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Research Article

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR DETERMINATION OF ESOMEPRAZOLE SODIUM BY HPLC

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ABSTRACTS

A very simple, accurate, specific, precise and rapid reverse phase high performance liquid chromatography technique has been built up as well as validated to determine the Esomeprazole Sodium. The method is applicable for the quantitative analysis of the drug substance. Chromatographic separation was achieved on a Supelco 250-mm, 4.6-mm, and 5-µm C-18 analytical column with a mixture of Buffer and Acetonitrile at a volume ratio of 55:45 (v/v) as mobile phase at a flow rate of 1.0 mL /min. The molecule eluted within a short runtime (within 7.0 min). The eluted compound was monitored at a detector wavelength of 302 nm and the column oven temperature was maintained at 25 °C. The developed method was validated according to ICH guidelines. The high recovery and low relative standard deviation confirm the suitability of the method for determination of Esomeprazole Sodium. The repeatability and intermediate precision, expressed by the % RSD, were less than 2%. Accuracy (% recovery: 98.00-102.00%) was found to satisfactory. The method was validated by determining its accuracy, precision, system suitability, linearity and robustness. Validation studies reveal that the method is simple, specific, rapid, reproducible, precise and accurate, which is useful for the routine determination of Esomeprazole Sodium.

Keywords: Method Development, Esomeprazole Sodium, HPLC, Validation.

INTRODUCTION

Esomeprazole Sodium[1-5] is the form of S-isomer of Omeprazole as Sodium salt, which act as proton pump inhibitor. Esomeprazole is actually protonated and then converted into sulfenamide in the parietal Cells of the stomach. This active achiral can bind some disulfide covalent bonds with an enzyme called proton pump hydrogen potassium adenosine triphosphatase or H+/K+ ATPase. Thus, it can inhibit the activity of the last step of the gastric acid production by inhibiting the entrance of the H+ ions into the gastric lumen. H+/K+ ATPase enzyme is the integral membrane protein of our gastric parietal cell. Chemical name of Esomeprazole Sodium is Sodium 5-methoxy-2-[(S) - (4-methoxy- 3, 5-dimethylpyridin-2-yl) methylsulfinyl] benzimidazol-1-ide (C17H18N3O3SNa) (figure 1) and the molecular weight is 367.397 g/mol. It is white to pale yellow powder and freely soluble in ethanol (96%) as well as very soluble in water.

MATERIALS & METHODS

Reagents and Chemicals

Dipotassium Hydrogen phosphate, Potassium dihydrogen phosphate, HPLC-grade Acetonitrile and Esomeprazole Sodium (active pharmaceutical substance and working standard) were gifted by Beximco Pharmaceuticals Ltd. HPLC-grade water was used to prepare all solutions.

Instruments

The chromatography was performed on liquid Chromatograph, Shimadzu LC 2010 dual detector equipped with an automatic injector with an injection volume of 100 µL. The HPLC system was equipped with LC solution Software.

METHOD DEVELOPMENT

Preparation of Buffer Solution

0.524 g of Dipotassium hydrogen phosphate and 2.669 g of Potassium dihydrogen phosphate were taken in a 1000 mL of volumetric flask.600 mL of water was added to dissolve the salts and finally water was added upto to 1000 mL and mixed well.

Preparation of Mobile Phase

Mixed buffer solution with Acetonitrile at a ratio of 55:45. It was filtered through a nylon filter having a nominal pore size not greater than 0.45 µm. Finally the mixture was degassed in an ultrasonic bath.

Preparation of Standard Solution

About 50 mg of Esomeprazole Sodium standard was taken in a 100 mL volumetric flask and 50 mL of the mobile phase was added to dissolve. Finally, mobile phase was added upto to 100 mL.5 mL of this solution was diluted to 50 mL with the mobile phase. It was filtered through a nylon filter having a nominal pore size not greater than 0.45 µm.

Preparation of Sample Solution

About 50 mg of the substance to be examined was taken in a 100 mL volumetric flask and 50 mL of the mobile phase was added to dissolve. Finally, mobile phase was added upto to 100 mL. 5 mL of this solution was diluted to 50 mL with the mobile phase. It was filtered through a nylon filter having a nominal pore size not greater than 0.45 µm.

