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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF OMEPRAZOLE IN CAPSULE DOSAGE FORM USING RP-HPLC METHOD

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ABSTRACT

A method for the determination of Omeprazole in capsule dosage form by reverse phase high performance liquid chromatography has been developed. This is a simple, rapid, precise and an accurate method. The method was developed on a Inertsil C85µ, (4.6×250 mm) column using acetonitrile and buffer in the ratio of 27:73 where the buffer was 14% w/v disodium hydrogen orthophosphate as a mobile phase which was pumped at a flow rate of 1.0 ml/min and detection was done at 305 nm. The retention time of the drug was found to be 21 min. The method was validated for accuracy, precision, linearity, specificity, robustness. The linearity was observed in the range of 9.6 – 15.6 µg/ml. 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 The results of recovery studies indicated that the method was accurate. Hence the developed method was found to be suitable for the estimation of Omeprazole in bulk and capsule dosage forms.

KEYWORDS: Omeprazole, RP-HPLC, Determination & Capsule dosage.

INTRODUCTION

Omeprazole (Namrata singh et al., 2012; Indian Pharmacopoeia., 2010; United States of Pharmacopoeia 2009) (RS)-6-methoxy-2-((4-methoxy-3, 5-dimethylpyridin-2-yl) methyl sulfinyl)-1H-benzo (d) imidazole is a proton pump inhibitor (PPI) and an anti-secretory compound. It suppresses gastric acid secretion by inhibiting the gastric H+ /K+ ATPase (hydrogen-potassium adenosine triphosphatase) at the secretory surface of the gastric parietal cell. Omeprazole has been characterized as a gastric acid-pump inhibitor, in that it blocks the final step of acid production. After rapid disappearance from plasma, Omeprazole can be found within the gastric mucosa for a day or more. It is freely soluble in ethanol and methanol, slightly soluble in acetone and isopropanol and very slightly soluble in water.

Chemically Omeprazole is 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl] sulfinyl]-1Hbenzimidazole 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]

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]

EXPERIMENTAL METHOD

Instruments and Reagents: 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. Disodium hydrogen orthophosphate and Orthophosphoric acid were of AR grade and acetonitrile and water of HPLC grade were used. A 20 µl hamitton injection syringe was used for sample injection.

Chromatographic conditions: A Inertsil C85µ, (4.6×250 mm) column and a mobile phase comprising of Acetonitrile and 14% w/v disodium hydrogen orthophosphate in the ratio of 77:23 v/v were used. The flow rate was maintained at 1.0 ml/min.

Selection of analytical wavelength: From standard stock solution further dilutions were prepared using mobile phase and scanned over the range of 200-400 nm and spectrum was overlain. It was observed that 305 nm was λmax of omeprazole and it was preferred as suitable wavelength for detection.

Preparation of Mobile phase: The mobile phase consisted of acetonitrile and 14% w/v disodium hydrogen orthophosphate buffer in the ratio of 77:23 v/v. The buffer was prepared by dissolving 14 g of disodium hydrogen orthophosphate in 100 mL of distilled water, followed by filtration and sonication. The mobile phase was mixed, filtered through a 0.45 µm membrane filter, and degassed before use.

STANDARD PREPARATION: 24 mg of omeprazole was weighed and dissolved in a 200 ml of mobile phase and from that stock solution diluted 5 ml to 50 ml with mobile phase containing 12 µg/ml of omeprazole was prepared.

SAMPLE PREPARATION: The powder containing 24 mg of omeprazole was weighed and dissolved in 150 ml of mobile phase, sonicated for 30 minutes and diluted with mobile phase to produce 200 ml, then mixed and filtered. 5 ml from the above solution was diluted to 50 ml with the mobile phase.

Construction of calibration curve: Solutions containing 9.6 – 15.6 µg/ml of Omeprazole were prepared. 20µl of each concentration were injected for 5 times and the mean area was calculated. A calibration plot was constructed between peak area and concentration. The regression equation obtained was y = 103352.0x - 75731 and the regression coefficient was found to be 0.9992. This equation was used later to estimate the amount of Omeprazole in capsule dosage form.

Method Validation

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.

Development and Validation

Linearity: Six different concentration levels (9.6 µg/mL, 10.8 µg/mL, 12 µg/mL, 13.2 µg/mL, 14.4 µg/mL and 15.6 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.

Accuracy: 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 (50%, 100% & 150%) 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.

Precision: 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 12 µg/mL of standard Omeprazole solutions using the proposed method.

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 Acetonitrile and 14% w/v disodium hydrogen orthophosphate for 30 minutes at ambient temperature at flow rate of 1 mL/min with UV detection at 305 nm. The injection volume was kept at 20 µL for standard and all samples. The retention time of Omeprazole was obtained at 21 ± 0.1 min (Fig. 1).

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. 1).

