IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
December 2020 Vol.:20, Issue:1

Stability Indicating Spectrophotometry and HPLC Method for Determination of Omeprazole and Domperidone Maleate in Combined Dosage Form

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Yashaswini K*, Ganesha Krishna P., Prashanth PH.

Assistant professor, Department of pharmaceutical chemistry Shree Devi college of pharmacy, Mangalore, India

Submitted: 12 November 2020
Revised: 02 December 2020
Accepted: 22 December 2020

Keywords: Omeprazole, Domperidone, RP-HPLC, Q-Absorbance ratio Method, Stability studies

ABSTRACT

A spectrophotometric method and an RP-HPLC method has been developed and validated for simultaneous estimation of Omeprazole and Domperidone in bulk and pharmaceutical formulation. The RP-HPLC Method for Omeprazole and Domperidone was developed using C18column (250 mm × 4.6 mm, 5 µm) as stationary phase and Potassium dihydrogen phosphate:Acetonitrile (65:35 v/v, pH 7.4) as mobile phase. The mobile phase was maintained at a flow rate of 1.5 ml/min and detection was carried out at 285 nm. The spectroscopic method utilizes methanol as a solvent and the λ max of Omeprazole and Domperidone selected for analysis was found to be 400-200 nm using U.V spectrophotometer, Shimadzu 1800. Linearity for Omeprazole was found in the concentration range of 5-30 µg/ml for the spectrophotometric method and 50-150 µg/ml for RP-HPLC method. Domperidone was found to be linear in the concentration range of 5-15 µg/ml for the spectrophotometric method and 50-150 µg/ml for RP-HPLC method. The accuracy and precisions were determined and found to comply with ICH guidelines and also the developed methods were compared with standard protocol for stability studies which suggested that there is no significant deviation in this method. So, the methods can be successfully applied for the routine analysis of Omeprazole and Domperidone in pharmaceutical formulation.

INTRODUCTION

A combination of Domperidone maleate and Omeprazole is used for treating various ulcers and in condition of gastric upset that is nausea, vomiting, gastroparesis etc. It is also helpful in patients having asthma and gastro-oesophageal reflux disease (GERD) who received anti-reflux treatment. Domperidone, acting as an anti-dopaminergic, results in increased prolactin secretion, and thus promotes lactation. Omeprazole is used in the infection caused by *Helicobacter pylori* and is the causative factor in the majority of peptic and duodenal ulcers and has an important use in acute tubulointerstitial nephritis, an inflammation of kidney**.** Domperidone is an antiemetic & prokinetic agent. It is a peripheral D-2 receptor antagonist. It blocks the Dopamine receptors, in the GIT causing increased gastric motility, in the CTZ showing antiemetic effect. Omeprazol**e** belongs to the category of proton pump inhibitor. It acts by inhibiting theH+K+-ATPase pump and inhibits the secretion from Histamine, Acetylcholine and Gastrin and thus there is inhibition in exchange of H^+K^+ ions. Thus excess of HCl is not liberated.

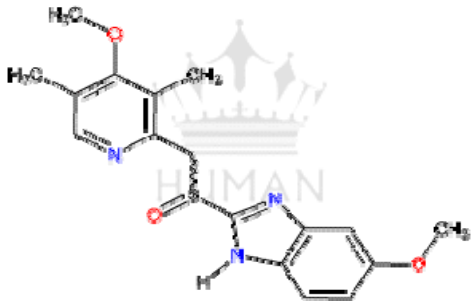

Figure No. 1: Structure of omeprazole(5-methoxy-2-[(4-methoxy-3,5-dimethyl-pyridin-2-yl)methylsulfinyl]-3H-benzimidazole)

Domstal RD (10mg Domperidone and 10 mg Omeprazole) is novel from conventional marketed formulations. Domstal RD is bilayer tablet of Domperidone and Omeprazole which is not enteric-coated. But it contains Sodium Bicarbonate in its formulation. So, during disintegration in stomach, sodium bicarbonate neutralizes the stomach fluid and omeprazole and domperidone will absorb from stomach[5]. Stability is important from Quality control perspective in the industry, because the instability of pharmaceuticals can cause a change in physical, chemical, pharmacological and Toxicological properties of the active pharmaceutical ingredients (API), thereby affecting its safety and efficacy and therefore any analytical method developed should preferably be stability-indicating. So Simultaneous

estimation for domperidone maleate and omeprazole has been carried out but no work has been reported for stability indicating assay method for this combination.

