INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

DEVELOPMENT AND VALIDATION OF METHOD FOR DETERMINATION OF ESOMEPRAZOLE BY HPLC

Sm Faysal Bellah[1], Momin Mohammad Abdul Motalib[2], Islam Mohammad Mostaharul[3], Khan Mohammad Salahuddin[2], Anisuzzaman Sharif Md.[2]*
[1]Department of clinical pharmacy and pharmacology, University of Dhaka-1000, Bangladesh
[2]Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh
[3]Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh

Article Received on: 09/04/12 Revised on: 20/05/12 Approved for publication: 11/06/12

*E-mail: shamim729@yahoo.com

ABSTRACT
A simple, selective and rapid reversed phase high performance liquid chromatographic (RPHPLC) method for the analysis of esomeprazole has been developed and validated. The separation was achieved from HPLC Column (Prevail C8, 5µ, 4.6 mm x 150 mm) with a mobile phase consisting of HPLC grade acetonitrile and phosphate buffer solution (35:65) at a flow rate of 1ml/min with UV detection at 280nm. The method was specific and it was observed that no interference with diluents. The proposed method was accurate with 99.12% recovery for esomeprazole and precise (%RSD of area of system precision, % RSD of assay of method precision and Intermediate precision were found to be 0.09%, 0.21% and 0.43% respectively). From the linearity study the correlation coefficient is found to be 1.0000, which indicated that the method was linear over 10% to 150% range. The method was found robust for possible changes. Therefore, this method can be used as a more convenient and efficient option for the analysis of esomeprazole to establish the quality of the drug substance during routine analysis with consistent and reproducible results.
KEYWORDS: Method Validation, RP HPLC, Esomeprazole, System suitability

INTRODUCTION

Esomeprazole magnesium trihydrate (ESO) is proton pump inhibitor used in the treatment of acid related disorders.[1] ESO, a single optical S-isomer of omeprazole provides better acid control than current racemic proton pump inhibitors and has a convenient pharmacokinetic profile in comparison to omeprazole.[2] Several UV and RP-HPLC methods have been conducted for the estimation of ESO alone and combination with other drugs. [3-11]

Method validation is an indispensable necessity from both regulatory and quality perspective.[12] As the analytical process varies widely; there is no universal regulation for method validation. But United States Food and Drug Administration and European Commission for medicinal products have developed general non-mandatory guidelines.[13, 14] The prime purpose for validation to guarantee that method and equipments meet the requirements to ensure safety, integrity, quality and strength of the product for use by the general public.[15, 16] The official method for estimation of esomeprazole by HPLC is included in United States Pharmacopeia. But the peak retention time is long, so the present work was undertaken with the aim to develop and validate a rapid and consistent reversed-phase high performance liquid chromatographic method in which the peak will appear in a short possible time according to ICH guideline.[17]

MATERIALS AND METHODS

Reagents and chemicals

HPLC grade Acetonitrile, Methanol and 85% Ortho-phosphoric acid were from Merck, Germany; analytical grade Monobasic, Dibasic and Tri-basic sodium phosphate were from Scharlau, Spain. USP Omeprazole and Esomeprazole Magnesium Trihydrate Reference Standard were from EP commission. Esomeprazole Magnesium Trihydrate USP working standard (WS) was from Glenmark generics Ltd. India. Purified water was used for the analytical purpose.

Instrumentation

A Waters 2487 Module binary system with dual λ detector, USA was used. In this HPLC method we used Prevail C8, 5µ, 4.6 mm x 150 mm column. Analytical Balance, pH meter from Mettler, UK and Micropipette from Fischer, Germany was used.

METHOD DEVELOPMENT

Preparation of Phosphate Buffer pH 7.6:

0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate were dissolved in 300 ml of purified water. Solution was diluted with purified water to 1000 ml. 250 ml of this solution was diluted to 1000 ml.

Preparation of mobile phase:

35 volumes of HPLC grade acetonitrile was mixed with 65 volumes of phosphate buffer (pH 7.6). Solution was filtered through a 0.45 µm membrane filter.

