RESEARCH ARTICLE JPAR 2020

Journal of Pharmaceutical Advanced Research

(An International Multidisciplinary Peer Review Open Access monthly Journal)

Available online at: www.jparonline.com

RP-HPLC methodology for the Assay of Omeprazole in Omeprazole Buffered Capsule

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Received: 01.09.2019 Revised: 12.09.2020 Accepted: 22.09.2020 Published: 30.09.2020

ABSTRACT: Background: Omeprazole works by decreasing the amount of acid your stomach makes. It belongs to a class of drugs known as proton pump inhibitors (PPIs). Sodium bicarbonate is an antacid that reduces stomach acid and helps Omeprazole to work better. This medication relieves symptoms such as heartburn, difficulty in swallowing, and persistent cough. It helps to heal the acid damage to the stomach and esophagus, helps prevent ulcers, and may help prevent cancer of the esophagus. **Aim:** To develop a HPLC methodology for assay of Omeprazole in Omeprazole and Sodium bicarbonate capsule formulation. **Method:** A stability indicating reverse phase HPLC method was developed and validated for the estimation of Omeprazole in Omeprazole buffered capsule. The analysis was performed on a Thermo HPLC system with a Phenomenex Gemini NX C_{18} 110Å, 250 × 4.6 mm; 5 µ column and isocratic elution consisting of 10 ml Triethylamine in 1000 ml water, pH adjusted to 7.4 ± 0.05 with o-phosphoric acid as the buffer. Mobile phase was prepared by mixing Buffer: Methanol: Acetonitrile in ratio 50: 30: 20. The detection wavelength was 302 nm with an acquisition time of 10 min. **Results:** The retention time of Omeprazole peak was observed to be approximately 5 min. The developed method was validated according to the ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the specified acceptance criteria. **Conclusion:** The proposed method was successfully applied to the capsule dosage form for routine analysis.

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Keywords: Omeprazole, Sodium bicarbonate, Antacid, Proton pump inhibitor, RP-HPLC, Enantiomer, Zegerid, Accuracy.

INTRODUCTION:

Omeprazole/sodium bicarbonate capsule is a combination of omeprazole, a proton-pump inhibitor and sodium bicarbonate, an antacid. Omeprazole is a substituted benzimidazole, 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole, a racemic mixture of two enantiomers that inhibits gastric acid secretion. Its empirical formula is $C_{17}H_{19}N_3O_3S$, with a molecular weight of 345.42. The structural formula is presented in Fig 1.

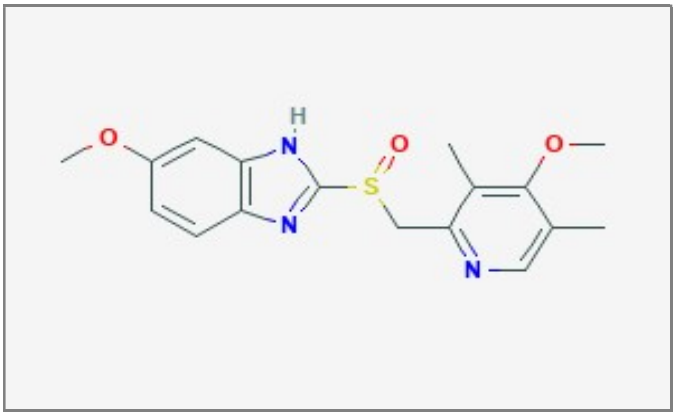

Fig 1. The chemical structure of Omeprazole.

Omeprazole belongs to a class of antisecretory compounds, the substituted benzimidazoles, which do not exhibit anticholinergic or H2 histamine antagonistic properties, but act by suppressing the gastric acid secretion by specific inhibition of the H^+/K^+ ATPase enzyme system at the secretory surface of the gastric parietal cell. Since this enzyme system is regarded as the acid (proton) pump within the gastric mucosa, Omeprazole has been characterized as a gastric acid-pump inhibitor, in that it blocks the final step of acid production [2]. This effect is dose related and leads to inhibition of both basal and stimulated acid secretion irrespective of the stimulus. Animal studies indicate that after rapid disappearance from plasma, Omeprazole can be found within the gastric mucosa for a day or more. Omeprazole is acid labile and thus gets rapidly degraded by the gastric acid. ZEGERID Capsules and Powder for oral suspension are the immediate-release formulations that contain sodium bicarbonate which raises the gastric pH and thus protects Omeprazole from acid degradation.

