International Journal of Drug Development & Research
| October-December 2012 | Vol. 4 | Issue 4 | ISSN 0975-9344 |
Available online http://www.ijddr.in
Covered in Official Product of Elsevier, The Netherlands
SJR Impact Value 0.03 & H index 2

Full Length Research Paper
Covered in Index Copernicus with IC Value 4.68 for 2010

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

Muhammad Tariq Khalil[1*], Muhammad Usman[1], Gul Majid Khan[2], Sattar Bakhsh Awan[3], Hafsa Bibi[4], Aisha Siddiqua[5]

1Mohi Ud Din Islamic Institute of Pharmaceutical sciences, Mirpur, AzadJammu & Kashmir
[2]Department of Pharmacy, Quaid e Azam University, Islamabad, Pakistan
[3]Faculty of Pharmacy; Gomal University, Dera Ismail Khan, Pakistan
[4]Department of chemistry, Gomal University, Dera Ismail Khan, Pakistan
[5]Department of chemistry, Gomal University, Dera Ismail Khan, Pakistan

Abstract

A reversed-phase high performance liquid chromatography (RP-HPLC) method was developed and validated for the estimation of esomeprazole in bulk and capsules dosage forms. The separation was achieved on C18 analytical column (250 mm × 4.6 mm i.d., 5.0 µm) using acetonitrile and phosphate buffer (pH 7.4 ± 0.05 adjusted with 5% potassium hydroxide) 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 302nm. The total chromatographic analysis time per sample was about 7.0min with esomeprazole eluting at retention time of about 6.5min. 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 25-150µg/mL with R^2 close to one (0.9991). The limit of detection (LOD) and limit of Quantitation (LOQ) obtained for esomeprazole were 0.015µg/mL and 0.04µg/mL, respectively. The developed and validated method was successfully applied for the quantitative analysis of Esoquin® capsules. The high recovery and low relative standard deviation confirm the suitability of the proposed method for the determination of esomeprazole in capsule dosage form.

*Corresponding author, Mailing address:
Muhammad Tariq Khalil, Lecturer
Mohi Ud Din Islamic Institute of Pharmaceutical sciences, Mirpur, Mohi Ud Din Islamic University, Nerain Sharif; Azad Jammu & Kashmir
e-mail: star82_hmtk@hotmail.com

Key words:

Analytical method development, Reversed phase HPLC method, ICH guidelines, Capsule dosage forms, Accuracy and precision

How to Cite this Paper:

Muhammad Tariq Khalil*, Muhammad Usman, Gul Majid Khan, Sattar Bakhsh Awan, Hafsa Bibi, Aisha Siddiqua "HPLC Method Development and Validation for the estimation of Esomeprazole in Bulk and Pharmaceutical Dosage Form" Int. J. Drug Dev. & Res., October-December 2012, 4(4): 252-256.

Article History:----------
Date of Submission: 03-10-2012
Date of Acceptance: 21-10-2012
Conflict of Interest: NIL
Source of Support: NONE

1. INTRODUCTION

Esomeprazole Magnesium (as trihydrate) belongs to the group of proton pump inhibitors (PPI). It is the enantiomer of omeprazole. Chemically it is 5-Methoxy-2- (S) [(4-methoxy-3, 5-dimethyl-2-pyridinyl) methyl] sulfinyl]-1H-benzimidazole magnesium salt trihydrate with molecular formula $C_{34}H_{36}MgN_6O_6S_2 \bullet 3H_2OC_{17}H_{18}N_3O_3S \bullet Na$.

Esomeprazole shows its pharmacological action by reducing the concentration of gastric acid by hindering enzyme action in gastric parietal cells, thus putting off movement of hydrogen ion into gastric lumen.(1)

The peak plasma concentration (C_{max}) for esomeprazole, after oral administration, can be achieved in 1.5 hours (t_{max}). The C_{max} for esomeprazole is directly proportional to the dose administered. The plasma protein binding for esomeprazole is approximately 97% which is constant over 2 to 20 µmol/L concentration range. It is metabolized by the cytochrome P450 (CYP) enzyme system of the liver. About 80% of the orally administered drug is excreted as inactive metabolites in the urine while the remaining inactive metabolites are found in feces.

Esomeprazole is indicated for various clinical conditions of the gastric problems. Mainly it is indicated for gastro esophageal reflux disease (GERD). Along with this, it is also used to treat stomach and small intestine ulcer, and heart burn. It is some time also considered as prophylaxis treatment of the esophageal cancer.

There are various methods in the literature for the qualitative and quantitative analysis of the esomeprazole in the bulk and the pharmaceutical dosage forms. The method was developed and validated under the light of International Conference on Harmonization (ICH) guidelines (2, 3). And for the statistical evaluation of results, standards guidelines were followed (4, 5). Hence, our aim was to establish an easy and convenient high pressure liquid chromatography (HPLC) technique, which not only useful for researcher but also for the analysts working in the pharmaceutical quality control labs.

