Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 5, 2012

Research Article

METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF OMEPRAZOLE AND DOMPERIDONE IN SOLID DOSAGE FORM BY RP-HPLC

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Received: 26 Mar 2012, Revised and Accepted: 10 May 2012

ABSTRACT

The present work describes a simple Reverse Phase HPLC method for the determination of Omeprazole and Domperidone from capsule formulations. The determination was carried out on a Gasco, ODS, C-18 (250× 4.5 mm, 5 micron) column using a mobile phase of Acetonitrile: 0.05M ammonium acetate buffer (pH- 4) in the ratio of (85:15). The flow rate and runtime were 1 ml/min and 10 min, respectively. The eluent was monitored at 280 nm. The method was reproducible, with good resolution between Omeprazole and Domperidone. The detector response was found to be linear in the concentration range of 4-12 µg/ml for Omeprazole and 8-16 µg/ml for Domperidone.

Keyword: Domperidone (DOM), Omeprazole (OME), Acetonitrile, Jasco HPLC (2080 Plus) and 0.05 ammonium acetate buffer (pH - 4)

INTRODUCTION

Domperidone is an antiemetic and antinauseant and acts on dopamine receptor system as an antagonist. Chemically it is 5-chloro-1-[1- (2, 3-dihydro-2-oxo-1H-benzimidazole-1-yl) propyl)-4-piperidyl]-2,3- dihydro-1H benzimidazol-2-one1. It is official in British Pharmacopoeia and European Pharmacopoeia where non-aqueous titration is the official method of assay.

Omeprazole is 5-methoxy-2-(4-methoxy-3, 5-dimethyl-2- Pyridinyl methyl sulfinyl)-1H-benzimidazole. It is official in IP 2, USP 3 and BP. Omeprazole is the proton pump inhibitor. In he acidic conditions of the stomach, omeprazole react with a cysteine group in H+/K+-ATPase, thereby destroying the ability of the parietal cells to produce gastric acid. Thus together both these drugs have synergistic effect in controlling the gastric ulcer diseases. [1-2]

Literature survey reveals that several methods like Spectrophotometry, HPLC, HPTLC and LC-MS were reported for the determination of Domperidone in combination with other drugs as well as in biological fluids but no method has been reported for simultaneous analysis of Domperidone and Omeprazole in its combination anywhere before. . These above developed methods are too expensive and time consuming. An attempt has been made to develop a simple, economical, precise, accurate and reproducible HPLC method for estimation of Domperidone and Omeprazole in bulk as well as pharmaceutical formulations. [3-4]

Instrumentation

Column Gasco C18, ODS (250× 4.5 mm, 5 micron).A Shimadzu UV/Visible spectrophotometer, model 1800 (Japan) was employed with spectral bandwidth of 2 nm and wavelength accuracy of ± 0.5 nm, with automatic wavelength correction employing a pair of quartz cells. A Shimadzu electronic analytical balance (AX-200) was used for weighing the sample

Reagents and chemicals

Pure Domperidone was obtained from Macro Lab, Bangalore, India and pure Omeprazole was obtained from Lupine Research Pvt. Limited, Pune. The commercial fixed dose Capsule formulations OMEZ D (DR.REDDY'S) containing 30 mg Domperidone and 20 mg of Omeprazole were procured from the local market. Aetonitrile reagents used were of HPLC grade. Acetic Acid of AR Grade, HPLC water and Ammonium Acetate were used during the experiment. Spectral and absorbance measurements were made on Analytical technologies spectrophotometer with 1 cm matched quartz cells.

Preparation of 0.05 Ammonium Acetate Buffer (PH - 4)

3.85 gm of Ammonium Acetate dissolved in HPLC water and volume made up to 1000 ml by HPLC water. . From this solution 50ml diluted to 1000 ml with 0.05M Acetic Acid. pH is adjusted to 4 with the Triethylamine.

