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Original Article

Stability Indicating Chromatographic Method Development and Validation for the Simultaneous Determination of Ilaprazole and Domperidone in Its Pharmaceutical Dosage Form by RP- HPLC

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Abstract

A specific, accurate, precise and reproducible RP HPLC method has been developed and subsequently validated for the simultaneous estimation 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 ìm particle size), and mobile phase consisting of water: Acetonitrile: Acetic acid (30:70:0.1) at a flow rate of 1.0 mL/ min. Quantitation was achieved with PDA detection at 255 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 3.753 min and 6.120 min respectively. The stability studies were carried out and 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: Ilaprazole; Domperidone; Stability Indicating Assay; HPLC.

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Introduction

For the development of stability-indicating assay method, the drug is subjected to Various ICH (International Conference on Harmonization) stress conditions such as photolytic, hydrolytic, thermal and oxidative. As per the ICH drug stability test guidelines Q1A (R2), validated stability-indicating assay method should be developed for the analysis of drug substance and drug product. Ilaprazole; 2-[[(4-Methoxy-3-methyl-2-pyridinyl)methyl]sulfinyl]-6-(1Hpyrrol-1-yl)-1H-benzimidazole is a new proton pump inhibitor used in the treatment of pepticulcer disease, dyspepsia, gastro esophageal reflux disease and duodenal ulcer which reduces acid secretion by inhibiting the parietal cell H+/K+ ATP pumpDomperidone (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. In literatures, few analytical methods have been reported of analysis of Ilaprazole such as UPLC, LC-MS/MS, HPLC-ESI-MS/MS, however no stability-indicating HPLC method have been reported for the analysis of Ilaprazole and domperidone in its combined dosage form. Hence the aim of the present study was to develop and validate stability-indicating HPLC method for determination of Ilaprazole (IPZ) and Domperidone bulk drug as per the ICH guideline. The developed method is stability-indicating and was successfully

utilized for the determination of Ilaprazole in tablet formulation.

Materials and Methods

Apparatus and Instrument

The analysis was carried out on a HPLC system (WATERS Milford USA) equipped with PDA 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.

Reagents and Materials

Ilaprazole and Domperidone were obtained as gratis sample from Accurate Pharmaceuticals, Godhra. Methanol HPLC Grade, Water HPLC Grade, A stock-standard solution of ILA and DOM was prepared by dissolving accurately weighed amount of pure drug in mobile phase.

Mobile Phase

The mobile phase water:Acetonitrile:Acetic acid (30:70:0.1) was filtered through Millipore filter paper type HV (0.45 µm) and degassed by sonication.

Chromatographic Conditions

Chromatographic analysis was carried out on an hypersil C-18 column, (5 µm, 250mm x 4.6mm i.d) LC-20 AT. The mobile phase consisted of water:Acetonitrile:Acetic acid (30:70:0.1). 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.

Preparation of Standard Stock Solution of Ilaprazole (100 mg/ml) and Domperidone (300mg/ml)

A 10 mg of standard Ilaprazole and 30 µg 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

Preparation of Combined Working Standard Solution Containing Ilaprazole and Domperidone in 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.

Degradation Studies

All stress studies for Ilaprazole and Domperidone were performed at concentration of 1mg/ml. The neutral (water) degradation study was performed by refluxing the drug solution at 800C for 8h. The alkaline degradation study was carried out by refluxing drug solution in 0.1N NaOHat 80C for 4h. The drug solution was refluxed with 0.1N HCl at 80C for 4h to conduct degradation study under acidic conditions. For degradation study in hydrogen peroxide (H2O2) drug solution was refluxed with 3% H2O2 at 80C for 8h. Photolytic stress degradation study was carried out by exposing the drug powder to UV light for 48h. Thermal degradation behavior of Ilaprazole and domperidone was studied by exposing the drug powder to dry heat in an oven at 80C for 24h. samples were withdrawn periodically and analyzed by HPLC after suitable dilution.

Method Validation

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).

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.

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.

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.

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.

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.

