Research Article

Development and Validation of RP-HPLC Method for Acid Resistance and Assay of Esomeprazole Magnesium in Esomeprazole Magnesium Pellets

Pathan Safia Khanam*, Nanda Kishore Agarwal and Yasmeen Sultana

Department of Pharmaceutical Analysis, Nimra College of Pharmacy, Vijayawada, India.

ABSTRACT

Esomeprazole magnesium is a gastric proton-pump inhibitor (PPI) used in treatment of gastric-acid related diseases. In present study simple, selective and accurate high performance liquid chromatographic (HPLC) method was developed and validated for the assay of esomeprazole magnesium in esomeprazole magnesium pellets 22.5%. The HPLC method was developed using grace altima C8, 250x4.6mmx5um or its equivalent column with mobile phase consisting phosphate buffer (P^H7.0 KOH) and ACN in the ratio of 55:45 at wavelength of 302nm. The method was validated by using various validation parameters like linearity, accuracy, precision, specificity, and robustness.

Keywords: Esomeprazole Mg pellets 22.5%, RP-HPLC, validation, proton pump inhibitor.

INTRODUCTION

Esomeprazole is chemically bis (5methoxy-2-[(S)-[(4-methoxy-3, 5-dimethyl-2-pyridinyl) methyl] sulfinyl]-1H-benzimidazole-1-yl). It is a gastric proton-pump inhibitor (PPI) used in treatment of gastric-acid related diseases. Its molecular formula is $(C_{17}H_{18}N_3O_3S)_2Mg$ x $3H_2O$ with molecular weight of 767.2 as a trihydrate and 713.1 on an anhydrous basis.[1,3,8] Esomeprazole provides better control of intragastric P^H than omeprazole, lansoprazole, pantoprazole and rabeprazole[2,4,7]. Consequently, esomeprazole produces higher healing rates of erosive oesophagitis and better symptom control than omeprazole in patients with gastro-oesophageal reflux disease.

Pharmacology

It is a prototype member of substituted benzimidazoles. The significant pharmacological action of esomeprazole is dose dependent suppression of gastric acid secretion; without anticholinergic or H_2 blocking action. It is a powerful inhibitor of gastric acid: can totally abolish HCl secretion, both resting as well as stimulated by any of the secretagogues, without much effect on pepsin, intrinsic factor, juice volume and gastric motility[5,6].

MATERIALS AND METHODS

Chemicals and reagents

An analytically pure sample of esomeprazole magnesium pellets 22.5% was procured as gift sample from Rachem pharma Ltd. (Hyderabad, India). HPLC grade Methanol and milli-Q grade Water was purchased from Merck. Potassium dihydrogen orthophosphate (AR grade, purity 99.5%) was purchased from Qualigens**.**

Instrumentation

A High performance liquid chromatography (Make: Schimadzu, Model: LC2010 CHT) equipped with Auto Sampler and UV detector [10] with the software of LC Solutions. All weighting's were done on single pan balance (Sartorius).

Chromatographic equipment and conditions

The liquid chromatographic system consisted of following components:

Column	: Grace Altima C8, 250x4.6mm, 5µm
Flow rate	: 1.0 ml/min
Wave length	: 302 nm
Injection Volume	: 20 µL
Run Time	: 10 minutes

Preparation of Mobile phase
Dissolve 0.288g of Dipotassium hydrogen orthophosphate and 1.469g of potassium dihydrogen orthophosphate in 550 ml of water, and then adjust the P^H to 7.0 with 10M potassium hydroxide. To this add 450 ml of Acetonitrile and mix. Filter and degas.

Preparation of Standard Solution
Accurately weighed and Transferred about 21.53mg of Esomeprazole magnesium trihydrate working standard in to a 50 ml volumetric flask add 5 ml DMF, sonicate for 10 minutes and dilute with the 0.1N sodium hydroxide to volume.[7,8] Mix and filter. (Standard Stock Solution). Transfer 3.0 ml of this solution to a 25 ml volumetric flask, dilute with mobile phase.

Preparation of Placebo Solution
Transfer an accurately weighed quantity of the placebo powder equivalent to about 20.0 mg of Esomeprazole Mg to a 50 ml volumetric flask, add 5 ml of DMF, sonicate for 30 minutes and cool the solution to room temperature and made up to the volume with 0.1N sodium hydroxide. Mix and Filter the solution through 0.45 μm Nylon filter. Transfer 3.0 ml of resulting solution in to a 25 ml volumetric flask dilute with Mobile phase to volume and mix.

Preparation of Sample Solution
Transfer an accurately weighed quantity of the powdered pellets equivalent to about 20.0 mg of Esomeprazole Mg (88.8 mg approximately) to a 50 ml volumetric flask, add 5 ml of DMF, sonicate for 30 minutes and cool the solution to room temperature and made up to the volume with 0.1N sodium hydroxide. Mix and Filter the solution through 0.45 μm Nylon filter. Transfer 3.0 ml of resulting solution in to a 25 ml volumetric flask and made up to the volume and mix.

METHOD VALIDATION
Once the HPLC method development is over, the method is validated in terms of parameters like linearity, system suitability, specificity, precision, accuracy, ruggedness and robustness.