Preparation of standard stock solution

A. Standard Domperidone stock solution (100 µg/ml)

Domperidone standard stock solution was prepared by weighing 10 mg of Domperidone, transferred to a 100 ml volumetric flask. Add 30 ml of methanol and volume was made up to 100 ml with Acetonitrile to get a concentration of 100µg/ml. This prepared solution is sonicated for 5 minutes and then filtered through the Whatman filter paper no.41. Again this filtered solution is filtered by vacuum filtration through 0.42 membrane filter paper. From this solution, an aliquot of 1 ml was withdrawn, and it was diluted to 10 ml with Acetonitrile to get the concentration of 10µg /ml solution. 3-6

B. Standard Omeprazole stock solution (100 µg /ml)

Omeprazole standard solution was prepared by weighing 10 mg of Omeprazole to a 100 ml volumetric flask.Add30 ml of methanol and volume was made up to 100 ml with Acetonitrile to get a concentration of 100 µg /ml. From this solution an aliquot of 1 ml was withdrawn and it was diluted to 10 ml with Acetonitrile. For calibration curve, stock solutions of Domperidone and Omeprazole were appropriately diluted to obtain concentration range of 8-16 µg /ml and 4-12 µg /ml respectively. [7-9]

Table 1: Optimised chromatographic conditions

Chromatograph	Jasco HPLC (2080 plus)
Column	Gasco, ODS C18(4.5mm×250mm)
Flow Rate	1 ml/minute
Detector	UV-2075 plus-jasco
Detection Wavelength	284 nm
Injection Volume	20µl
Temperature	Ambient (25±2^{0}C)
Mobile Phase	Acetonitrile:0.05M ammonium acetate buffer (pH - 4) (85:15).

