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Research Article

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A novel RP-HPLC method development and validation for the simultaneous estimation of domperidone and pantoprazole in bulk and pharmaceutical formulations

J. Subba Rao*, S. Vidyadhara, A. Ramu, B. Venkateswara Rao and Vanaja

Department of Pharmaceutical Analysis, Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowdavaram, Guntur, A.P., India

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ABSTRACT

A simple, precise, accurate, reproducible, economical and sensitive reverse phase liquid chromatography method was developed and validated for the simultaneous estimation of Domperidone and Pantoprazole in bulk and marketed formulations. Evaluation of drugs in this combination was performed with a C18 column [Kromasil column. 250mm × 4.5 mm]using mobile phase of composition Acetonitrile and phosphate buffer (60:40 v/v, pH 7).The flow rate was 0.8 ml/min, and the effluents were monitored at 288nm. The retention time of Domperidone and Pantoprazole were 3.46 min and 5.34 min respectively. The method was found to be linear over a range of 10-50 µg/ml for Domperidone and 40-200µg/ml for Pantoprazole. The established method proved as reproducible one with a %RSD value of less than 2 and having the robustness and accuracy within the specified limits. Assay of marketed formulation was determined and find with 99.08% and 99.87% for Domperidone and Pantoprazole respectively. The method was validated according to the guidelines of International Conference on Harmonization (ICH) and was successfully employed in the estimation of commercial formulations. This liquid chromatographic method can be applied for the qualitative and quantitative determination of selected drugs by the modern chemist.

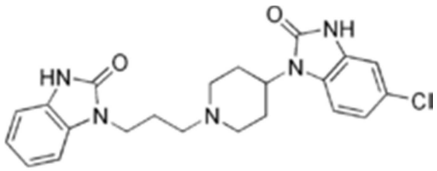
Keywords*:* Domperidone, Pantoprazole, RP-HPLC and Method Validation.

INTRODUCTION

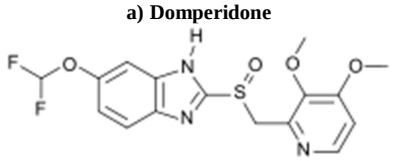
Domperidone is chemically 5-chloro-1-[1-[3-(2-oxo-1,3- dihydrobenzoimidazol-1-yl)propyl]-4-piperidyl] -1,3-dihydrobenzoimidazol-2-one and it is used as gastrointestinal emptying (delayed) adjunct, a peristaltic stimulant, and also as an antiemetic and dopaminergic blocking agent[1,2].The drug comes in many different trade names such as Costi, Motinorm, and Motillium. Its molecular formula is $C_{22}H_{24}ClN_5O_2$ and its molecular weight of 425.911g/mol. Domperidone is a An off-white to creamish white powder used for the treatment of antiemetic

Pantoprazole is chemically designated as 6-(difluoromethoxy)-2-{[(3, 4-dimethoxypyridin-2-yl) methane] sulfinyl}-1H-1,3-benzodiazole [1-2]. The molecular formula is $C_{16}H_{15}F_2N_3O_4S$, and molecular weight is 383.371g/mol. Pantoprazole is a proton pump inhibitor (PPI) that suppresses the final step in gastric acid production by forming a covalent bond at the sites of the (H^+,K^+)-ATPase enzyme system at the secretary surface of the gastric parietal cell. This effect is dose-related and leads to inhibition of the both basal and stimulated gastric acid secretion irrespective of the stimulus.

Extensive literature survey reveals that very few analytical methods were reported for the simultaneous estimation of Domperidone and Pantoprazole [3-15]. So an attempt was made to develop a modified, simple, less time consuming, sensitive liquid chromatographic method for the simultaneous estimation of selected drugs in bulk and in commercial formulations.



a) Domperidone



b) Pantoprazole

Fig 1: Chemical Structures of a) Domperidone and b) Pantoprazole

EXPERIMENTAL SECTION

Equipment used

The chromatographic separation was performed on Agilent 1120 compact liquid chromatographic system integrated with a variable wavelength programmable UV detector and a Rheodyne injector equipped with 20µl fixed loop. A reverse phase C18 [Kromasil column. 250mm × 4.5 mm]was used. Lab India 3000^{+}double beam UV-visible spectrophotometer and Axis AGN204-PO electronic balance were used for spectrophotometric determinations and weighing purposes respectively.

