



Analytical method development and validation of HPLC method for the determination of omeprazole in capsule dosage form

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ABSTRACT

Omeprazole is chemically 5-methoxy-2-[[[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl] sulfinyl]-1H benzimidazole. It works by blocking acid production in the stomach. This medication is known as a proton pump inhibitor (PPI). It is used to treat acid-related stomach and throat (esophagus) problems and also used in the treatment of dyspepsia, peptic ulcer disease (PUD), Gastro esophageal reflux disease (GORD/GERD), and Laryngopharyngeal reflux (LPR) and Zollinger–Ellison syndrome. From the literature survey conducted there is no simple method reported for the determination of Omeprazole in capsule dosage form. Hence an attempt has been made to develop a HPLC method for the determination of Omeprazole in capsule dosage form and validate the developed method. The method was validated with respect to linearity, precision, system suitability, and specificity. The response was linear in concentration range of 9.6µg/ml to 15.6µg/ml. The value of correlation coefficient found to be 0.9992. The R.S.D% value for repeatability precision studies was 0.8500. For system suitability it is essential for the assurance of the quality performance of chromatographic system so five injections of standard drug solution were given to the system. The %RSD value for system suitability was 0.1645.

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Introduction

Omeprazole is a proton pump inhibitor used in the treatment of dyspepsia, peptic ulcer disease (PUD), Gastro esophageal reflux disease (GORD/GERD), and Laryngopharyngeal reflux (LPR) and Zollinger–Ellison syndrome. Omeprazole is chemically 5-methoxy-2-[[[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl] sulfinyl]-1H benzimidazole[1]. Decreasing excess stomach acid can help relieve symptoms such as heartburn, difficulty swallowing, persistent cough, and trouble sleeping. It can also prevent serious acid damage to your digestive system (e.g., ulcers, cancer of the esophagus). This medication may be used in combination with antibiotics to treat certain types of ulcers caused by bacterial infection.

From the literature survey conducted it was found there are few HPLC methods have been reported for determination of Omeprazole in pharmaceutical dosage forms. There is no simple method reported for the determination of Omeprazole in capsule dosage form. Hence in this present investigation an attempt has been made to develop a HPLC method for the determination of Omeprazole in capsule dosage form and validate the developed method by the International Conference on Harmonization (ICH) [2].

Omeprazole is a white crystalline powder that melts with decomposition about 155°C and it is a weak base freely soluble in ethanol and methanol and slightly soluble in acetone and isopropanol and very slightly soluble in water. This drug has a half-life of 1 to 2 hours and protein binding capacity is 95%, molecular mass is 345.4 g/mol the bio-availability is 35 to 60%, excretion is 80% renal and 20% fecal [3]. It was first marketed in the US in 1989 by Astra Zeneca as the magnesium salt omeprazole magnesium under the brand names Losec and Prilosec, and is now also available from generic manufacturers

under various brand names. Omeprazole is one of the most widely prescribed drugs internationally and is available over the counter in some countries. Its structural formula is (Fig. 1).



Fig. 1. Structure of Omeprazole

Methodology and Experimental Conditions:

Instrumentation and chromatographic conditions:

Omeprazole Capsule BP 20mg containing omeprazole was estimated by a Shimadzu HPLC with UV detector at 305 nm. Injection volume was about 40µl in the flow rate of 1.0ml/min on a USP L7, Octylsilane chemically bonded to porous silica particles, Inertsil C8 5µ, (4.6×250mm) column kept at ambient temperature using the mobile phase of a mixture of acetonitrile and buffer in the ratio of 27:73 where the buffer was 14% w/v Disodium hydrogen orthophosphate. A Shimadzu electronic analytical balance (AX-200) and Cyber lab Sonicator was used for weighing and mixing the sample. HPLC grade and high purity chemicals and reagents are used as per GLP (Good Laboratory Practice) regulations mentioned in the SOP

(Standard Operation Procedure) as this technique is a precision method requiring high degree of purity and specific methodology .[4,5]

Standard preparation:

Weigh 24 mg of omeprazole in a 200 ml volumetric flask dissolve in mobile phase and makeup to the volume with mobile phase. Dilute 5 ml from the solution to 50 ml with mobile phase. (12 mcg/ml)

Sample preparation:

Weigh a quantity of the powder containing 24 mg of omeprazole in a 200 ml volumetric flask add 150 ml of mobile phase, sonicate for 30 minutes dilute with mobile phase to produce 200 ml, mix and filter. Dilute 5 ml from the above solution to 50 ml with the mobile phase.