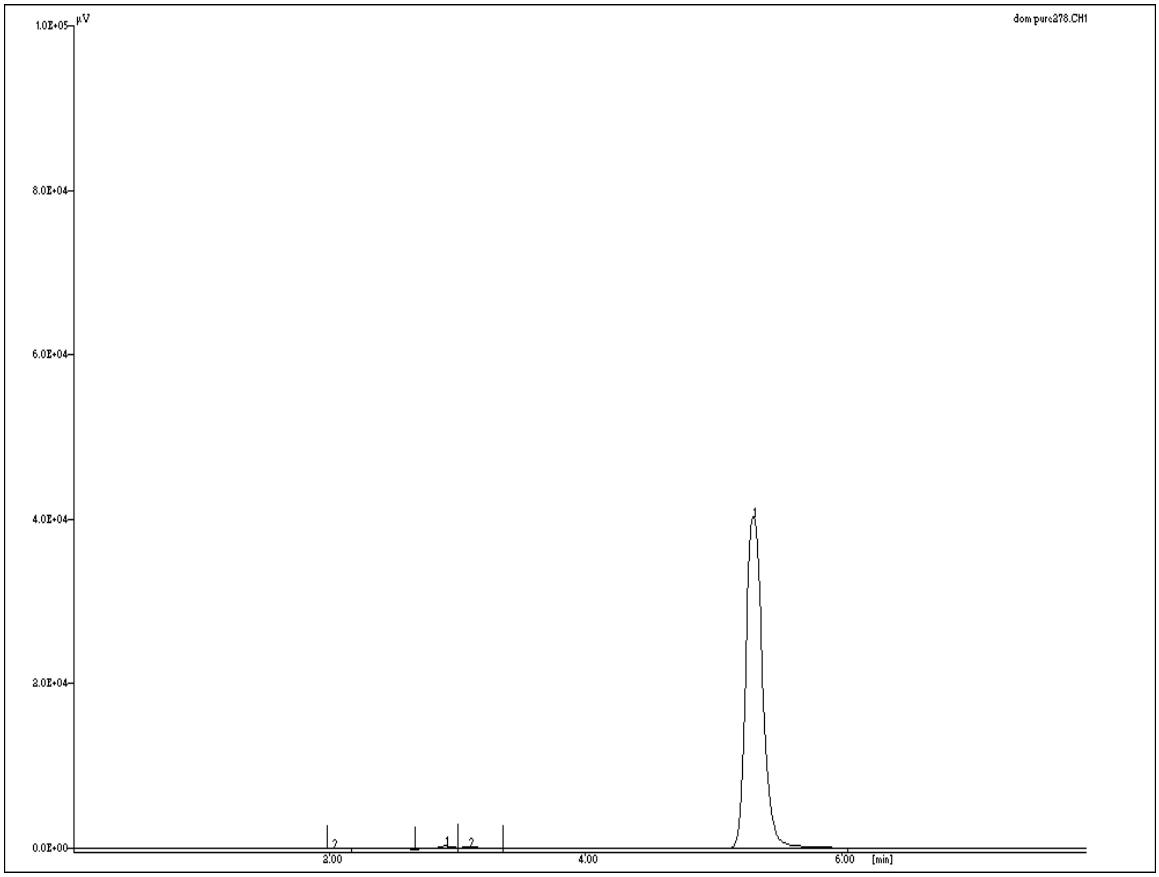

Fig. 1: Typical Chromatogram of Standard Domperidone (Rt = 5.24 min.)

