Development and Validation of Analytical Method for Determination of Esomeprazole in Pharmaceutical Effluents Using Reverse Phase High Performance Liquid Chromatography

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ABSTRACT

A simple, sensitive and accurate analytical has been developed to estimate esomeprazole in pharmaceutical effluents, which are releasing from the pharmaceutical industries into aquatic environment by using RP-HPLC with UV detection. the developed method is highly reproducible and sensitive to determine the esomeprazole in less than 10 ppm level. A reversed-phase high performance liquid chromatography (RP-HPLC) method was developed and validated for the estimation of esomeprazole in effluents or pharmaceutical industry washouts. The separation was achieved on C18 Gemini NX column (150mm × 4.6mm i.d., 5.0µm) using a mixed buffer of sodium dihydrogen phosphate monohydrate and dibasic sodium phosphate anhydrous having a pH of 7.3 as buffer, and the mobile phase is a mixture of 350 mL buffer: 500 mL acetonitrile: 150 mL of water in isocratic mode as mobile phase and at a flow rate of 0.8 mL/min. Detection was carried out using a UV detector at 302 nm. The total chromatographic analysis time per sample was about 10.0 min with esomeprazole eluting at retention time of about 5.0 min. The method was validated for accuracy, precision, specificity, linearity, and sensitivity. Validation studies demonstrated that this HPLC method is accurate, specific, rapid, reliable, and reproducible. Linearity was observed for esomeprazole in the concentration range of 0.025–10 µg/mL (R2 > 0.95). The limit of detection (LOD) and limit of quantitation (LOQ) was found to be 0.003 µg/ml and 0.009 µg/ml respectively for esomeprazole, the method was validated as per ICH guidelines. The RSD for intra-day and inter-day precision were found to be less than 5%. The percentage recovery was in good agreement and the method is simple, specific, precise, and accurate for the determination of esomeprazole in the pharmaceutical industry washouts.

Key Words: *Esomeprazole, Estimation of residual amount, liquid chromatography*

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INTRODUCTION

The pharmaceutical production has begun in large scale many years ago, however in recent years, the scientific studies has been focused more on the waste generation and its effect on the environment. Several studies have been confirmed that the presence of pharmaceutical substances in the water bodies of different aquatic environments like lakes, drinking water etc. the ongoing urbanization and population growth has further increased the requirement for clean water, which leads to the increase in the volume of effluent to be treated.

On the other hand, as the need increases for new products across several therapeutic areas like antibiotics, anti-retroviral, anti-cancer etc., the contamination of domestic water further increases by producing large volumes of pharmaceutical products. Recent studies confirmed that

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antibiotics and anti-retroviral were ranked among the pharmaceutical substances as major risk group because of their high toxicity to algae and bacteria, even at low level concentrations. These risks not only cause an increase in the occurrence of fatal cases of hospital borne infections with such pathogens, but also develop resistance towards antibiotics.

Studies on the published databases of different environmental agencies show inconsistency in monitoring of the waste management and their non-compliance to the effluent discharge regulations. Inspections in several industries has been revealed that the waste management is in its preliminary stage and large volume production is causing more inconsistency in effluent composition. The inconsistency in the composition of waste highly affects the treatment systems as well as the different analytical techniques employed for their estimation during effluent treatment.

As there is very limited scientific work on the determination of pharmaceutical substances, the author has selected the esomeprazole, one of the drugs with large volume consumption in the pharmaceutical therapeutic category, and developed a simple and rapid reverse phase chromatographic analytical method. The same can be employed for the estimation of the drug substance in various pharmaceutical washouts or effluents.

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 of $C_{34}H_{36}MgN_6O_6S_2 \cdot 3H_2O$ $C_{17}H_{18}N_3O_3S \cdot Na$. 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 (fig. 1) 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 sometimes 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, standard guidelines were followed [4, 5]. Hence, our aim was to establish an easy and convenient high-pressure liquid chromatography (HPLC) technique, which is not only useful for researchers but also for the analysts working in the pharmaceutical quality control labs. However, no HPLC analytical method has been reported for estimation of residual esomeprazole in industrial wastes.