The standard and the sample solutions were injected into the HPLC system and record the peak areas for major peaks. From the chromatograms the RSD, tailing factor and theoretical plate for standard and sample solution were calculated.

Calculation:

The content of the drug per average of the capsule

$$\frac{\text{Sample Area}}{\text{Standard Area}} \times \frac{\text{Standard dilution}}{\text{Sample dilution}} \times \frac{\text{Standard Purity}}{100} \times \text{Average weight}$$

Method Validation:

The method was validated to meet the acceptance criteria of industrial guidance for the bio-analytical method validation.

System Suitability:

System suitability parameters are evaluated by following ICH guidelines injecting five replicates of 12µg/ml concentration of standard omeprazole solution. Resolution factor, theoretical plate and tailing factor were evaluated by following ICH guidelines.

Specificity:

The specificity of the method was determined by preparing the placebo which contained all the ingredients except active ingredients in the same proportion as present in the formulation and it was injected into the HPLC system as is, to demonstrate the purity of the drug peak. Also, any interference at the retention time of in the chromatogram.

Linearity:

The drug solutions were prepared in the concentration range of 9.6µg/ml to 15.6µg/ml. The solution were injected in triplicate into HPLC system using the mobile phase of a mixture of acetonitrile and buffer in the ratio of 27:73 where us the buffer was 14% w/v Disodium hydrogen orthophosphate.

Precision:

Peak area response was determined by making six measurements at six different concentration points in the range of 349.2, 350.3, 350.5, 351.7, 348.4, 348.9 mg/ml of sample Omeprazole respectively, were given on the same day and the values of relative

Standard deviation was calculated to determine intra-day precision.

Results and Discussion:

System Suitability:

It is essential for the assurance of the quality performance of chromatographic system. Five injections of standard drug solution were given to the system. The mean area, standard deviation and %RSD were calculated and mentioned in Table no 1. The system suitability parameters such as retention time, tailing factor, number of theoretical plate and peak area response

were also be calculated and mentioned in Table no 2. It was observed that all the values are with in the limits.

Specificity:

The complete separation of Omeprazole was noticed in presence of capsule excipients. In addition there was no significant interfering peaks from endogenous compounds were observed at the retention times of analyte. The method was indicating sufficiently specific to the drug. This shows that the peaks of analyte were pure and excipients in the formulation does not interfere the analyte. The results are shown in the Table no 3. The peak area for placebo and sample were shown in figure 2-3.

