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Development and Validation of a RP-HPLC Method for the Quantification of Omeprazole in Pharmaceutical Dosage Form

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Abstract

A rapid and highly sensitive reversed phase high performance liquid chromatographic method has been developed for quantitative estimation of omeprazole in pharmaceutical preparations. The method has been validated according to FDA and USP guidelines with respect to accuracy, precision, specificity and linearity. The method was developed by using a gradient condition of mobile phase comprising 90% aqueous acetonitrile to 100% acetonitrile for 10 minutes at a flow rate of 0.7 mL/min over C-18 (ODS, 250 x 4.6 mm) column at ambient temperature. More than 97% recovery demonstrated the accuracy of the protocol. Intra-day and inter-day precision studies of the new method were less than the maximum allowable limit (RSD% ≤ 2.0 according to FDA). The method showed linear response with correlation coefficient (r^2) value of 0.998. Therefore, it was found to be accurate, reproducible, sensitive and less time consuming and can be successfully applied for the assay of omeprazole formulations.

Keywords: HPLC; Method development; Gradient condition; Validation; Omeprazole.

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1. Introduction

Chemically omeprazole is 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl] sulfinyl]-1*H*benzimidazole and it is used as proton pump inhibitor for the treatment of gastro-oesophageal reflux disease (GERD), peptic ulcer, Zollinger-Ellison syndrome and other conditions caused by excess stomach acid. It is also used to promote healing of erosive esophagitis [1-3].

Omeprazole may also be given together with antibiotics to treat gastric ulcer caused by infection with *Helicobacter pylori*.

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Omeprazole is a racemate. It contains a tricoordinated sulfinyl sulfur in a pyramidal structure and therefore can exist in equal amounts of both the (*S*)- and (*R*)-enantiomers. In the acidic conditions of the canaliculi of parietal cells, both are converted to achiral products (sulfenic acid and sulfenamide configurations) which reacts with a cysteine group in H^+/K^+ ATPase, thereby inhibiting the ability of the parietal cells to produce gastric acid [4].

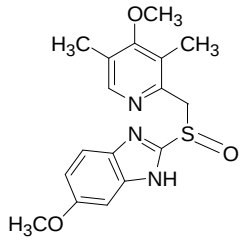

Fig. 1. Chemical structure of omeprazole.

Like other drugs omeprazole also requires some absolute parameters like quality, potency etc. to serve its best activities. It is obvious that change in the formulation or variations in the manufacturing process or use of low quality materials can affect the stability and efficacy of the product. Therefore, quality and quantity assessment ensure their safety and efficacy which can be ensured by analyzing the products during and after manufacturing and at various intervals during the shelf life of the product. Effective process validation contributes significantly to assuring drug quality. The basic principle of quality assurance is that a drug should be produced that is fit for its intended use and does not expose the consumers to risks.

Although several methods have been reported previously for determination of omeprazole in the pharmaceutical formulations [4-8], some of the methods described the use of chiral columns for RP-HPLC analyses [9-10]. The chiral column is expensive and has limitations in analytical uses. To overcome the limitations, the objective of the present work was to develop a simpler, accurate and rapid liquid chromatographic analytical method utilizing widely used and common column for the assay of omeprazole in pharmaceutical formulations and to validate the method in accordance with the guidelines of FDA, USP and ICH with respect to accuracy, reproducibility, linearity and specificity [11-15].

We, herein, describe a gradient HPLC method comprising of 90% aqueous acetonitrile to 100% acetonitrile for 10 minutes with UV detection at 302 nm on a C-18 bonded silica column. To the best of our knowledge, this is the first report of using non-buffer containing 90% aqueous acetonitrile to assay omeprazole of pharmaceutical preparations.