Upon reviewing the literature, there are no HPLC methods reported for the determination of esomeprazole in pharmaceutical effluents at ppm (part per million) to nano level. However, only one spectrophotometric method is available, which is not highly sensitive method to detect the drug substance at the lower concentration due to its operational limitations, that is, it can't detect the drug substance below mg/mL concentration due to lower absorbance, where reproducibility is the issue. In the present work, the author has developed a simple, rapid, accurate and sensitive HPLC method for the determination of esomeprazole by using HPLC technique, and the method has been validated as per ICH guidelines [27].

MATERIALS AND METHODS

- **Reagents& Chemicals:**

Sodium dihydrogen phosphate, dibasic sodium phosphate, acetonitrile (HPLC grade), and ortho phosphoric acid were obtained from Merck (India). All chemicals were of an analytical grade and used as received.

Experimental

- **Instrumentation:**

Chromatographic separation was achieved by a Waters 2489 UV 2695 pump, Waters 2998 PDA 2695 pump Software Empower2 photodiode array detector was used.

Buffer preparation

Buffer was prepared by dissolving 13.7 g of sodium dihydrogen phosphate and 7.0 g of dibasic sodium phosphate anhydrous in 1000 mL of purified water. The pH was adjusted to 7.3 (±0.05) with dilute ortho phosphoric acid solution. The solution was filtered through 0.45 µm membrane filter.

Mobile phase preparation

A filtered and degassed mixture of buffer, acetonitrile and water in respectively the ratio of 350:500:150 v/v was prepared.

Diluent preparation

A degassed mixture of borax solution (prepared by dissolving 3.8 g/L of borax) and ethanol was used in the ratio of 20:80 v/v.

Preparation of Swab Blank:

Transfer 10mL of diluent into a clean test tube. Place one cleaned Swab in the test tube and sonicate for 5 minutes. Filter through 0.45µm PVDF filter.

Standard preparation:

About 25.0 mg of omeprazole (omeprazole and esomeprazole are optical isomers and they have same physico-chemical properties except the difference in optical rotation. Since esomeprazole is highly hygroscopic, omeprazole was used as standard) was accurately weighted and transferred each into a 100 mL volumetric flask; 60 mL of mobile phase was added to the flask, and sonicate to dissolve. The solution was cooled to room temperature and diluted to volume with diluent. 2 mL of the above solution was transferred into a 100 mL volumetric flask, diluted to volume with diluent, and mixed. since esomeprazole is highly hygroscopic and both omeprazole and esomeprazole are optical isomers, omeprazole was used as standard as omeprazole is more stable than esomeprazole.

Sensitivity Standard preparation:

5 mL of the standard solution was transferred into a 50 mL volumetric flask, diluted to volume with diluent, and mixed.

Sample preparation

The effluent samples were collected from different locations, diluted with diluent and sonicated for 5 min.

Chromatographic conditions

A Gemini NX- C-18 (Make: Phenominex, 150 mmx4.6 mm I.D; particle size 5 µm) Column was used for analysis at ambient column temperature. The mobile phase was pumped through the column at a flow rate of 0.8 mL/min. The sample injection volume was 10 µL. The photodiode array detector was set to a wavelength of 302 nm for the detection and chromatographic runtime was 10 minutes.

RESULTS AND DISCUSSION

Method development [27-28]

To develop a suitable and robust LC method for the determination of esomeprazole, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Symmetry C-18 (Make: Waters; 150mm x 4.6mm I.D; particle size 3 µm with the following mobile phase). About 2.72 g of potassium di-hydrogen phosphate monohydrate was added to 1000 mL of purified water and mixed. The pH was adjusted to 3.0 (±0.05) with dilute Ortho phosphoric acid solution. The solution was filtered through 0.45 µm membrane filter. A filtered and degassed mixture of buffer and acetonitrile was prepared respectively in the ratio of 500:500 v/v.