Results and Discussion

Method Development

The HPLC isocratic programming was utilized to analyze drug and its degradation products. The separation was achieved with mobile phase consisting of water: methanol initially in ratio 50:50 but no peak were observed so mobile phase water:Acetonitrile (30:70) was tried and Ilaprazole peak single was observed. Then again using isocratic conditions water:Acetonitrile:acetic acid were carried out. After considering the varying combinations of various mobile phases, Water: Acetonitrile: Acetic acid (30:70:0.1) was finalized as it was showing good peak shapes and a significant amount of resolution.

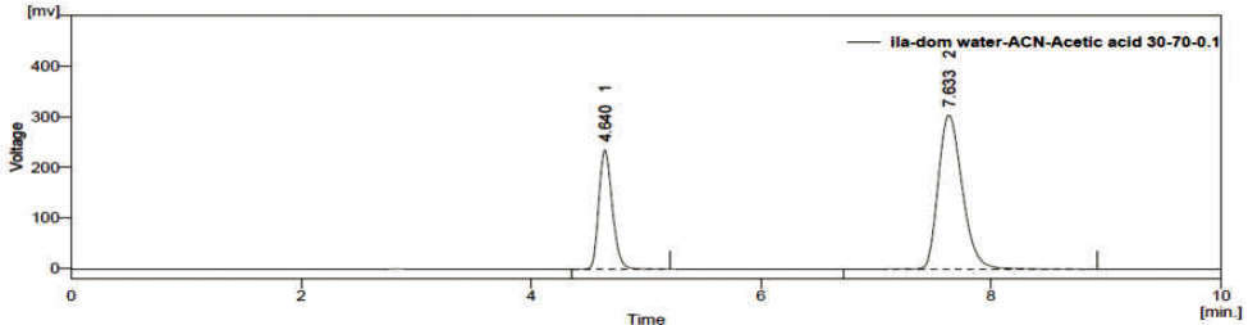

Fig. 3.1: Chromatogram of ilaprazole and domperidone in water: acetonitrile: acetic acid (30:70:0.1 v/v) (Flow rate-1.0 ml/min)

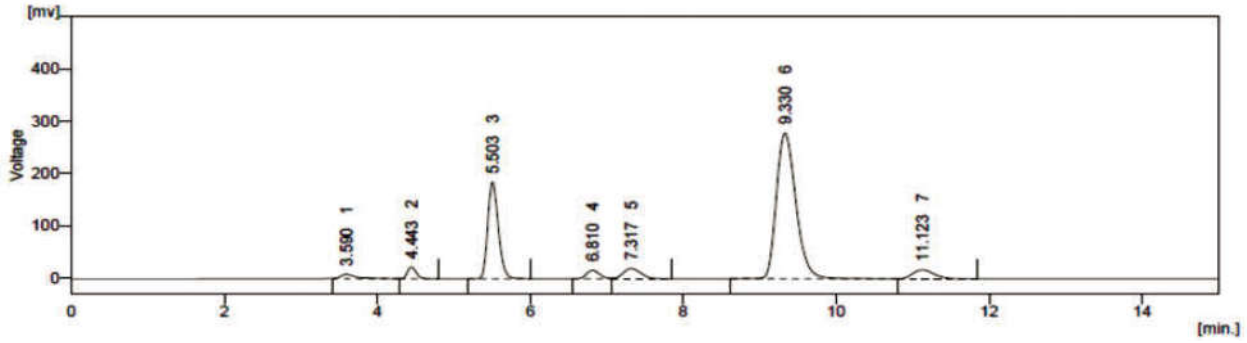

Fig. 3.2: Domperidone and ilapazole acid degradation

Degradation behavior of IPZ and DOM

Ilaprazole was subjected to above mentioned stress conditions and showed following degradation behavior.