Preparation of Phosphate Buffer (pH 11):

11 ml of 0.25 M tri-basic sodium phosphate was mixed with 22 ml of 0.5 M dibasic sodium phosphate and diluted with water to 100 ml.

Chromatographic conditions

We used a 4.6 mm x 15 cm column in HPLC method that contains 5-µm packing L7 injection volume 20µl. Detection was carried out at 280 nm and the flow rate was 1 ml/min.

Standard solution

Accurately weighed 10.0 mg of omeprazole USP reference standard was taken into 200 ml volumetric flask. 10 ml of HPLC grade methanol was added to dissolve the standard and then 10 ml of buffer solution was added (pH 11). Solution was diluted to 200 ml with purified water.

Preparation of test solution:
10.0 mg of the test sample was taken into a 200 ml volumetric flask. 10 ml of HPLC grade methanol was added to dissolve the sample and then 10 ml of buffer solution (pH 11) was added. Solution was diluted to 200 ml with purified water.

System Suitability study
The standard solution was used as system suitability solution and 20 µl of six replicate injections of standard solution was injected. Chromatogram was recorded & system suitability parameters for each of the injection and %RSD of area (within 1.0 %) and %RSD of retention time (within 1.0 %) were calculated.

METHOD VALIDATION

System suitability
The relative standard deviation (%RSD) of the peak area responses for esomeprazole from six replicate injections of standard solution should be not more than 1.0%, The tailing factor, theoretical plate counts in standard solution and %RSD of retention time should not be more than 2.0, less than 2000 and within 1.0 % respectively.

Specificity, linearity and system precision
For specificity study identification, placebo (diluents) interference and RT ratio of sample and standard were observed. The linearity was carried out by observing the correlation coefficient (r) of standard solution. System Precision was carried out by performing six replicate injections at 100% of the test concentration and calculating the % RSD of the measured area.

Method Precision
Method precision was assessed by performing six replicate injections of the sample at 100% of the test concentration and % RSD of the assay result is calculated.

Intermediate Precision (Ruggedness)
Intermediate precision or ruggedness study of an analytical method is the degree of reproducibility of the test results obtain by the analysis of the same samples under a variety of normal test conditions i.e. different instrument, analysts, column, days, laboratories etc. Sample for intermediate precision was assessed by performing replicate assays (n=6) of sample at 100% of the test concentration and difference between mean assay of two different analysts. %RSD of assay results (twelve assay results) was calculated in this study.

Accuracy
Study was carried out over a range of 50%, 80%, 90%, 100%, 120%, 130% and 150 %(3 replicates each of the total analytical procedure) of test concentration. The % recovery and RSD of % recovery of each concentration were measured.

Range
Data generated in linearity, precision and accuracy were considered for establishing the range of the analytical method.

Robustness
Robustness of the method was investigated by changing flow rate (± 2%), column temperature (± 5ºC), ratio of components of mobile phase and pH (±0.2).

Stability Study
The solution stability experiments were performed under room temperature at intervals of 0 hr, 6 hrs, 12 hrs, 18 hrs, 24 hrs, 30 hrs and 48 hrs.

RESULTS AND DISCUSSION

System suitability
System suitability is an integral part of analytical procedures. In optimized chromatographic conditions %RSD of area of Omeprazole and %RSD of retention time were found 0.22, 0.07 respectively. Average tailing factor and theoretical plate count were 1.29 and 8056 respectively .Table-1 shows the system suitability data. The results from six consecutive injections of the standard solution indicates a good system for analysis.

Specificity
Specificity of an analytical method is its ability to assess unequivocally the analyte in the presence of components that may be expected to be present. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures.[18] From the specificity study, it was observed that the chromatogram for esomeprazole sample with omeprazole standard show positive response and blank (diluents) has no response. The RT ratio of sample & standard was 1.0 (limit 0.95-1.05) which also indicated the specificity of the method.

Linearity
The linearity of an analytical method is its ability to elicit test results directly proportional to the concentration of the analyte in samples within given range.[19] Linearity of the method was evaluated from the correlation coefficient of calibration curves that were constructed from mean peak area of omeprazole at different concentrations level (10%, 20%, 30%, 50%, 80%, 90%, 100%, 120%, 130% and 150%). Correlation coefficient was 1.0000 which proved that the method was linear. (Table-2) (Figure-1).