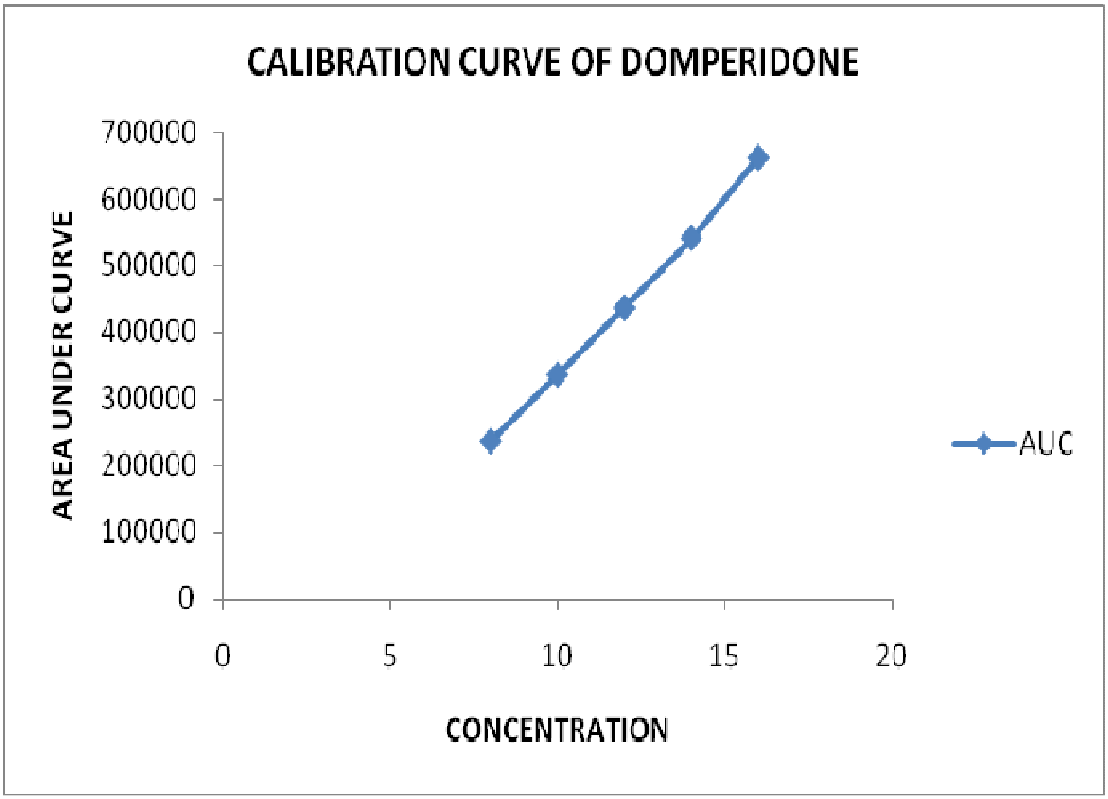

Fig. 2: Calibration curve of Domperidone

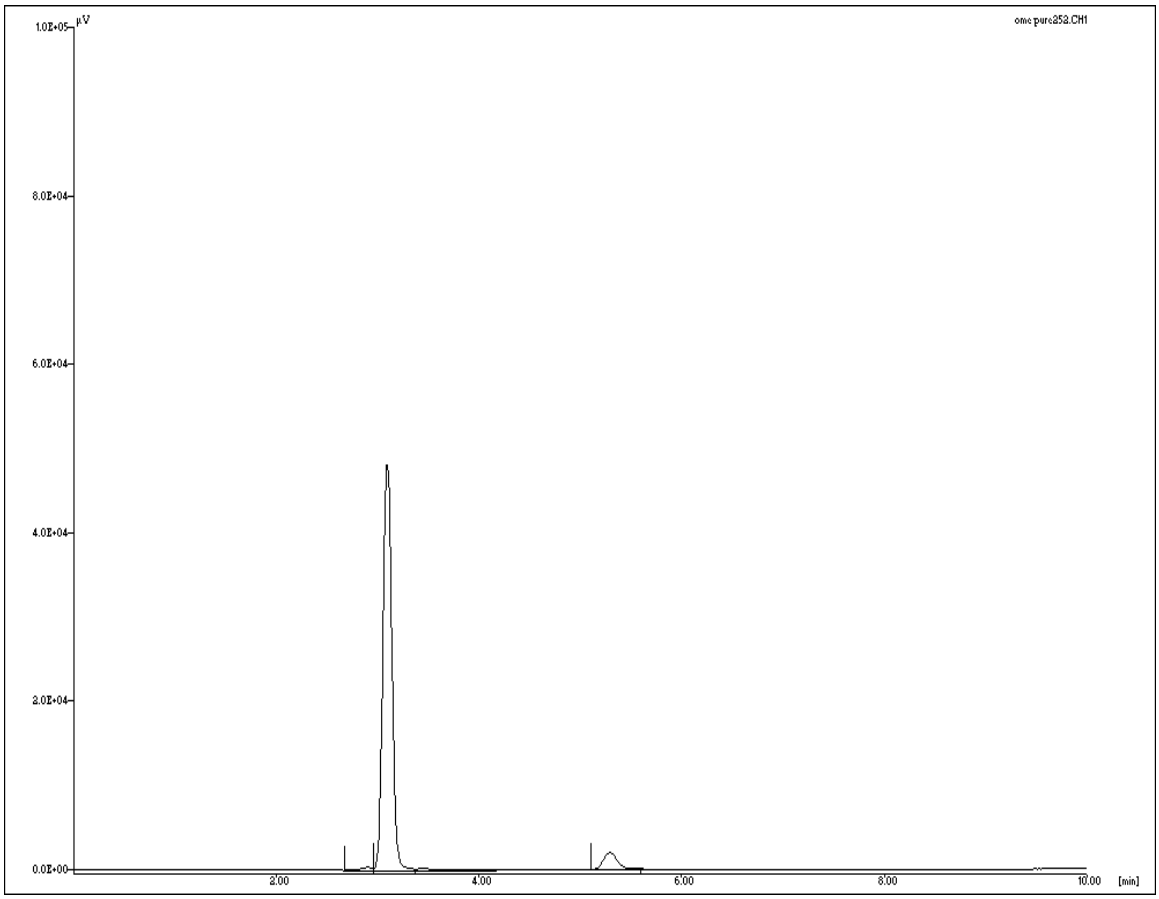

Fig. 3: Typical Chromatogram of Standard Omeprazole (Rt = 3.092 min.)