Hydrolytic Studies

Acid decomposition studies were performed by refluxing the 1 ml of stock solution and transferred in to 10 ml of volumetric flask. 2 ml of 0.1 N HCl solutions was added and mixed well and put for 80°C for 4 hrs. Then the volume was adjusted with diluent to get Ilaprazole (10 µg/mL) and Domperidone (30 µg/mL) Basic decomposition studies were performed by refluxing the 1 ml of stock solution and transferred in to 10 ml of volumetric flask. 2 ml of 0.1 N NaOH solutions was added and mixed well and put for 4 hrs at 80°C. Then the volume was adjusted with diluent to get Ilaprazole (10 µg/mL) and Domperidone (30 µg/mL).

Oxidative Degradation

Oxidative decomposition studies were performed by refluxing the 1 ml of stock solution transferred in to 10 ml of volumetric flask. 2 ml of 3% H_2O_2 solutions was added and mixed well and put for 8hrs at 80°C. Then the volume was adjusted with diluent to get Ilaprazole (10 µg/mL) and Domperidone (30 µg/mL).

Thermal Degradation

Thermal Degradation studies were performed 1 ml of stock solution was transferred in to 10 ml of volumetric flask. The volumetric flask was stored in oven at 80°C for 24 hrs. Then the volume was adjusted with diluent to get Ilaprazole (10 µg/mL) and Domperidone (30 µg/mL).

Photo Degradation

Photo Degradation studies were performed by taking 1 ml of stock solution and transferred in to 10 ml of volumetric flask. The volumetric flask was kept in presence of UV for 48 hrs. Then the volume was adjusted with diluent to get Ilaprazole (10 µg/mL) and Domperidone (30 µg/mL).

