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Research Article

Method development and validation for the simultaneous determination of omeprazole in sold dosage form by RP-HPLC

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ABSTRACT

The present work describes a simple Reverse Phase HPLC method for the determination of Omeprazole from Capsule formulations. The determination was carried out on a Gasco, ODS, C-18 (250×4. 5mm, 5 microns) column using a mobile phase of Methanol: HPLC water (80:20). The flow rate and runtime were 1 ml/min and 10 min, respectively. The eluent was monitored at 280 nm. The method was reproducible, with good resolution and sharp peak of omeprazole. The detector response was found to be linear in the concentration range of 6-20 µg/ml for omeprazole.

Keywords: Omeprazole; Mehanol; Jasaco HPLC (2080 Plus).

INTRODUCTION

Omeprazole is 5-methoxy-2-(4-methoxy-3, 5-dimethyl-2- Pyridinyl methyl sulfinyl)-1H-benzimidazole. It is official in IP 2, USP 3 and BP. Omeprazole is the proton pump inhibitor. In the acidic conditions of the stomach, omeprazole react with a cysteine group in H+/K+- ATPase, thereby destroying the ability of the parietal cells to produce gastric acid. Thus together with domperidone these drugs have synergistic effect in controlling the gastric ulcer diseases. (Patel B., Patel M., 2007) Literature survey reveals that several methods like Spectrophotometry, HPTLC and LC-MS were reported for the determination of Omeprazole in combination with other drugs as well as in biological fluids but no method has been reported for this combination anywhere before. Literature survey reveals that no HPLC method has been reported for simultaneous estimation of Omeprazole from capsule dosage form. These above developed methods are too expensive and time consuming. An attempt has been made to develop a simple, economical, precise, accurate and reproducible HPLC method for estimation of Omeprazole in capsule dosage form. (The United States pharmacopoeia XXIV and National Formulary XIX, 2000)

INSTRUMENTATION

Gasco, ODS, C-18 (250×4. 5mm, 5 micron) column. A Shimadzu UV/Visible spectrophotometer, model 1800 (Japan) was employed with spectral bandwidth of 2 nm and wavelength accuracy of ± 0.5 nm, with automatic wavelength correction employing a pair of quartz cells. A Shimadzu electronic analytical balance (AX-200) was used for weighing the sample.

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REAGENTS AND CHEMICALS

Pure omeprazole was obtained from Lupine Research Pvt. Limited, Pune. The commercial fixed dose Capsule formulation of Brand Name Ompizole Capsule (Ranbaxy) containing 20 mg of Omeprazole were procured from the local market. Methanol reagents used were of HPLC grade. HPLC water was used during the experiment. Spectral and absorbance measurements were made on Analytical technologies spectrophotometer with 1 cm matched quartz cells.

PREPARATION OF STANDARD STOCK SOLUTION OF OMEPRAZOLE (100 µg/ml)

Omeprazole standard solution was prepared by weighing 10 mg of Omeprazole and transferred to a 100 ml volumetric flask and volume was made up to 100 ml with methanol to get a concentration of 100 µg/ml. This prepared solution is sonicated for 5 minutes and then filtered through the Whatman filter paper no. 41. Again this solution was filtered by vacuum filtration using 0.42 membrane filter paper. From this solution an aliquot of 1 ml was withdrawn and it was diluted to 10 ml with Methanol. For calibration curve, stock solutions of Omeprazole were appropriately diluted to obtain concentration range of 6-20µg/ml respectively. (Murokami, Fabio, Sep. 2007 and Iuga C., Moldovan M., Popa A, 2008)

SELECTION OF ANALYTICAL WAVELENGTH:

The stock solutions of Omeprazole were separately diluted with Methanol to get a concentration of 10 µg/ml of Omeprazole respectively and scanned in the wavelength range of 200-400 nm on the Shimadzu UV/Visible spectrophotometer, model 1700 (Japan)

with spectral bandwidth of 2 nm and wavelength accuracy of ± 0. 5 nm, with automatic wavelength correction employing a pair of quartz cells. From the spectra of Omeprazole, the wavelengths observed is 284nm (λ max of Omeprazole). The sample solution run on HPLC instrument at above given wavelength and the sharp peak with minimum consumption of time are obtained at 284 nm. Therefore this wavelength is selected for the present work. (Raval P. B., Puranik M 2008 and Lakshmi S, 2003 and Lakshmanprabhu S., Shirwaikar A, 2008)

ANALYSIS OF FORMULATION

Twenty Capsules of brand OMPIZOLE (RANBAXY) containing 20 mg of Omeprazole were weighed, average weight determined and finely powdered in mortar. Appropriate quantity of powder from each tablet equivalent to20 mg of Omeprazole was accurately weighed transferred to a 100ml volumetric flask and volume was made up to 100 ml with Methanol. Shaken vigorously for 15 min and sonicated for 5 minutes and filtered through the Whatman filter paper no. 41. Again this solution is filtered by vacuum filter through 0.42 membrane filter paper. Necessary dilutions of filtrate were made with Methanol to get final concentration 20 µg/ml of Omeprazole. This solution were injected and run on HPLC instrument. Results are shown in table no 3. (Patel BH, Patel MM, Patel JR, Suhagia BN 2007)

VALIDATION OF ANALYTICAL METHOD

Validation of the developed method was done according to the USP 2006, Asian edition and ICH Guideline 2005.