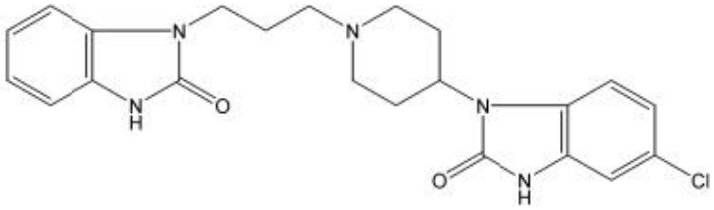

Figure No. 2: Structure of domperidone (5-chloro-1-{1-[3-(2-oxo-1, 3-dihydrobenzoimidazol-1-yl) propyl]-4 piperidyl}-1,3-dihydrobenzoimidazol-2-one Maleate)

MATERIAL AND METHODS

Instrumentation

HPLC method for determination of omeprazole and domperidone maleate in combined dosage form was done by using Shimadzu LC–2010-HT system on a symmetry C18 column (250 mm × 4.6 mm, 5 µm) was used. Injector used for injecting the sample was Rheodyne – 7725i (Fixed Capacity Loop of 20 µl). To investigate the appropriate wavelength for simultaneous determination of OME and DOM, the solutions of the same in the mobile phase were scanned separately by UV–Visible spectrophotometer (UV spectrophotometer Shimadzu 1800, Software Version 1) in the range of 190-400 nm and the overlain spectrum were recorded.

Preparation of phosphate buffer

Accurately weighed 6.8 gm of KH_2PO_4 was transferred in the 1000 ml volumetric flask and volume were made up to the mark with HPLC grade water. After dissolving properly, adjust the pH of this buffer solution to 7.4 with 0.2 N NaOH.

Preparation of mobile phase

Mobile phase was prepared by mixing 650 ml of KH_2PO_4 buffer (pH 7.4) and 350 ml of Acetonitrile and filtered through 0.2 µm Supor 200 membrane filter using Vacuum Pump and ultrasonicated for 10 min for degassing.

Preparation of standard stock solution

Standard stock solution of OME

Weigh accurately about 40.00 mg of Omeprazole working/reference standard into a 100 ml standard volumetric flask, add to it around 70 ml of Methanol and sonicate to dissolve, dilute up to mark with diluents, mix thoroughly.

Standard stock solution of DOM

Weigh accurately about 50.90 mg of Domperidone Maleate working/reference standard into a 100 ml standard volumetric flask, add to it around 70 ml of Methanol and sonicate to dissolve, dilute up to mark with diluents, mix thoroughly.

Combined standard stock solution of OME and DOM

Mix 5 ml of Domperidone Maleate Standard Stock solution and 10 ml of Omeprazole Standard stock solution in 100 ml volumetric flask and dilute up to mark with diluents, mix thoroughly.

Preparation of calibration curve for OME and DOM

Weigh and transfer accurately about 50.9 mg of Domperidone maleate and 80 mg of Omeprazole working standard into 100 ml volumetric flask Add 70 ml of methanol and sonicate to dissolve, make up the volume with mobile phase and mix well.

Dilute standard stock solution as given below to get desired concentrations.

Method validation

Validation of RP-HPLC method

Accuracy

Accuracy shall be determined over the range 50 % to 150 % of the working concentration. A calculated amount of Domperidone maleate & Omeprazole reference standard shall be added in placebo to attain 50 %, 100 % and 150 % of working concentration**.**

Level 1 (50 %):

Weigh accurately and take 5 placebo tablets (without API) & weigh and transfer accurately about 31.8 mg of Domperidone maleate and 50mg of Omeprazole working standard into 500 ml volumetric flask. Add 350 ml of methanol and sonicate for 35 min, make up the volume with the mobile phase, and mix well. Centrifuge at 3500 rpm for 15 min. Dilute 5 ml of this solution to 25 ml with the mobile phase, and mix. Prepare samples in triplicate at 50 % level and inject each preparation in duplicate.

Level 2 (100 %):

Weigh accurately and take 5 placebo tablets (without API) & Weigh and transfer accurately about 63.61 mg of Domperidone maleate and 100 mg of Omeprazole working standard into 500 ml volumetric flask add 350 ml of methanol and sonicate for 35 min, make up the volume with mobile phase and mix well. Centrifuge at 3500 rpm for 15 minutes. Dilute 5 ml of this solution to 25 ml with the mobile phase, and mix. Prepare samples in triplicate at 100 % level and inject each preparation in duplicate**.**

Level 3 (150 %):

Weigh accurately and take 5 placebo tablets (without API) & weigh and transfer accurately about 95.41 mg of Domperidone maleate and 150 mg of Omeprazole working standard into 500 ml volumetric flask add 350 ml of methanol and sonicate for 35 min, make up the volume with mobile phase and mix well. Centrifuge at 3500 rpm for 15 min. Dilute 5 ml of this solution to 25 ml with the mobile phase, and mix Prepare samples in triplicate at 150% level and inject each preparation in duplicate. The average % recovery shall be calculated at each level.

Acceptance criteria:

1) % Recovery and % mean recovery at each level should be between 98-102 %.

