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STABILITY INDICATING RP-HPLC MEHOD DEVELOPMENT AND VALIDATION OF DOMPERIDONE IN BULK AND PHARMACEUTICAL DOSAGEFORM

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ABSTRACT

A specific, accurate, precise and reproducible stability indicating HPLC method has been developed and subsequently validated for Domperidone in commercial tablets. The proposed HPLC method utilizes Agient eclipse XDB C18 column (150 mm - 4.6 mm i.e., 5 µm) and mobile phase consisting of methanol-Water(50:50, v/v) at a flow rate of 1.0 mL/min. Quantitation was achieved with UV detection at 270 nm based on peak area with linear calibration curves at concentration range 12.5-200.0µg/mL for Domperidone (R2 > 0.999). The method was validated in terms of accuracy (% recovery 99.6%), precision (%RSD 0.04), linearity, limits of detection (4.1 ng/ml), limits of quantitation (12.6ng/ml), assay (100.5%), and robustness. This method has been successively applied to pharmaceutical formulation and no interference from the tablet excipients was found Domperidone drug product were exposed to acid, base and neutral hydrolysis, oxidation, dry heat and photolytic stress conditions and the stressed samples were analyzed by the proposed method. As the proposed method could effectively separate the drug from its degradation products, it can be employed as stability-indicating method for the determination of instability of these drugs in bulk and commercial pharmaceutical formulations.

KEYWORDS: HPLC method.

INTRODUCTION

Domperidone acts on peripheral dopamine D2 receptors as selective antagonist, with gastro prokinetic and antiemetic properties.[1,2] It also used as gastrointestinal emptying adjunct, peristaltic stimulant. Chemically Domperidone is 5-chloro-1-[1-[3-(2-oxo-1,3-dihydrobenzoimidazol-1-yl) propyl]-4-piperidyl] -1,3-dihydrobenzoimidazol-2-one.[3] Structure of Domperidone was shown as fig:1.[4]

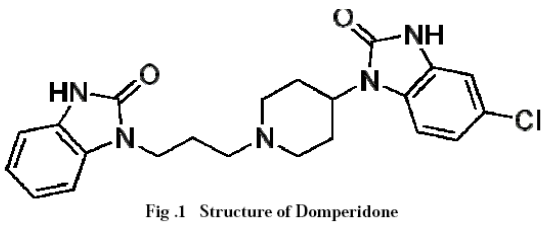

Fig .1 Structure of Domperidone

In this research work we developed, optimized, and validated the method using HPLC. Drug was assayed by stability testing method. The main objective of this method should be time saving and cost effective.

MATERIALS AND METHOD

Chemicals and Reagents

Reference standard was obtained from sigma Aldrich laboratories. The formulation used for assay is Domperidone manufactured by MOREPEN and the solvents used in this method were acetonitrile, methanol, and water of HPLC grade.

Instrumentation

Stability indicating method development and validation was carried out by HPLC (Shimadzu Tokyo, Japan) with PDA detector module with auto-sampler. Column used was Agilent Eclips XBD (150*4.6 * 5µm), and data recorded using LC Solutions software.
DILUENT: Water: Methanol (50:50)

Preparation of Standard

Weigh accurately about 100mg of working standard drug and transferred into 100 ml volumetric flask to it added 30 ml of diluent, sonicated 5 minutes and finally made up the volume with diluent. Pipette out 1 mL above solution into 10 mL volumetric flask to it made up to volume with diluent. Obtained standard concentration is 100µg/ml solution.

Preparation of Sample

Twenty tablets of Domeperidone were taken and powdered, weigh accurately about 10 mg of equivalent weight of drug and transferred into 100 ml volumetric flask to it added 30 ml of diluent, sonicated 5 minutes and finally made up the volume with diluent .Pipette out 1 mL above solution into 10 mL volumetric flask to it

made up to volume with diluent. Obtained standard concentration is 100µg/ml solution.

Method Optimization
Based on literature of domeperidone and its combinations, one method was developed after conducting several trials and developed method was optimized.[5,6,7]

Validation
Developed method was validated for different parameters like specificity-Stability, Accuracy, Precision, linearity, LOD, LOQ, and robustness as per ICH guidelines Q_2R_1.[8]

System Suitability
By injecting it six times into the system, the chromatograms of 100µg/ml were analyzed. Form chromatogram the system suitability parameters like plate count, tailing factor, capacity factor and reproducibility were determined.