Drug combinations are single preparations containing two or more active pharmaceutical ingredients (APIs) for concurrent administration as a fixed dose drug [3]. Most multi-component drug formulations usually contain two or more active ingredients which are responsible for a combined therapeutic activity of the drug. However, monographs in most official pharmacopoeia are for single component drugs; hence, pharmaceutical manufacturing companies in the analysis of multi-component drug formulations use methods that involve multiple and repeated extractions to extract each active component before their quantification using spectrophotometry or titrimetry [4,5]. Such methods are laborious and cumbersome. This has led to researchers developing various methods to help facilitate easy and quick analysis of multi-component drugs. With HPLC being a method of choice, many researchers have worked at developing various RP-HPLC methods for the estimation of various active components in multi-component drugs [6-10].

The main objective of this work is to develop and validate a new, simple, accurate, linear, precise, specific, robust, sensitive and cost effective RP-HPLC method for estimation of Omeprazole in Omeprazole/Sodium bicarbonate capsules 40/1100 mg in multi-component capsule dosage form.

MATERIALS AND METHODS:

Chemicals and reagents:

Omeprazole working standard used was from the lot that was available in Oman Pharmaceutical Products L.L.C. Capsule formulation containing Omeprazole and Sodium bicarbonate 40/1100 mg was taken from the R&D department of Oman Pharmaceutical Products L.L.C. HPLC grade Acetonitrile and Methanol was procured from Merck Ltd. All other chemical reagents were of analytical grade.

Preparation of Diluent (0.1M Methanolic NaOH):

Accurately 4 g of sodium hydroxide pellets was weighed and transferred in a 1000 ml volumetric flask. To the flask, 100 ml of water was added. The mixture was sonicated to dissolve and then the solution was diluted up to the mark with methanol and mixed well.

Preparation Omeprazole standard solution:

Accurately 40 mg of Omeprazole working standard was weighed and transferred in a 200 ml amber colored volumetric flask. To the flask, 100 to 120 ml of diluent was added. The mixture was sonicated to dissolve and then the solution was diluted up to the mark with diluent. The concentration of Omeprazole in standard solution was 200 µg/ml.

Preparation of Sample solution:

Accurately weighed 5 intact capsules. One by one, the 5 capsules were opened and the contents were transferred along with the empty shells into a 200 ml amber colored volumetric flask. To the flask, about 150 ml diluent was added. The mixture was sonicated for 20 min with occasional swirling. Then the content of the solution was cooled to room temperature and finally it was diluted up to the mark with diluent. The mixture was mixed well and kept aside for about 15 min to settle down. From the above sample solution, 5 ml was transferred into 25 ml amber colored volumetric flask, diluted up to the mark with diluent and mixed well. Further, the sample was filtered using 0.45 µm PVDF syringe filter into a HPLC

vial after discarding the first few ml of the filtrate and injected into the chromatograph. The concentration of omeprazole in the injected solution was 200 µg/ml.

Procedure for HPLC study:

The HPLC column was equilibrated with the mobile phase for sufficient time until a stable base line was obtained. Separately injected equal volumes (5 µL) of blank in single, standard preparation in six replicates and each sample preparation duplicated into the chromatographic system (Table 1) and the chromatograms was recorded [11-12].

Table 1. Chromatographic conditions for HPLC study of Omeprazole.