All peaks were not separated and the peak of esomeprazole was eluted closely with blank peak. For next trial, the mobile phase composition was changed slightly. The mobile phase composition was buffer and acetonitrile in isocratic mode. The observed peaks were a little separated, but the peak shape was a little broad.

Again, the mobile phase was modified by changing a mixed buffer with acetonitrile and water in isocratic mode at flow rate of 0.8 mL/min. UV detection was performed at 302 nm. The retention time of esomeprazole was about 5 min. and the peak shape was good (Fig 4.).

The chromatogram of esomeprazole with all components using the proposed method is shown in Fig-4. System suitability results of the method are presented in Table 1. Esomeprazole shows significant UV absorbance at Wavelength of 302 nm. Hence this wavelength has been chosen for detection in analysis of residue in esomeprazole.

3.0 Method Validation

3.1 System Suitability

To demonstrate system suitability, the standard solution prepared as per method and injected six replicate injections into the HPLC system as per methodology. The system suitability parameters were evaluated from the standard solution and found to be within the acceptance criteria. The % RSD for esomeprazole peak areas from six replicate injections of standard solution was found to be within the limits. The results are summarized in Table 1 and Figures 2, 3 and 4.

Specificity

Blank interference

A study was conducted to demonstrate the noninterference of swab; triplicate swab blanks of each plate (Stainless steel (SS) plate,) were prepared and injected into the HPLC system as per the proposed test method. The interference of swab blanks was evaluated at the retention time of esomeprazole peak and no peaks were found at the retention time of esomeprazole peak. Typical chromatogram shown figures 2 and 5 indicate that there is no interference of blank at the retention time of esomeprazole.

Establishment of Limit of Detection and Limit of Quantification

A study was conducted to establish the limit of detection (LOD) and limit of quantification (LOQ) of esomeprazole based on slope method. The series of solutions were prepared from 1% to 200% of standard concentration of esomeprazole. These solutions were injected into the HPLC system as per methodology.

A graph was plotted by taking concentration on X-axis and area on Y-axis; the standard error and slope of the

calibration curve were calculated. The predicted LOQ concentration and LOD concentration are calculated by using formula given below. The results are summarized in the Table 2.

$$LOQ = \frac{10 \times \sigma}{S}, \quad LOD = \frac{3.3 \times \sigma}{S}$$

σ = Standard Error of the calibration curve
S = Slope of the calibration curve

LOQ Precision

Precision at LOQ concentration was established for esomeprazole. Six samples were prepared by diluting standard stock solution to obtain the LOQ concentration and injected in to HPLC system as per methodology. The % RSD for esomeprazole was calculated in ppm. The results were found to be within the acceptance criteria and the data were summarized in Table 2.

Linearity

Linearity is carried out under LOD-LOQ establishment experiment; the same linearity establishment data can be used to deduce the linearity from LOQ level to 200% specification level. A graph was plotted to concentration in ppm on X-axis versus response on Y-axis. % y-intercept and correlation coefficient were calculated. The results and the linearity graph is presented in Figure 6 and Table 3.

Method Precision (Repeatability)

A study of repeatability of esomeprazole from the surfaces was conducted in six preparations by spiking 1 mL of esomeprazole sample stock solution over 10'' x 10" sq. cm stainless steel (SS) plate. The sample was dried, by blowing warm air. The stainless-steel surface was swabbed horizontal, vertical, and diagonal. The liquid absorbed by swabs was squeezed out into test tube having 10 mL of diluent, mixed, sonicated for 5 minutes and swabs were discarded. The solution was injected into HPLC system as per methodology. The results are summarized in Table 4.

Intermediate Precision

The intermediate precision of same sample was performed by different analyst, different instrument, different column and on different day.