2. Experimental

2.1. *Materials and methods*

Working standard of omeprazole (potency 99.93%) was a kind gift of Drug International Ltd., Dhaka, Bangladesh. For the estimation of omeprazole formulated as tablets, samples

manufactured by different manufacturers were purchased from retail pharmacies on random basis and were coded as Sample-1, Sample-2 and Sample-3. HPLC grade acetic acid and acetonitrile were obtained from Active Fine Chemicals Ltd., Bangladesh.

2.1.1. *Apparatus*

HPLC system: High Performance Liquid Chromatographic system (Shimadzu-UFLC Prominence), equipped with an auto sampler (Model- SIL 20AC HT) and UV-Visible detector (Model-SPD 20A) was used for the analysis. The data was recorded using LC-solutions software.

Column: Analytical reversed phase C-18 column [Luna C-18(2), 5 µ, 250 x 4.6 mm, Phenomenex, Inc] was used to analyze the samples.

Mobile phase: Nano pure water was sonicated for 10 minutes and then it was filtered through a 0.22 µm millipore filter and degassed. HPLC grade actonitrile was also filtered and degassed before using.

Chromatographic condition: All analyses were done at ambient temperature under gradient condition. The mobile phase comprised of 90% aqueous acetonitrile to 100% acetonitrile for 10 min at a flow rate of 0.7 mL/min. The injection volume was 20 µL for standard and samples. Before analysis, every standard and sample were filtered through 0.45 µm filter tips. The column eluate was monitored with UV detection at 302 nm.

Preparation of standard solutions: Solutions of the standard drug was prepared by dissolving 12.01 mg of omeprazole powder (equivalent to 12.0 mg omeprazole) in a 50 mL volumetric flask by using 10 mL of acetonitrile. Then the volume was made up to the mark with the same solvent. The final concentration obtained was obtained as 240 µg/mL. Appropriate volume from this solution was further diluted to get standards of varying concentrations (6, 8, 10, 12 and 14 µg/mL).

Preparation of test sample: Ten tablets in each of the samples (Sample-1, Sample-2 and Sample-3) were weighed, made into fine powder in a mortar with pestle and average weight was taken. Accurately weighed powder equivalent to average weight of each tablet (20 mg of omeprazole) was taken in a 200 mL volumetric flask, and 100 mL of acetonitrile was added and sonicated to mix uniformly. The final volume was adjusted with the same solvent to get the concentration of 100 µg/mL. The solution was further diluted to get the concentration of 10 µg/mL, filtered through 0.45 µm filter tips, and aliquots of 20 µL from this solution was injected into the HPLC by using an auto injector. The average content of the tablets was determined by using the calibration curve.

2.2. *Method validation*

2.2.1. *Specificity*

The specificity of the LC method was evaluated to ensure that there was no interference from the excipients present in the pharmaceutical product. The specificity was studied by injecting the excipients, standard solution and pharmaceutical preparation of omeprazole.

2.2.2. *Linearity*

Five different concentration levels (6 µg/mL, 8 µg/mL, 10 µg/mL, 12 µg/mL and 14 µg/mL) were prepared from standard solution. Then 20 µL from each solution was injected into the HPLC using auto-sampler and the analyses were monitored at 302 nm and repeated four times. The average peak areas were plotted against concentrations. The linearity of the proposed method was evaluated by using calibration curve to calculate coefficient of correlation, slope and intercept values.

2.2.3. *Accuracy/ recovery*

The accuracy of an analytical method expresses the nearness between the expected value and the value found. It is expressed by calculating the percent recovery (%R) of analyte recovered. In this case, to evaluate the accuracy of the proposed method, successive analysis (n = 3) for three different concentrations (7 µg/mL, 10 µg/mL and 14 µg/mL) of standard omeprazole solution were carried out using the proposed method. The data of the experiment were statistically analyzed using the formula [% Recovery = (Recovered conc. /Injected conc.) x 100] to study the recovery and validity of the proposed method.