System precision
System Precision was carried out by performing six replicate injections at 100% of the test concentration and calculating the % RSD of the measured area. . From the data (table-3) it was observed that the % RSD of area is 0.09% which is well within the acceptance limit of 1.0%. Hence the system is precise.

Method Precision
The % RSD of six sample assay results was found 0.21 % which was within the acceptance limit. (Table-4)

Intermediate precision or ruggedness
The intermediate precision of the method was evaluated using different analyst and different instrument in the same laboratory. Assay results by two different analysts at different days have been found very much close to each other and with a difference of only 0.74 % and the % RSD of two analysts (12 samples) is 0.43% which is well within acceptance criteria. So the method can be considered to be rugged enough. (Table-5)

Accuracy
The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The average % recovery at different accuracy label was found 99.12 %, and % RSD for individual % recovery meets the acceptance criteria. Hence the method is Accurate. (Table-6)

Range
The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of

analyte within the extremes of the specified range of the analytical procedure.

Based on the linearity, precision and accuracy results, the range of the method was determined as 50% to 150% of the target concentration. (Table-7)

Robustness

The robustness of an analytical method is a measure of its capacity to remain unchanged by little but intentional variation in method parameters and provides an indication of its reliability during normal usage.[20] Robustness of the method was investigated by changing flow rate (± 2%), changing column temperature (± 5ºC), ratio of components of mobile phase, changing pH (±0.2).(Table-8)

From the above results it is clear that the system suitability criteria meet with the acceptance limit. Hence the method is robust.

Stability study

From the solution stability study it was observed that the test sample solution is found to be stable up to 48 hours at ambient condition. (Table-9)

CONCLUSION

The method adopted for estimation of Esomeprazole by HPLC is precise, linear, accurate, rugged and robust enough. The sample solution was found to be stable up to 48 hours at ambient condition. Hence this method can be considered for routine use to establish the quality of the drug substance during routine analysis with consistent and reproducible results.

REFERENCES

1. Andersson T, Hassan-Alin M, Hasselgren G, Rohss K, Weidolf L. Pharmacokinetic studies with esomeprazole, the (S)-isomer of omeprazole. Clin Pharmacol 2001; 40:411-426.

2.Scott L.J., Dunn C.J., Mallarkey G, Sharpe M. Esomeprazole- a review of its use in the management of acid-related disorders. Drugs 2002;, 62:1503-1538

3. Hultman I., Stenhoff H., Liljeblad M. Determination of esomeprazole and its two main metabolites in human, rat and dog plasma by liquid chromatography with tandem mass spectrometry. J Chromatogr B 2007; 848:317-22.

4. Johnson D.A., Roach A.C., Carlsson A.S,. Karlsson A.A., Behr D.E.. Stability of esomeprazole capsule contents after in vitro suspension in common soft foods and beverages. Pharmacotherapy 2003; 23:731-734.

5.Li X.Q., Anderson T.B., Ahlstrom M., Weidolf L.. Comparison of inhibitory effects of the proton pump-inhibiting drugs omeprazole, esomeprazole, lansoprazole, pantoprazole and rabeprazole on human cytochrome P450 activities. Drug Metab Dispos 2004;32:821-7.

6. Shetty R, Subramanian G, Kumar R.A., Pandey S, Udupa N. Estimation of esomeprazole in human plasma by reverse phase high performance liquid chromatography. Indian Drug s2005; 42:158-61.

7.Magesh A.R., Vijayalakshmi R, Satyavati D, Sravanthi Devi G, Dhanaraju M.D.Validated spectrophotometric estimation of esomeprazole using hydrotrophic solubilisation technique. Orie J Chem 2010; 26:1191-3.

8. Onal A,, Oztunc A. Development and validation of high performance liquid chromatographic method for the determination of esomeprazole in tablets. J Food Drug Anal 2006; 14:12-8.