Specificity
Interference from Blank: By injecting the mobile phase in to the system we can determine the interference from blank. The mobile phase used in this method was 50:50 V/V water and methanol.

Interference from Excipients: The excipients from the tablet should not show any response (peak) at the retention time of the drug.

Interference from Impurities: It includes interference from the degradation product generated during stress testing. The drug peak should be homogenous and there should be no co-eluting peaks. Peak purity for drug peak should pass.

Stress Testing[9]
Acid Degradation
Accurately weighed equivalent to 10mg of domeperidone was weighed and dissolved in to it added 30 ml of 0.1 M HCL, sonicated 5 minutes and finally made up the volume with 0.1 M hydrochloric acid, this solution was transferred to a round bottom flask(RBF) and refluxed at 80 C. The resulted concentration of the sample solution was 100µg/ml. Aliquots were withdrawn initially (0hr) and at different time points (30mins, 1,2,4 and 8 hr); neutralized to pH 7.0 with counter base 0.1 M sodium hydroxide and then diluted to 10 µg/ml with mobile phase and injected into the HPLC system. The standard solution of 10µg/ml was considered as 100 % and the percentage degradation of drug was calculated by area normalization method.

Base Degradation
Accurately weighed equivalent to 10mg of domeperidone dissolved in 100 ml volumetric flask to it added 30 ml of 0.1M NaOH and the volume was made to 100 ml with 0.1 M sodium hydroxide, it was then kept aside at room temperature. The resulted concentration of the sample solution was 100 µg/ml. Aliquots were withdrawn initially (0 hr) and at different time points (30mins, 1,2,4 and 8 hr); neutralized to pH 7.0 with counter acid 0.05M hydrochloric acid and then diluted with mobile phase to 10 µg/ml and injected into the HPLC system. The standard solution of 10µg/ml was considered as 100 % and the percentage degradation of drug was calculated by area normalization method.

Oxidative Degradation
Accurately weighed equivalent to 10mg of domeperidone dissolved in 100 ml volumetric flask to it added 30 ml of 10% v/v hydrogen peroxide and the volume was made to 100 ml with10% v/v hydrogen peroxide, it was then kept aside at room temperature. The resulted concentration of the sample solution was 100 µg/ml. Aliquots were withdrawn initially (0 hr) and at different time points (30mins, 1, 2, 4 and 8 hr); diluted to 10 µg/ml with mobile phase and injected into the HPLC system. The standard solution of 10µg/ml was considered as 100 % and the percentage degradation of drug was calculated by area normalization method.

Thermal Degradation
Sufficient amount of Domeperidone was placed in a petridish and kept in the hot air oven at 80^{o}C for 24 hours. Aliquots were withdrawn initially (0 hr) and at different time points (30mins, 1, 2, 4 and 8hr); diluted to 10 µg/ml with mobile phase and injected into the HPLC system. The standard solution of 10µg/ml was considered as 100 % and the percentage degradation of drug was calculated by area normalization method.

Photostability
Assay sample Solution in methanol and water (50:50, v/v) was prepared and the resultant solution was exposed to natural sunlight during the day time for 8 h. The degraded sample was then filtered using syringe filters and injected into HPLC system.

Linearity
A series of solutions were prepared at concentration levels as 12.5 µg/ml 25 µg/ml,50 µg/ml, 100µg/ml, 125 µg/ml, 150µg/ml, and200 µg/ml. A 10µlvolume from each concentration of solutions were injected twice into the HPLC system. Chromatograms were recorded under optimized chromatographic conditions. A graph was plotted considering peak areas on Y-axis and concentration on X-axis. The linear equation, Y-intercept, slope of regression line and regression constant (r^2) were calculated.

Accuracy
A series of solutions were prepared in triplicate by spiking the known standard concentrations of Domperidone in the range of 50-150% on the tablet solution and analyzed. The accuracy of method was provided at three different concentration levels at 5, 10, and 15 µg/ml of Domperidone standard. The percentage

recoveries of three different concentrations were found to be within the range of 98 to 102 % as per ICH Q_2R_1 guidelines.

Precision

Repeatability or intra-day precision: The peak areas of 100 µg/ml were analyzed on the same day by injecting it six times into the system. The chromatogram was recorded and RSD was calculated.

Limit of Detection and Limit of Quantitation

LOD and LOQ can be calculated based on the signal to noise ratio approach, visual evaluation and standard deviation of the response and slope of the calibration curve. The slope (S) is calculated from the equation of straight line in calibration curve of the analyte. The standard deviation (σ) is calculated based on its blank response or they-intercepts of regression line. Formulas were given below.