Parameters	Specification
Instrument	HPLC
Column	Phenomenex Gemini NX-C_{18} 110Å 250 × 4.6mm ; 5µ (Part No : OOG-4454-EO)
Buffer	10 ml Triethylamine in 1000 ml o-phosphoric acid. Mix well. Filter through 0.45 µ nylon membrane filter
Mobile phase	Buffer : Methanol : Acetonitrile = 50 : 30 : 20
Flow	1.5 ml/min
Detection	302 nm
Injection volume	5 µL
Sampler temperature	5°C
Column oven temperature	40 °C
Run Time	10 min
Elution time	Approx. 5 min

System Suitability:

The % RSD of area obtained for Omeprazole from six replicate standard injections should not be more than 2.0. Tailing factor obtained for Omeprazole from standard injections should not be more than 2.0. Theoretical plates obtained for Omeprazole from standard injections should not be less than 2000.

RESULTS AND DISCUSSIONS:

The developed method for determination of assay of Omeprazole in Omeprazole/Sodium bicarbonate capsules 40/1100 mg was validated by using the following parameters.

System suitability:

Followed the procedure described in the methodology and established the system suitability before starting the analysis. The system suitability data is given in Table 2.

Table 2. System suitability of RP-HPLC study.

System suitability			
Injection #	Omeprazole standard		
	Area	Asymmetry	Plates
1	293701	1.08	6961
2	295480	1.08	6962
3	295084	1.09	6951
4	294741	1.10	6951
5	294753	1.08	6951
6	294323	1.08	6993
Mean	294680	1.09	6961.50
SD	616.14	--	--
% RSD	0.21	--	--

Specificity:

There were no interfering peaks at the retention time of the Omeprazole from the excipient components present in the formulation. Further, to demonstrate the specificity of the method, the sample was subjected to acid, base, oxidation, thermal and photolytic degradation. This was evaluated by the peak purity value of Omeprazole. The result of specificity is presented in Fig 2 to Fig 9 for the chromatograms and Table 3 for the peak purity analysis data.

Table 3. Force degradation study of Omeprazole.

Sample name	Assay	Degra-dation	Peak purity
As such (Unstressed)	101	-	
Acid degradation	82.2	18.8	0.9976
Alkali degradation	89.97	11.0	0.9977
Peroxide degradation	67.97	33.0	0.9987
Thermal degradation	84.98	16	0.9976
Photolytic degradation	90.48	10.5	0.9971

Data are presented in %.

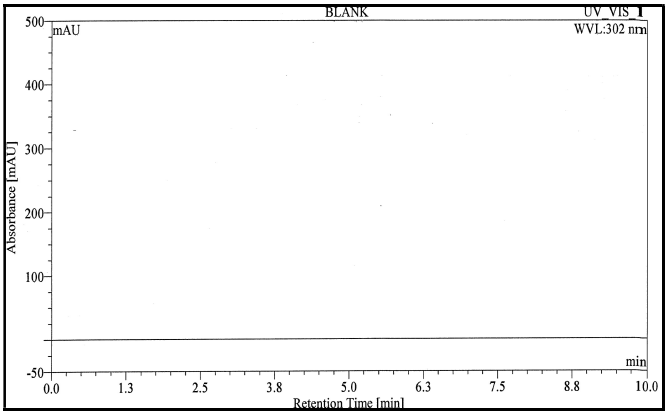

Fig 2. Reference chromatogram of Blank.

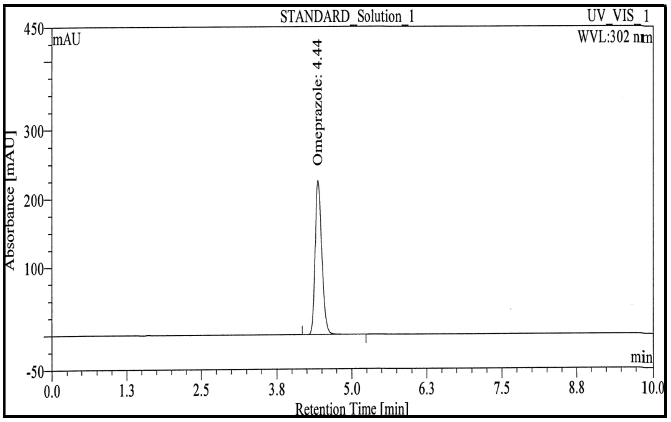

Fig 3. Reference chromatogram of Standard Solution.