9.Prabu S.L., Shirwaikar A., Shirwaikar A., Kumar C.D., Joseph A., Kumar R.. Simultaneous estimation of esomeprazole and domperidone by UV spectrophotometric method. Indian J Pharm Sci 2008; 70:128-31.

10. Patel B.H., Suhagia B.N., Patel M.M., Patel J.R. Determination of pantoprazole, rabeprazole, esomeprazole, domperidone and itopride in pharmaceutical products by reverse phase liquid chromatography using single mobile phase. Chromatographia 2007; 65:743-748.

11.Zanitti L, Ferretti R, Gallinella B, Torre F.L., Sanna M.L., Mosca A. Direct HPLC enantioseparation of omeprazole and its chiral impurities: Application to the determination of enantiomeric purity of esomeprazole magnesium trihydrate. J Pharm Biomed Anal, 2010; 52:665-671.

12. Fourman G.L.and Millen M.V. Pharma, Technol 1993; 17: 54.

13. SOP Validation studies. Indian pharma guidance academy Nagpure, 1996; p. 1-3.

14. Willar H.H.L.L., Dean, J.L., Settle F.A. Instrumental method analysis, 7th edition, CBS publishers and distributions, New Delhi,1986; p.60-75.

15. Chowdary K.P.K., Himabindul G.Validation analytical method, Eastern Pharmacist, 1999; 39-41.

16. Snyder L.R. & Kirkland J.J. Practical HPLC Method development, Wiley inter science publications, New York; 1997. p. 685-712.

17. International Conferences on Harmonization. Guidance on Validation of Analytical Procedure: Text and methodology. Q2A (R1). Federal Register, step 4 versio, 2005.

18.Rockville, M.D.Validation of compendial methods, United States Pharmacopeia XXIII, National Formulary, , The United States Pharmacopeial Convention; 1995..p.1612–1710.

19.ICH guidelines .International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of analytical procedures: Methodology,ICH-Q2B, Geneva,1996.

20. Ravichandran V., Shalini S., Sundramk.M. and Harishrajak .Validation of analytical methods- Strategies and importance. International Journal of Pharmacy and Pharmaceutical Sciences 2010; 2(3):18-22.

Table – 1: System suitability Study

Injection	Area	Retention Time	USP Tailing Factor	Theoretical Plate Number
1.	1449028	5.95	1.29	7943
2.	1446999	5.94	1.29	8085
3.	1451887	5.94	1.30	8072
4.	1454273	5.94	1.30	8068
5.	1455190	5.94	1.29	8063
6.	1453127	5.93	1.30	8106
Average (n=6)	1451751	5.94	1.29	8056
SD	3165.29	0.01	-	-
%RSD	0.22	0.07	-	-
Acceptance Criteria	**NMT 2.0%**	**NMT 1.0%**	**NMT 2.0**	**NLT 2000**

Table – 2: Linearity study

Level	Concentration in % (X -axis)	Area (Y - axis)
1.	10	128821
2.	20	260444
3.	30	381725
4.	50	645212
5.	80	1013319
6.	90	1143045
7.	100	1270226
8.	120	1532850
9.	130	1658616
10.	150	1916998
Correlation coefficient (r)		1.0000
Acceptance Criteria : Correlation coefficient, r = ≥ 0.9990		

Table 3: System precision study

Injection	Retention Time (mins)	Area
1.	5.96	1299248
2.	6.00	1299052
3.	5.97	1297044
4.	5.96	1299421
5.	5.96	1299162
6.	5.96	1300751
Average	5.97	1299113
SD	0.02	1190.21
% RSD	0.30	0.09

Table 4: Method precision

Sr. No.	Wt. of Standard	Area of Standard	Wt. of Sample	Area of Sample	% of Assay
1.	10.05	1449286	10.01	1300037	100.88
2.			10.00	1299925	100.97
3.			10.03	1298730	100.58
4.			10.02	1299260	100.72
5.			10.02	1300562	100.82
6.			10.01	1304228	101.20
Average (n=6)					100.86
SD					0.22
%RSD					0.21
Acceptance Criteria: %RSD of Assay NMT 2.0%					

