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HPLC Method Development and Validation for the Estimation of Esomeprazole in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A reversed-phase high performance liquid chromatography (RP-HPLC) method was developed and validated for the estimation of esomeprazole in bulk and tablet dosage forms. The separation was achieved on Thermo C_{18} analytical column (250 mm × 4.6 mm i.d., 5.0 µm) using acetonitrile and methanol in the ratio 50:50 v/v as mobile phase and at a flow rate of 1.0 ml/min. Detection was carried out using a UV detector at 300nm. The total chromatographic analysis time per sample was about 8.0min with esomeprazole eluting at retention time of about 6.863±0.3 min. The method was validated for accuracy, precision, specificity, linearity and sensitivity. Validation studies demonstrated that this HPLC method is simple, specific, rapid, reliable and reproducible. The standard curve was linear over the concentration range of 5-25µg/ml with r^2 close to one (0.999). The limit of detection (LOD) and limit of quantitation (LOQ) obtained for esomeprazole were 0.100µg/ml and 0.314µg/ml respectively. The developed and validated method was successfully applied for the quantitative analysis of ESOMZ 20 Tablet. The high recovery and low relative standard deviation confirm the suitability of the proposed method for the determination of esomeprazole in tablets dosage form.

Keywords: Analytical method development, Reversed phase HPLC method, ICH guidelines, Tablet dosage forms, Accuracy and precision

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INTRODUCTION

Esomeprazole magnesium trihydrate[1] (ESO), bis(5-methoxy-2-[(S)- [(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl] sulfinyl]-1-H-benzimidazole-1- yl)magnesium trihydrate [Figure 1], is a compound that inhibits gastric acid secretion. ESO is cost-effective in the treatment of gastric oesophageal reflux diseases. ESO is the S-isomer of omeprazole, the first single optical isomer proton pump inhibitor, generally provides better acid control than current racemic proton pump inhibitors and has a favorable pharmacokinetic profile relative to omeprazole[2]. Several methods have been employed for the estimation of ESO alone and combination with other drugs such as UV and RP-HPLC methods[3-11]. But there is no simple and easy method for the analysis of esomeprazole. Hence, it is necessary to develop a rapid, accurate and validated RP-HPLC method for the determination of ESO in tablet dosage form. The method proved to be simple model since it does not contain a buffer system. This paper describes the development and validation of reliable, simple, robust, time and money saving reversed phase HPLC method, using PDA detection, for the estimation of ESO in tablet dosage forms. The developed method validated according to ICH guidelines [12].

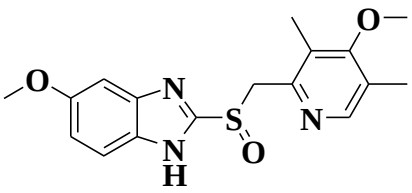


Figure 1 Chemical structure of esomeprazole

MATERIALS AND METHODS

Instrumentation

Liquid chromatographic system from Waters model no 784 comprising of manual injector, water 515 binary pump for constant flow and constant pressure delivery and UV-Visible detector connected to software Data Ace for controlling the instrumentation as well as processing the generated data.

Weighing was done on a Digital Micro Balance (CX-265) manufactured by Citizen Scale (I) Pvt. Ltd.

Reagents and chemicals

Analytically pure sample of ESO was a generous gift from Glenmark Pharma Ltd., Baddi, along with their analytical reports. HPLC grade methanol and acetonitrile was obtained from Merck (India) limited. All other chemical used were of analytical grade. Triple distilled water was used for whole experiment was generated in house. Tablets ESOMZ 20, 20 mg Dev Life Corporation Mumbai, India was purchased from local market.