2) % RSD of %Recovery at each level should not be more than 2.0 %.

Precision

Repeatability (method precision)

Method precision shall be established by analyzing six-sample preparation as per test

Procedure under the same conditions. Six replicate of sample shall be prepared by one analyst and injected on the same equipment and same day.

Individual % assay value, % mean assay value, 95 % confidence interval, SD and % RSD shall be calculated and recorded.

Intermediate precision

The individual, mean assay value and % RSD shall be calculated and recorded. % Mean assay value obtained in intermediate precision will be compared with the mean assay value obtained in method precision study. The difference of the % mean assays obtained, 95 % confidence interval, SD and % Overall RSD shall be calculated and recorded.

Acceptance criteria:

i) RSD of % assay of six replicate sample preparations of intermediate precision study should not be more than 2.0%.

ii) The difference in the % mean assay value obtained in intermediate precision study and method precision study should not be more than 2.0%.

iii) Overall RSD of % assay of six replicates sample preparation of method precision and six replicates sample preparation of intermediate precision should not be more than 2.0%.

Linearity and Range

Linearity shall be determined over the range of 50 % to 150 % of working concentration. The standard stock solution shall be prepared and further diluted to attain concentration at about 50 %, 75 %, 100 %, 125 % and 150 % of working concentration.

Acceptance criteria

The correlation coefficient value should not be less than 0.995 over the working range.

Limit of detection (LOD) and Limit of quantitation (LOQ)

The limit of detection and the limit of quantification of the drug were calculated using the following equations as per ICH guidelines.

$$LOD = 3.3 \times N/S \quad LOQ = 10 \times N/S$$

Where N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

Robustness:

Procedure:

Following parameters shall be changed one by one and their effect on system suitability test shall be observed.

1) Change in flow rate by ± 0.1 ml/min. [use flow rate 1.4 ml and 1.6 ml]

2) Change in the minor components in the mobile phase by ± 2 % absolute [Use Composition (a) Buffer: Acetonitrile (663:337), Composition (b) Buffer: Acetonitrile (637:363)]

3) Change in the pH of Buffer ±0.2 (Use Buffer (a) 7.2 pH and (b) 7.6 pH)

4) Change in Temperature ±5°C (Use Temp. (a) 20°C and (b) 30°C)

Acceptance criteria:

i) A number of theoretical plates should not be less than 3000 for Domperidone peak and 3000 for Omeprazole peak.

ii) Tailing factor for Domperidone and Omeprazole peak should not be more than 2.

iii) Resolution between Domperidone peak and Omeprazole peak should not less than 3.

iv) Relative standard deviation for five replicate injections of the standard solution should not be more than 2.0% for Domperidone and Omeprazole.

STABILITY STUDIES

Standard and sample preparation shall be prepared as per method and assay shall be determined as per method. Solutions shall be stored up to 24 h. % Assay of standard and 24 h against freshly prepared standard shall be determined. The assay obtained at that time intervals shall be compared with the initial assay value and recorded. Solution stability period for sample and standard preparation shall be determined.

Acceptance criteria

The difference in the assay values should not be more than 2.0 % from the initial value.

Forced degradation studies

The placebo of Domperidone and Omeprazole (Domstal RD) tablets was subjected to acid,

base, oxidation, and thermal degradation. For each degradation study, a blank was prepared separately. The stress conditions were adjusted such that a maximum of 30 % degradation was achieved.

RESULTS AND DISCUSSION

Preliminary analysis of OME and DOM such as Description, Solubility, Identification test and Assay were performed according to available literature survey and IP 2010, respectively.

The standard solutions of OME (100 µg/ml) and DOM (100 µg/ml) in mobile phase were scanned in the UV region of 190-400 nm and the overlain spectra were recorded. It was observed that both the drugs showed the absorbance at 285 nm. So, the wavelength of detection used was 285 nm which is shown below.