Six samples were prepared by spiking 1 mL of esomeprazole sample stock solution over 10'' x 10" sq. cm stainless steel (SS) plate. The sample was dried by blowing warm air. The stainless-steel surface was swabbed horizontal, vertical, and diagonal. The liquid absorbed by swabs was squeezed out into test tube having 10 mL of diluent, mixed, sonicated for 5 minutes and swabs were discarded. The solution was injected into HPLC system as per methodology. The results are summarized in Table 5 and representative chromatogram was presented in fig 7.

Accuracy

A study of recovery of esomeprazole from the surfaces was conducted in triplicate preparations by spiking 1 mL of esomeprazole to obtain about 2.5 ppm (for 50% recovery), about 5 ppm (for 100% recovery) and about 7.5 ppm (for 150% recovery) from sample stock solution over 10'' x 10" SS plate. The sample was dried by blowing warm air. The sample swabbed horizontal, vertical, and diagonal. The liquid absorbed by swabs was squeezed out into test tube having 10 mL of diluent, mixed, sonicated for 5 minutes and swabs were discarded. The solution was injected into HPLC system as per methodology. The results are summarized in Table 6 and representative chromatogram was presented in fig 8.

Solution Stability

A study to was conducted establish bench top stability of esomeprazole in sample solution and standard solution at initial, 1 day and 2 days. The % recovery of esomeprazole in sample solution and standard solution was estimated each time against freshly prepared standard. The difference in % recovery of standard and sample solutions from initial to 1 day and 2 days was calculated each time against freshly prepared standard and results are summarized in Table 7.

Bench Top Stability of Mobile Phase:

A study was conducted to establish the bench top stability of mobile phase at Initial, day 3. The mobile phase was prepared as per the test method, analyzed, and kept on bench top in well closed condition. Standard solution was prepared as per test method and injected into HPLC system with the mobile phase kept on bench top at day 3. The System suitability parameters were found to be within the limits. The results are summarized in Table 8.

Robustness

Similarly, robustness also was evaluated and found that the method is robust enough for various robustness parameters such as flow variation, column temperature variation, and mobile phase composition variation.

All the system suitability criteria are meeting all the robust parameters; this indicates that the proposed analytical method is robust enough for the estimation of esomeprazole by using the analytical method.

CONCLUSION

A simple, economic, accurate and precise HPLC method was successfully developed. This method was carried out by using Gemini Nx-C18, (150× 4.6mm) with 5 µm particle size. Injection volume of 10 µl is injected and eluted with the mobile phase in the ratio of buffer (pH was adjusted to 7.3 with dilute ortho phosphoric acid)

acetonitrile and water in the ratio of 350:500:150 v/v as mobile phase over isocratic program, which is pumped at a flow rate of 0.8 ml/min. Detection was carried out at 302 nm. All the compounds were well resolved from blank peak and there was no interference from blank. The results obtained were accurate and reproducible. The method developed was statistically validated in terms of selectivity, accuracy, linearity, precision, robustness, stability of solution and mobile phase stability.

For Selectivity, the chromatograms were recorded for standard and sample solutions of esomeprazole. Selectivity studies revealed that the peaks were well separated from each other. Therefore, the method is selective for the determination of esomeprazole.

The limit of detection (LOD) and limit of quantitation (LOQ) was found to be 0.017 µg/ml and 0.053 µg/ml respectively for dilute gravir Sodium. The linearity results for esomeprazole in the specified concentration range are found satisfactory, with a correlation coefficient greater than 0.95. Calibration curve was plotted and correlation co-efficient for esomeprazole was found to be more than 0.95.

The accuracy studies were shown as % recovery for esomeprazole at 50%, 100% and 150%. The limit of % recovered shown is not less than 80% and the results obtained were found to be within the limits. Hence, the method was found to be accurate. The accuracy studies showed % recovery of the esomeprazole in the range 93-100% respectively.

For Precision studies six (6) replicate injections were performed. % RSD was determined from the peak areas of esomeprazole. The acceptance limit should be not more than 10 %RSD, and the results were found to be within the acceptance limits. For intermediate precision, the bias is not more than ± 1.0.