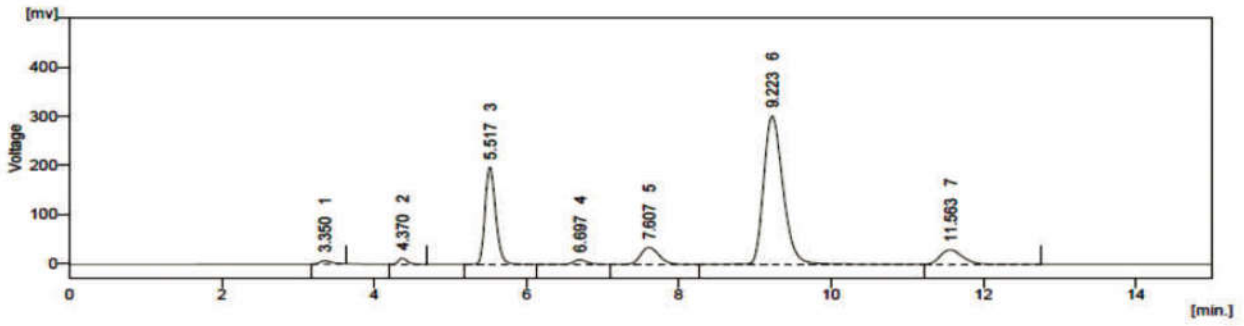

Fig. 3.3: Domperidone and ilapazole base degradation

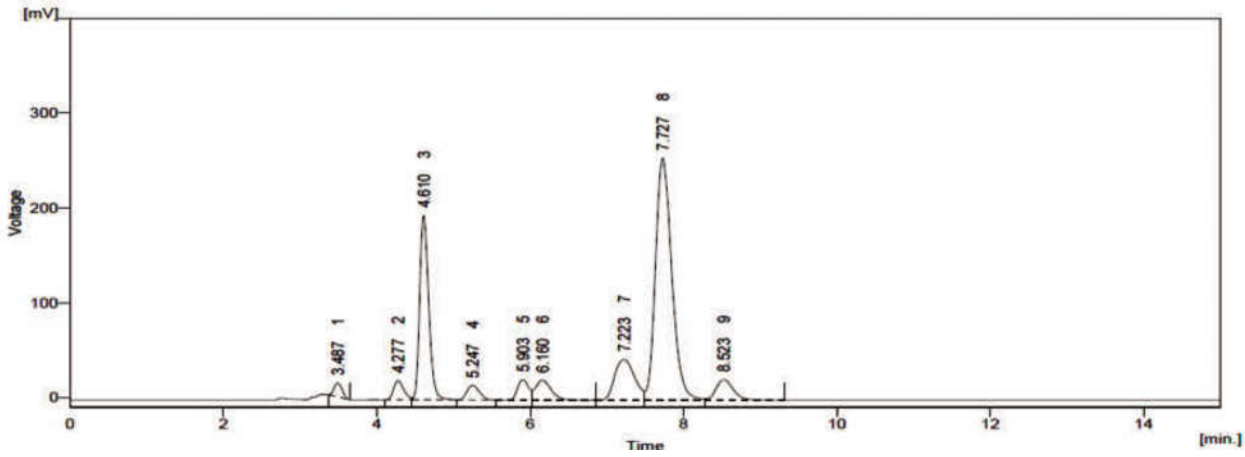

Fig. 3.4: Domperidone and ilaprazole oxidation degradation

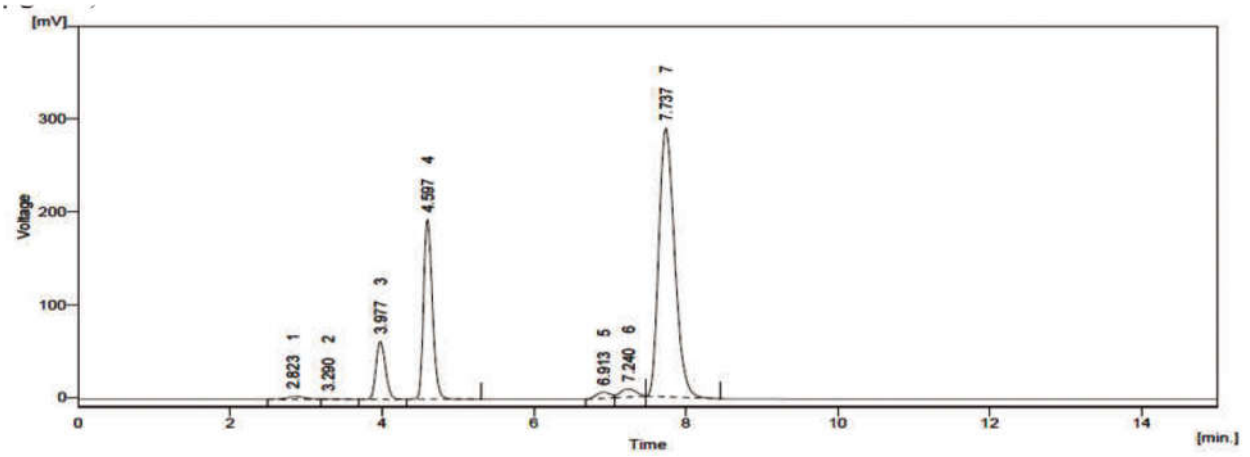

Fig. 3.5: Domperidone and ilaprazole thermal degradation

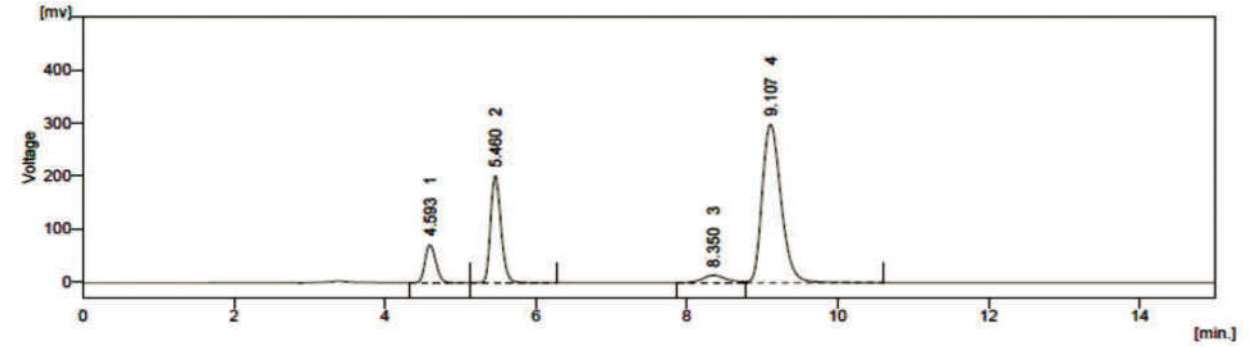