1) System suitability
Prepared a system suitability solution i.e. standard solution (This solutions contain 0.0526 mg/ml of Esomeprazole Mg) in diluent as per methodology and inject six replicate injections into the chromatograph and expressed results as %RSD of peak area.

2) Linearity
Linearity of Esomeprazole Mg over the concentration range of 80%-120% of specification level was established using standard stock solution.

3) Accuracy
The accuracy of the method was determined by analyzing standard solution containing Esomeprazole Mg API spiked to the placebo at approximately 80%, 100% and 120% of the working strength of product. Each solution was analyzed in triplicate.

4) Precision
The method precision was performed by analyzing six individual sample preparations of Esomeprazole Mg pellets (0.048 mg/ml in diluent) at working concentration for both Assay and acid resistance.

5) Specificity
To demonstrate that diluents and placebo are not interfering with analytic peak. Solutions of blank, standard, sample and placebo were prepared individually and run chromatogram. The peak purity of analyte peak should be not less than 0.999.

6) Ruggedness (Intermediate Precision)
Evaluating the variability of results obtained with the analysis six individual sample preparations of Esomeprazole Mg pellets (0.048 mg/ml in diluent) at working concentration for both Assay and Acid resistance by different analysts on different days with different instruments and different columns assessed the method ruggedness.

7) Robustness
The robustness of proposed method was determined by analysis of aliquots from

homogenous lots by differing physical parameters like flow rate (±0.1ml/min), Buffer composition (±5%) and buffer P^H (±0.2) which may differ but the responses were still within the specified limits.

RESULTS AND DISCUSSIONS

Optimization of the mobile phase was performed based on resolution, asymmetric factor & peak area obtained for Esomeprazole Magnesium. The Mobile phase Acetonitrile: buffer (Phosphate buffer, 45:55) was found to be satisfactory and gave symmetric and well resolved peak for Esomeprazole Magnesium and results for system suitability and acid resistance were summarized in Table.1 and 2.

A calibration curve was made and concentration examined within the detection range of 80% to 120µg/ml with correlation coefficient was found to be 0.999 and results were summarized in Table.3. The recovery experiment values obtained were summarized in Table.4. The precision (expressed as the relative standard deviation) was determined for Esomeprazole Magnesium for repeated analysis and the values are presented in Table.5.

There is no interference observed due to blank and placebo at retention time of analyte peak and the method is specific and chromatograms were shown in fig.2 and 3. The reliability of the method was determined by conducting ruggedness and robustness studies and results were summarized in Table.6 and 7.

CONCLUSION

A method was developed for the determination of Esomeprazole Magnesium in Pellets which is rapid, stable and specific. The results indicate that the described method can be used for quantitative analysis of the compounds.

ACKNOWLEDGMENT

We are grateful to RAchem Pharma, Nacharam, Hyderabad for providing gift samples of Esomeprazole Magnesium. The authors are also thankful to prof. N. Duraiwel, principle Nimra College of pharmacy for providing all required facilities.

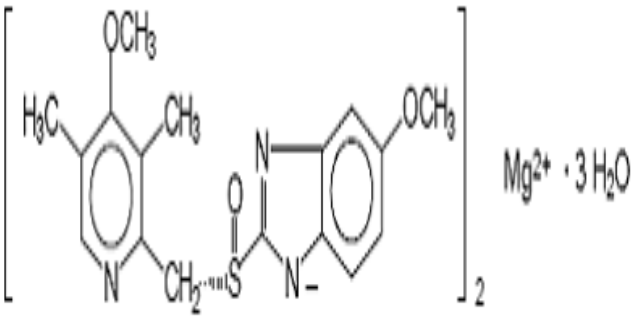

Fig.1: Structure of Esomeprazole Mg

Table 1: Chromatogram Values for System suitability

Property	Esomeprazole Mg	Required Limits
Retention time (R_T)	3.582 min	RSD ≤ 2.0%
Theoretical plates (N)	>4000	N > 2000
Tailing factor (T)	0.89	≤2.0%

Table 2: Results for precision of Acid resistance

Injection No.	Acid Resistance (%)
1	103.3
2	103.7
3	99.6
4	102.6
5	100.0
6	103.6
Mean	**102.0**
SD	**1.84**
%RSD	**1.80**

Table 3: Linearity for Esomeprazole Mg

Concentration (mg/ml)	Peak Area
0.0420	1887932
0.0473	2126272
0.0526	2360203
0.0578	2584132
0.0631	2812824
Slope	43789236
Intercept	52710.35
Correlation Coefficient	1.0000

Table 4: Accuracy of Esomeprazole Mg

Level	Actual Conc. Of Esomeprazole Mg (%)	Added Conc. (mg/ml)	Recovered Conc.(mg/ml)	% Recovery	%RSD
80%	76.4	0.0395	0.0387	98.0	**0.12**
	76.8	0.0397	0.0390	98.2	
	76.2	0.0394	0.0387	98.2	
100%	98.3	0.0508	0.0499	98.2	**0.59**
	98.6	0.0510	0.0501	98.2	
	97.7	0.0505	0.0501	99.2	
120%	116.6	0.0603	0.0596	98.8	**0.36**
	116.2	0.0601	0.0591	98.3	
	118.8	0.0614	0.0608	99.0	

