Optimized and Validated RP-HPLC Method for the Determination of Esomeprazole Magnesium in Pharmaceutical Formulation

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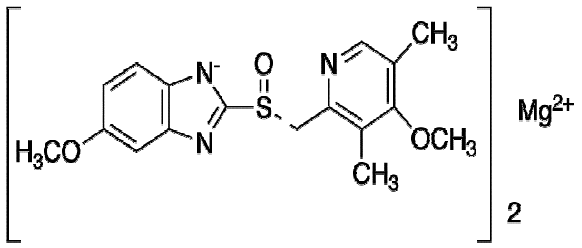
ABSTRACT: A simple, precise, accurate, rapid and reproducible reversed phase-High Performance Liquid Chromatographic (RP-HPLC) method has been developed for the determination of esomeprazole magnesium in pharmaceutical tablet formulation. Chromatography was carried out on a reversed phase C_8 column (250 mm × 4.6 mm i.d., 5 µm particle sizes). Optimum separation was achieved at 7.5 min using a mobile phase containing acetonitrile - phosphate buffer (pH 7.6) at a ratio 35:65 with 1 ml/min flow rate and the detection was done at 280 nm. The method produced linear responses in the concentration range from 25.40-76.20 µg/ml of esomeprazole magnesium with correlation coefficient of 1, accuracy of 99.15% and precision of 0.845%. The method was found to be reproducible for analysis of the drug in pharmaceutical formulations. The results of the analysis were tested and validated for various parameters according to ICH guidelines and recovery studies confirmed the accuracy of the proposed method.

Key words: Esomeprazole, RP-HPLC, validation, proton pump inhibitor

INTRODUCTION

Esomeprazole is the *S*-enantiomer of omeprazole. Chemically esomeprazole magnesium is magnesium, bis [5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H benzimidazole].[1,2] The *S*- and *R*-isomers of omeprazole are protonated and converted in the acidic compartment of the parietal cell forming the active inhibitor, the achiral sulphenamide.[3,4] It was approved in 2001 for use as a new pharmacological entity designed to improve the clinical outcome of available proton pump inhibitors in the management of acid-related disorders.[5] Now, it is used in the treatment of peptic ulcer disease, stomach infection caused by *Helicobacter pylori* along with certain antibiotics, NSAIDS associated ulceration and Zollinger-Ellison syndrome. It has a favorable pharmacokinetic profile relative to omeprazole.[6] In addition, on January 26, 2015, the U.S. Food and Drug Administration (FDA) approved the use of this drug to treat gastroesophageal reflux disease (GERD).[7]



Structure of Esomeprazole magnesium

Esomeprazole, an over the counter drug is available as tablet, capsule as well as parenteral dosage form in Bangladesh.[8] To maintain the maximum quality of the medicines, accurate, precise,

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easy and cost effective methods are necessary to analyze the drug in the respective dosage forms.

Literature review reveals that several methods such as UV spectrophotometry[9-12], HPLC[5,13-18], HPTLC[19], differential scanning calorimetry[20], and capillary electrophoresis[21] have been reported for the estimation of esomeprazole alone as well as combination with other drugs in pharmaceutical dosage forms. In order to determine esomeprazole, HPLC method is found as the best option due to its more accuracy, precision, sensitivity as well as less time requirement. Most of the HPLC methods were found to deploy methanol along with water as mobile phase whereas acetonitrile was also used in some cases. As mobile phase we have chosen acetonitrile over methanol due to its superiority in several aspects like its lower absorbance than methanol leading to lower noise in the HPLC chromatogram. Besides, the pressure experienced by the column in case of acetonitrile is lower than that of methanol. Furthermore, the elution strength is also higher in case of acetonitrile.

This present study aims to develop a simple, reliable, sensitive, accurate and precise RP-HPLC method for the determination of esomeprazole magnesium in the pharmaceutical formulation. The developed method has been validated by the evaluation of the system suitability, specificity, linearity, limits of detection and quantification, robustness, precision and accuracy according to the ICH guidelines.[22]

MATERIALS AND METHODS

Drugs and chemicals. HPLC grade acetonitrile (Merck, Germany), analytical grade monobasic sodium phosphate (Scharlau, Spain), analytical grade anhydrous dibasic sodium phosphate (Scharlau, Spain), analytical grade tribasic sodium phosphate, distilled water were used for preparing the mobile phase. Pure (92.112% as esomeprazole) esomeprazole magnesium (Glenmark Pharmaceuticals Ltd., India) was used as working standard without further purification. A commercial esomeprazole magnesium tablet was purchased from local pharmacy.

