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A SIMPLE HPLC METHOD FOR THE DETERMINATION OF OMEPRAZOLE *IN VITRO*

Tanveer Akhtar Malik[1], Kazi Muhammad Ashfaq[1], **Asif Hanif Chauhdry*[2]**, Muhammad Shafiq[3], Muhammad Aslam[4], Muhammad Saeed Akhtar[5]

[1]Drugs Control and Traditional Medicine Division, National Institute of Health, Islamabad.
[2]GARL, GSP, Islamabad.
[3]Department of Chemistry, GC University, Faisalabad.
[4]Pharmaceutical Research Centre, Pakistan Council for Science and Technology, Karachi.
[5]IAQ Lab, Qarshi Industries (Pvt) Ltd., Hattar

Corresponding author*: asif_hanif101@yahoo.com
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ABSTRACT:
Omeprazole is used for the treatment of stomach and gastroesophageal reflux disease. Omperazole is administered in the form of oral dose as independent drug or in combination with other antibiotics. Present study was carried out to develop and validate a simple HPLC methtod of determination of omeprazole in vitro. Mobile phase empolyed was acetonitrile: phosphate buffer (65:35), pH 6.8 and C_{18} column was used. UV detector was used using 300nm eluted with the mobile phase at a flow rate of 1.0 mL/min. percentage recovery was 99.92%±0.4%. This method was used for the routine analysis of omeprazole in raw material & finished product.
Keyword: Omeprazole, Proton Pump Inhibitor, High Performance Liquid Chromatography, Validation, Vitro study

1. Introduction

Omeprazole (5-methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2yl) methyl] sulphinyl]-1H-benzimidazole) is proton pump inhibitor, deccreases the amount of acid produced in the stomach. Omeprazole is used to treat symptoms of gastroesophageal reflux disease (GERD) and promote healing of erosive esophagitis[1-4]. Omeprazole formulations must be administered in the form of enteric preparations to prevent degradation in acidic medium [oral] [5].

Various methods for the quantification of omeprazole independently or in combination with other drugs of same group or different group [6-9]. Reverse phase HPLC methods were also reported for the determination of omeprazole [10].

All these methods were based on various difficult and time consuming procedures especially when large numbers of samples were needed in biological experiments like bioequivalence study. Therefore there was requirement of simple and fast method to analyze the omeprazole.

2. Experimental

2.1 Chemicals and Reagents: Double distilled water was used. AR grade solvents were used during the whole study. HPLC grade acetonitrile (Merck) was used for the mobile phase. Disodium hydrogen phosphate and sodium dihydrogen phosphate were of also AR grade (Merck). Reference standard was provided by the Pharmagen Pvt. Ltd. Commercial available capsules of omeprazole were collected from the local market.

2.2 Instrument: Estimation of omeprazole done on the HPLC using isocratic elution system. HPLC assay was performed on Shimadzu LC-10A HPLC system (Kyoto, Japan) using UV detector, UV wavelength set at 300 nm. Shimadzu CLC ODS-(C-18) – 150 x 6.0 mm column was used. Mobile phase was consisting of acetonitrile and phosphate buffer in the ratio 35: 65 v/v (pH-6.7) with the flow rate of 1.0 ml/ mint. Mobile phase was filtered through 0.45 micron filter and degas it by sonicator for 10 minutes.

2.3 Drug Standard Stock Solution: Internal standard stock solution was prepared by dissolving 100mg of reference standard in the 100 ml of AR grade ethanol. This stock standard solution was further diluted up to the fine concentration of 20µg/ml. This stock solution was further diluted to make different concentration of 4, 8, 12, 16 and 20 µg/ml.

2.4 Preparation of sample Solution: Twenty capsules were taken. Granules were collected and ground to fine powder. Powder equivalent to 100mg of omeprazole was taken and transferred

to 100ml flask and make solution with AR grade ethanol. This solution was further diluted and makes the concentration of 20 µg/ml.

2.5 Robustness of Analytical Method: Robustness of HPLC method was assessed by changing the chromatographic conditions i-e pH and composition of mobile phase.

2.6 Stability: Stability of different parameters for the newly developed analytical method was observed by examining the samples Kept at room temperature up to 12 and 24 hours.

2.7 Linearity: Linearity of newly developed method was observed by analyzing the eight solutions (4, 8, 12, 16, 20, 24, 28, 32, 36 µg/ml). These solutions were prepared in triplicate.

2.8 Precision and Accuracy: Precision of the method was assessed by repeatability both by intra day and inter day. Samples were analyzed five times a day. Accuracy of the method was ascertained by reproducibility and evaluated by adding known concentration of omeprazole then calculated it by using this newly developed method.