Reagents and chemicals

Pharmaceutical grade pure Domperidone and Pantoprazole gift samples were procured from Mylan Laboratories, Hyderabad. Marketed Formulation Tablets with a dose of 10mg of Domperidone and 40mg of Pantoprazole were purchased from local market. HPLC grade Acetonitrile and Water were purchased from Merck Specialties private Limited, Mumbai.

Chromatographic conditions

Kromasil 100-5C_{18} column [250mm x 4.6mm]was used for the chromatographic separation at a detection wavelength of 288 nm. The mobile phase of composition Acetonitrile and Phosphate buffer pH7 in a ratio of 60:40 v/v was selected for elution and the same mixture was used in the preparation of standard and sample solutions. Flow rate was adjusted to 0.8ml/min, and the injection volume was 20µl.

Preparation of Mobile Phase

Phosphate buffer pH7 was prepared by Dissolve 6.804gm of Potassium dihydrogen phosphate in 250ml of HPLC grade water and adjust the pH to 7.0. Acetonitrile and Phosphate buffer in the ratio of (60:40) was filtered through the 0.45µ membrane filter and sonicated for 20 minutes.

Preparation of Standard Solutions

25mg each of Domperidone and Pantoprazole was accurately weighed and transferred into two 25ml volumetric flasks, dissolved using mobile phase and the volume was made up with the same solvent to obtain primary stock solutions A (Domperidone) B (Pantoprazole) of concentration 1000µg/ml of each drug. From the primary stock solutions , 0.5ml and 2ml were pipette out from A and B respectively, transferred to a 10ml volumetric flask and the volume was made up with the mobile phase to obtain final concentrations of 50 µg/ml and 200µg/ml of Domperidone and Pantoprazole respectively and this solution is (working stock solution A).

Preparation of Sample Solution

Twenty tablets of Domperidone and Pantoprazole were weighed and crushed. Tablet powder equivalent to 10mg of Domperidone and 40mg of Pantoprazole was weighed accurately and transferred to a 25ml volumetric flask. The content was dissolved with 10ml of mobile phase and then sonicated for 15min. The volume was made up with the mobile phase and filtered with the 0.45µ membrane filter and sonicated for 20min. 0.5ml of this solution was pipetted out and transferred to a 10ml volumetric flask, and the volume was made up with the mobile phase to obtain a concentration of 50µg/ml of Domperidone and 200µg/ml of Pantoprazole (working stock solution B).

Optimization of RP-HPLC method

The HPLC method was optimized with an aim to develop a simultaneous estimation procedure for the assay of Domperidone and Pantoprazole. For the method optimization, different mobile phases were tried, but acceptable retention times, theoretical plates and good resolution were observed with Acetonitrile ,Phosphate buffer pH7 (60:40 v/v) using Kromasil 100-5C_{18} column [250mm x 4.6mm].

Validation of the RP-HPLC method

Validation of the optimized method was performed according to the ICH Q2 (B) guidelines.

System suitability

System suitability was carried out with five injections of a solution of 100% concentration having 50 µg/ml of Domperidone and 200µg/ml of Pantoprazole into the chromatographic system. A number of theoretical plates (N) obtained and calculated tailing factors (T) were reported in Table 1.

Linearity

For the determination of linearity, appropriate aliquots were pipetted out from working stock solution A to a series of 10ml volumetric flasks and volume was made up with the solvent to obtain concentration ranging from 10-50µg/ml of Domperidone and 40-200µg/ml of Pantoprazole. Each solution was injected in triplicate. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients. The calibration curves for Domperidone and Pantoprazole were shown in figure 3 and figure 4 their corresponding linearity parameters were given in Table 2.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were calculated from the slope(s) of the calibration plot and the standard deviation (SD) of the peak areas using the formulae LOD = 3.3 σ/s and LOQ = 10 σ/s. The results were given in Table 2.

Precision

The repeatability of the method was verified by calculating the %RSD of six replicate injections of 100% concentration (50 µg/ml of Domperidone and 200µg/ml of Pantoprazole) on the same day and for intermediate precision % RSD was calculated from repeated studies on different days. The results were given in Table 3.

