperidone in Bulk and Solid Dosage Form

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ABSTRACT

A Simple, Accurate, rapid& selective Stability indicating high performance thin layer chromatography(HPTLC) method was developed for the simultaneous estimation of Ilaprazole (ILA) and Domperidone (DOM) in pure form and in their combined dosage form. Study was performed on precoated silica gel $60F_{254}$ TLC plates using Toluene: Methanol (8:2, v/v) as the mobile phase with saturation time 20 min. ILA and DOM showed Rf values 0.35±0.006 and 0.64± 0.004 respectively. Followed by the optimum wavelength for detection and quantitation used was 229 nm. Linear response was obtained in the concentration range 50-250 ng/band for ILA and 150-750 ng/band for DOM with correlation coefficient value of 0.999 for both the drugs. The analytical percent recovery was found to be 100.43±0.94% for ILA and99.83±0.58% for DOM. The intra and interday precision with percent relative standard deviation (%RSD) values in the range of 0.74-0.95 for ILA and 0.56-0.79 for DOM. The method was validated as per ICH guidelines. The analytical performance of the proposed HPTLC method was statistically validated with respect to linearity, ranges, precision, accuracy, selectivity, robustness, detection and quantitation limits.

Keywords: Ilaprazole, Domperidone, Stability indicating, High Performance Thin Layer Chromatography (HPTLC), Forced degradation study.

INTRODUCTION

Ilaprazole is chemically2-[4-methoxypropoxy]-3-methyl-2-pyridinyl] methyl] sulfinil] 6-(1H-pyrrol-1—yl)-1H-benzimidazole. Ilaprazole is a new proton pump inhibitor that suppress gastric acid secretion by specific inhibition of the enzyme system of Hydrogen/Potassium adenosine triphosphate ($H^+K^+ATP^{ase}$) at the secretory surface of the gastric parietal cell and is used in the treatment of various gastric disorders such as, gastric and duodenal ulcers, gastro esophageal reflux disease and in pathological hypersecretory conditions. Molecular basis of ilaprazole reveals that it is a complex for the estimation by UV method and the -OCH_3, Benzimidazole, pyridine are responsible for its therapeutic activity and quality control parameters[1-2].

Domperidone is Chemically 5-chloro-1-[1-[3-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl) Propyl]-piperidine-4-yl]-1,3-dihydro-2H-benzimidazol-2-one.Domperidone acts as a gastrointestinal emptying(delayed adjunct and peristaltic stimulant). The gastroprokinetic property of domperidone is related to its peripheral dopamine receptor blocking properties [3].

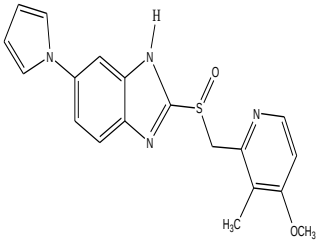

Figure 1: Structure of Ila

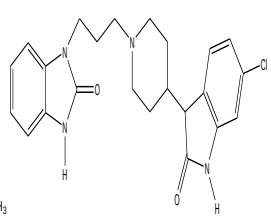

Figure 2: Structure of Dom

As per literature survey there are various analytical methods described for determination of ILA by UV spectrophotometry[4-6] HPLC[7] and DOM by UV[8-10] RP-HPLC [11-17] HPLC[18] FT-IR[19] have been reported alone or in combination with other drugs. But there is no method developed for Stability indicating HPTLC for simultaneous estimation of ILA and DOM in solid dosage form (capsule). The objective of present work was to develop simple, rapid, economic, accurate and precise analytical method for simultaneous estimation of Ilaprazole and Domperidone in bulk and solid dosage form (capsule). The developed method was validated in accordance with ICH guidelines and successfully employed for the assay of ILA and DOM in combine dosage form (capsule).

MATERIALS AND METHODS

Pure analytical sample of ILA and DOM was procured from Lupin Pharmaceuticals, Mumbai and Wockhardt Pharmaceuticals, Aurangabad as a gift sample. The pharmaceutical capsule dosage form Lupila-D (Labelled contain 10 mg of Ilaprazole and 30 mg Domperidone)was purchased from local market. All solvents and chemicals used in the study were Merck analytical grade.

Instrumentation:

Camag HPTLC system consisting:

- Camag Linomat– V sample applicator
- Camag TLC Scanner 3
- Win CATS software Version- 1.4.2
- Merck TLC plates Pre-coated with silica Gel $60F_{254}$
- Hamilton syringe (100 µl)

Selection of mobile phase and chromatographic conditions

Chromatographic separation studies were carried out on the working standard solution of ILA and DOM. Initially, trials were carried out using Methanol: Ethyl Acetate, Benzene: Methanol, Chloroform: methanol, Toluene: methanol in various proportions, to obtain the desired system suitability parameters. After several trials, Toluene: methanol (8:2 v/v), was selected as the mobile phase with chamber saturation time of 20 min, which gave good resolution and sharp peaks. Other chromatographic conditions like run length, sample application volume, sample application positions, distance between tracks, detection wavelength, were optimized to give reproducible R_f values and symmetrical peak shape for drug peak.