LOD= (3.3X SD)/Slope
LOQ= (10 X SD)/Slope

Robustness and Ruggedness

The robustness of a method is its ability to remain unaffected under changes in parameters. Robustness was carried out by altering the flow rate (±0.2ml/min) and mobile phase (60:50 & 50:60) .The standard solution comprising of Domperidone (100µg/ml) was injected six times and the %RSD was calculated for the resultant area of the peak.

Assay

Twenty tablets of Domeperidone were taken and powdered, Weigh accurately equivalent about 10 mg of lable drug and transferred into 100 ml volumetric flask to it added 30 ml of diluent, sonicated 5 minutes and finally made up the volume with diluent. The solution was then injected into the HPLC system. The sample was prepared in triplicates.

$$\% \text{ Assay} = \frac{(\text{Area of unknown X Conc Of standard}) \text{ X } 100}{(\text{Area of standard X Conc of unknown})}$$

RESULTS AND DISCUSSION

Optimization of Chromatographic: Developed method was optimized for different parameters. Parameters were given in Table.1.

Table 1: Optimized conditions.

S.No	Parameter	Results
1	Instrument	Shimazu
2	Stationary Phase	Zorbax C18 150*4.6 5µ
3	Mobile Phase	Water : Methanol (50:50 v/v)
4	Injection Volume	20µL
5	Wavelength	280
6	Run Time	8
7	Retention time	3.741
8	Flow Rate	1.0 mL/min
9	Temperature	Ambient

Stability Indicating Assay Method

Stability studies were conducted under different conditions like acidic, alkaline, oxidation, and photo stress studies were performed. Domperidone was highly degraded in photo stability study due the instability and polymorphism of chromophoric group present in Domperidone. In oxidative stress and thermal stress conditions moderate degradation was observed, partially degraded in acid and alkaline stress conditions because proton pump inhibitors are highly stable under acidic and alkaline conditions. The degradation results were shown in the table:2. Comparison of degradation results were given in Fig: 2.

Table 2: Results of stress degradation of Domperidone.

Stress Condition	Drug substance % degradation	Drug product % degradation
Acidic	1	0.09
Alkaline	5	2.8
Oxidative	31	28
Thermal	54	46
Photo stability	24	18

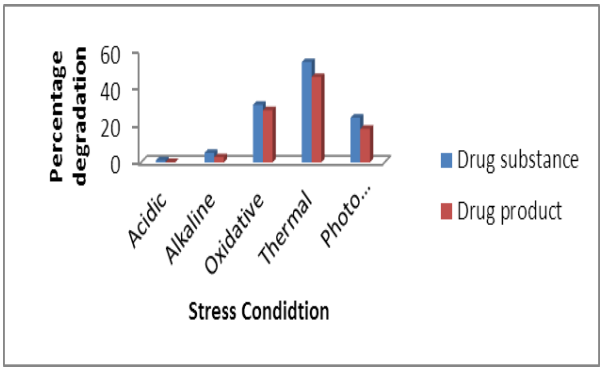

Fig 2: Comparative study of degradation.

Linearity

The calibration curve was made by plotting the concentration on X-axis against peak area on Y-axis. A series of Domperidone standard solution were prepared in the range of 12.5 to 200µg/ml. The correlation coefficient of the curve was found to be 0.999 with a regression equation of Y=317228x - 248444. This is shown in figure-2 and results were given in table: 3.

Table 3: Linearity results of Domperidone.

Concentration	Area
12.5	104532
25	359066
50	688031
100	1010831
125	1332999
150	1674281
200	1973524

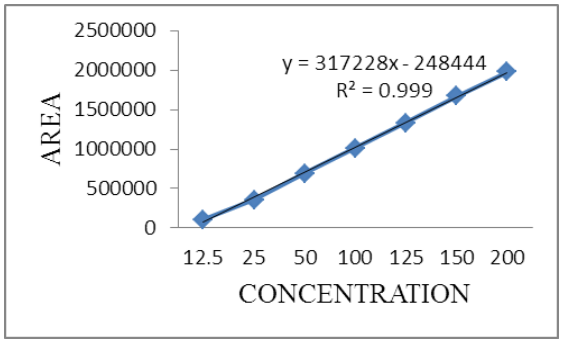

Fig 3: Linearity of Domperidone.