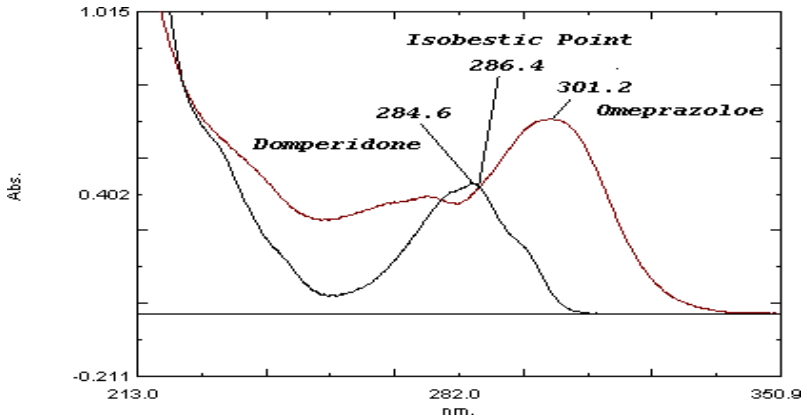

Figure No. 3: Overlain spectra of OME (100µg/ml), DOM (100µg/ml) in mobile phase

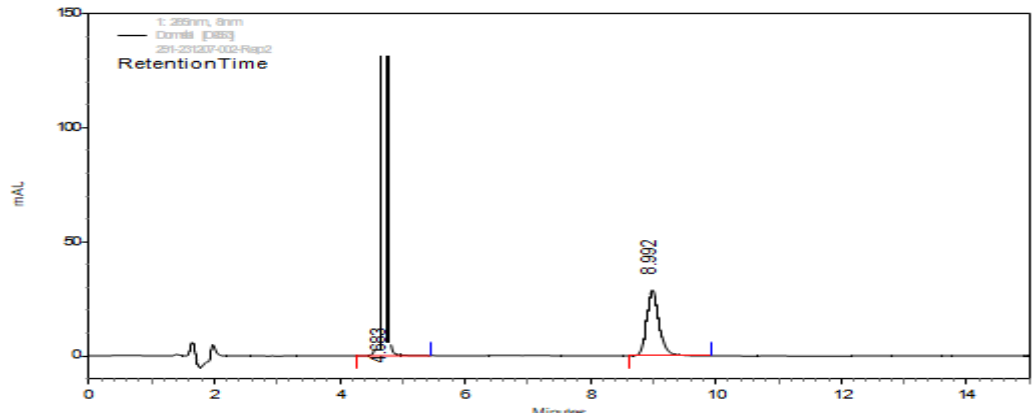

Figure No. 4: HPLC Chromatogram of OME and DOM with corresponding retention time at 285 nm

Table No. 1: Accuracy of OME

Levels	Conc. (%)	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Mean % Recovery	% RSD
1	50	0.02115	0.02148	101.6	100.3	0.1
		0.02120	0.02151	101.5		
		0.02124	0.02159	101.6		
2	100	0.04189	0.04246	101.4	101.0	0.4
		0.04182	0.04204	100.5		
		0.04186	0.04232	101.1		
3	150	0.06112	0.06137	100.4	100.4	0.1
		0.06120	0.06146	100.4		
		0.06118	0.06137	100.3		
Overall Result					**100.98**	**0.19**

Table No. 2: Accuracy of DOM

Levels	Conc. (%)	Amount added (µg/ml)	Amount found (µg/ml)	Recovery (%)	Mean % Recovery	% RSD
1	50	0.00774	0.00784	101.3	101.3	0.2
		0.00770	0.00781	101.4		
		0.00776	0.00784	101.1		
2	100	0.01591	0.01616	101.6	100.9	0.6
		0.01590	0.01600	100.6		
		0.01600	0.01608	100.5		
3	150	0.02488	0.02508	100.8	100.3	0.5
		0.02499	0.02496	99.9		
		0.02490	0.02499	100.3		
Overall Result					**100.83**	**0.42**

Accuracy is within the range 50 % to 150 % of the working concentration

Table No. 3: Linearity data of OME

Linearity Level	Conc. (%)		Area	Average area
	Conc. (%)	Actual Conc. (µg/ml)		
1	50	20.23	891410	892999
			891410	
			894069	
2	75	30.35	1289661	1290115
			1288986	
			1291699	
3	100	40.46	1727571	1726337
			1725141	
			1726298	
4	125	50.56	2177503	2176901
			2177921	
			2175280	
5	150	60.69	2572732	2576059
			2578931	
			2576514	
Correlation coefficient (r^2)			0.99975	
Slope of regression line			42053.5	
Y-intercept			31082.6	
LOD(µg/ml)			0.587	
LOQ(µg/ml)			1.264	
Residual sum of squares			899144379.4	