Preparation of Linearity Solution

About 50 mg of the substance to be examined was taken in a 100 mL volumetric flask and 50 mL of the mobile phase was added to dissolve. Finally, mobile phase was added upto to 100 mL. 5 mL of this solution was diluted to 50 mL with mobile phase.1.0 mL, 1.6 mL, 2.0 mL, 2.4 mL, 3.0 mL of this was transferred into six different 10-mL volumetric flasks to achieve 50%, 80%, 100%, 120% and 150% of the nominal concentration (50 µg/mL for Esomeprazole Sodium) respectively. After that volume up to the mark with mobile phase and mixed well. These were filtered through a nylon filter having a nominal pore size not greater than 0.45 µm.

Preparation of Blank Solution

Mobile phase was used as blank.

Chromatographic Conditions

Supelco C18 (250 x 4.6mm; 5µm) column was used for separation. The mobile phase consists of a mixture of Buffer and Acetonitrile at a volume ratio of 55:45 and filtered through a 0.45 nm nylon filter. The mobile phase delivered in isocratic mode at a flow rate of 1.0 ml/min quantified at 302 nm.

VALIDATION OF THE PROPOSED HPLC METHOD

For validation of analytical method[6-10], the guidelines of the International Conference on the Harmonization have suggested some essential validation characteristics. These validation characteristics are given below:

1. System Suitability Test
The HPLC system was equilibrated with the initial mobile phase composition trailed by 6 injections of the same standard. The system was considered suitable when relative standard deviation (%RSD) is not more than 2.0%, tailing factor is not more than 2.0, and theoretical plate count is not less than 2000.

2. Specificity
Specificity study was resolved by comparison of the chromatograms of blank solution, standard solution and sample solution.

3. Linearity
Linearity was determined from concentration 50-150% of the nominal concentration for a total of 7 seven different concentrations. The calibration curve was created by plotting the response factor (peak area) against the various concentrations of Esomeprazole Sodium.

4. Range
Linearity, accuracy and precision data were measured for establishing the range of this analytical system.

5. Accuracy
The accuracy of the method was formed by the recovery experiments and these experiments were carried out six times.

6. Precision
Repeatability (Method Precision)
Equilibrated the system and performed 6 consecutive injections of Sample Solution against a standard solution to determine relative standard deviation (RSD of 6 injections).
Intermediate Precision
A second analyst performed the same experiment as a repeatability experiment on different days and different equipment. For determination of intermediate precision, calculated the %RSD of two analyst's results.

7. Robustness
The robustness was conducted by changing two different parameters (Flow rate and Temperature) of the method. Inject 20 µL of the standard Solution maintaining chromatographic conditions. Change the chromatographic condition by changing the Flow rate, from 1.0 mL/min to 1.2 mL/min and to 0.8 mL/min. Again change the chromatographic condition by changing the Temperature of the column from 25°C to 30°C and later to 20°C.

RESULTS

System Suitability
Chromatograms integrated automatically and then six system suitability injections were calculated. The relative standard deviation (%RSD) of the peak areas and retention times were 0.146 (NMT 2.0%) and 0.048 (NMT 2.0%) respectively. The mean tailing factor was 1.01 (NMT 2.0) and the average theoretical plate was 6953 (NLT 2000) in Table 1.

Specificity
The specificity of the analyte peak was determined from that of the blank injection. The chromatograms of Blank injection, Standard injection and sample injection were justified to find the specificity of target analyte and found no peak at the same time in the blank chromatogram. Necessary chromatograms are presented from Figure 2 to 4.

Linearity
The actual concentrations of the seven standards against the respective peak areas were computed and the linear regression curve using Microsoft Office Excel® was generated. A linear relationship was determined through calculation of a regression line by the method of least squares (or similar technique). A plot of the data as well as the correlation coefficient, y-intercept and slope of the regression line were presented in Figure 5 and Table 2.

Range
Linearity, accuracy and precision data were measured for establishing the range of this analytical system in Table 6.

Accuracy
The % of recovery and a standard deviation of % recovery were calculated as well as presented in (Table 3). The mean recovery and %RSD were found to be 99.2 % and 0.342% of Esomeprazole Sodium indicating very good reproducibility of the developed HPLC method of Esomeprazole Sodium.