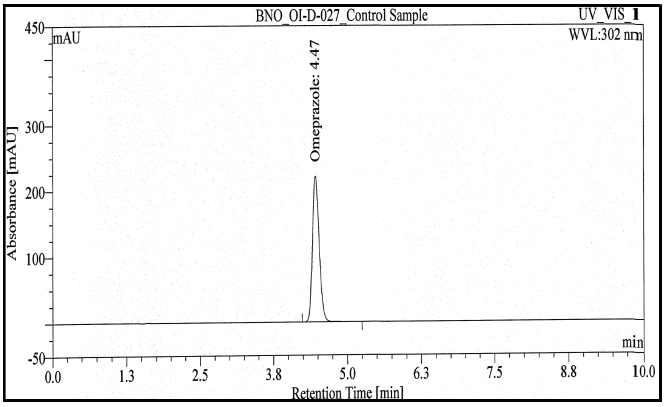

Fig 4. Reference chromatogram of as such sample.

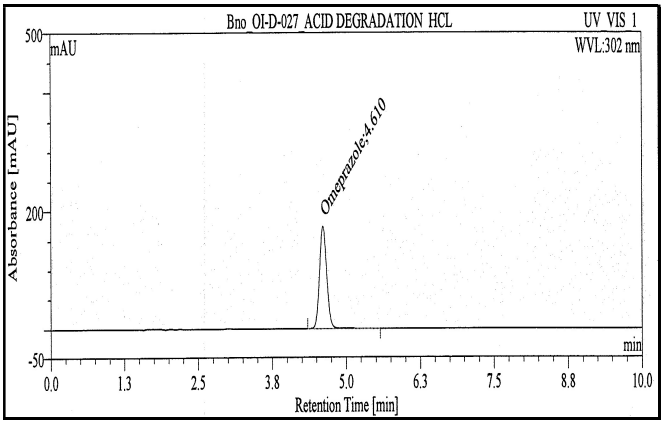

Fig 5. Reference chromatogram of Acid degradation.

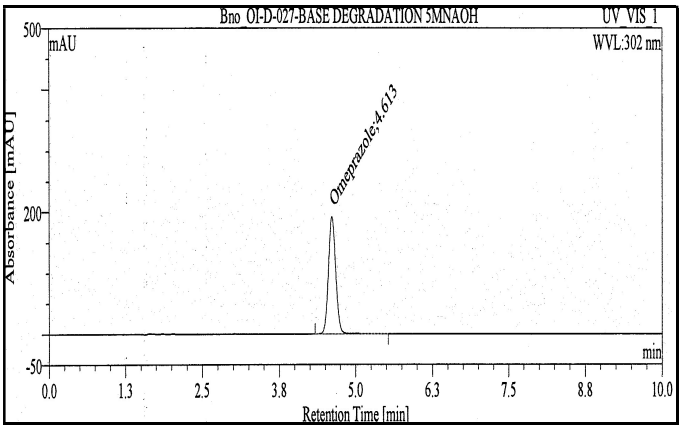

Fig 6. Reference chromatogram of base degradation.

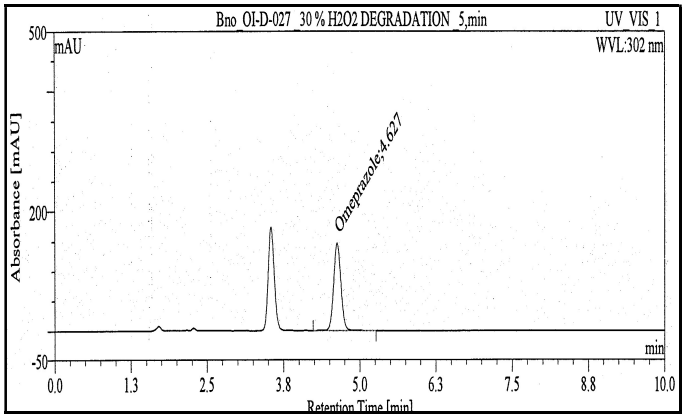

Fig 7. Reference chromatogram of Peroxide degradation.