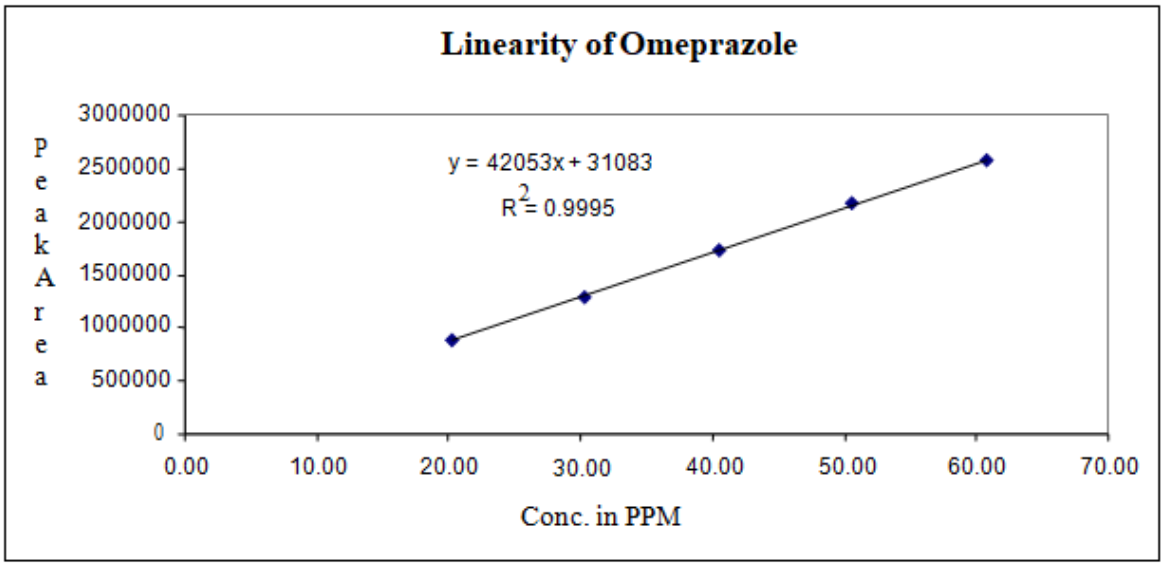

Figure No. 5: Calibration curve for OME

Table No. 4: Linearity data of DOM

Linearity Level	Conc.(%)		Area	Average area
	Conc. in %	Actual Conc. in µg/ml		
1	50	9.98	438503	441476
			442013	
			443913	
2	75	14.97	645162	643658
			641896	
			643916	
3	100	19.96	865661	864762
			863374	
			865252	
4	125	24.95	1091384	1089592
			1087802	
			1089591	
5	150	29.93	1290407	1290421
			1291474	
			1289381	
Correlation coefficient (r^2)			0.99980	
Slope of regression line			42969.8	
Y-intercept			8452.7	
LOD(µg/ml)			0.679	
LOQ(µg/ml)			1.382	
Residual sum of squares			186566997.2	

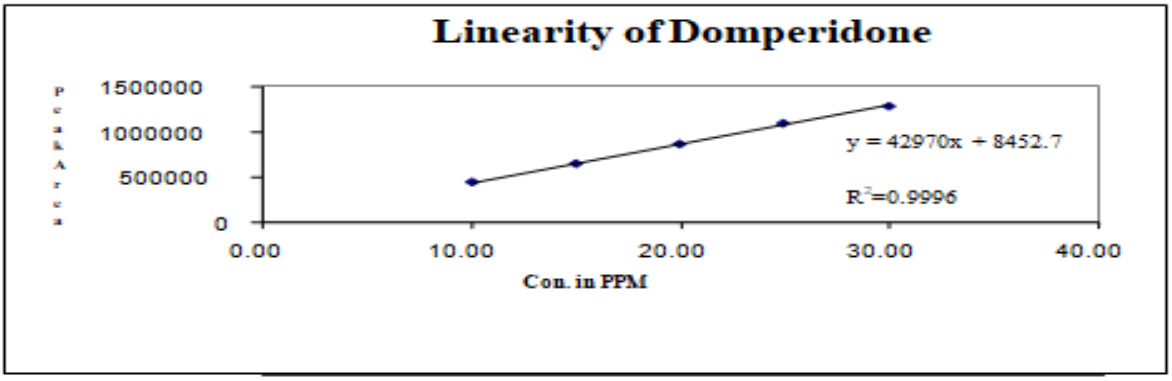

Figure No. 6: Calibration Curve for DOM

Table No. 5: Percentage assay of OME and DOM

Sample preparation	% Assay	
	OME	**DOM**
1.	103.5	94.7
2.	102.6	94.9
3.	103.4	95.8
4.	102.8	94.5
5.	103.3	94.9
6.	103.1	95.1
Mean	**102.1**	**95.2**
RSD (%)	**1.11**	**0.62**
95% Confidence Interval (CI)	**0.284**	**0.359**

Repeatability

OME and DOM were found to be linear in the concentration range of 50-150 µg/ml and 50-150 µg/ml, respectively.

Percentage of recoveries of OME and DOM were found in the range from 100.3-101.5% and 99.9-101.1 %, respectively. The precision of the method was determined by % RSD found among intra-day precision, inter-day precision, repeatability. It was found to be less than 2 %.