Instruments. An HPLC system (Shimadzu, Japan) equipped with a UV-visible detector (Model: SPD-20Av), a C_8 reversed phase column (250 mm × 4.6 mm i.d., 5 µm particle size), SIL 20 AC HT manual injector, LC-20 AT binary pump with software LC solution of version 1.2 was employed in the study.

Preparation of mobile phase

Preparation of buffer solution A. 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate were dissolved in 300 ml of distilled water in a 1000-ml volumetric flask. Finally, distilled water was added up to the volume 1000 ml and pH was adjusted to 7.6 with phosphoric acid.

Preparation of buffer solution B. 11 ml of 0.025 M tribasic sodium phosphate was mixed with 22 mL of 0.5 M dibasic sodium phosphate and diluted with distilled water up to 100 ml.

Mobile phase. A freshly prepared 35:65 v/v mixture of acetonitrile and buffer solution A was used as the mobile phase. The mixture was filtered through a filter (Pall corporation, India) having a nominal pore size not greater than 0.45 µm.

Preparation of standard stock solution. About 25 mg of esomeprazole magnesium working standard was taken in a 50 ml volumetric flask and dissolved in 10 mL of methanol. 10 mL of buffer B was then added and diluted up to 50 mL with distilled water.

Preparation of analytical standard solution. 5 ml of the standard stock solution was diluted to 50 ml with distilled water to make the concentration 50 µg/ml. Then the solution was filtered through a filter having a nominal pore size not greater than 0.45 µm.

Preparation of sample solution. Twenty tablets were weighed accurately and ground into fine powder. An amount of the powder equivalent to standard solution of esomeprazole magnesium was weighed and then dissolved in 10 ml of methanol in a 50 ml volumetric flask. 10 ml of buffer B was added and sonicated for 15 minutes in an ultrasonic bath. The sample was cooled to the room temperature. This

was diluted up to 50 ml with distilled water to make the concentration 50 µg/ml. The solution was filtered through a filter having a nominal pore size not greater than 0.45 µm. All solutions were stored at room temperature. These solutions were shown to be stable during the period of study.

Validation of the developed method. The developed method for the determination of esomeprazole magnesium was validated as per ICH guidelines (ICH 2005).[22]

System suitability test. System suitability was established by injecting 20 µL each for six replicate injections of system suitability solution prepared as analytical standard solution. Using six peak areas, relative standard deviation (% RSD) and mean tailing factor were calculated.

Linearity and range. Appropriate dilutions of standard stock solution (25□75µg/ml) were assayed as per the developed method for esomeprazole magnesium. To establish linearity of the proposed method, seven separate series of solutions of esomeprazole magnesium were prepared from the stock solutions and analyzed.

Precision. Precision was done by (i) repeatability or intra-assay precision and (ii) intermediate precision.

i) Repeatability (intra-assay precision). Repeatability was determined from six test samples by injecting 20 µL of each sample.

ii) Intermediate precision. A second analyst performed the same experiment as repeatability experiment. For determination of method precision, analyst 1 repeatability (n=6) was combined with analyst 2 precision (n=6) and expressed as method precision (n=12).

Accuracy. To check the accuracy of the developed method and to study the interference of formulation additives analytical recovery experiments were carried out by standard addition 80%, 90%, 100%, 110% and 120% of the label claim. Accuracy was conducted by adding known amounts of esomeprazole magnesium to the sample matrix and five different concentrations of test sample were prepared. Duplicate injections were made for each concentration.

Robustness. The robustness of this validation was conducted by changing two different parameters (Temperature: 30^0C and 40^0C and flow rate: 1 ml/min and 1.2 ml/min) of the method by using the same concentration of test solution of repeatability test.

Specificity. Specificity is determined by injecting separately blank, placebo, standard and sample solution of esomeprazole magnesium in duplicate.

RESULTS AND DISCUSSION

System suitability. Chromatograms were automatically integrated and visually inspected for an acceptable integration. The percentage RSD of the peak areas (0.605), the mean tailing factor (0.918) for six system suitability injections were calculated. The system suitability parameters were within the limits (Table 1).

Table 1. System suitability test of the developed RP-HPLC method for the determination of esomeprazole magnesium in the pharmaceutical formulation.

Replicate	Peak area	Tailing factor	%RSD		Tailing factor		Pass/Fail
			Limit	Results	Limit	Results	
1	1690630	0.920	NMT 2.00	0.605	NMT 2.00	0.918	Passed
2	1702933	0.919					
3	1703040	0.918					
4	1681426	0.917					
5	1679951	0.920					
6	1696971	0.917					

Linearity and range. A good linear relationship (r^2=1) was observed between the concentration of esomeprazole magnesium and the respective ratio of peak areas. The regression curve was constructed by linear regression and its mathematical expression was y=33248x + 5345 (where y is the ratio of peak areas of the drug to that of reference standard and x is the concentration of esomeprazole magnesium) (Figure 1). The lower limit of quantitation (LLOQ) was defined as the lowest concentration within the linear range (25.40µg/mL). The upper limit of quantitation (ULOQ) was defined as the highest concentration within the linear range (76.20µg/ml) (Table 2).