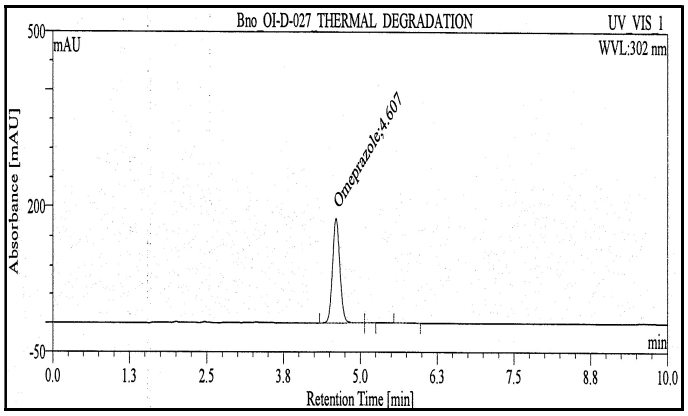

Fig 8. Reference chromatogram of Thermal degradation.

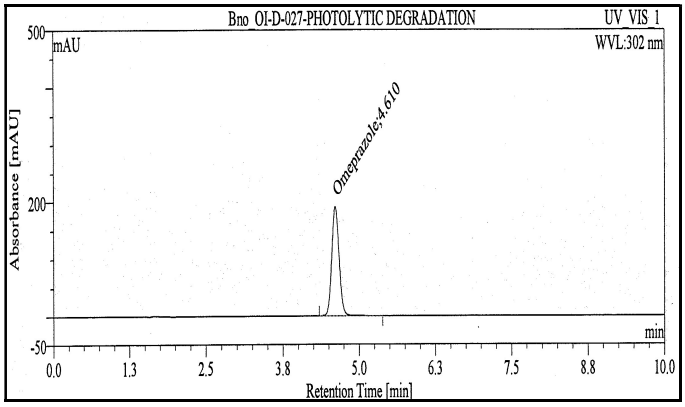

Fig 9. Reference chromatogram of UV degradation.

Linearity:

The linearity levels were prepared by diluting the linearity stock solutions in a stepwise manner to obtain five different concentrations (50, 80, 100, 120 and 150 % of the target concentration). Each linearity level was injected in duplicate and the average peak areas were plotted against concentrations. Further, the calibration curve was evaluated to calculate coefficient of correlation, slope and intercept. In general, a value of correlation coefficient (r^2) > 0.999 is considered as the evidence of an acceptable fit for the data to the regression line.

The linearity results obtained are presented in Table 4 and the result shows that the current method was linear for the analyte in the ranges of 50 to 150 %, with a correlation coefficient better than 0.999. The linearity plots have been shown in Fig 10.

Table 4. The Linearity data of Omeprazole HPLC study.

Level No.	Concentration (µg/ml)	Mean area
1	101.16	141228
2	161.85	229910
3	202.32	294425
4	242.78	348116
5	303.48	434392
Slope		1452.087
Intercept		-3874.634
CC		0.9997
R^2		0.9994

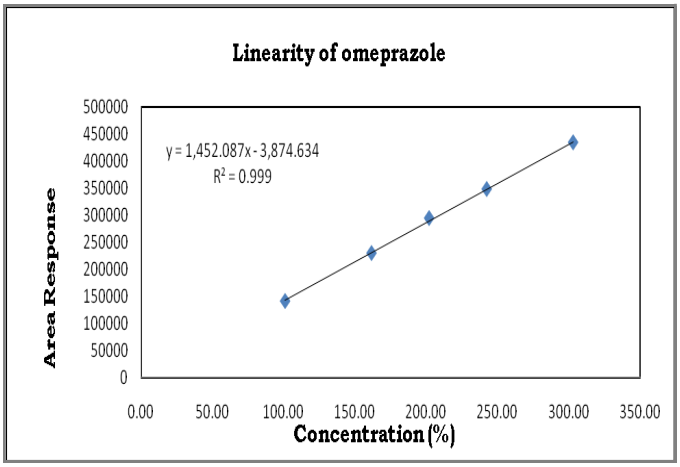

Fig 10. Linearity plot of Omeprazole.