Precision
Repeatability (Method Precision)
Concentration values were calculated from the corresponding peak areas for six concentrations and relative standard deviation (%RSD) was found 0.103% in Table 4.

Intermediate Precision

Analysis results which were carried out two different analysts found very similar results and their %RSD of % recovery were 0.103% and 0.261% respectively in Table 5.

Robustness

There was no significant effect was observed for accuracy and repeatability because of the change of flow rate and temperature. The specificity of this method remains unaffected by these changes (Table 7). So the method is considered robust.

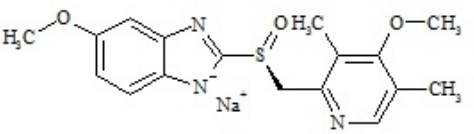

Figure 1: structure of esomeprazole sodium

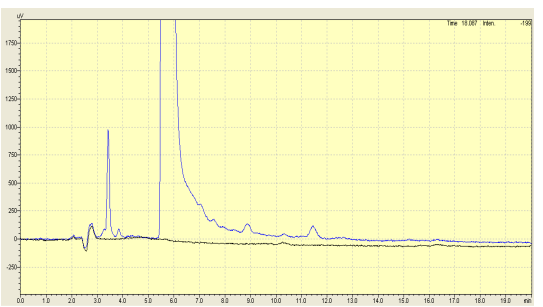

Figure 2: chromatogram comparison between blank and sample

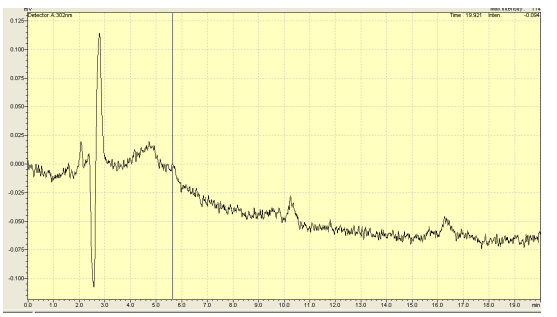

Figure 3: chromatogram of blank

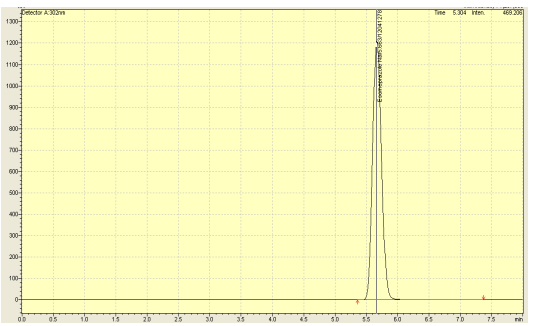

Figure 4: chromatogram of standard

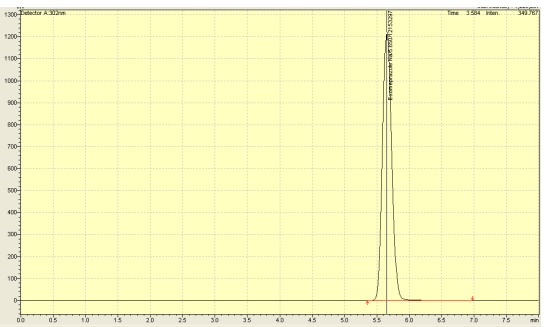

Figure 5: chromatogram of sample

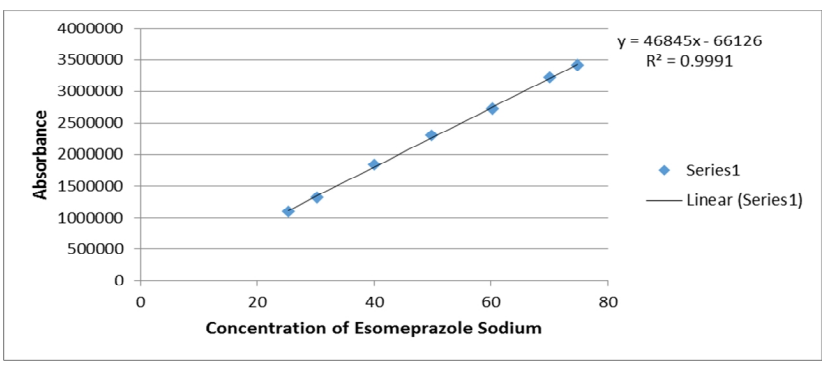

Figure 6: linearity curve

Table 1: System Suitability Study

Injection	Peak area	Retention Time	Theoretical plate	Tailing Factor
1	2249890	5.938	6677	0.99
2	2247643	5.945	6859	1.01
3	2242139	5.944	7061	1.02
4	2242534	5.942	7023	1.02
5	2242743	5.944	7041	1.02
6	2243032	5.946	7055	1.01
Average (n=6)	2244664	5.943	6953	1.01
%RSD	0.146	0.048	N/A	

Table 2: Linearity study

No.	Nominal value (%)	Conc. of sample (µg/mL)	Peak areas
1	50	25.3	1106780
2	60	30.2	1322940
3	80	40.1	1849453
4	100	49.9	2301441
5	120	60.2	2729801
6	140	70.1	3233082
7	150	74.8	3417609
Correlation coefficient (R^2)			0.9991

Table 3: Accuracy Study

Nominal Value (%)	Wt. of sample (mg)	Peak area of Sample	Wt. of standard (mg)	Peak area of standard	Recovery (%)
80%	40.1	1849453	49.8	2295842	99.1
	40.0	1831334			98.4
	40.2	1855661			99.2
100%	50.0	2301441			98.9
	49.9	2310143			99.5
	50.0	2310112			99.3
120%	60.2	2779801			99.3
	60.1	2779201			99.4