Selection of detection wavelength

From the standard stock solution further dilutions were done using methanol and scanned over the range of 200-400 nm and the spectra was obtained. It was observed that both the drug showed considerable absorbance at 229 nm. is shown in fig.3.

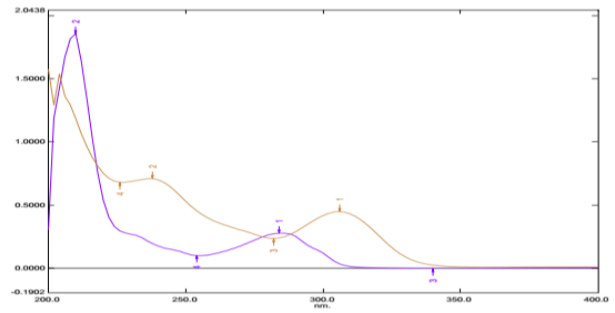

Figure 3: Overlain Spectrums of Ila and DOM

Preparation of standard stock solutions:

Standard stock solution of ILA was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000µg/ml from which 0.5 ml was further diluted with methanol to get the final concentration 50ηg/ml.

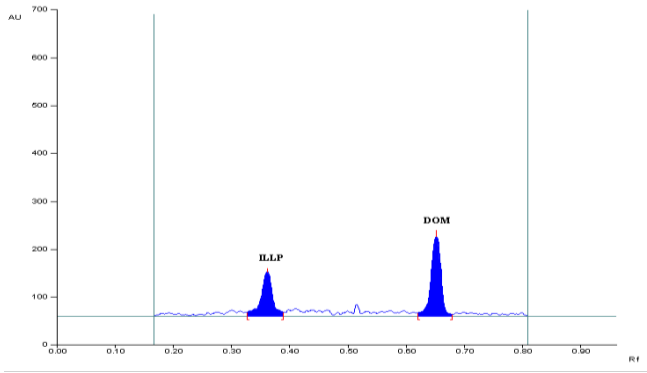

Figure 4: Representative Densitogram of Mixed Standard Solution of Ila (150ng/band,R_f=0.35±0.006) and DOM (450 ng/band,R_f,0.64±0.004

Standard stock solution of DOM was prepared by dissolving 30 mg of drug in 10 ml of methanol to get concentration of 3000µg/ml from which 0.5 ml was further diluted with methanol to get the final concentration 150ηg/ml.

Analysis of capsule formulation

Twenty capsules were weighed accurately and finely powdered. A quantity of powder equivalent to 10 mg of ILA and 30 mg of DOM was weighed and transferred to a10 ml volumetric flaskcontaining approximately 7 ml methanol. The mixture was sonicated for 5 min and diluted to volume with methanol. The solution was filtered and one mililitre of the above solution was further diluted with methanol to obtain the concentration 50 ηg/band for ILA and 150 ηg/band for DOM. Two microliter volume of this solution was applied on TLC plate to obtain final concentration 100 ηg/band for ILA and 300 ηg/band for DOM. After chromatographic development the peak areas of the bands were measured at 229 nm and the amount of the each drug in each sample was determined from the respective calibration plots. The analytical procedure was repeated six times for the homogenous powder sample. The % drug content (Mean±S.D) was found to be 99.70±1.21 for ILA and 100.08±1.36 for DOM. The results obtained are shown in Table 1.

Table 1: Analysis of capsule formulation

Drug	Amount taken (ng/band)	Amount found (ng/band)	% Drug content	S.D.*	% R.S.D.*
ILA	100	99.70	99.70	1.21	1.21
DOM	300	300.26	100.08	4.08	1.36

*n=6

Analytical Method Validation

Preparation of Calibration Curve (Linearity)

For the preparation of calibration curve, standard stock solutions of ILA (50 ηg/µl) and DOM (150 ηg/µl) were applied by over spotting on HPTLC plate in range of 1,2,3,4 and 5 µl with the help of CAMAG 100 µl sample syringe, using Linomat 5 sample applicator to obtain final concentration 50-250 ηg/band for ILA and 150-750 ηg/band for DOM. The plate was developed and scanned under above established chromatographic conditions. Each standard in six replicates was analysed and peak areas were recorded. Calibration curves of ILA and DOM were plotted separately of peak areas were Vs respective concentration. Linear response was observed in the concentration range 50-250 ηg/band for ILA and 150-750 ηg/band for DOM. Excellent correlation exists between peak area and concentration of drugs within the concentration range indicated above. The results obtained are shown in Table 2

Table 2: Linear regression data for calibration plot of Ilaprazole and Domperidone (*n*=6).