2.9 Specificity: The specificity of the method was fined by analysis of drug and standard. Solution of standard and drug prepared as mentioned procedure.

2.10 Statistical Analysis: Statistical analysis of method was also carried out & uncertainty between different measurements of varying absolute magnitude were determined through relative standard deviation by using following formula;

[RSD (%) = (Standard deviation/Mean) x 100 %]

3. Results and Discussion

A reverse phase HPLC method has been developed & validated for determination of Omeprazole by using mobile phase comprising of acetonitrile & phosphate buffer in the ratio of 65% & 35% (v / v) at ambient temperature at a flow rate of 1.0mL/min. The injection volume was kept at 20µg/mL & pH of the mobile phase was adjusted at 6.8 using phosphate buffer. Method which implies with greater change in the pH of mobile phase can degraded the structure of omeprazole. Current method is optimized for the excellent sensitive, repeatability, stability, time consumption and cost effectiveness. Retention time for the elution of omeprazole was obtained at 5.8 min.

Robustness of proposed method was performed by changing the pH as well as composition ratio (acetonitrile : phosphate buffer) of mobile phase. It was observed that change in pH of the reaction medium from 6.0 to 6.8 & 7.2 has no significant effect on percentage recovery of omeprazole. As shown in Table-1. However an increase in concentration of acetonitrile resulted in a decrease in retention time i.e. retention time decreased from 6.5min to 5.0min with an increase in concentration of acetonitrile from 30% to 60%. However these results are also in close agreement with each other & show that newly developed method is robust enough to withstand slight variations in pH & composition of mobile phase.

Stability of newly developed method and working samples solutions were evaluated. Stability studies were done for short term stability for 12hrs and long term stability of 24hrs. There is no significant variation in the stability of omeprazole even after 24hrs.

Precision of the essay was estimated with respect to repeatability as well as reproducibility. The precision of an analytical method expresses the closeness of agreement among individuals test result where the method is applied repeatedly to multiple samplings. Precision was evaluated by the intraday (repeatability) and inter day (reproducibility) of responses after replicate injections for 3-consective days & expressed as RSD% amongst responses. The %RSD values for intra- and inter-day assays of omeprazole performed in the same laboratory did not exceed more than 0.7% (Table 2).

The accuracy was evaluated at three different concentrations; which were conducted in successive analysis using the proposed method and the value was expressed as percentage of recovery between the mean concentrations found and added concentration for omeprazole. The average percentage of recovery was found to be 99.33%, 98.88% and 98.60% for 12 µg/mL, 24 µg/mL & 24µg/mL respectively (Table 3). All experimental results were in close agreement with respect to precision and accuracy, which indicated that the developed method is sensitive enough and accurate for determination of omeprazole.

Table 1. Robustness of the Assay

Experimental Conditions Applied Mobile Phase	**Retention Time**	**Percentage Recovery (%)**
Acetonitrile : Phsosphate Buffer (35:65) Reaction medium pH 6.8	5.8	100.12
Acetonitrile : Phsosphate Buffer (30:70) Reaction medium pH 6.8	6.5	100.23
Acetonitrile : Phsosphate Buffer (60:40) Reaction medium pH 6.8	5.0	100.65
Acetonitrile : Phsosphate Buffer (60:40) Reaction medium pH 6.0	6.1	100.29
Acetonitrile : Phsosphate Buffer (60:40) Reaction medium pH 7.2	6.3	100.25

Table 2. Precision of the Assay

Injected Conc. (µg/mL)	**% Recovery**			**RSD% (Inter Day)**
	Day 1	**Day 2**	**Day 3**	
4	99.2	99.4	99.7	0.25
8	99.5	99.7	100.1	0.31
12	99.7	100.2	100.5	0.4
16	99.9	100.5	100.8	0.46
20	100.1	99.8	101.1	0.68
Mean	99.68	99.92	100.44	
RSD% (Intra Day)	0.35 (12Hrs)	0.4(24Hrs)	0.55(36Hrs)	

Table 3. Recovery Efficiency of Omeprazole

Injeccted Conc (µg/mL)	**Amount Recovered (µg)**	**Recovery (%)**	**RSD (%)**
12	11.92	99.33	0.13
24	23.73	98.88	0.36
36	35.50	98.60	0.47

Conclusion

A simple, novel HPLC method has been developed & validated successfully for estimation of omeprazole in pharmaceutical formulations. This is an excellent method for routine quality control of omeprazole in raw materials, in-process and finished product. In current developed method a small quantity of mobile phase is required which can favor the cost effectiveness of method. Therefore developed method was found to be simpler, accurate, reproducible, efficient & economic and was applied successfully for the study of omeprazole formulations.

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