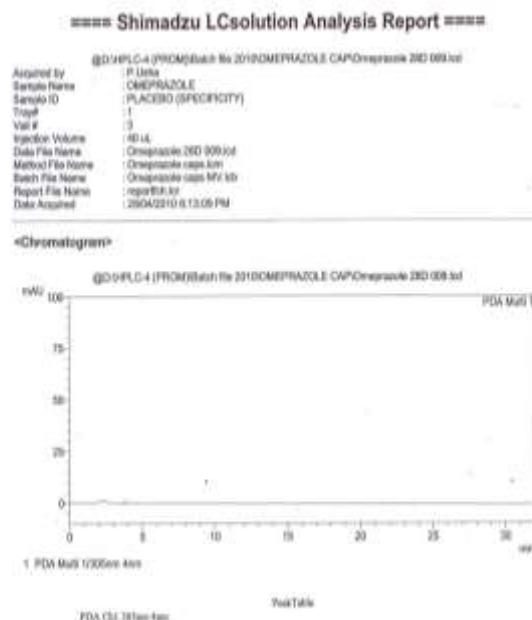


Fig. 2. Chromatogram for the study of specificity of placebo

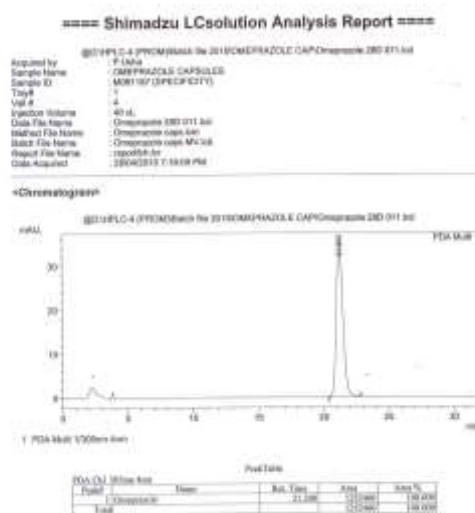


Fig. 3. Chromatogram for the study of specificity of sample Omeprazole capsule

Table 1. System Suitability for Omeprazole

S.No.	Sample	Concentration($\mu\text{g/ml}$)	Area
1.	Standard Injection-1	12 $\mu\text{g/ml}$	1190641
2.	Standard Injection-2	12 $\mu\text{g/ml}$	1195452
3.	Standard Injection-3	12 $\mu\text{g/ml}$	1192739
4.	Standard Injection-4	12 $\mu\text{g/ml}$	1195189
5.	Standard Injection-5	12 $\mu\text{g/ml}$	1193505
MEAN			1193505.20
SD			1963.16
RSD			0.1645

Table 2. System suitability parameters for Omeprazole

S.No.	System suitability parameters	Omeprazole
1.	Retention time	21.1688
2.	Tailing factor	1.2222
3.	Number of theoretical plate	8228.287
4.	Peak area response	1195452

Table 3. Specificity for Omeprazole

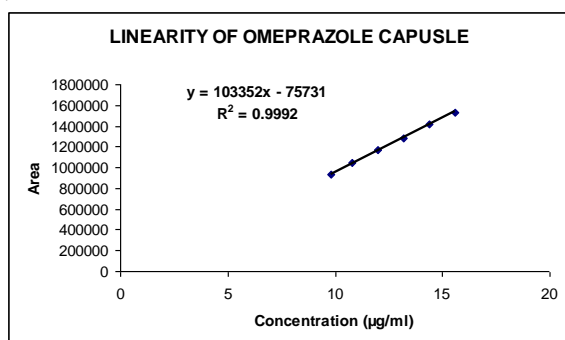
S.No.	Sample Name	No. of solutions	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	1196442
4.	Sample	1	1252460

Table 4. Repeatability for Omeprazole

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

Linearity of Response:

The linearity of this method was determined at six concentration levels from 9.6 $\mu\text{g/ml}$ to 15.6 $\mu\text{g/ml}$ and the plot of peak area against concentration for omeprazole were found (Fig. 4) to be linear in the range of 80% -130% of operating concentration. From the linear studies the correlation coefficient for omeprazole was 0.9992, typically, the regression equation was found to be $Y=10335x-75731$. It was observed that correlation coefficient and regression analysis are within the limits.

**Fig. 4. Linearity of Omeprazole Capsule****Precision:**

Repeatability is the precision of a method under the same operating conditions over a short period of time. One aspect of this is instrumental precision. A second aspect is sometimes

termed intra-assay precision and it involves multiple measurements of the same sample by the same analyst under the same conditions. The % relative standard deviation (%RSD) of Omeprazole was found to be 0.8500. These %RSD values were well within the generally acceptable limit of 2%, confirming good precision of the assay method. Result is given in table no 4.

Conclusions:

The proposed study shows a HPLC Method for the Determination of Omeprazole in Capsule Dosage Form. The application of this method in routine analysis can be justified since easy sample preparation steps are involved with simple reagents and solvents. The method was validated as per ICH guidelines and found to be simple, linear, precise, suitable and specific. Therefore the proposed method can be successfully used for the routine analysis of determination of Omeprazole from its pharmaceutical formulation and bulk drug.

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