2. MATERIALS & METHODS:

2.1 Chemicals & Reagents:

The working standards of Esomeprazole (99.95% purity) was received from Global Pharmaceuticals Pvt. Ltd, Islamabad, as gift sample. The Esoquin capsule (Vision Pharmaceuticals, Islamabad) claiming enteric coated granules equivalent to 40mg were purchased from the local pharmaceutical market. The acetonitrile used in the research was of HPLC grade while potassium Di-hydrogen phosphate, Di-potassium hydrogen phosphate, sodium hydroxide and potassium hydroxide were of high purity analytical grades. All the chemicals purchased from the local franchise of Sigma Aldrich.

2.2 Apparatus & Chromatographic Conditions:

An isocratic elution HPLC system of Shimadzu with LC20AD pump and SPD-20A UV-visible detector was used working via Lab-Solution software. The separation was carried on column (*thermolab)* with C_{18} packaging and 250 x 4.6mm dimensions (5µm internal diameter). The analysis of elution was completed at 302nm on ambient temperature. The run time was set at 10 minutes for this analysis at flow rate of 1ml/minute.

2.3 Preparation of Mobile Phase:

The mobile phase was prepared by mixing buffer and acetonitrile in 50:50 (v/v) ratios. For the preparation of buffer, 2.72g of potassium di-hydrogen phosphate and 0.525g of Di-potassium hydrogen phosphate were dissolved in distilled water and final volume was made up to 1000ml with same solvent. Then the pH of the buffer was adjusted to the 7.4 by using 5% solution of potassium hydroxide. Then, the buffer was mixed with acetonitrile. The final mobile phase was then filtered by passing through 0.5µm membrane filter and degassed before use.

2.4 Preparation of Standard Solution:

Standard solution was prepared by dissolving esomeprazole magnesium trihydrate equivalent to 100mg of esomeprazole in 100 mL of 0.1N sodium hydroxide solution (final concentration, 1 mg/mL). Then, 1ml of the above solution was diluted to 50ml using the mobile phase (final concentration, 20µg/mL). This solution was filtered through 0.2µm membrane filter and 20 µL of this solution was injected for HPLC analysis. Unknown assay samples were quantified based on the AUC of the above standard.

2.5 Capsule Sample Preparation:

For the assay of esomeprazole, 20 capsules were weighed; their contents were crushed into fine powder and mixed thoroughly. An amount of capsule powder equivalent to 100mg of esomeprazole was accurately weighed and transferred in a 100mL volumetric flask and 25 mL of 0.1N sodium hydroxide solution was added. This mixture was subjected to sonnication for 10 min for complete extraction of drugs and the solution was made up to the mark with 0.1N sodium hydroxide solution. Then, 1ml of the above solution was diluted to 50ml using the mobile phase. This solution was filtered through 0.2µm membrane filter and 20 µL of this solution was injected for HPLC analysis.

3. RESULTS & DISCUSSION:

3.1 System Suitability:

Before performing the main analysis, the system suitability was evaluated. For this purpose, various parameters were calculated as per their standard procedure e.g. retention time (for esomeprazole), theoretical plates number of the column (for column efficiency), tailing factor, relative standard deviation of peak area and retention time. The table 1 shows the result for these parameters. The column efficiency was much better for analysis i.e. ≥2000. The tailing factor was also within range i.e. ≥1.2. Moreover, the calculated relative standard deviation for the retention time and peak area (mean of 6 replicates) also within acceptance criteria. Depending on all these information, it reflects that the proposed method will be suit able for routine analysis.

3.2 Accuracy:

In order to check the accuracy of the method, solution of esomeprazole with different concentration (25, 50, 75, 100, 125, and 150%) was prepared and then analyzed over HPLC with the help of developed method. During this step, six samples of each concentration were prepared and their mean was used for further calculations. These calculations (percentage recovery) are shown in the table 2. The results in the given table show that the recovery of esomeprazole from the prepared samples ranges from 99.40% to 100.56% i.e. within ±1% range. Moreover, the RSD (relative standard deviation) also lies within acceptance range i.e. ≤2.0. These all observations and calculations indicate the method's accuracy due to narrowness of theoretical and actual yields.

3.3 Precision:

The precision of the method was checked by inter day and intraday repeatability and reproducibility. The repeatability of method was analyzed by replicate analysis (n=6) by injecting the sample solution into the HPLC system. The results are shown in the table 3 which indicates that the proposed method is good with high precision. Moreover, the low RSD values indicate the high degree of correctness of method.