Table 5: Results for Method Precision

Injection No.	%Assay
1	103.9
2	103.5
3	103.7
4	103.8
5	103.6
6	103.4
Mean	**103.7**
SD	**0.19**
%RSD	**0.18**

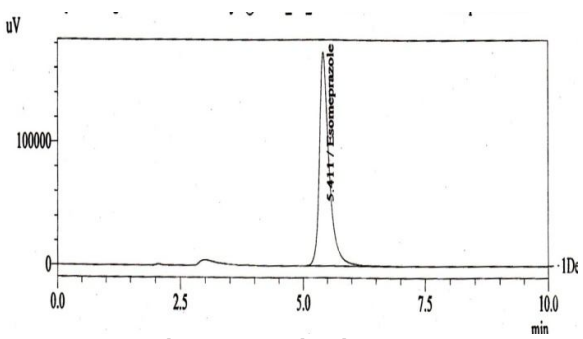

Fig. 2: Sample chromatogram

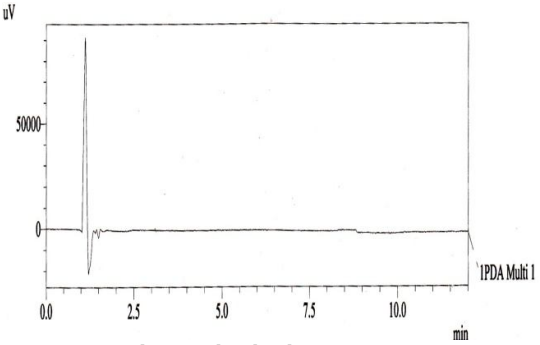

Fig.3: Blank chromatogram

Table 6: Assay Result for Ruggedness

Sample ID	Analyst(1)/Day(1)	Analyst(2)/Day(2)	Bias (%)
1	103.9	104.7	-
2	103.5	104.1	-
3	103.7	103.7	-
4	103.8	104.0	-
5	103.6	104.8	-
6	103.4	105.3	-
Mean	**103.7**	**104.4**	**-0.7%**
SD	**0.19**	**0.60**	**-**
%RSD	**0.18**	**0.57**	**-**

Table 7: Assay Result for Robustness

Parameter Condition	RT of Esomeprazole Mg	%Assay
Actual	5.31	103.7
High Flow: 1.1 ml/min	4.84	104.5
Low Flow :0.9 ml/min	5.91	104.0
High Buffer :60	6.59	104.5
Low Buffer :50	4.47	103.9
High Buffer pH:7.2	5.20	103.8
Low Buffer pH:6.8	5.45	104.6

Table 8: Validation Parameters

Parameters	RP-HPLC
Detection wavelength (nm)	302nm
RT (MIN)	3.582± 0.004 min
Calibration Range (µg/ml)	80%-120%
Slope	30946
Intercept	1723.1
Regression equation (y*)	y = 30946x +1723.1
Correlation coefficient(R^2)	0.999
Accuracy	98.0%-99.2%
Intraday method precision	0.58
Interday method precision	1.19
Limit of detection (µg/ml)	0.057
Limit of quantitation (µg/ml)	0.19

REFERENCES

1. Gisbert, J.P, Pajares, J.M. Esomeprazole-based therapy in Helicobacter pylori eradication: a meta-analysis Digestive and Liver Disease 36 (2004) 253–259.
2. Sharma s.; Sharma M.C. Densitometric method for quantitative determination of esomeprazole and domperidone in dosage form, Am.-Eurasian J. Toxicological Sci. 3(3): 143-148, 2011.
3. Vibha Guptaa, Durgesh Mishrab, Kapil Rajc, Ramniwas Dwiwedia, Dev Rishika Sainia, development & Validation of HPLC Method for the determination of Esomeprazole in Pharmaceuticals :Indo Global Journal of Pharmaceutical Sciences, 2012; 2(2): 191-196.
4. Nafisur Rahman,* Zehra Bano and Syed Najmul Hejaz Azmi Spectrophotometric Determination of Esomeprazole Magnesium in Commercial Tablets Using 5-Sulfosalicylic Acid and N-BromosuccinimideJournal of the Chinese Chemical Society, 2008, 55, 557-566 557.
5. Kulkarni S., Tripathi S., Mehta P D., Lodhi N.S., Sengar N.P.S. Esomeprazole In The Treatment Of Acidic Disorder : An Overeview, Asian Journal of Biochemical and Pharmaceutical Research Issue 2 (Vol. 1) 2011 562 disorders. Ann. Pharmacotheraphy 36: 655-663.
6. Scott, L. J.; Dunn, C. J.; Mallarkey, G.; Sharpe, M. Drugs 2002, 62, 1503.
7. International Conference on Harmonisation, ICH Harmonised Tripartite Guideline – Text on Validation of Analytical Procedures, Fed. Regist. 1995, 60, 11260.