2.2.4. *Precision/ reproducibility*

Precision of the assay was assessed with respect to both repeatability and reproducibility. The precision of an analytical method is the degree of agreement among individuals test result where the method is applied repeatedly to multiple samplings. It was checked by intra- and inter-day repeatability of responses after replicate injections and expressed as RSD % amongst responses using the formula [RSD (%) = (Standard deviation/Mean) x 100 %]. In the current method development and validation protocol, the precision was determined by five replicate analyses at the concentration of 9 µg/mL of standard omeprazole solutions using the proposed method.

3. Results and Discussion

A reversed phase HPLC method has been developed and validated as per USP and FDA guidelines for determination of omeprazole in pharmaceutical formulations by using a gradient mobile phase comprising 90% aqueous acetonitrile to 100% acetonitrile for 10 minutes at ambient temperature at flow rate of 0.7 mL/min with UV detection at 302 nm. The injection volume was kept at 20 µL for standard and all samples. The retention time of omeprazole was obtained at 5.0 ± 0.1 min (Fig. 2).

The specificity of the method was monitored by analyzing the placebo (containing all the ingredients of the formulation except the analyte), standard solution and market

preparation containing omeprazole. No peak was detected close to the retention time of omeprazole which proved the high degree of specificity of the method (Fig. 2).

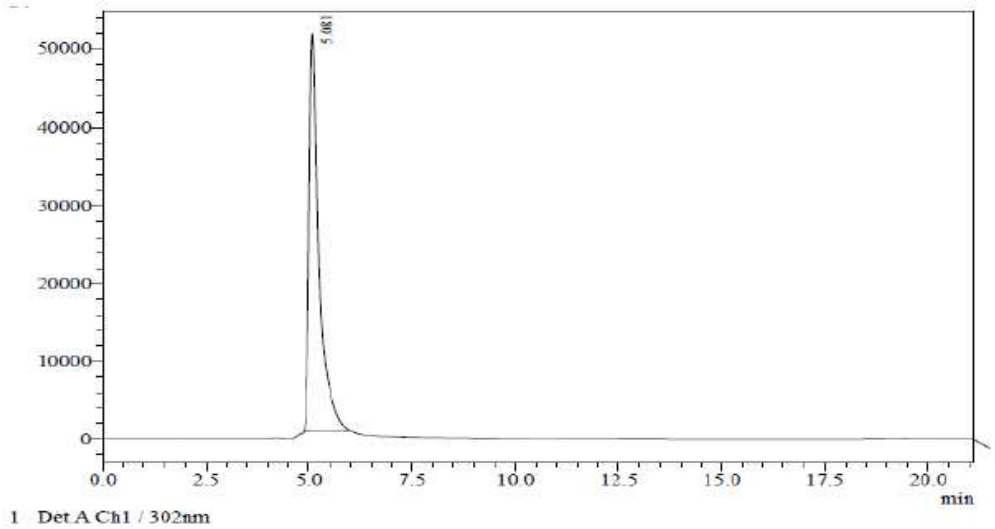

Fig. 2. HPLC chromatogram of omeprazole standard.

When peak area (*y*) was plotted against concentration levels of 6 µg/mL, 8 µg/mL, 10 µg/mL, 12 µg/mL and 14 µg/mL, a linear relation with good correlation coefficient was obtained. For the equation of calibration curve, correlation coefficient (r^2) was obtained as 0.998 which was within the accepted range of the official published guidelines and showed good linear relationship of the newly developed method with the slope (m) and intercept (c) of the calibration curve as 71351 and 1887, respectively (Table 1, Fig. 3) by using the equation, $y = mx + c$.

Table 1. Linearity of the method.