	60.1	2779949			99.4
Average (n=9)					99.2
% Relative standard deviation					.342

Table 4: Repeatability (Method Precision) Study

No.	Wt. of sample (mg)	Peak area of sample	Wt. of standard (mg)	Peak area of standard	Recovery (%)
1	50.1	2308610	50.4	2325451	99.0
2	50.1	2313501			99.2
3	49.4	2279684			99.1
4	49.2	2273009			99.2
5	50.3	2325685			99.3
6	49.9	2304594			99.2
Average (n=6)					99.2
% Relative standard deviation					0.103

Table 5: Intermediate precision Study

Analyst	No.	Wt. of sample (mg)	Peak areas of Sample	Wt. of standard (mg)	Peak area of standard	Assay
Analyst 1	1	50.1	2308610	50.4		99.0
	2	50.1	2313501			99.2
	3	49.4	2279684			99.1
	4	49.2	2273009			99.2
	5	50.3	2325685			99.3
	6	49.9	2304594		2325451	99.2
Average (n=6)						99.2

% Relative standard deviation						0.103
Analyst 2	1	51.1	2358298	49.4		99.1
	2	50.3	2330530			99.5
	3	50.6	2345958			99.6
	4	50.7	2337827			99.0
	5	50.9	2349510			99.1
	6	50.9	2358299		2279840	99.5
Average (n=6)						99.3
% Relative standard deviation						0.261

Table 6: Range Study

Parameter	Concentration Range	Acceptance Limit	Result
Linearity	50% to 150%	$R^2 > 0.995$.	0.9991.
Accuracy	80% to 120%	% Recovery: 98.0% to 102.0% and % RSD NMT 2.0%.	99.2% and 0.342%.
Repeatability	100%	%RSD of % Recovery NMT 2.0%.	0.103%.
Intermediate precision	100%	%RSD of two analysts NMT 2.0%.	%RSD of 1st analyst and 2nd analyst were 0.103% and 0.261% respectively.

Table 7: System Suitability Study (Robustness Study)

Parameter	% RSD of Area	Theoretical Plate number	Tailing factor
Flow rate + 0.2	0.445	6943	1.01
Flow rate - 0.2	0.348	7053	0.99
Column temperature at 30ºC	0.124	6983	1.04
Column temperature at 20ºC	0.139	7004	0.98

DISCUSSION

From the above test parameters, it is proved that the System Suitability, Linearity Range, Accuracy, Precision (Repeatability, Intermediate), Specificity were found okay and within the required range. Therefore, this method is validated and suitable for the assay of Esomeprazole Sodium.

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