Accuracy

To ensure the reliability and accuracy of the method recovery studies were carried out by standard addition method. A known quantity of pure drug was added to pre-analyzed sample and contents were reanalyzed by the proposed method and the percent recovery was reported. The results were given in Table 4.

Specificity

Specificity of a method was determined by testing standard substances against potential interferences. The method was found to be specific when the test solution was injected, and no interferences were observed because of the presence of excipients. The optimized chromatogram of Domperidone and Pantoprazole without any interference was shown in figure 2.

Robustness

Robustness of the method was verified by altering the chromatographic conditions like mobile phase composition, wavelength detection, flow rate, etc. and the % RSD should be reported. Small changes in the operational conditions were allowed, and the extent to which the method was robust was determined. A deviation of ±2nm in the detection wavelength and ±0.2ml/min in the flow rate, were tried individually. A solution of 100% test concentration with the

specified changes in the operational conditions were injected into the instrument in triplicate. %RSD was reported in Table 5.

Assay of Marketed Formulations

20µl of sample solution of concentration 50 µg/ml of Domperidone and 200 µg/ml of Pantoprazole was injected into the chromatographic system and the peak responses were measured. The solution was injected three times into the column. The amount of drug present and percentage purity was calculated by comparing the peak areas of the standards with that of test samples. A typical chromatogram for assay of the marketed formulation was shown in figure 5, and the obtained values were reported in table 6.

RESULTS AND DISCUSSION

After some trials with mobile phases of different composition, Acetonitrile, Phosphate buffer pH 7 in the ratio 60:40v/v was selected as a mobile phase because of better resolution and symmetric peaks. Domperidone and Pantoprazole were found to show appreciable absorbance at 288nm when determined spectrophotometrically and hence it was selected as the detection wavelength. An optimized chromatogram indicating the separation of Domperidone and Pantoprazole at different R_Ts was illustrated in figure 2.