Table No. 6: Result of robustness study: Change in flow rate

Flow Rate (ml/min)	Analyte	Mean peak area (n=3)	±SD	% RSD	% Assay
1.4	OME	1830289	827.50	0.01	103.4
	DOM	915760	99157.40	0.1	94.3
1.5	OME	1732727	6579.50	0.11	103.5
	DOM	854544	20868.35	0.14	94.7
1.6	OME	1597780	1323.60	0.05	103.7
	DOM	801907	509737.50	0.2	94.4

Table No. 7: Change in the mobile phase

Mobile phase ratio	Analyte	Mean peak area (n=3)	±SD	%RSD	%Assay
350:650	OME	1732727	6579.50	0.11	103.5
	DOM	854544	2086.80	0.14	94.7
363:637	OME	1708144	1497.60	0.08	103.5
	DOM	852262	6489.20	0.02	94.6
337:663	OME	1695916	9912.90	0.04	103.5
	DOM	844531	3490.70	0.05	94.4

Table No. 8: Change in pH

pH	Analyte	Mean peakarea (n=3)	±SD	%RSD	%Assay
7.2	OME	1691101	5678.40	0.08	103.4
	DOM	854737	5768.10	0.19	94.2
7.4	OME	1732727	6579.50	0.11	103.5
	DOM	854544	2086.80	0.14	94.7
7.6	OME	1697484	5647.90	0.01	103.7
	DOM	857249	1739.50	0.13	94.6

Table No. 9: Change in temperature

Temp. (°C)	Analyte	Mean peak area (n=3)	±SD	%RSD	%Assay
20	OME	1715875	6579.50	0.08	103.3
	DOM	854694	2086.80	0.20	94.5
25	OME	1732727	1489.30	0.11	103.5
	DOM	854544	3892.10	0.14	94.7
30	OME	1705536	4993.30	0.07	103.6
	DOM	858350	5488.10	0.06	94.5

For robustness study, the effect change in the pH (0.2 units) of mobile phase, mobile phase ratio (3 %) and flow rate (1 %) on the Mean peak area, % RSD, and % Assay were studied. Standard solutions of OME (100 µg/ml), DOM (100 µg/ml) were prepared and analyzed at different pH (7.2,7.4,7.6) of the mobile phase, different mobile phase ratio (650:350,637:363,663:337v/v) and at different flow rate (1.4, 1.5, 1.6 ml/min) and Change in temperature $\pm 5^0$c. The percentage RSD of each peak in all variables was found to be less than 3%. LOD and LOQ of OME were found to be 0.587 and 1.264 µg/ml, respectively. LOD and LOQ of DOM were found to be 0.679 and 1.382 µg/ml, respectively.

Table No. 10: % Degradation

Conditions	Name of peak	Peak purity	% Degradation
Sample preparation (As such)	OME	1.00000	NA
	DOM	1.00000	NA
Sample preparation (Acid degradation)	OME	1.00000	7.5
	DOM	1.00000	0
Sample preparation (Alkali degradation)	OME	0.99643	24.1
	DOM	1.00000	2.3
Sample preparation (Peroxide degradation)	OME	1.00000	68.1
	DOM	1.00000	67.1
Sample preparation (Thermal degradation)	OME	1.00000	1.8
	DOM	1.00000	0