Precision:

Precision was determined by preparing the standard and sample as per the methodology. The sample was prepared in six replicates and injected into the chromatograph. The % Assay value of each preparation was calculated and finally the % RSD of the six replicate preparations was deduced.

Table 5. Precision study data of Omeprazole.

Sample No.	% Omeprazole
1	101.0
2	101.0
3	100.7
4	100.3
5	101.0
6	102.6
Mean	101.0
SD	0.82
RSD (%)	0.81

The data obtained for six replicate standard injections and the six sample preparations have been presented in Table 5. The precision result revealed that the adopted method for analysis of Omeprazole in the capsule was highly precise.

Ruggedness:

Ruggedness of the method was demonstrated by preparing the standard and sample as per the methodology by a different analyst on a different day, using a different column lot and using a different HPLC system. The sample was prepared in six replicates and injected into the chromatograph. The % Assay value of each preparation was calculated and finally the % RSD of the twelve replicate preparations (6 precision and 6 ruggedness) were deduced. The data obtained for six replicate standard injections and the six sample preparations have been presented in Table 6 and 7.

Table 6. Ruggedness data of Omeprazole assay study.

Sample No.	% Omeprazole
1	98.0
2	98.4
3	98.3
4	98.3
5	98.1
6	98.3
Mean	98.2
SD	0.14
RSD (%)	0.15

Table 7. Ruggedness studies comparison.

Sample No.	% Omeprazole	
	Precision	Ruggedness
1	101.0	98.0
2	101.0	98.4
3	100.7	98.3
4	100.3	98.3
5	101.0	98.1
6	102.6	98.3
Mean	101.0	98.2
SD	0.82	0.14
RSD (%)	0.81	0.15
Overall mean	99.6	
Overall SD	1.4	
Overall RSD (%)	1.40	

Accuracy:

Accuracy of the proposed method had been demonstrated by the recovery study that was performed by the standard addition method at levels 50, 100 and 150 % of the target concentration. The data obtained had been presented in Table 8.

Table 8. Accuracy data of Omeprazole RP-HPLC study.

Sl. No.	Level	Sample	Amount recovered (µg/ml)	Amount added (µg/ml)	% Recovery	% RSD in each level
1	50 %-1	1	101.96	101.19	100.8	Avg: 100
2	50 %-2	2	100.87	100.85	100.0	SD 0.85
3	50 %-3	3	100.11	101.06	99.1	% RSD: 0.9 %
4	100 %-1	1	197.85	199.98	98.9	Avg: 98.4
5	100 %-2	2	196.91	200.58	98.2	SD: 0.473
6	100 %-3	3	196.28	200.22	98.0	% RSD: 0.5
7	150 %-1	1	296.94	299.63	99.1	Avg: 98.8
8	150 %-2	2	297.69	300.30	99.1	SD: 0.520
9	150 %-3	3	294.42	299.67	98.2	% RSD: 0.5

The result showed that the assay method was very accurate in quantification of Omeprazole in the capsules.

CONCLUSION:

This intended study can be concluded as the proposed method is economical, simple, ultra-fast, sensitive and reliable and is found to be accurate, precise, specific, stability indicating and rugged. Hence it can be employed for the routine estimation of Omeprazole in Omeprazole/Sodium bicarbonate 40/1100 mg capsules.

ACKNOWLEDGEMENT:

Authors wish to thank the management of Oman Pharmaceutical Products Co. LLC, for providing laboratory facility to carry out this research study.

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Conflict of Interest: None
Source of Funding: Nil
Paper Citation: Das P*, Shukla A, Maity A. RP-HPLC methodology for the Assay of Omeprazole in Omeprazole Buffered Capsule. J Pharm Adv Res, 2020; 3(9): 988-993.