Parameters	Ilaprazole	Domperidone
Linearity range (ng/band)	50-250	150-750
Correlation coefficient (R^2)	0.999	0.999
Slope	13.65	4.562
Intercept	62.08	1419
LOD	5.23	41.47
LOQ	15.87	125.69

Precision

The intra-day precision (%RSD) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Inter-day precision (%RSD) was assessed by analyzing drug solutions within the calibration range on three different days over a period of a week.

Table: 3 Intra-day and Inter-day precision results for Ilaprazole and Domperidone.

Drug	Ilaprazole			Domperidone		
Concentration (ng)	100	150	200	300	450	600
			Intra-day precision			
Mean drug content (%)*	99.82	100.00	99.99	99.53	100.17	99.70
% R.S.D.*	0.6432	0.7269	0.6200	1.1763	0.6859	0.6128
			Inter-day precision			
Mean drug content (%)*	100.38	100.34	99.79	99.24	99.85	100.12
% R.S.D.*	0.7474	0.9570	0.8935	0.7904	0.6101	0.5669

*n=3

Accuracy

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out at three levels of 80, 100 and 120 % in triplicate and the percentage of recovery was calculated and it shown in Table 4. The mean recovery of the drugs was found to be in the range of 98-102 % and % RSD is less than 2, indicating a high degree of accuracy for the developed method.

Table 4: Recovery Study

Drug	Amount taken (ng/band)	Amount added (ng/band)	Total amount found (ng/band)	% Recovery	S.D.	% R.S.D.*
ILA	100	80	180.97	100.53	0.69	0.68
	100	100	202.36	101.16	0.93	0.92
	100	120	219.16	99.61	1.23	1.23
DOM	300	240	537.70	99.57	0.55	0.55
	300	300	599.22	99.87	0.53	0.53
	300	360	660.38	100.05	0.66	0.66

*n=3

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 0.991, indicating the no interference of any other peak of degradation product or impurity.

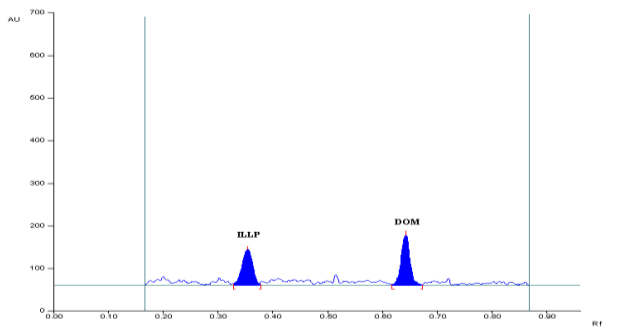

Figure 5: Densitogram of Sample (LUPILA-D) (ILA of 100 ng/band,Rf = 0.34±0.006) (DOM of 300 ng/band, Rf = 0.64 ± 0.004)

Robustness

The robustness of the method was studied during the method development by small but deliberate variations in mobile phase composition and chamber saturation time were altered. One factor at a time was changed at a concentration level of 250 ng/band for ILA and 750 ng/band for DOM respectively, to study the effect on the peak area of the drugs. The results obtained are shown in Table 5.

Table 5: Robustness Study

Sr. No.	Parameters	Variation	% RSD	
			ILA	DOM
1.	Mobile phase composition	± 1% Methanol	0.58	0.38
2.	Chamber saturation period	± 10 %	0.35	0.36

Forced Degradation Study

Table 6: Results of Stress Degradation Studies of ILA and DOM

Stress conditions	ILA		DOM	
	% Assay of active substance	Rf values of degraded products	% Assay of active substance	Rf values of degraded Products
Acid/ 0.1 N HCl/Reflux at 60^{0}C for 4 hr.	87.54	---	79.38	0.73, 0.78
Alkali/0.1N NaOH/ Reflux at 60^{0}C for 4 hr.	77.72	0.48	83.33	0.56
Oxidative /3 % H_2O_2 / Reflux at 60^{0}C for 4 hr.	76.70	022, 0.45	88.83	---
Neutral/H_2O/ Reflux at 60^{0}C for 4 hr.	84.02	0.27	91.83	----
Dry heat/ 80^{0}C/ 2hr.	92.48	---	89.16	0.75
Photolysis	86.29	0.19	91.62	0.52

CONCLUSION

Stability indicating HPTLC method has been developed and validated for the simultaneous determination of ILA and DOM in combined capsule dosage form. The developed method is simple, precise, accurate, and reproducible and can be used for simultaneous quantitative analysis of ILA and DOM in pharmaceutical dosage form as well as for routine analysis in quality control laboratories. The proposed method would be suitable for analysis of ILA and DOM without any interference from the excipients and can be successfully used to estimate the amount of drugs in the formulations by easily available low cost materials.

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