Precision. The repeatability and intermediate precision study of the developed method demonstrated RSD 1.072% for analyst-1 and RSD 0.609% for analyst-2 where RSD value for 12 samples was 0.845% which was not more than 2.0 %. That indicated the developed method had excellent repeatability (Table 3) and intermediate precision (Table 4).

Accuracy. The validity and reliability of proposed method were assessed by recovery studies by standard addition method. The mean % recovery of 99.15% and the mean % RSD of 0.474% were found to be within limit (NMT 2%). This result revealed that any small change in the drug concentration in the solution could be accurately determined by the developed analytical method (Table 5).

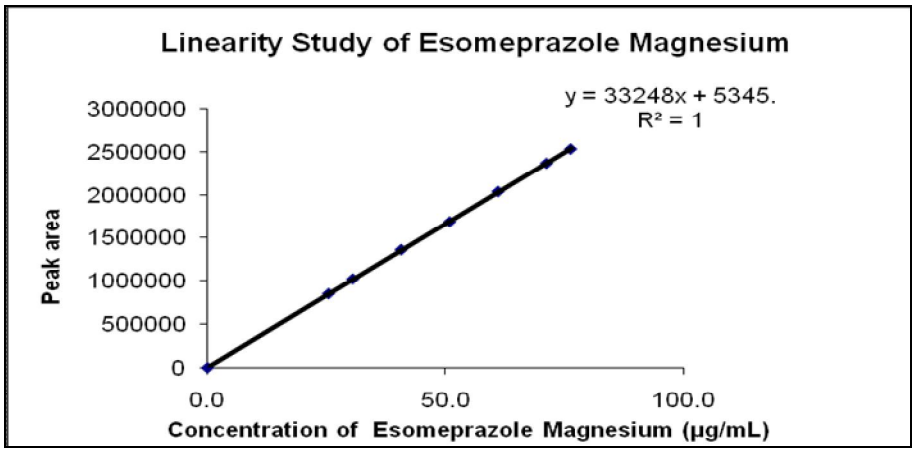

Figure 1. Linearity of esomeprazole magnesium.

Table 2. Linearity and range test of the developed RP-HPLC method for the determination of esomeprazole magnesium in the pharmaceutical formulation.

% of nominal value	Conc. of Std (µg/ml)	Peak area	Statistical analyses	Pass/Remark
50%	25.4	851032	Regression correlation coefficient (R^2)= 1 y-intercept = 5345 Slope of regression line = 33248	Passed
60%	30.48	1023118		
80%	40.64	1363104		
100%	50.80	1687024		
120%	60.96	2039353		
140%	71.12	2367987		
150%	76.20	2534148		
Lower limit of quantitation (LLOQ)				25.40 µg/ml
Upper limit of quantitation (ULOQ)				76.20 µg/ml

Table 3. Repeatability test of the developed RP-HPLC method for the determination of esomeprazole magnesium in the pharmaceutical formulation.

Sample	Peak area of sample	Average peak area of sample	Assay, (%)	%RSD	RSD limit
1	1756340 1765609	1760975	98.52	1.072	NMT 2.0%
2	1697341 1697044	1697193	99.71		
3	1647575 1697341	1672458	100.1		
4	1735918 1734588	1735253	98.75		
5	1750458 1754163	1752311	99.91		
6	1726739 1737207	1731973	101.5		
Average of assay			99.75%		

Table 4. Intermediate precision test of the developed RP-HPLC method for the determination of esomeprazole magnesium in the pharmaceutical formulation.

	Analyst-1			Analyst-2		
Sample	Peak area of sample	Average peak area of sample	Assay, (%/tablet)	Peak area of sample	Average peak area of sample	Assay (%/tablet)
1	1756340 1765609	1760975	98.52	1730301 1731705	1731003	99.20
2	1697341 1697044	1697193	99.71	1700284 1704883	1702584	99.16
3	1647575 1697341	1672458	100.1	1754949 1743335	1749142	98.85
4	1735918 1734588	1735253	98.75	1702725 1683775	1693250	99.63
5	1750458 1754163	1752311	99.91	1780667 1785853	1783260	100.6
6	1726739 1737207	1731973	101.5	1724775 1719714	1722245	99.45
RSD for analyst-1			1.072%	RSD for analyst-2		0.609%
RSD for 12 sample				0.845%		

Robustness. Robustness of the method was checked by deliberate changes in the chromatographic conditions. Column temperature and flow rate of the mobile phase were varied to ensure the robustness. The method was found to be robust enough as the % RSD of peak area, tailing factor were not apparently affected by variation in the chromatographic conditions (Table 6).

