JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimationof Ilaprazole and Domperidone in their Combined Tablet Dosage Form

Jain Pinkikumari*, Mandev B. Patel

Student of Quality Assurance, A-One Pharmacy College, Anasan, Ahmedabad, Gujarat, India
HOD Quality Assurance, A-One college of Pharmacy, Anasan, Ahmedabad, Gujarat, India

ABSTRACT:

A simple, rapid, economical, precise and accurate stability indicating RP-HPLC method for simultaneous estimation of Ilaprazole and Domperidome in their combined dosage forms has been developed. A reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Ilaprazole and Domperidon in their combined dosage form has been developed. The separation was achieved by C18 (25cm x .46 cm) column and water: Acetonitrle: Acetic acid (30:70:0.1) as mobile phase, at a flow rate of 1ml/min. Detection was carried out at 255 nm retention time of Ilaprazole and Domperidone were found to be 4.640 min and 7.633 min, respectively. The method has been validated for linearity, accuracy and precision. Linearity observed for Ilaprazole 5-15 ug/ml and for Domperidone 15-45 ug/ml. The percentage recoveries obtained for Ilaprazole and Domperidone were found to be in range of 100.36-100.83% and 100.90-101.45%, respectively. Developed method was found to be accurate, precise and rapid for simultaneous estimation of Ilaprazole and Domperidone in their combined dosage form. The drug was subjected to stress condition of hydrolysis, oxidation, photolysis and Thermal degradation, Considerable Degradation was found in alkaline degradation. The proposed method was successfully applied for the simultaneous estimation of both the drugs in commercial combined dosage form.

Key words: Ilaprazole, Domperidone, stability indicating RP-HPLC method, validation.

Article history:

Received 10 March 2016
Revised 14 March 2016
Accepted 28 March 2016
Available online 01 May 2016

Citation:

Jain P, Patel M. B. Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimationof Ilaprazole and Domperidone in their Combined Tablet Dosage Form. **J Pharm Sci Bioscientific Res**. 2016, 6(3):415-419

For Correspondence:

Jain Pinkikumari
Student of Quality Assurance,
A-One college of Pharmacy, Anasan,
Ahmedabad, Gujarat, India.

(www.jpsbr.org)

INTRODUCTION:

Ilaprazole act by irreversibly blocking the hydrogen/potassium adenosine triphosphatase enzyme system (the H+/K+ ATPase, or, more commonly, the gastric proton pump) of the gastric parietal cells. The proton pump is the terminal stage in gastric acid secretion, being directly responsible for secreting H+ ions into the gastric lumen, making it an ideal target for inhibiting acid secretion.Targeting the terminal step in acid production, as well as the irreversible nature of the inhibition, results in a class of drugs that are significantly more effective than H2 antagonists and reduce gastric acid secretion by up to 99%. ("Irreversibility" refers to the effect on a single copy of the enzyme; the effect on the overall human digestive system is reversible, as the enzymes are naturally destroyed and replaced with new copies.)Domperidone is a peripheral dopamine (D2) and (D3) receptor antagonist. It provides relief from nausea by blocking receptors at the chemo-receptor trigger zone (a location in the nervous system that registers nausea) at the floor of the fourth ventricle (a location near the Brain). It increases motility in the upper gastrointestinal tract to a moderate degree

and increases lower esophageal sphincter pressure by blocking dopamine receptors in the gastric antrum and the duodenum. It blocks dopamine receptors in the posterior pituitary gland increasing release of prolactin which in turn increases lactation. Domperidone may be more useful in some patients and cause harm in others by way of the genetic characteristic of the person, such as polymorphisms in the drug transporter gene ABCB1, the potassium channel KCNH2 gene, and α1D-adrenoceptor ADRA1D gene.

MATERIALS AND METHODS:

Apparatus

Model: TSP, Column: C_{18} (25 cm × 0.46 cm) Hypersil BDS, Injector: 20µL fixed loop, Detector: UV Detector, Software: LC,Analytical balance: Electronic analytical balance (Shimadzu).

Reagents and Materials:

Ilaprazole was procured from accretion pharma; Domperidone was procured from accretion pharmaceutical, Water, Methanol, Acetonitrile, Hydrogen phosphate

Chromatographic Conditions

Column: C_{18} (250 mm × 4.6 mm i.d.0.5µm), Mobile Phase: Water: Acetonitrile: Acetic acid, Flow Rate: 1.0 ml/min, Detection Wavelength: 255 nm, Run time: 10.0 min, Injection volume: 20.0 µl.