Fig. 3.6: Domperidone and ilapazole photo degradation

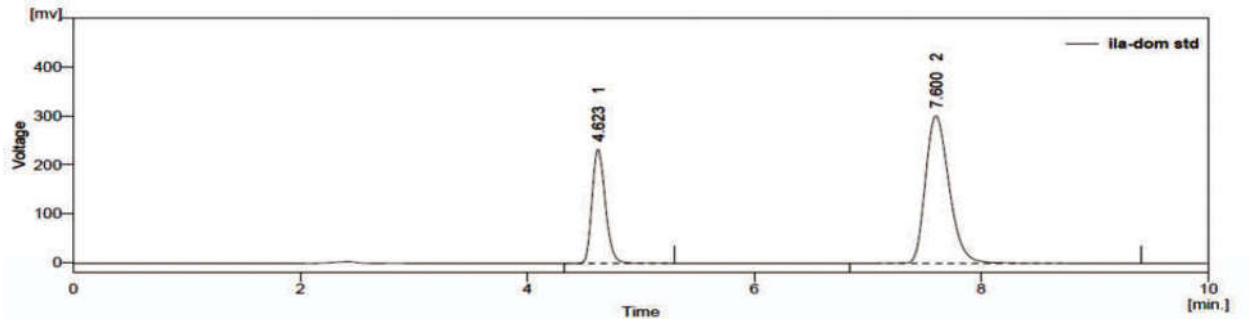

Fig. 3.7: Chromatogram of ilaprazole and domperidone standard

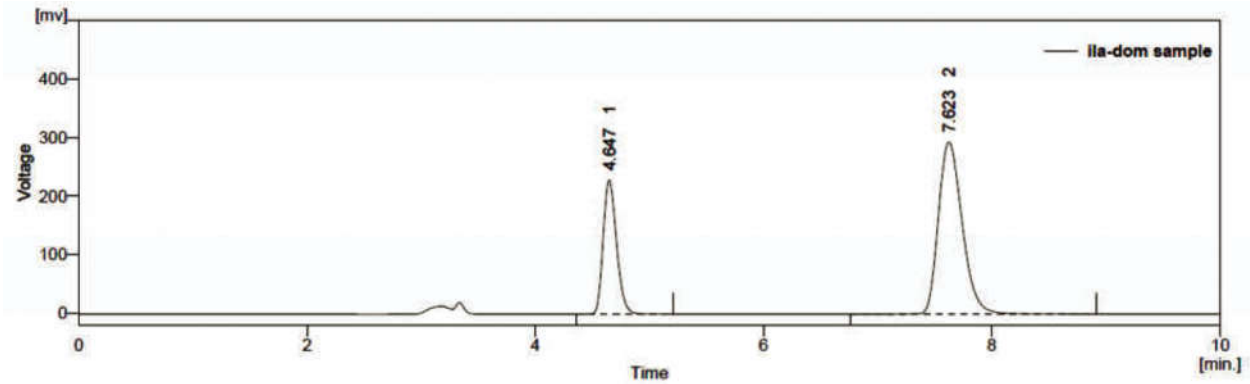

Fig. 3.8: Chromatogram of ilaprazole and domperidone sample

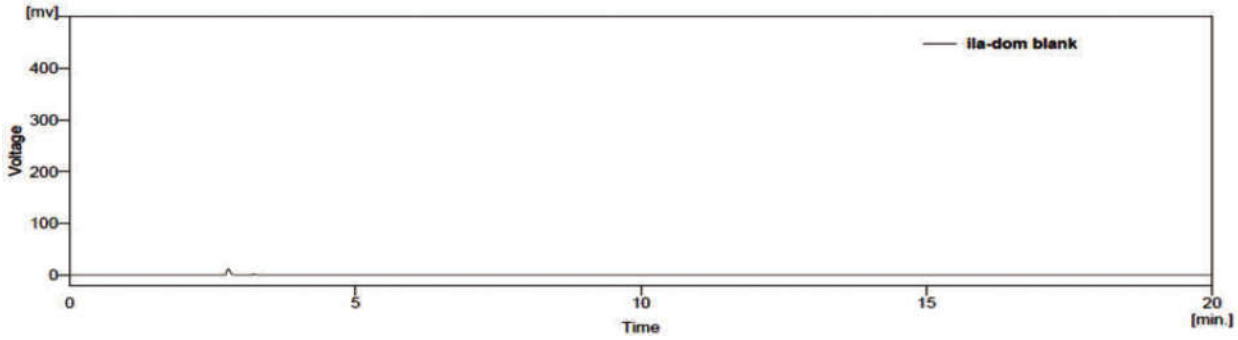