LINEARITY

The linearity of the method is its ability to elicit test results that are directly proportional to the concentration of the analyte in samples. The calibration curve was taken in the range of 6-20µg/ml for Omeprazole at the respective λmax. The correlation coefficient of the linearity was found to be 0. 999 at each wavelength for both drugs as shown in table 2. (ICH, Q2A validation of analytical procedure, October 1994)

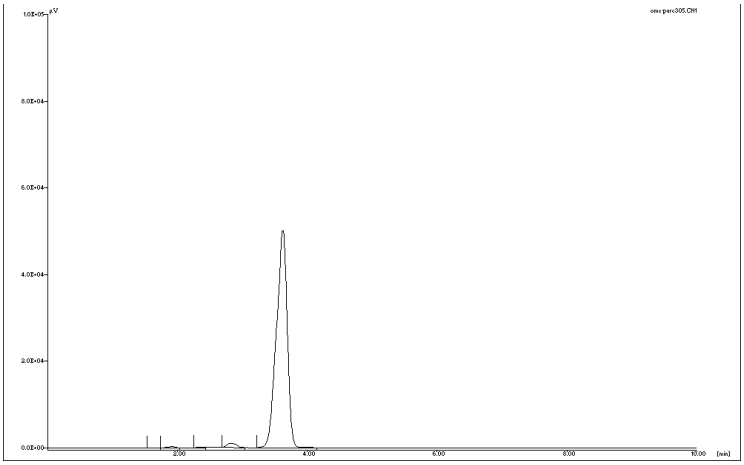

Figure 1: Typical Chromatogram of Standard Omeprazole (Rt = 3. 62 min)

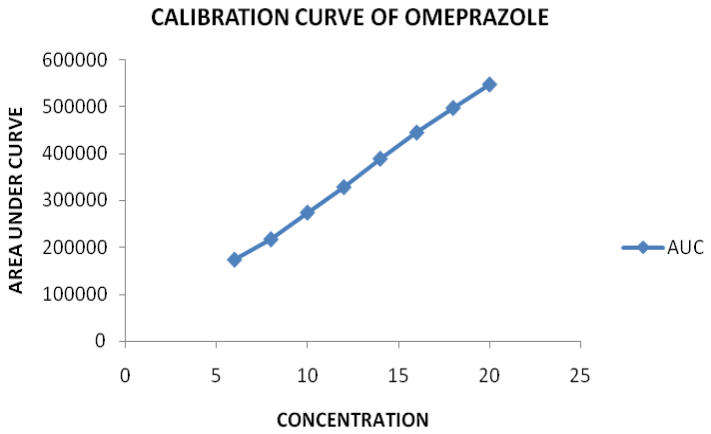

Figure 2: Calibration curve of Omeprazole

RECOVERY STUDIES

In order to ensure the reliability and suitability of the proposed method, recovery studies were carried out. It was done by mixing known quantity of standard drug with formulation sample and the content was reanalyzed by the proposed method. To a quantity of formulation equivalent to 20 mg of Omeprazole, standard drugs of Omeprazole were added at 80%, 100% and 120% levels. This was extracted, diluted and reanalyzed

Table 1: Optimized chromatographic conditions (Murokami, Cruz et al, 2007)

Chromatograph	Jasco HPLC (2080 plus)
Column	Gasco, ODS, C18(4.5mm×250mm)
Flow Rate	1 ml/minute
Detector	UV-2075 plus-jasco
Detection Wavelength	280 nm
Injection Volume	20µl
Temperature	Ambient (25±2^{0}C)
Mobile Phase	Methanol: HPLC Water (80:20)

Table 2: System suitability parameter (Raval P. B., Puranik M, 2008)

PARMETER	OMEPRAZOLE
Calibration Range	6-20µg/ml
Theoretical Plate	2410
Resolution	3
Asymmetry	1
Slop	27233.55
Intercept	4809.438
Regression Coefficient (r^2)	0.9995
Retention Time	3.62 min
Capacity	434
Selectivity	1