Preparation of Standard Solutions

(A) Domperidone standard stock solution: (300 µg/mL)

A 30 mg of Domperidone was weighed and transferred to a 100 mL volumetric flask and volume was made up to the mark with methanol.

(B) Ilaprazole standard stock solution: (100 µg/mL)

A 10 mg of Ilaprazole was weighed and transferred to a 100 mL volumetric flask and volume was made up to the mark with methanol.

(C) Preparation of standard solution of binary mixtures of Domperidone (30 µg/mL) and Ilaprazole (10 µg/mL)

Take 1 mL from the Domperidone stock solution and 1mL from Ilaprazole stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

Preparation of Mobile Phase

Mobile phases were prepared by Water: Acetonitrile: Acetic acid (30:70:0.1)

Selection of Detection wavelength

Standard solution of Domperidone (30 µg/mL) and Standard solution of Ilaprazole (10 µg/mL) in Methanol were scanned between 200-400 nm using UV-visible spectrophotometer. Both solutions were scanned between 200 - 400 nm. Wavelength was selected from the overlay spectra of above solutions.

Preparation of Calibration Curves

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, 5,7.5,10,12.5,15 ml solutions were pipette out from the Stock solution of Domperidone (300 µg/ml) and Ilaprazole (100 µg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 15,22.5,30,37.5 and 45 µg/ml and 5,7.5,10,12.5 and 15 µg/ml for Domperidone and Ilaprazole respectively in term of slope, intercept and correlation co-efficient value. The graph of peak area obtained verses respective concentration was plotted.

Estimation of Ilaprazole and Domperidone in marketed tablet

Take Crushed Capsule powder equivalent to 30 mg of Domperidone and 10 mg of Ilaprazole was transferred to a 100 ml volumetric flask and made up volume up to the mark with mobile phase. The solution was filtered through Whatman filter paper no. 42 and first few drops of filtrate were discarded 10 ml of this solution was diluted to 100 ml with mobile phase. The solution was injected 20 µl. The areas of resulting peak were measured at 255 nm.

METHOD VALIDATION

As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantitation.

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**

Accuracy

For Domperidone:

15 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 255 nm. The amount of Domperidone was calculated at each level and % recoveries were computed.

For Ilaprazole:

5 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 255 nm. The amount of Ilaprazole was calculated at each level and % recoveries were computed.

Precision

Results should be expressed as Relative standard deviation (RSD) or coefficient of variance.

A.Repeatability: Standard solution containing Domperidone (30 µg/ml) and Ilaprazole (10 µg/ml) was injected six times and areas of peaks were measured and % R.S.D. was calculated.

B. Intra-day precision: Standard solution containing (15,30,45 µg/ml) of Domperidone and (5,10,15 µg/ml) of Ilaprazole were analyzed three times on the same day and % R.S.D was calculated.

C. Inter-day precision: Standard solution containing (15,30,45 µg/ml) of Domperidone and (5,10,15 µg/ml) of Ilaprazole were analyzed three times on the different day and % R.S.D was calculated.

Limit of Detection and Limit of Quantification

LOD:

The LOD was estimated from the set of 3 calibration curves used to determination method linearity.

The LOD may be calculated as,

LOD = 3.3 × (SD/Slope)

Where,
SD= Standard deviation of Y-intercepts of 3 calibration curves.
Slope = Mean slope of the 3 calibration curves.
LOQ:
The LOQ was estimated from the set of 3 calibration curves used to determine method linearity.
The LOQ may be calculated as,

LOQ = 10 × (SD/Slope)

Where,
SD = Standard deviation of Y-intercepts of 3 calibration curves.
Slope = Mean slope of the 3 calibration curves.

RESULTS AND DISCUSSION

Stability Indicating RP-HPLC Method Development And Validation For Simultaneous Estimation of Domperidone and Ilaprazole in their Combined Dosage Form.

Identification of drugs

Melting point of Domperidone is 240-242 °C and Melting point of Ilaprazole is 150-152 °C. This Value is same as that of the literature citation.

Infrared spectroscopy**:** A pellet of the drug and KBr (Spectroscopic grade) was prepared using hydraulic pellet press at a pressure of 7-10 tones. FT-IR was scanned from 400-4000 cm^{-1}. Following peaks were observed.