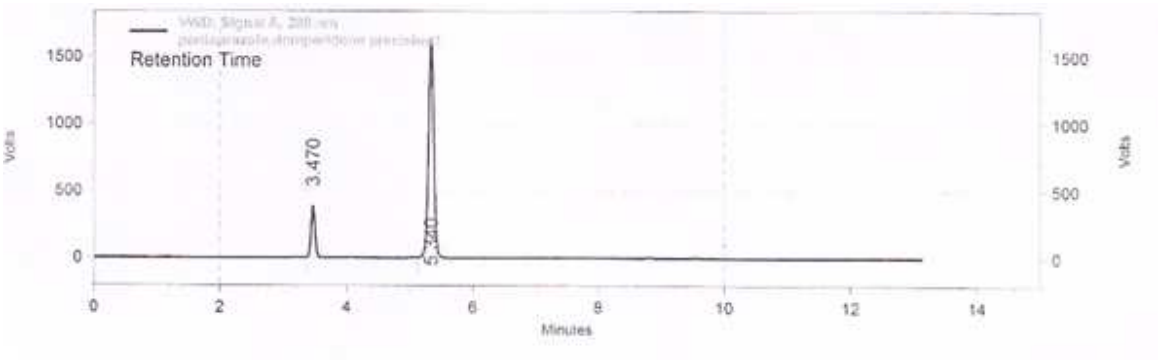


Fig 2: Optimized Chromatogram of Domperidone and Pantoprazole

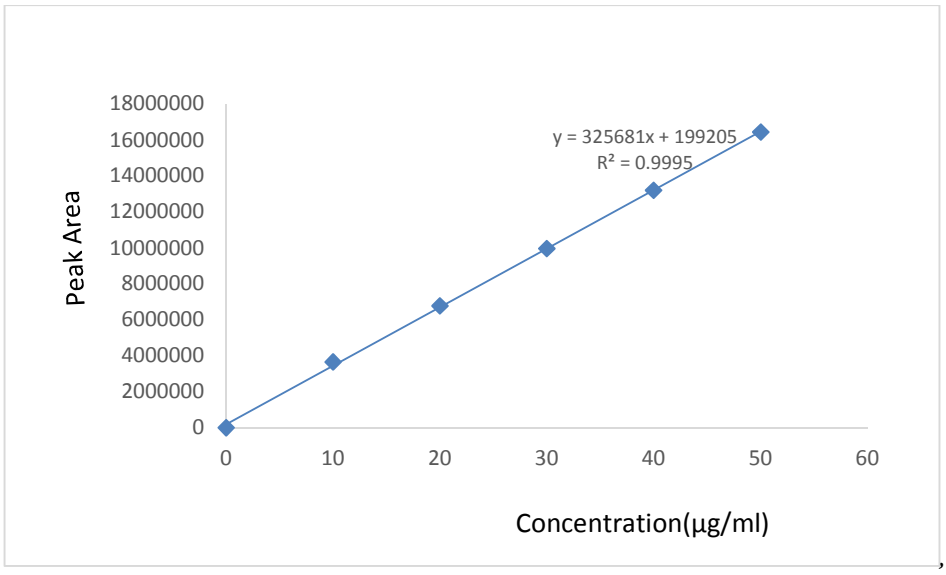


Fig 3: Calibration plot of Domperidone

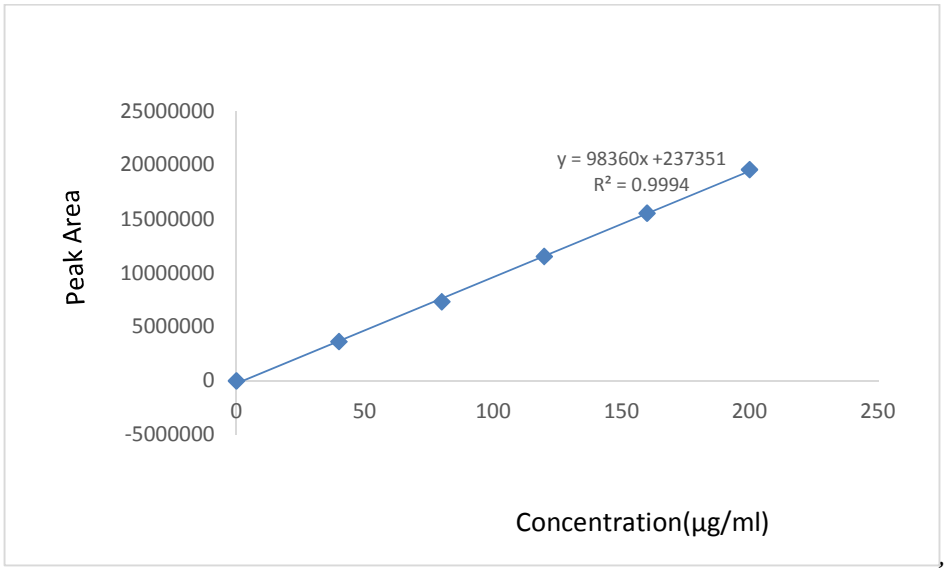


Fig 4: Calibration plot of Pantoprazole

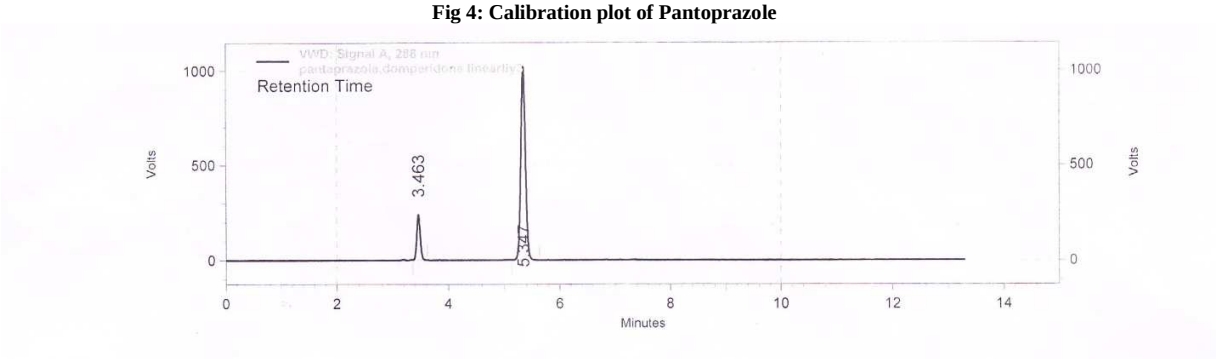


Figure 5: A typical chromatogram for assay of marketed formulation containing 50µg/ml of Domperidone and 200µg/ml Pantoprazole

Table 1: system suitability parameters (n=5)