Fig. 3.9: Chromatogram of ilaprazole and domperidone blank

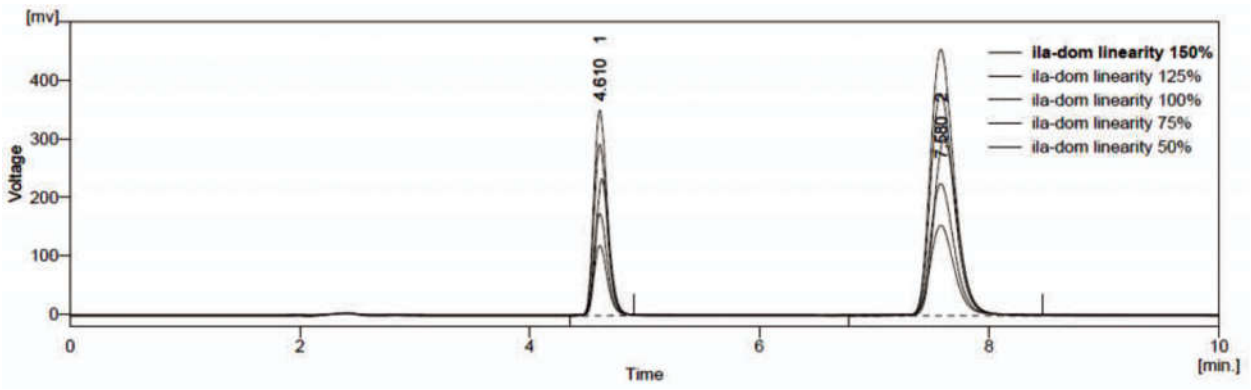

Fig. 3.10: Overlay chromatogram of different concentrations of binary mixtures of ilaprazole and domperidone

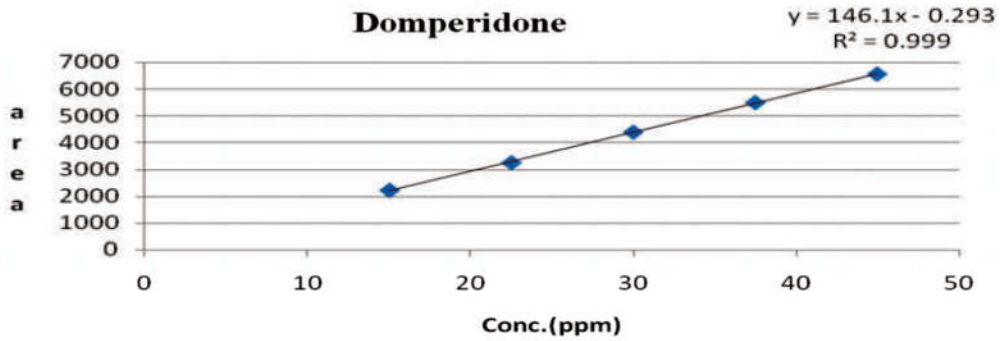

Fig. 3.11: Calibration curve of domperidone (15-45 ìg/ml)