Method Development for Simultaneous Estimation of Domperidone and Ilaprazole by RP-HPLC:

Selection of Elution Mode:

Reverse phase chromatography was chosen because of its recommended use for ionic and moderate to non-polar compounds. Reverse phase chromatography is not only simple, convenient but also better performing in terms of efficiency, stability and reproducibility. C_{18} column is least polar compare to C_4 and C_8 columns. Here, C_{18} (250 mm × 4.6 mm i.d.0.5µm) column of 5.0 µm

packing was selected for separation of Domperidone and Ilaprazole. Isocratic mode was chosen due to simplicity in application and robustness with respect to longer column stability.

Selection of wavelength:

Both Domperidone and Ilaprazole show reasonably good response at 255 nm.

Selection of Mobile Phase:

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.

Table-1: Linearity studies of Ilaprazole and Domperidone

Parameter	Ilaprazole	Domperidone
Linearity range	5-15ug/ml	15-45ug/ml
Coefficient of correlation (r^2)	0.999	0.999
Regression Equation	y = 188.9 x + 7.213	y = 146.1 x -0.293
slope	188.9	146.1

Table-2: System suitability and validation parameters

Parameter	Ilaprazole	Domperidone
Retention Time	3.743	6.120
Theoretical plates	7058	6238
Tailing Factor	1.367	1.404
Resolution	9.877	9.877
LOD (µg/ml)	0.229ug/ml	0.668ug/ml
LOQ(ug/ml)	2.025ug/ml	0.693ug/ml

Table-3: Repeatability studies of Ilaprazone and Domperidone

Drug	Concentration (ug/ml)	%RSD
Ilaprazole	10	1.047
Domperidone	30	1.025

Table-5: Intraday and Interday studies of Ilaprazone and Domperidone

Drug	Concentration (ug/ml)	%RSD	
		Intraday	**Interday**
Ilaprazole	15	0.503	1.055
	30	0.210	0.706
	45	0.407	0.893
Domperidone	15	0.355	1.753
	30	0.720	1.234
	45	0.492	0.893

Table-6: Estimation of Ilaprazone and Domperidone in marketed tablet

Brand	Label Claim (mg/ Capsule powder)	Assay (% of label claim*) Means ± S.D.	
	Ilaprazole: Domperidone	Ilaprazole	Domperidone
Lupila-D Capsule	10:30	99.901 ± 0.763	98.975 ± 0.808 2.218124

Table-4: Recovery studies of Ilaprazone and Domperidone

Spike Level %	Amount of Standard Drug Added (µg/ml)	% Mean Recovery	% RSD

	Ilaprazole	Domperidone	Ilaprazole	Domperidone	Ilaprazole	Domperidone
80	4	12	100.900	100.837	0.858	1.020
100	5	15	101.458	101.365	0.264	0.499
120	6	18	101.119	100.566	0.405	0.669

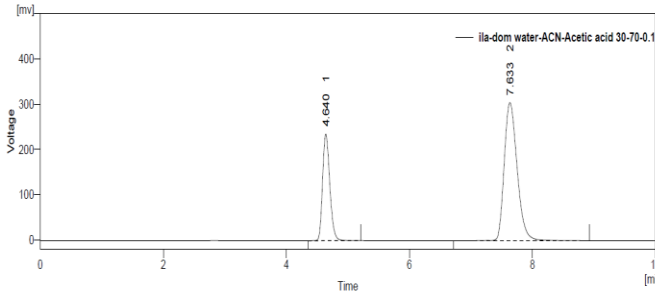

Figure-1: Typical Chromatogram of Ilaprazone and Domperidone

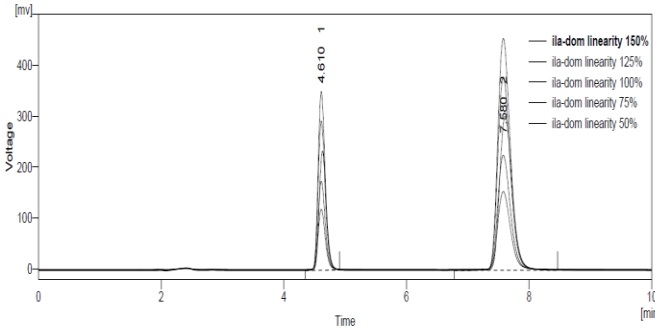

Figure-2: Overlay Chromatogram of different concentration of binary mixture of Ilaprazone and Domperidon

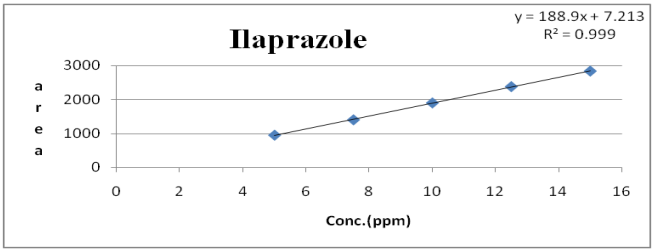

Figure-3: Calibration Curve of Ilaprazole (5-15 µg/ml).