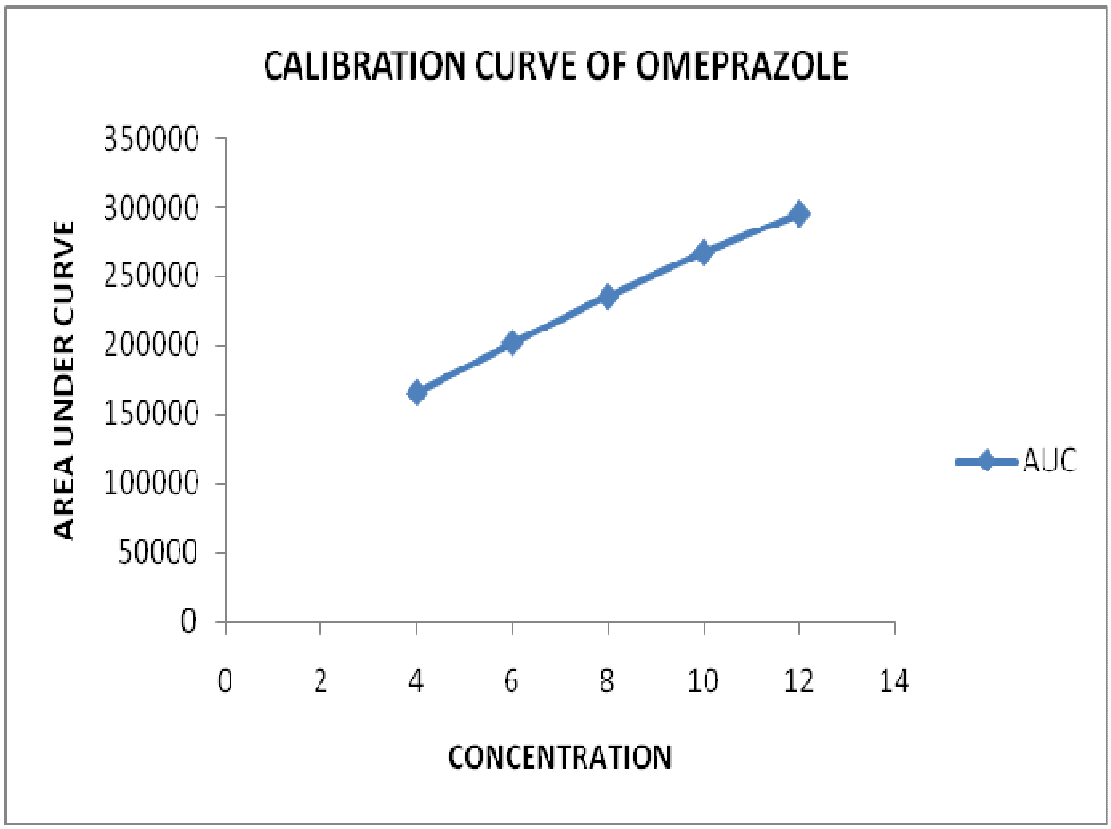

Fig. 4: Calibration curve of Omeprazole

Table 2: System suitability parameter

Parameter	Domperidone	Omeprazole
Calibration Range	8-16µg/ml	4-12µg/ml
Theoretical Plate	9432	7456
Resolution	12	1
Asymmetry	1	1
Slop	52594.74	16180.54
Intercept	-135054	103861.7
Regression Coefficient (r2)	0.999	0.9989
Retention Time	5.24 min	3.092 min
Capacity	636	370
Selectivity	2	1

Selection of Analytical Wavelength

The stock solutions of Domperidone and Omeprazole were separately diluted with Acetonitrile to get a concentration of 10 µg /ml of Domperidone and 10 µg /ml of Omeprazole respectively and scanned in the wavelength range of 200 -400 nm on the Shimadzu UV/Visible spectrophotometer, model 1700 (Japan) with spectral bandwidth of 2 nm and wavelength accuracy of ± 0.5 nm, with automatic wavelength correction employing a pair of quartz cells. From the overlain spectra of both drugs, the wavelengths observed are 290.9 nm (isoabsorptive point) , 280 nm (λ max of Domperidone) and 301.2 nm(λ max of Omeprazole).The sample solution run on HPLC instrument at above given three different wavelength but the sharp peak with minimum consumption of time are obtained at 280 nm. Therefore this wavelength is selected for the present work. [9-11]

Analysis of formulation

Twenty Capsules of brand OMEZ D (SR) (DR. REDDY'S) containing 20 mg of Omeprazole and 30 mg of Domperidone were weighed, average weight determined and finely powdered with the help of mortar and pestle. Appropriate quantity of powder from each tablet equivalent to 30 mg of Domperidone was accurately weighed transferred to a 100ml volumetric flask and volume was made up to 100 ml with Methanol and Acetonitrile in the proportion of 30:70. Shaken vigorously for 15 minutes and filtered through the Whatman filter paper no 41.Again this solution is filtered by vacuum filtration through 0.45 membrane filter paper. Necessary dilutions of filtrate were made with acetonitrile to get final concentration 6.7 µg/ml of Omeprazole and 10 µg/ml of Domperidone. This solution were injected and run on HPLC instrument. Results is shown in the following table. [12-15]