Table-5: Table for Intermediate precision or Ruggedness study

Analyst Name	**Analyst 1**			**Analyst 2**		
Location	Instrument Room-I			Instrument Room-I		
Instrument	HPLC System I			HPLC System II		
Date of analysis	09.02.10			11.02.10		
Area of Std.	1449286			1446769		
Sr. No.	Weight of sample (mg)	Area	Assay (%)	Weight of sample (mg)	Area	Assay (%)
1.	10.01	1300037	100.88	9.99	1290286	100.10
2.	10.00	1299925	100.97	10.00	1295512	100.40
3.	10.03	1298730	100.58	10.05	1295966	99.94
4.	10.02	1299260	100.72	10.03	1295176	100.08
5.	10.02	1300562	100.82	10.04	1293823	99.87
6.	10.01	1304228	101.20	10.01	1295698	100.32
	Mean Assay (n=6)		100.86	Mean Assay (n=6)		100.12
	Standard deviation n=6		0.22	Standard deviation n=6		0.21
	% RSD (n=6)		0.21	% RSD (n=6)		0.21
i. Difference (Difference between mean assay of Two different analysts) → 0.74% ii. %RSD of assay results (twelve assay results) → 0.43%						
Acceptance Criteria: i. ± 2% Difference of each other ii. %RSD of assay results (twelve assay results): NMT 2.0%						

Table-6: Accuracy study

Level	Weight of sample (mg)	Area of Sample	% of Recovery	% RSD of Individual recovery
50%	4.98	637834	99.49	0.32
	4.99	640901	99.76	
	5.00	638058	99.12	
80%	7.95	1010522	98.73	0.86
	7.98	1013916	98.69	
	8.02	1034520	100.19	
90%	8.97	1145799	99.22	0.27
	8.99	1144773	98.91	
	9.00	1143363	98.68	
100%	10.00	1274461	98.99	0.12
	9.99	1275038	99.14	
	9.96	1272459	99.24	
120%	11.98	1526959	99.00	0.06
	11.99	1527580	98.96	
	12.00	1530572	99.07	
130%	12.98	1652022	98.86	0.28
	12.96	1651859	99.00	
	12.98	1661019	99.40	
150%	14.95	1911661	99.26	0.25
	15.00	1911226	98.97	
	14.99	1906121	98.77	
Average (n = 21)			**99.12**	-
Standard Deviation (SD)			0.37	-
%Relative Standard Deviation (%RSD)			0.37	-
Acceptance Criteria : % Recovery: (98 –102)% and %RSD: NMT 2.0%				

Table-7: Range study

Parameter	Concentration Range	Acceptance limit	Result
Linearity	10 % to 150%	R NLT 0.9990	R =1.0000
Method Precision	100%	%RSD of Assay NMT 2.0%	0.21%
Intermediate Precision	100%	%RSD of two analyst NMT 2.0 ± 2% Difference of each other	% RSD of 2 analyst =0.43% Difference of assay =0.74%
Accuracy	50% to 150%	% Recovery: (98 –102)% and %RSD: NMT 2.0%	% Recovery: 99.12 %, %RSD: NMT 2.0%

Table-8: Data of System suitability (Robustness study)

Parameter	% RSD of Area	Theoretical Plate Number	Tailing Factor
Flow rate + 2%	0.08%	7976	1.31
Flow rate - 2%	0.16%	8416	1.33
Column Temperature at 30˚C	0.08%	8537	1.30
Column Temperature at 35˚C	0.05%	8758	1.32
Buffer : HPLC grade Acetonitrile (63 : 37)	0.09%	8064	1.34
Buffer : HPLC grade Acetonitrile (67 : 33)	0.11%	8726	1.35
Buffer pH 7.40	0.05%	8338	1.35
Buffer pH 7.80	0.07%	7922	1.33