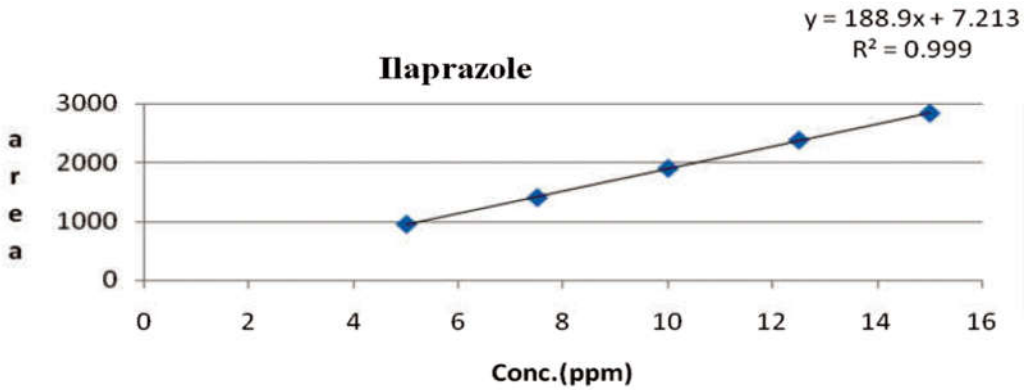

Fig. 3.12: Calibration curve of ilaprazole (5-15 ìg/ml)

Validation

Specificity

The Chromatograms of Ilaprazole and Domperidone standards and Ilaprazole and Domperidone sample show no interference with the Chromatogram of Ilaprazole and Domperidone Blank, so the Developed method is Specific.

Linearity and Range

The linearity for Domperidone and Ilaprazole were assessed by analysis of combined standard solution in range of 15-45 µg/ml and 5-15 µg/ml respectively. Correlation co-efficient for calibration curve Domperidone and Ilaprazole was found to be 0.999 and 0.999 respectively. The regression line equation For Domperidone: **y = 146.1x - 0.293** and For Ilaprazole : **y = 146.1x - 0.293.**

Table 1: Linearity data for domperidone

Sr. No	Concentration(µg/ml)	Area
1	15	2213.258
2	22.5	3247.411
3	30	4389.165
4	37.5	5500.228
5	45	6566.309

Table 2: Linearity data for Ilaprazole

Sr. No	Concentration(µg/ml)	Area
1	5	958.938
2	7.5	1408.078
3	10	1902.745
4	12.5	2380.279
5	15	2835.108

Precision

Repeatability

The data for repeatability of peak area measurement for Ilaprazole and Domperidone, based on six measurements of same solution of Ilaprazole and Domperidone are depicted in table 2 and 3. The % RSD for Ilaprazole and Domperidone was found to be 0.704 and 0.747 respectively.

Table 3: Repeatability data for domperidone

		Domperidone		
Sr. No.	Conc (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	30	5375.432	5341.141±39.900	0.747
		5380.794		
		5308.947		
		5311.033		
		5375.328		
		5295.311		

Table 4: Repeatability data for ilaprazole

SR. NO.	Conc. (µg/ml)	Domperidone Area Mean ± S.D. (n=3)	% R.S.D	Conc. (µg/ml)	Ilaprazole Area Mean ± S.D. (n=3)	% R.S.D
1	15	2675.239 ± 8.307	0.310	5	1158.236± 9.977	0.861
2	30	5314.804± 50.483	0.950	10	2317.879± 4.673	0.202
3	45	8001.386± 20.746	0.259	15	3469.973± 15.576	0.449

Table 5: Intraday precision data for estimation of ilaprazole and domperidone

Sr. No.	Conc. (µg/ml)	Domperidone Area Mean ± S.D. (n=3)	% R.S.D	Conc. (µg/ml)	Ilaprazole Area Mean ± S.D. (n=3)	% R.S.D
1	15	2675.239 ± 8.307	0.310	5	1158.236± 9.977	0.861
2	30	5314.804± 50.483	0.950	10	2317.879± 4.673	0.202
3	45	8001.386± 20.746	0.259	15	3469.973± 15.576	0.449