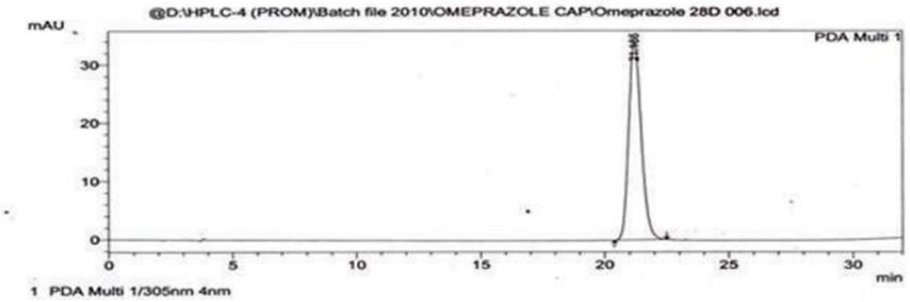


Fig 1: HPLC Chromatogram of Omeprazole Standard.

When peak area (y) was plotted against concentration levels of 9.6 µg/mL, 10.8 µg/mL, 12 µg/mL, 13.2 µg/mL, 14.4 µg/mL and 15.6 µg/mL a linear relation with good correlation coefficient was obtained. For the equation of calibration curve, correlation coefficient (r2) 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 103352 and 75731, respectively (Table 1, Fig. 2) by using the equation, y = mx + c.

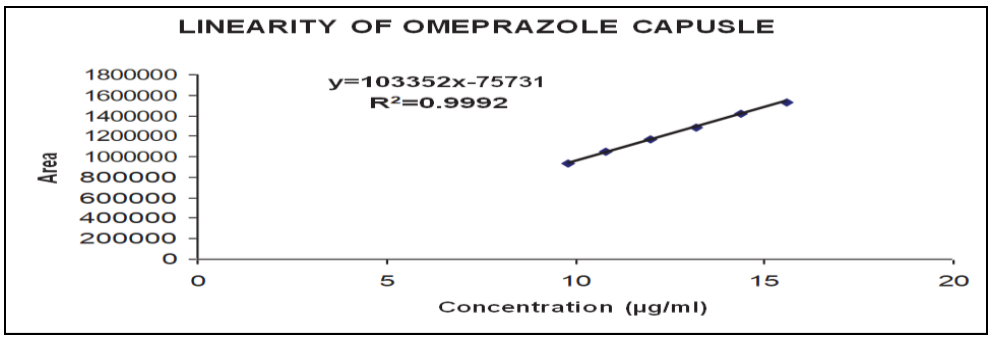


Fig 2: Calibration Curve of Omeprazole Standard.

Table 1: Linearity of the Method.

Linearity Level	Concentration (µg/ml)	Volume of stock solution(ml)	Area	Mean
Linearity-1	9.6	4.0	930883	930030
			929177	
Linearity–2	10.8	4.5	1047798	1048047
			1048296	
Linearity–3	12.0	5.0	1167784	1167267
			1166750	
Linearity–4	13.2	5.5	1280711	1283088
			1285465	
Linearity–5	14.4	6.0	1420497	1418964
			1417432	
Linearity-6	15.6	6.5	1534152	1532271
			1530390	

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 99.94%, 100.06% and 99.82% for 50%, 100% & 150%, 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.

Target Level	Area	Amount of drug Present (mg)	Drug Recovery (%)
50% Sample-1	1466747	12.8006	99.6438
50% Sample-2	1466646	12.7987	99.9953
50% Sample-3	1474317	12.9441	100.1581
100% Sample-1	2635895	24.9606	100.1214
100% Sample-2	2632829	23.8024	100.0049
100% Sample-3	2634186	23.9282	99.6570
150% Sample-1	4161213	36.7813	99.9637
150% Sample-2	4165898	36.9601	99.8232
150% Sample-3	4169443	36.0273	99.7820
Average			**99.9054**
%RSD			**0.20007%**

The precision of the proposed method was checked by intra-day and inter-day repeatability of responses after replicate injection (n = 3) of standard solutions (12 µg/mL). The standard solution of 12 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).

Sample Name	Wt. taken in mg	No. of Injections	Area	%Assay
Standard	23.9	2	1233317	----
Sample–1	349.2	1	1239251	99.08
Sample–2	350.3	1	1239121	98.76
Sample–3	350.5	1	1245648	99.23
Sample–4	351.7	1	1268094	100.67
Sample–5	348.4	1	1249950	100.17
Sample–6	348.9	1	1246910	99.78
Mean				**99.44**
Standard Deviation				**0.0085**
% of RSD				**0.8500**

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

Area	Avg. area	Amt of drug present(mg)	Drug recovery
2677734	2682741	687	99.7442
2679354			
2691134			
2683694	2686875	686	100.0435
2698261			
2678670			
2681683	2686542	688.6	99.6534
2697579			
2680366			
Mean			**99.8137**
Standard Deviation			**0.2041**
%RSD			**0.2045%**

CONCLUSION

The present work refers to the fact that the most accurate, precise, and robust HPLC method was developed and validated for estimation of solifenacin in pharmaceutical dosage form in accordance with the ICH parameters. The method was validated and found to be simple, accurate, and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of Omeprazole in its dosage form.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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