Chromatographic conditions

The isocratic mobile phase consisted of methanol: acetonitrile (50:50 v/v), flowing through the column at a constant flow rate of 1.0 ml/ min. The mobile phase was filtered through nylon 0.22 µm membrane filters and was degassed before use (30 min). A Thermo (C-18) column (5 µm, 250mm x 4.60mm) was used as the stationary phase. By considering the chromatographic parameter, sensitivity and selectivity of method for drugs, 300 nm was selected as the detection wavelength for UV-Visible detector.

Standard preparation

Standard stock solution

Accurately weighed 10 mg of ESO was transferred into 10 ml volumetric flask, dissolved in 5ml of methanol and volume was made up to 10ml with methanol to get concentration of solution 1000 µg/ml (Stock-A), 5ml of stock-A was taken and diluted up to 50ml to get concentration of 100µg/ml (Stock-B).

Working standard solution

Working standard solutions were prepared by taking dilutions ranging from 5-25 µg/ml for ESO.

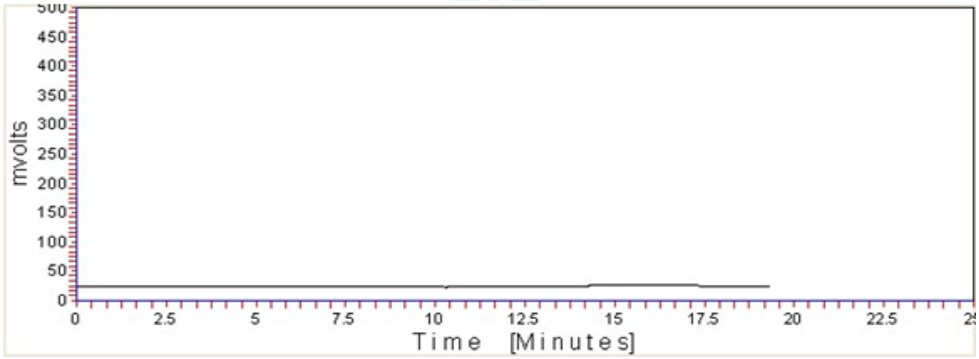
Sample preparation

Commercial formulations ESO of ESOMZ 20mg was selected for analysis. Twenty tablets were taken and their average weight was determined. They are crushed to fine powder; amount equal to 10 mg of ESO was taken in 100-ml volumetric flask. The volume is made up to the mark by methanol and filtered by whatmann filter paper (no.41) and Then different concentration of solution were prepared by serial dilution technique, as per standard and each dilution was analyzed.

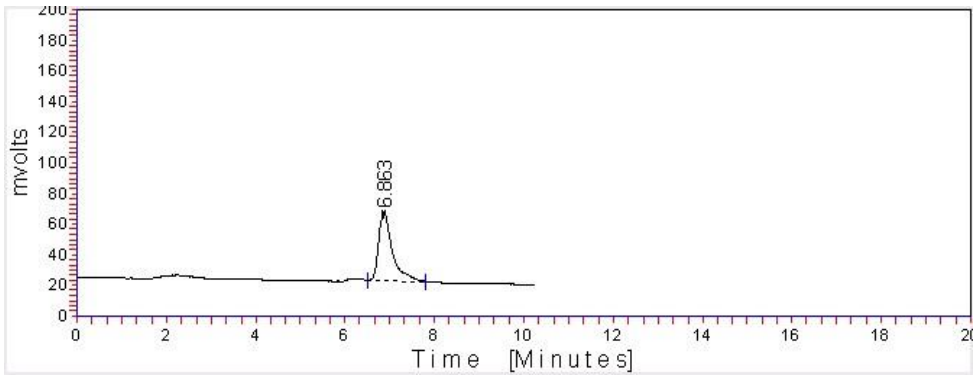
RESULTS AND DISCUSSION

Chromatography

The mobile phase was chosen after several trials with methanol, isopropyl alcohol, acetonitrile, water and buffer solutions in various proportions and at different pH values. A mobile phase consisting of methanol: acetonitrile (50:50v/v) was selected to achieve maximum separation and sensitivity. Flow rates between 0.5 and 1.5 min were studied. A flow rate of 1 ml/min gave an optimal signal-to-noise ratio with a reasonable separation time. Using a reversed-phase C_{18} column, the retention times for ESO was observed to be 6.863 ± 0.3 min. Total time of analysis was less than 8 min. The maximum absorption of ESO was detected at 300nm and this wavelength was chosen for the analysis Fig. 2