Table 6: Interday precision data for estimation of ilaprazole and domperidone

Sr. No.	Conc. (µg/ml)	Domperidone Area Mean ± S.D. (n=3)	% R.S.D	Conc. (µg/ml)	Ilaprazole Area Mean ± S.D. (n=3)	% R.S.D
1	15	2679.123±16.261	0.607	5	1163.900±7.726	0.664
2	30	5326.325±46.950	0.881	10	2315.576±12.951	0.559
3	45	7986.264±36.150	0.453	15	3450.463±26.217	0.759

Intraday Precision

The data for intraday precision for Ilaprazole and Domperidone is shown in Table 4. The % R.S.D. for Intraday precision was found to be 0.259-0.950. for Domperidone and 0.202-0.861 for Ilaprazole.

Interday Precision

The data for intraday precision for Ilaprazole and Domperidone is shown in table 5. The % R.S.D. for interday precision was found to be 0.893-1.753 for Domperidone and 0.706-1.055 for Ilaprazole.

Table 7: Recovery data for domperidone

Sr. No.	Conc. Level (%)	Sample Amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	15	12	12.21	101.75	100.11± 1.89
2		15	12	12.06	100.53	
3		15	12	11.77	98.05	
4	100 %	15	15	14.90	99.31	100.85 ± 1.08
5		15	15	14.94	99.57	
6		15	15	15.19	101.29	
7	120 %	15	18	18.15	100.81	101.06 ± 0.29
8		15	18	18.25	101.38	
9		15	18	18.18	100.99	

Table 8: Recovery data for ilaprazole

Sr. No.	Conc. Level (%)	Sample Amount	Amount Added	Amount Recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	5	4	4.05	101.35	100.24 ± 1.56
2		5	4	4.04	100.90	
3		5	4	3.94	98.45	
4	100 %	5	5	5.02	100.45	100.51 ± 0.30
5		5	5	5.01	100.25	
6		5	5	5.04	100.84	
7	120 %	5	6	6.02	100.36	100.18 ± 0.48
8		5	6	5.98	99.63	
9		5	6	6.03	100.54	

Table 9: Robustness data for domperidone

Sr. No.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (-0.2)	Area at pH (+0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
1	2482.653	2151.774	2130.257	2245.255	2527.745	2114.007
2	2485.057	2116.911	2125.967	2247.819	2530.193	2103.401
3	2472.598	2092.614	2119.684	2233.800	2517.503	2113.944
% R.S.D	0.266	1.402	0.250	0.333	0.267	0.289

Table 10: Analysis of marketed formulation

Capsule	mg/ Capsule powder		Assay (% of label claim*) Mean ± S. D.	
	Domperidone	Ilaprazole	% Domperidone	% Ilaprazole
Lupila-D	30	10	95.96±0.898 2.218124 2.218124 ± 1.5289	97.253 ± 0.572

LOD and LOQ

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration curve

LOQ = 10 * SD/slope of calibration curve

Where,

SD = Standard deviation of intercepts

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. The results are shown in table 6 and 7. Percentage recovery for Domperidone was 100.900-101.458 %, while for Ilaprazole, it was found to be in range of 100.365-100.837 %.

Robustness

The effect of changes was found to be within the acceptance criteria as shown in Table 8. The % RSD should Be less than 2%.

Analysis of Marketed Formulation by Developed Method

Applicability of the proposed method was tested by analyzing the commercially available

Capsule formulation Lupila-D. The results are shown in Table 9.

The assay results were comparable to labeled value of each drug in Capsule dosage form. These results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

Conclusion

The stability-indicating assay has been developed and validated for the determination of Ilaprazole and Domperidone in bulk drug and tablet dosage form. The degradation behavior of Ilaprazole and Domperidone was studied as per ICH recommended conditions. The proposed method is simple, precise, accurate, specific, and is able to separate drug from its degradation products. The developed method could also be extended to the analysis of stressed marketed formulation of Ilaprazole and Domperidone, as there is no interference from excipients or other components observed.

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