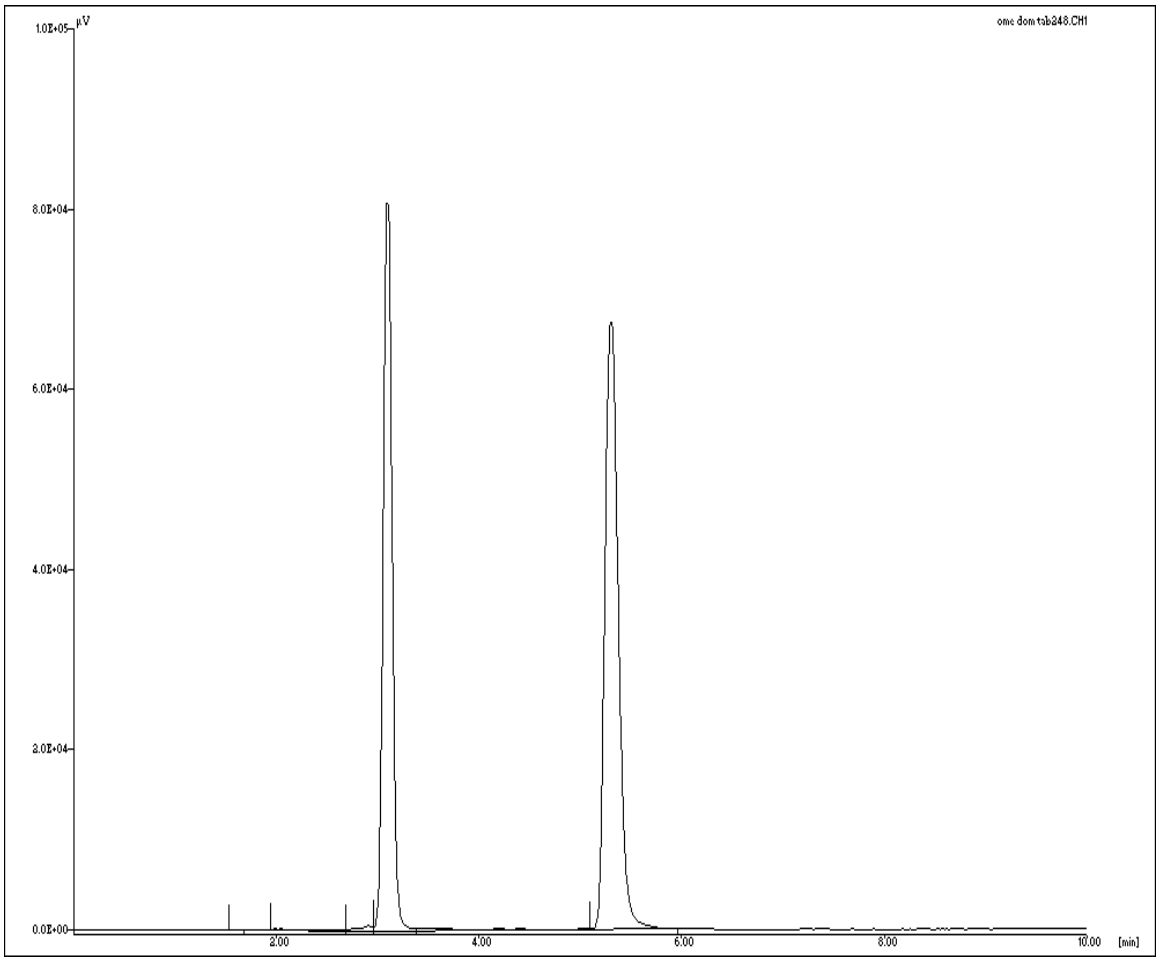

Fig. 5: Typical Chromatogram of Omeprazole and domperidone in capsule formulation

Table 3: Result of Capsule Analysis

Drug Name	AUC (Area under curve)*	RT (Retention time in minute)	Lable Claim (mg)	% Lable Claim Found*	Amount Found (mg)	S.D	% RSD OR %COV
DOM	386728.69	5.275	30	99.26	29.78	0.051	0.0514
OME	216902.56	3.092	20	98.65	19.73	0.085	1.2925

* It indicates averages of three readings.

DOM = Domperidone, OME = Omeprazole.