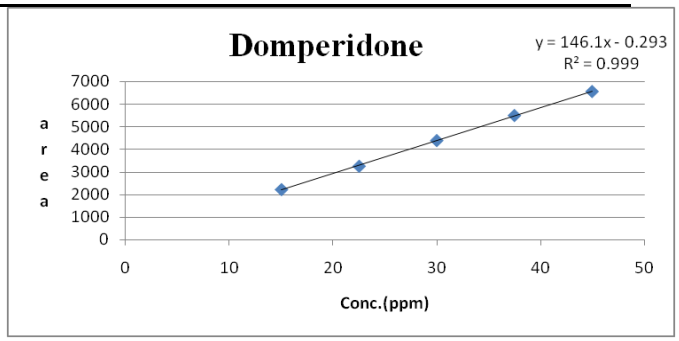

Figure-4: Calibration Curve of Domperidone (15-45 µg/ml).

CONCLUSION

The proposed RP-HPLC method is simple, precise, accurate, economic and rapid for the determination of Ilaprazole and Domperidone in bulk drug and in combined Capsule dosage form. Analysis of authentic sample containing Ilaprazole and Domperidone showed no interference from the common additives and excipients. It can be successfully adopted for routine quality control analysis of Ilaprazole and Domperidone in combined capsule dosage form without any interference from common excipients and impurityThe degradation behavior of Ilaprazole and Domperidone was investigated under different stress degradation (hydrolytic, oxidative, photolytic and thermal) conditions recommended by International Conference on Harmonization (ICH) using HPLC. Stability- indicating RP-HPLC method was developed that could separate drug from degradation products formed under various stressed conditions. Ilaprazole and Domperidone were found to degrade significantly in acidic and alkaline conditions as well as in photolytic degradation and under oxidative condition. Resolution of drug and the degradation products formed under different stress studies were successfully achieved on a BDS hypersil C18 (250mm × 4.6mm, 5µ (particle size), utilizing Water: Acetonitrile:Acetic acid (30:70:0.1) Flow rate was 1ml/min and at the detection wavelength of 255 nm.The method was validated with respect to linearity, precision, accuracy, selectivity.A simple, accurate and precise RP-HPLC method has been developed and validated as per

ICH guideline for Simultaneous Estimation of Ilaprazole and Domperidone in Capsule Dosage Form. Validation parameters like Linearity, Accuracy, Precision, Robustness, System suitability, were tested. Observation of all these parameters leads to the point that developed RP-HPLC method is linear, accurate, precise, specific and robust.

ACKNOWLEDGEMENT

The authors are thankful to RPG Life Sciences Ltd., Maharashtra, India for providing gift sample of standard Spironolactone and Furosemide for research.

REFERENCES

1. ICH, Validation of Analytical Procedures; Methodology, Q2 (R1), International Conference on Harmonization, IFPMA, Geneva 1996.

2. Indian Pharmacopoeia, The Indian Pharmacopoeia Commission Ghaziabad Vol.II, 2010, pp 1225-1226.

3. Dilip G, Priti D., "Simultaneous Determination of Esomeprazole and Domperidone in Combined dosage form by HPLC" Int. j. of sci., 2011, 9,187-194.

4. Kalirajan R, Rajagopal KA, Mary S., "Simultaneous Determination of Rabeprazole and Domperidone in Combined dosage form by HPLC" Rasayana J. Chem,2008, 1,232-235.

5. Shirisha N and kumara S., "Simultaneous Determination of Cinnarizine and Domperidone in Combined dosage form by HPLC"J of Pharma. chem., 2013, 2, 46-50.

6. Haque MA, Shahria M, Parvin N and Ashraful Islam SM., "Validated RP-HPLC Method for Estimation of Ranitidine Hydrochloride, Domperidone and Naproxen in Solid Dosage Form" Asian J. Pharm. Ana. 2011; 1(3)59-63.