Concentration (µg/mL)	Mean area (*y*) (*n*=4)	Intercept (*c*)	Slope (*m*)	Correlation coefficient (r^2)
6	436552.3			
8	569134.3			
10	713235	1887	71531	0.998
12	846888			
14	1011188			

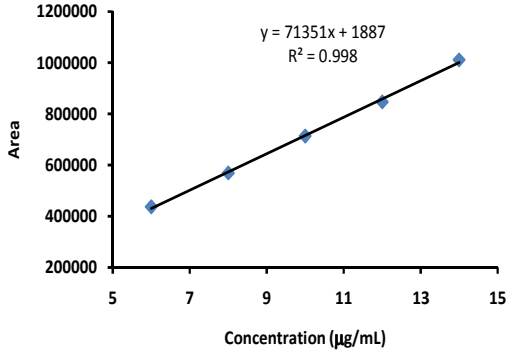

Fig. 3. Calibration curve of omeprazole standard.

The accuracy was evaluated at three different concentrations which were conducted in successive analysis (n = 4) using the proposed method and the value was expressed as percentage of recovery between the mean concentration found and added concentration for omeprazole. The average percentage of recovery was found to be 99.54%, 102.06% and 96.82% for 7 µg/mL, 10 µg/mL and 14 µg/mL, respectively (Table 2). All experimental results were in the range of the acceptability for precision and accuracy [15], which indicated that the developed method is sensitive enough and accurate for determination of omeprazole.

Table 2. Accuracy of the developed method.

Injected conc. (µg/mL)	Mean peak area	Slope (m)	Intercept (c)	Mean recovery (µg/mL)	%Recovery
7	499051.67			6.96787	99.541
10	730117.33	71351	1887	10.20631	102.063
14	969011.00			13.554457	96.817

The precision of the proposed method was checked by intra-day and inter-day repeatability of responses after replicate injection (n = 5) of standard solutions (9 µg/mL). The standard solution of 9 µg/mL concentration was analyzed for 3 days. The precision analysis was carried out for five times within the same day (intra-day variation) and three other days (inter-day variation). The precision was expressed in %RSD and it was found to be less than 2 for both intra-day and inter-day analyses and were within the acceptable range (Tables 3 and 4).

Table 3. Determination of precision (intra-day).

Day	Injected conc. (µg/mL)	Area	Slope (m)	Intercept (c)	Recovered (µg/mL)	Mean recovered (µg/mL)	SD	%RSD
1	9	639901	71351	1887	8.941907	8.739897	0.160398	1.835235
		633873			8.857423			
		622509			8.698154			
		620079			8.664097			
		611075			8.537904			
2	9	628774	71351	1887	8.78596	8.78702	0.006469	0.07362
		628744			8.785539			
		628283			8.779078			
		628882			8.787473			
		629566			8.79706			
3	9	610642	71351	1887	8.531836	8.703937	0.133705	1.536145
		619518			8.656235			
		620694			8.672717			
		636138			8.889168			
		627616			8.76973			

Table 4. Determination of precision (inter-day).

Injected conc.	Day	Inter-day mean recovered (µg/mL)	Mean	SD	%RSD
9	Day-1	8.739897	8.743619	0.041667	0.476546
	Day-2	8.787022			
	Day-3	8.703937			

By employing this method, the content of omeprazole was determined in marketed tablets. The omeprazole formulations available in Bangladeshi market were purchased on random basis from retail pharmacies and coded as Sample-1, Sample-2, and Sample-3, and the content of drug was found as 100.70%, 102.15% and 97.55%, respectively (Table 5), which were also in the acceptable range [13-15].

Table 5. Drug content found in the omeprazole tablets.

Formulation code	Amount of omeprazole claimed per tablet (mg)	Amount of omeprazole found (mg)	% of omeprazole found
Sample-1	20	20.14	100.70%
Sample-2	20	20.43	102.15%
Sample-3	20	19.51	97.55%

4. Conclusion

As a part of new analytical method development, a rapid and sensitive reversed phase high performance liquid chromatographic method has been developed and validated according to the guidelines of FDA, ICH and USP with respect to accuracy, precision, specificity and linearity. The newly developed method was found to be simple, accurate, reproducible, efficient and less time consuming, and can be successfully applied for the study of omeprazole formulations.

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