Accuracy

A series of solutions were prepared in triplicate by spiking the known standard concentrations of Domperidone in the range of 50-150% on the tablet solution and analyzed. The accuracy of method was provided at three different concentration levels at 5, 10, and 15 µg/ml of Domperidone standard. Each concentration triplicate samples were injected and average % recovered was calculated. The average % recovery was found to be 99.6%. Results were given in the Table: 4

Table 4: Accuracy results of Domperidone.

Spiked Levels	Area	Drug Added	Drug Recovered	%Recovered
50	504405	5	4.99	99.8
100	1001734	10	9.91	99.1
150	1514225	15	14.98	99.9
AVG				99.6

Precision

Repeatability or intra-day precision: The peak areas of 100µg/ml were analyzed on the same day by injecting it six times into the system. %RSD was calculated. The %RSD was found to be 0.004. Results were given in table: 5

Table 5: Intraday precision of Domperidone.

RT	Area
3.711	1037362
3.722	1037332
3.711	1037252
3.732	1037342
3.712	1037266
3.714	1037312
3.721	1037321
Avg	1037312
Sdv	39.99107
% RSD	0.004

LOD and LOD

LOD and LOQ were calculated from linearity graph. The limit of Detection and limit of Quantification were found out to be 4.1ng/ml and 12.6 ng/ml respectively.

Assay

Tablet solution was injected into the HPLC system for three times and % assay of drug was found to be 99.9%. These results were tabulated in Table: 6.

Table 6: Assay results of Domperidone.

Sample Area	Standard Area
1016151	1010831
1015216	
1017132	
Avg	1016166
% Assay	100.5

Robustness & Ruggedness

Change in the Mobile Phase: On evaluation of the results, it can be concluded that the variation in mobile phase affected the method significantly. Hence it indicates that method was not affected even by change in the mobile phase. The system suitability parameters were within the limit. The results were given in table: 7.

Table 7: Results of robustness (Change in the mobile phase).

S. No	Change in Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	60:50	5501	0.91
2	50:50	5358	0.94
3	50:60	5511	0.91

Change in the Flow Rate

Results for actual flow (1.0 ml/min) have been considered from Assay standard. System suitability parameters were studied and the results were within the limit. These results were shown in the table: 8.

Table 8: Results of robustness (Change in the Flow rate).

S. No	Change in Flow Rate	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	4935	0.93
2	1.0	5358	0.94
3	1.2	5543	0.95

CONCLUSION

An easy, rapid and efficient reverse-phase HPLC method was developed for quantitative estimation of Domperidone in drug product and drug substance. The method was validated as per ICH Q2 (R1) guideline. A precise, accurate, linear, robust and rugged method was found during validation. In the assay 100.5% drug was found in the drug product. Limits of detection (4.1

ng/ml) and limits of quantitation (12.6ng/ml) also determined. A stability-indicating HPLC method has been developed for the estimation of Domperidone in the presence of degradation products. The above method for Domperidone was found to be selective and stability indicating under different stress conditions.

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REFERENCES

1. Prakash A, Waqstaff AJ, Domperidone. A review of its use in diabetic gastropathy. Drugs, 1998 Sep; 56(3): 429-45.
2. M C Champion, M Hartnett, Domperidone, a new dopamine antagonist CMAJ, 1986 Sep 1; 135(5): 457–461.
3. G. Srinivasa Rao, A. B. N. Nagewara Rao, RP-HPLC method for simultaneous estimation of Domperidone and pantoprazole in capsule dosage form. Der Pharmacia Lettre, 2012; 4(6): 1712-1720.
4. www.drugbank.com.
5. V. Krishnaiah and Y. V. Rami Reddy, Development and validation of HPLC method for simultaneous determination of omeprazole and domeperidone. Der Pharma Chemica, 2012; 4(1): 455-459.
6. R.A Tamboli, V.C. Chauhan, Development and Validation of RP-HPLC Method for Simultaneous Estimation of Ilaprazole and Domperidone in Pharmaceutical Dosage Form. Pharma Tutor, (July-2014) ISSN: 2347–7881.
7. T. Sivakumar, R. Manavalan, development and validation of a reversed-phase HPLC method for simultaneous determination of Domperidone and pantoprazole in pharmaceutical dosage forms, acta chromatographica, 18: 2007.
8. Interna ICH of technical requirements for the registration of pharmaceuticals for human use, validation of analytical parameters; methodology adopted in, Geneva, 1996.
9. M.S. Charde, Jitendra Kumar, Review: Development of forced degradation studies of drugs. International Journal of Advances in Pharmaceutics, 2013; 2(3).