(A)



(B)

Figure 2 Chromatograms of (A) Blank mobile phase (B) ESO (15µg/ml) as reference substances

System suitability

System suitability parameters such as number of theoretical plates, HETP and peak tailing are determined. The results obtained are shown in Table 1. The number of theoretical plates for ESO was 45674.

Table 1 Results of system suitability parameters

Parameters	Esomeprazole
AUC*	1247.152
No. of Theoretical Plates	45674
Tailing Factor*	0.955
Retention time*	6.863
Calibration range (µg/ml)	5-25

*Each value is the mean ± SD of six determinations

Linearity

The calibration curve was linear over the concentration range of 5-25 µg/ml for ESO. The linearity was represented by a linear regression equation as follows:

Y (ESO) = 50.18 conc+8.069 (r^2 = 0.999)

Accuracy

Method accuracy was performed by adding known amounts of ESO to the preanalysed tablet solution and then comparing the added concentration with the found concentration. Three levels of solutions were made which correspond to 80%, 100% and 120% of the nominal analytical concentration (15µg/ml for ESO). Each level was made in triplicate table 2. The mean percentage recoveries obtained for ESO was 99.78% and RSD was less than 1.

Table 2 Results of recovery study

% Level	% Mean±SD*
	Esomeprazole
80%	100.22±0.244
100%	99.95±0.170
120%	99.78±0.170

***** Value of three replicate and three concentrations.

Precision

Repeatability

Five dilutions in three replicates were analyzed in the same day for repeatability and results were found within acceptable limits (RSD < 2) as shown in table 3.

Intermediate precision

Five dilutions in three replicates were analyzed on two different days and by two analysts for day-to-day and analyst-to-analyst variations and results were found within acceptable limits (RSD < 2) as shown in table 3.

Robustness

As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, methanol: acetonitrile (50:50 % v/v), to (45: 55% V/V) and method is found robust as RSD is again found < 2.0 table 3.

Table 3 Statistical data for precision and robustness

Statistical parameter	Esomeprazole		
	Mean*	**S.D***	**R.S.D***
Repeatability	99.74	0.028	0.028
Intermediate Precision (I) (A day to day)	99.27	0.041	0.383
(II) Analyst to Analyst	99.62	0.99	0.99
Robustness	99.45	0.065	0.546

*Mean of 15 determinations (three replicates at five concentration level)

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve table 4.

Table 4 LOD and LOQ

Name	LOD (µg/ml)	LOQ (µg/ml)
Esomeprazole	0.100	0.314

Analysis of tablets

The assay value of drugs was close to 100, SD and % RSD are less than 2 indicate the no interference of excipients in the estimation of drug table 5.

Table 5 Assay of tablet formulation

S. No.	Parameter	Esomeprazole
1.	**Mean**	99.98
2.	**S. D.**	0.125
3.	**% RSD**	0.145

Mean of nine determinations

CONCLUSION

The proposed HPLC method was validated as per the International Conference on Harmonisation (ICH) Q2B Guidelines, and was found to be applicable for routine quantitative analysis of ESO by HPLC in pharmaceutical dosage form. The results of linearity, precision, accuracy and specificity, were proved to be within the limits. The method provides selective quantification of ESO with no interference from other formulation excipients. The proposed method was highly reproducible, reliable, rapid, robust and specific. Therefore, a high percentage of recovery and the run time of less than seven minutes allow its application for the routine determination of ESO in the pharmaceutical dosage form.

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