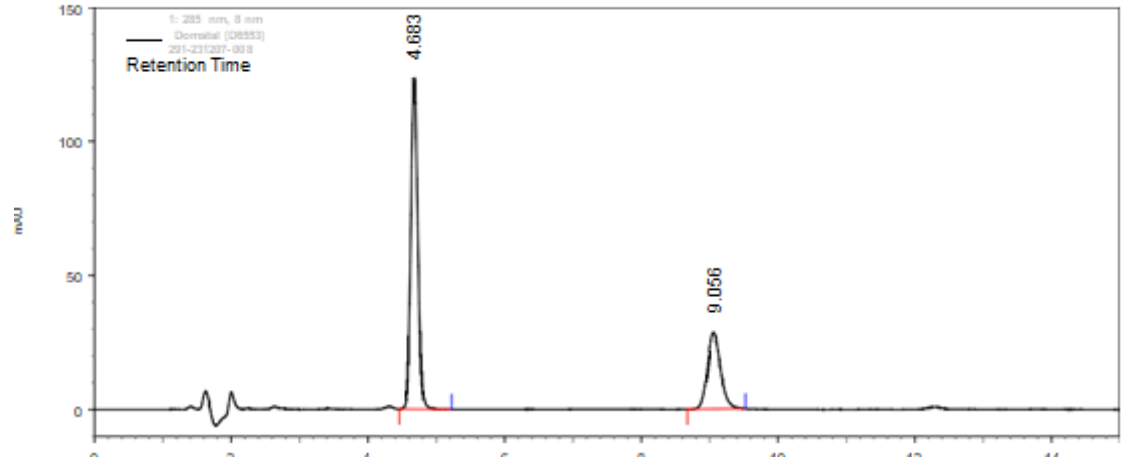

Figure No. 7: HPLC Chromatogram of sample preparation acid degradation at 285 nm

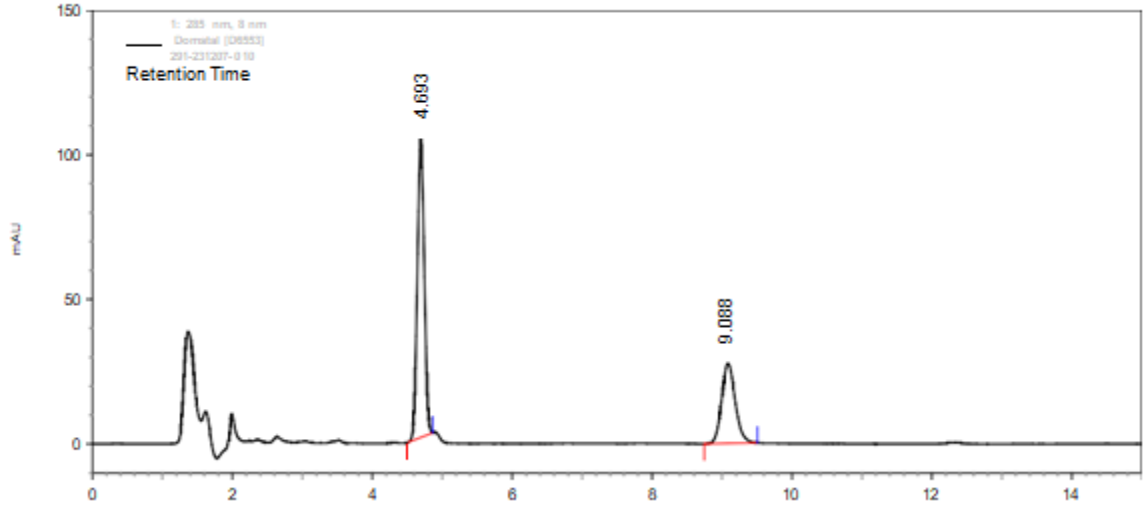

Figure No. 8: HPLC Chromatogram of sample preparation alkali degradation at 285 nm

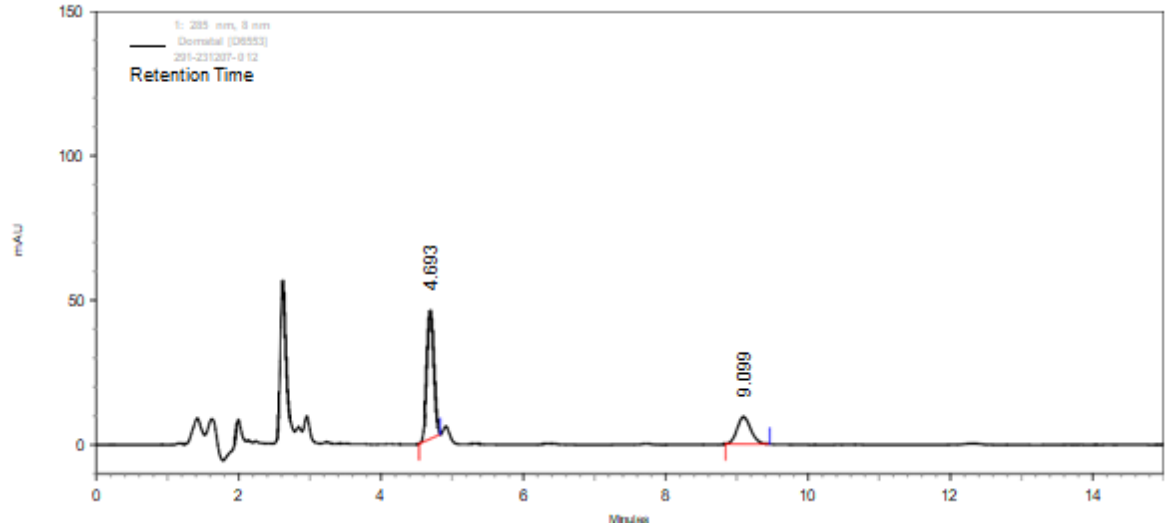

Figure No. 9: HPLC Chromatogram of sample preparation peroxide degradation at 285 nm