Table-9: Solution stability study

Duration of Sample Solution (Hour)	% of Assay	% Difference from initial
At initial (0 hr)	100.47	-
After 6 hrs	100.81	- 0.34
After 12 hrs	100.88	- 0.41
After 18 hrs	100.90	- 0.43
After 24 hrs	99.92	0.55
After 30 hrs	100.07	0.40
After 48 hrs	100.11	0.36
% RSD	0.42	-
Acceptance Criteria: ≤ 2.0% change from initial		

Figure-1: Graphical Representation of Linearity

Correlation Coefficient, R	1.0000

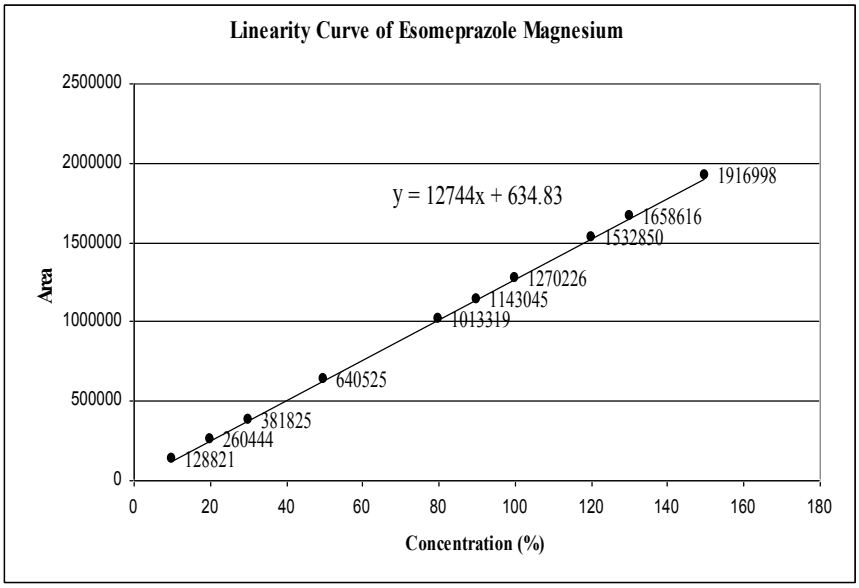
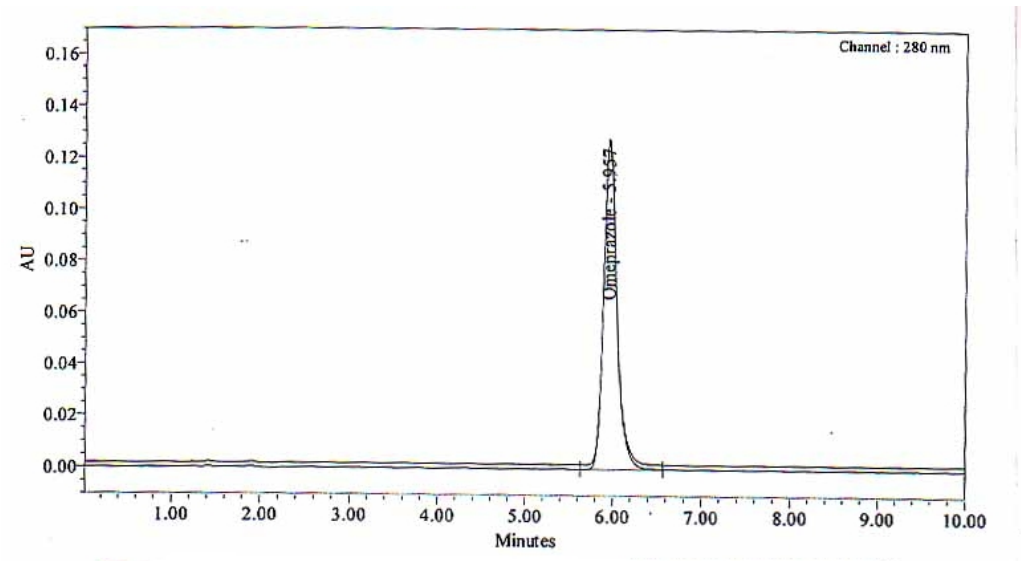


Figure 2: Representative HPLC chromatogram of Esomeprazole



Source of support: Nil, Conflict of interest: None Declared