Parameters	Domperidone	Pantoprazole
Retention time (min)	3.4	5.3
Theoretical plates (N)	11456	10366
Tailing factor (T)	1.2	1.4
Resolution (R_s)	1.9	

Table 2: Results for Linearity (n=3)

Parameters	Domperidone	Pantoprazole
Slope	311179	920580
Wavelength	288nm	288nm
Correlation coefficient r^2	0.999	0.999
Regression Equation	Y=325681x+199205	Y=983602x+233735
Linearity range	10-50µg/ml	40-200µg/ml
LOD	0.16µg/ml	0.33µg/ml
LOQ	0.49µg/ml	1.01µg/ml

**n= No. of determinants*

Table 3: Results of precision (n=6)

Drug	Intraday Precision (%RSD)	Inter-day Precision (%RSD)
Domperidone	0.502	0.78
Pantoprazole	0.16	0.770

**n= No. of determinants*

System suitability was carried out by injecting five replicate injections of 100% test concentration, the number of theoretical plates, HETP and resolution were satisfactory. The chromatograms confirm the presence of Domperidone and Pantoprazole at 3.46min and 5.34min respectively without any interference. The parameters were given in Table 1.

The concentration range of 10-50µg/ml for Domperidone and 40-200µg/ml of Pantoprazole were found to be linear with correlation coefficients 0.999 and 0.999 for Domperidone and Pantoprazole respectively. The results were given in Table 2.

The limits of detection for Domperidone and Pantoprazole were found to be 0.16µg/ml and 0.33µg/ml respectively, and the Limits of Quantitation were 0.49µg/ml and 1.01µg/ml respectively. Values were represented in Table 2.
The proposed method was found to be precise and reproducible with %RSD of 0.502 and 0.16 for Domperidone and Pantoprazole respectively. %RSD was reported in table 3.

An accuracy of the method was verified by performing recovery studies by standard addition method. The percent recovery of the standard added to the pre-analysed sample was calculated, and it was found to be 95.5% to 97.4% for Domperidone and 98 to 99.99% for Pantoprazole. This indicates that the method was accurate and the values obtained were given in Table 4.

The method was found to be robust after changing the conditions like detection wavelength (± 2nm) and flow rate (± 0.2 ml). %RSD was calculated for each variation and reported. Values obtained were given in Table 5.

The method was found to be specific for the combination of interest after verifying the chromatograms showing no interference of the excipients present. Hence, the method was well suitable for the estimation of the commercial formulations of the selected combination with a percentage purity of 99.08% for Domperidone and 99.87% for Pantoprazole. The typical chromatogram for assay of marketed formulations was shown in figure.5 and values obtained were given in Table 6.

Table 4: Results for Accuracy (n=3)

Recovery level	Domperidone				Pantoprazole			
	Amount Added (µg/ml)		Amount Found (µg/ml)	% Recovery	Amount Added (µg/ml)		Amount Found (µg/ml)	% Recovery
	std	test			std	Test		
80%	10	30	38.2	99.5	60	100	158.1	98.8
100%	20	30	48.7	97.4	100	100	196	98
120%	30	30	57.2	95.3	140	200	239.98	99.99
Mean recovery	95.3%-99.5%				98%-99.99%			

**n= No. of determinant*

Table 5: Results for Robustness (n=3)

Parameters (n=3)	%RSD	
	Domperidone	Pantoprazole
Detection wavelength at 286nm	0.66	0.12
Detection wavelength at 290nm	0.17	0.93
Flow rate 0.6ml/min	0.376	0.137
Flow rate 1.0ml/min	0.394	0.33

**n= No. Of determinant*

Table 6: Results for Assay (n=3) of Marketed formulation

Drug	Label claim (mg/tab)	Amount recovered	% Amount found in drug
Domperidone	10	9.98%	99.08%
Pantoprazole	40	39.8%	99.87%

**n= No. of determinants*

CONCLUSION

The RP-HPLC method developed and validated allows a simple and fast quantitative determination of Domperidone and Pantoprazole from their formulations. All the validation parameters were found to be within the limits according to ICH guidelines. The proposed method was found to be specific for the drugs of interest irrespective of the excipients present, and the method was found to be simple, accurate, precise, rugged and robust. So the established method can be employed in the routine analysis of the marketed formulations.

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