Similarly, for reproducibility was checked by replicate analysis (n=18) of samples over 3 consecutive days. From results (given in table 3), the low calculated RSD reflects that the method has a good inter-day reproducibility.

3.4 Linearity:

The linearity of the method was checked by preparing different strengths solution of esomeprazole from 25% to 150%. Then, a linear regression equation was derived by plotting the graph between the sample dissolved and recovered by the method. From the

observation and calculation (given in table 4), it is cleared that the correlation coefficient (R^2) equal to unity and comes under t he acceptance criteria ($R^2 \geq 0.999$). Moreover, the calculated Y-intercept is 0.1687 which is also less than ±2%. Therefore, depending upon calculated values of R^2 and Y-intercept, the developed method should be considered having a high degree of linearity.

3.5 Limit of Quantification (L.O.Q) and Limit of Detection (L.O.D):

Calibration curves were constructed in a very low concentration region (0.05 to 1.0% of the target concentration) of esomeprazole (0.10 to 0.20µg/mL) for the calculation of the limit of detection (LOD) and the limit of quantification (LOQ) using Eqs. (1) and (2), respectively.

$$LOD = \frac{3.3\sigma}{S} \quad (1)$$

$$LOQ = \frac{10\sigma}{S} \quad (2)$$

Where σ is the residual standard deviation of the regression line, S is the slope of the standard curve. The LOD and LOQ obtained for esomeprazole were 0.015µg/mL and 0.04µg/mL, respectively".

3.6 Application to Pharmaceutical dosage Form:

The proposed method was also applied to the pharmaceutical dosage (Capsules in this case) form of the esomeprazole. For this purpose 3 batches were selected and 6 replicates of each batch were analyzed by the HPLC, from the results (Table 5), it was observed that the obtained results are in good agreement with the claimed amount of esomeprazole by the manufacturer.

4 Conclusion:

A simple isocratic RP-HPLC method has been developed for the determination of esomeprazole in bulk and capsule dosage form, using a UV detector. The method was validated for accuracy, precision, specificity and linearity. The method has a relatively short run time (6.5min) that allows quantifying a large number of samples in routine and quality control analysis of capsules. In order to reduce cost of analysis and to increase sample throughput during routine analysis, the method is being further optimized, employing statistical experimental design.

Table 1: System suitability

Sl. No.	Parameters	Esomeprazole
1	Retention time (min)	6.5
2	Plate number	3225
3	Tailing factor	0.982
4	RSD of peak area (n=6)	0.88
5	RSD of retention time (n=6)	0.90

Table 2: Accuracy of Method

S. No:	Concentration level (%age)					
	25	50	75	100	125	150
1	99.65	99.81	99.77	100.33	100.11	99.73
2	99.72	100.21	99.87	99.90	100.24	99.81
3	100.0	100.24	99.69	99.78	100.40	100.36
4	100.23	100.35	100.34	100.29	99.79	99.95
5	99.44	99.67	100.56	100.09	99.83	99.48
6	99.87	100.11	99.40	100.05	99.65	100.30
Mean	99.81	100.06	99.43	100.07	100.00	99.93
%RSD	0.28	0.27	1.42	0.21	0.34	0.34

Table 3: Inter day and intraday precision of the method

S. No:	Recovery (%age)		
	Day 1	Day 2	Day 3
1	100.33	100.72	100.28
2	99.56	100.57	100.64
3	99.78	100.34	100.20
4	99.94	100.66	99.76
5	100.11	99.87	99.92
6	100.44	99.56	99.82
Mean	100.02±0.33	100.29±0.46	100.10±0.33
Inter day (n=18)	100.136±0.14		

Table 4: Linearity of the Method

S. No:	Drug Dissolved	Drug Recovered
1	25	25.21
2	50	50.11
3	75	74.90
4	100	100.12
5	125	125.33
6	150	149.88
Correlation Coefficient (R^2)=0.9991		
Y-intercept=0.1687		
Regression Equation: 0.1687+0.9991x		

Table 5: Assay Results of 4mg Esomeprazole Capsule (Esoquin)

B. No:	Drug Recovered (mg)±SD
1	40.21±0.134
2	40.11±0.564
3	34.96±0.883

Note: n=6; SD=Standard Deviation

References:

1) Michael, D.R., Stephen, P.H.A., & David, A.K., *Pharmacology*, Pharmaceutical Press, London, Chicago 2009.
2) International Conference on Harmonization, Guideline on Validation of Analytical Procedure-Methodology, Geneva, Switzerland, 1996.
3) Pharmaceutical Process Validation; 2nd edition, Editors: I. R. Berry and R.A. Nash, 1993
4) Guidelines on General Principles of Process Validation, CDER, US-FDA 1987
5) ICH, Q2 (A). Validation of analytical procedures: text and methodology International Conference on Harmonization. Geneva: 2005:1- 13.