Table 5. Accuracy test of the developed RP-HPLC method for the determination of esomeprazole magnesium in the pharmaceutical formulation.

% of nominal value	Peak area of sample	Average peak area of sample	% Recovery
80%	1351516 1344692	1348104	99.17
90%	1483950 1499154	1491552	99.49
100%	1669093 1669047	1669070	98.23
110%	1790269 1794532	1792401	99.26
120%	2047596 2043746	2045671	99.35
Mean			99.15%
RSD			0.474%

Table 6. Robustness test of the developed RP-HPLC method for the determination of esomeprazole magnesium in the pharmaceutical formulation.

Temperature (°C)	Flow rate (ml/min)	% RSD of peak area	Tailing factor	Theoretical plate
30	1	0.605	0.918	7320.628
30	1.2	0.821	1.001	7768.805
40	1	1.011	0.852	7759.372
40	1.2	0.925	1.202	7821.520

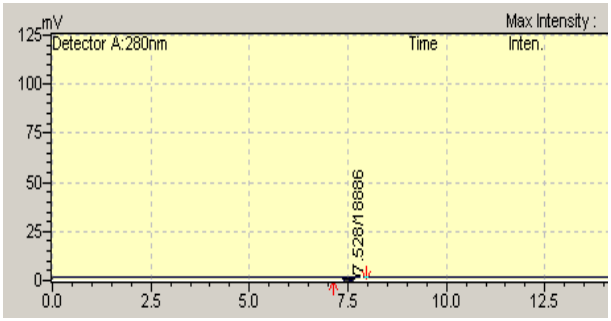

Figure 2. Chromatogram of blank.

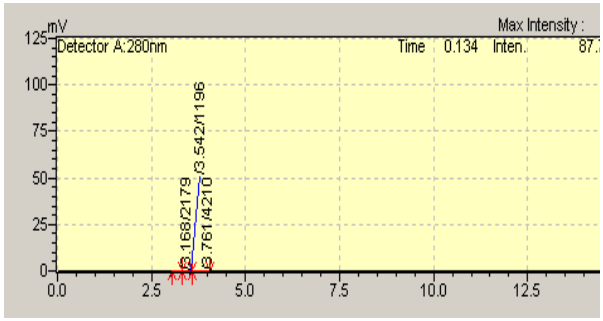

Figure 3. Chromatogram of placebo.

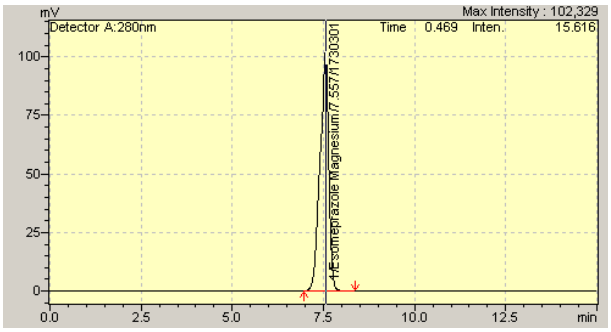

Figure 4. Chromatogram of standard.

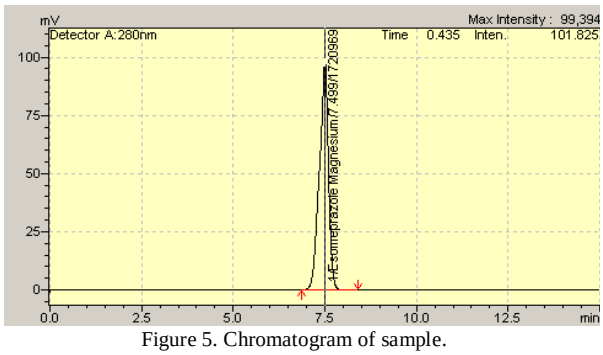

Figure 5. Chromatogram of sample.

Specificity. Specificity of the analyte peak was determined from that of the vehicle and blank injection. From the chromatogram of blank (Figure 2), placebo (Figure 3), standard (Figure 4) and sample (Figure 5), we found that there was no interference from the inactive ingredients which are presented below:

CONCLUSION

The RP-HPLC method developed for quantitative determination of esomeprazole magnesium is precise, accurate, and selective. The method is completely validated and satisfactory results are obtained for all the method validation data tested. Percent 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 Esomeprazole Magnesium in tablet dosage form.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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