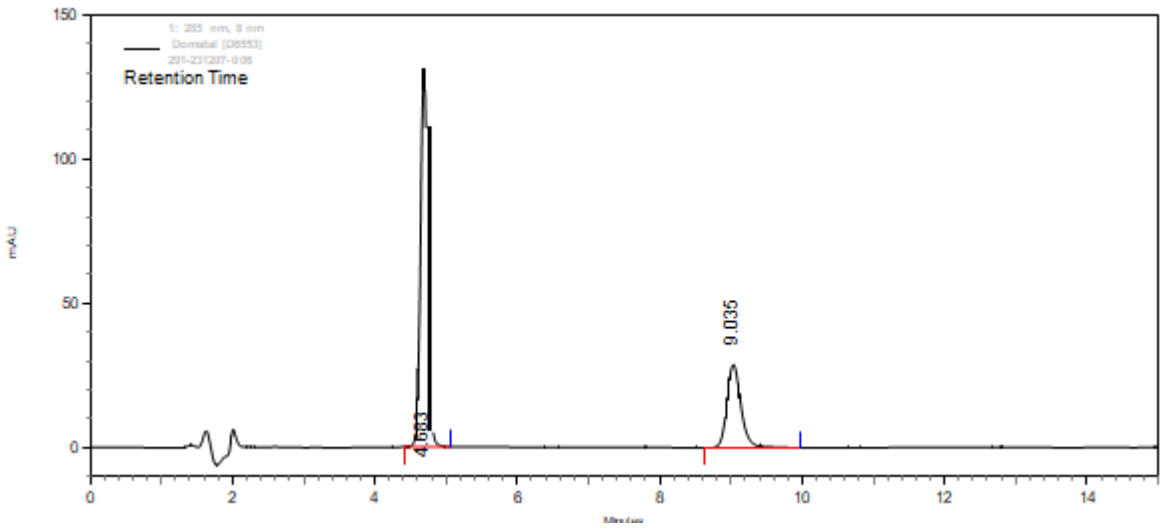

Figure No. 10: HPLC Chromatogram of sample preparation thermal degradation at 285 nm

The solution stability was checked for the sample preparation and standard preparation at 12 h. The results obtained are well within the acceptance criteria. Therefore, the standard preparation and sample preparation are stable in solution form upto 12 h at room temperature.

CONCLUSION

An attempt has been made to develop the Reverse Phase High-Performance Liquid Chromatographic Method for the simultaneous estimation of Omeprazole and Domperidone in dosage form and to validate the developed methods according to ICH guidelines. The RP-HPLC method for estimation of Omeprazole and Domperidone in dosage form was developed. The quantification was carried out by using C_{18} column (250mm×4.6mm,5□m) as stationary phase and KH_2PO_4 buffer: acetonitrile (65:35 v/v) as mobile phase where pH of the KH_2PO_4 buffer was adjusted to 7.4 using 0.2N NaOH. The mobile phase was maintained at a flow rate of 1.5 ml/min. The UV detector was operated at 285nm. The developed methods were compared with standard protocol for stability studies which suggested that there is no significant deviation in this method. So the methods can be successfully applied for the routine analysis of Omeprazole and Domperidone in pharmaceutical formulation.

REFERENCES:

1. Kumar R, Reddy P, Emmadi C, Koneru S. Simultaneous Determination 0f Naproxen Sodium and Pantoprazole Sodium in Bulk and Pharmaceutical Dosage Form by Validated Ultra-Violet Spectrophotometric Method. Int J of Chem and Analytical Science 2012; 3(10): 1569-1572.

2. El-Sherif ZA, Mohamed AO, El-Bardicy MG, El-Tarras MF. Developed Reversed-Phase High Performance Liquid Chromatographic Method for the Determination of Lansoprazole, Omeprazole and Pantoprazole Sodium Sesquihydrate in Presence of Their Acid-Induced Degradation Products. Chem Pharm Bull 2012;54(6):814-8.
3. Rao G, Rao A, Reddy G, Shoeb M, Olabamiwo O, Rao G. RP-HPLC method for simultaneous estimation of domperidone and pantoprazole in capsule dosage form, Der Pharmacia Lettre 2012: 4 (6):1712-1720.
4. Ricardo MO, Pierina SB. Simple and efficient method for enantioselective determination of omeprazole in human plasma. J Chromatogr B 2012 ;795(2):227-235.
5. Kumar R, Singh H, Singh P. Development of UV Spectrophotometric method for estimation of Pantoprazole in pharmaceutical dosage forms. J Chem Pharm Res 2011; 3(2):113-7.
6. Jitesh S, Jadhav, Swetal P, Jain H. Development and validation of a RP-HPLC method for simultaneous determination of pantoprazole and cinitapride in antiulcer formulation. Int J Pharm Pharm Sci 2011; 4(4):657-659.
7. Kang WK, Kim DS, Kwon KI. Advanced method for determination of omeprazole in plasma by HPLC. ArchPharmRes2007;22(1):86-8.
8. Naser LR, Kevin CB, Angela DM. A simple and sensitive bioanalytical assay for simultaneous determination of omeprazole and its three major metabolites in human blood plasma using RP-HPLC after a simple liquid–liquid extraction procedure. J Chromatogr B 2006; 844(2):314- 321.
9. Sivakumar T, Manavalan R, Valliappan K. Development and Validation of a Reverse Phase HPLC method for simultaneous determination of Domperidone and Pantoprazole in Pharmaceutical dosage form. ActaChromatog2006;78(5):215-220.