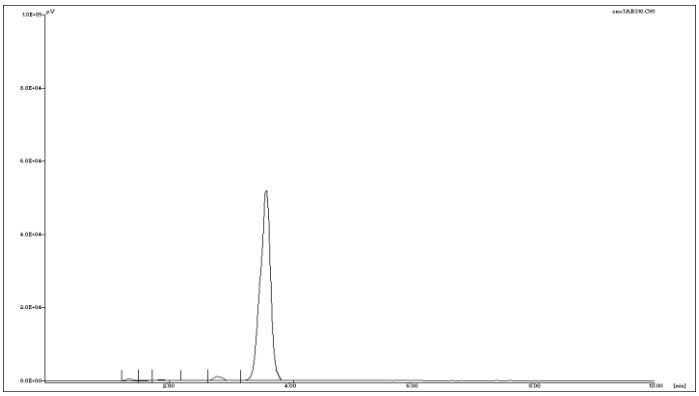

Figure 3: Typical Chromatogram of Omeprazole in capsule formulation

Table 3: Results of capsule analysis

Drug Name	AUC (Area under curve)*	RT (Retention time in minute)	Label Claim (mg)	% Label Claim Found*	Amount Found (mg)	S.D	% RSD OR %COV
OME	550457.41	3.627	20	100.2	20.04	0.059	0.2935

*Denotes the average of three estimations; OME=Omeprazole; S.D=Standard deviation; RSD=Relative Standard deviation; COV=Coefficient of variance

as per the formulation procedure. Absorbances were noted at respective wavelength.

Recovery studies were repeated for six times and the results are shown in table no. 4. (ICH, Q2B Validation of analytical procedure, March 1996 and Indian Pharmacopoeia, 1996)

PRECISION

The precision of an analytical method is determined by assaying a sufficient number of aliquots of a homogeneous sample to be able to calculate statistically valid estimate of % Relative Standard Deviation (%RSD). Intermediate precision was done to express within laboratory variation, on different days. Five replicates of 20µg/mL concentration of the working standard mixture and sample solution were analyzed %RSD was found to be less than 2%. Results are shown in table no. 5. (Reynolds, J. E. F, 2002 and Validation of Chromatographic Methods, 1994)

SPECIFICITY

Results of tablet solution showed that there is no interference of the excipients when compared with the

Table 4: Recovery study of Omeprazole

Recovery Level	Concentration µg/ml*	% Recovery*	S.D	% RSD OR % COV
80%	18	98.49	0.04725	0.2660
100%	20	99.53	0.0152	0.0767
120%	22	99.18	0.1047	0.4799

* Is the average of three estimations; S. D=Standard deviation; RSD=Relative Standard deviation; COV=Coefficient of variance.

Table 5: Intermediate Precision study of Omeprazole

Intermediate Precision	% of label claim estimated	SD	% RSD OR %COV
Interday	99.70%	0.054772	0.274600
Intraday	99.80%	0.022360	0.112027

S. D=Standard deviation; RSD=Relative Standard deviation; COV=Coefficient of variance.

Table 6: LOD and LOQ result of Omeprazole

Validation Parameter	Omeprazole
LOQ (µg/ml)	0.00004319
LOD (µg/ml)	0.00001425

LOD= Limit of detection; LOQ= Limit of quantification

working standard solution. Thus, the method was said to be specific. (Validation of Chromatographic Methods, Center for Drug Evaluation and Research (CDER), 1994)

LIMIT OF DETECTION

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. Limit of detection can be calculated using following equation as per ICH guidelines. (Gandhi SV, Khan SI, 2009). Results are shown in table no. 6.

$$LOD = 3.3 \times N/S$$

Where,

N = Standard deviation of the response and

S = Slope of the corresponding calibration curve.

LIMIT OF QUANTIFICATION

It is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. Limit of quantification can be calculated using following equation as per ICH guidelines. (ICH Harmonized Tripartite Guidelines, 2005). Results are shown in table no. 6.

$$LOQ = 10 \times N/S$$

Where,

N = Standard deviation of the response and

S = Slope of the corresponding calibration curve

RESULTS AND DISCUSSION

The aim of present study was to develop an accurate specific and reproducible RP-HPLC method for simultaneous estimation of Omeprazole from capsule dosage form. Various blends of solvent systems in varying proportions were tried as mobile phase. However mobile phase consisting of Methanol: HPLC Water in the ratio of 80:20 v/v was found to be more suitable and scanning wavelength of 280 nm. The selection of wavelength was based on maximum absorbance for optimum sensitivity.

The proposed method was found to be linear in the concentration range of 6-20 µg/ml for Omeprazole. The method was specific since excipients in the formulation did not interfere in the accurate estimation of Omeprazole. The accuracy of method was indicated by recovery values of 98.49 to 99.53%. Precision is reflected by % RSD values less than 2. The %LOD for Omeprazole was found to be 0.00001425 µg/ml. The % LOQ for Omeprazole was found to be 0. 00004319µg/ml respectively. These low values suggest sensitivity of the developed method. Hence, the developed RP-HPLC method is accurate, precise for routine analysis of Omeprazole from its dosage form. (ICH Harmonized Tripartite Guidelines, 2005)

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