Validation

Validation of the developed method was done according to the USP 2006, Asian edition and ICH guideline. [18-24]

Linearity

The linearity of the method is its ability to elicit test results that are directly proportional to the concentration of the analyte in samples. The calibration curve was taken in the range of 8-16 µg /ml for Domperidone and 4-12 µg /ml for Omeprazole at the respective λmax on HPLC instrument. The correlation coefficient of the linearity was found to be 0.999 at each wavelength for both drugs as shown in table 1. [15-17]

Recovery study

In order to ensure the reliability and suitability of the proposed method, recovery studies were carried out. It was done by mixing known quantity of standard drug with formulation sample and the content were reanalyzed by the proposed method. To a quantity of formulation equivalent to 30 mg of Domperidone, standard drugs of Domperidone and Omeprazole were added at 80%, 100% and 120% levels. This was extracted, diluted and reanalyzed as per the formulation procedure. Absorbance were noted at respective wavelength. Recovery studies were repeated for six times and the results are shown in the table no.4 and 5. [18-20]

Table 4: Recovery study of Omeprazole

Recovery Level	Concentration µg/ml	% Recovery	S.D	% RSD OR %COV
80%	18	98.72	0.1682	0.9466
100%	20	99.22	0.1457	0.7347
120%	22	99.26	0.0984	0.4509

Table 5: Recovery study of Domperidone

Recovery Level	Concentration µg/ml	% Recovery	S.D	% RSD OR %COV
80%	18	98.11	0.09539	0.540169
100%	20	98.45	0.06670	0.33896
120%	22	97.99	0.01000	0.000464

S.D = Standard deviation, RSD = Relative Standard deviation,

COV = Coefficient of varience.

Table 6: Intermediate Precision study of Omeprazole and Domperidone

Intermediate Precision	% of lable claim estimated (Mean± % RSD)	
	Domperidone	Omeprazole
Interday	99.33 ± 0.25674	98.90 ± 1.179
Intraday	99.43 ± 0.25649	99.46 ± .2055

RSD=Relative Standard deviation

Precision

The precision of an analytical method is determined by assaying a sufficient number of aliquots of a homogeneous sample to be able to calculate statistically valid estimate of % Relative Standard Deviation (%RSD). Intermediate precision was done to express within laboratory variation, on different days. Five replicates of10 µg/ml concentration of the working standard mixture and sample solution were analyzed %RSD was found to be less than 2%. [20-21]

Specificity

Results of tablet solution showed that there is no interference of the excipients when compared with the working standard solution. Thus, the method was said to be specific.

Limit of Detection

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. Limit of detection can be calculated using following equation as per ICH guidelines. [21-22]

$$LOD = 3.3 \times N/S$$

Where,

N = Standard deviation of the response and

S = Slope of the corresponding calibration curve.

Results are shown in the table no.7

Limit of Quantification

It is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. Limit of quantification can be calculated using following equation as per ICH guidelines.[23-24]

$$LOQ = 10 \times N/S$$

Where,

N = Standard deviation of the response and

S = Slope of the corresponding calibration curve

Results are shown in the table no.7

Table 7: LOD and LOQ Result of Omeprazole and Domperidone

Validation parameter	Omeprazole	Domperidone
LOD(µg/ml)	0.00001743	0.00000320
LOQ(µg/ml)	0.00005280	0.00000969

LOD= Limit of Detection; LOQ= Limit of Quantification

RESULT AND DISCUSSION

The proposed methods for simultaneous estimation of Domperidone and Omeprazole in combined capsule dosage form were found to be simple, accurate, economical and rapid. The % RSD was found to be less than 2% in the developed method. Hence proposed method may be used for routine analysis of these drugs in combined dosage forms.

ACKNOWLEDGEMENT

The authors are thankful to Lupine Pharmaceuticals Pvt Ltd. and Macro Lab (Banglor) for providing Omeprazole and Domperidone as gift samples for this work. And also Thanks to Prof.K.B.Burade (Principal) GOVERNMENT COLLEGE OF PHARMACY, KARAD, for providing the required facilities for my research work. I am also grateful of Mrs.A.S.Kulkarni (Assistant Professor) for guiding and supporting me.

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