Hence, the chromatographic method developed for esomeprazole is rapid, simple, sensitive, precise, and accurate. Therefore, the proposed method can be successfully applied for the routine analysis of the active pharmaceutical ingredients for assurance of its presence in pharmaceutical effluents.

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Table 1: Summary of system suitability

S. No.	Name of the compound	Tailing factor	Theoretical plates
1	Omeprazole	1.1	7339

Table 2. Precision at LOD and LOQ for Esomeprazole

Sample No.	LOD Concentration (ppm)	LOQ Concentration (ppm)
	Area Response	
1	1700	7211
2	2001	7344
3	1600	7101
4	1500	7634
5	1652	7642
6	1647	7069
Average	**1683**	**7334**
% RSD	**10.08**	**3.47**

Table 3- : Linearity

Esomeprazole				
Name of the level	Concentration			Area Response
	% level	in %	in ppm	
Level-1	loq	0.0025	0.025	7334
Level-2	10	0.005	0.500	16612
Level-3	50	0.025	2.500	80698
Level-4	100	0.050	5.000	161395
Level-5	150	0.075	7.500	234591
Level-6	200	0.100	10.000	322172
			Slope	3193730
			Intercept	69
			Res sum of squ	3028
			CC (r)	0.9998
			RSQ (r2)	0.9995
			LOD	0.003
			LOQ	0.009

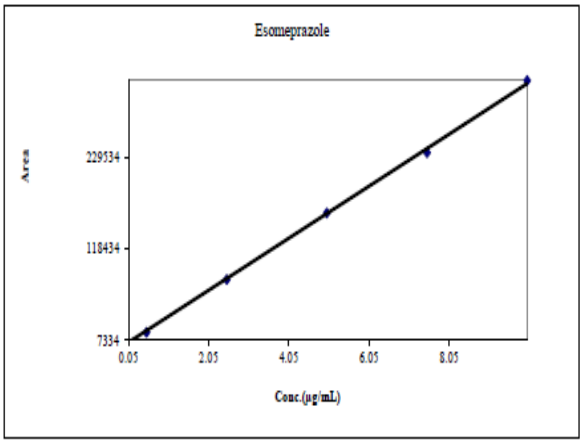

Table 4: Method Precision (Repeatability)

Sample No.	% Recovery
	Esomeprazole
1	95.8
2	101.2
3	97.6
4	100.4
5	98.6
6	99.4
Average	**99.0**
% RSD (Limit NMT 10.0)	**1.98**

Table 5: Intermediate Precision (Repeatability)

Sample No.	% Recovery
	Esomeprazole
1	94.9
2	95.1
3	97.7
4	93.9
5	89.4
6	93.4
Average	**94.0**
% RSD (Limit NMT 10.0)	**2.90**

Table 6: Recovery on SS plate for Esomeprazole

Sample No.	% Recovery		
	50%	100%	150%
1	100.7	94.5	93.7
2	96.9	99.2	98.4
3	94.8	97.6	95.9
Average (NLT 80.0%)	**97.47**	**97.10**	**96.00**
% RSD (NMT 10)	**3.1**	**2.5**	**2.4**
Overall Average	**96.9**		

Table 7: Bench Top Stability of Standard and Sample Solution for Esomeprazole

Time in days	% Assay of Standard preparation	Difference from Initial (NMT 2.0)	% Assay of sample preparation		Difference from Initial (NMT 2.0)	
			Sample-1	Sample-2	Sample -1	Sample-2
Initial	99.1	-	99.5	100.6	-	-
1	99.2	0.1	98.2	99.2	0.7	1.4
2	99.7	0.6	99.1	98.9	0.4	1.7

Table 8: Bench Top Stability of Mobile Phase

System Suitability Parameters	Initial	Day-3	Acceptance Criteria
The USP Tailing factor for Esomeprazole from standard solution.	1.1	1.1	NMT 2.0
The USP plate count for Esomeprazole peak from standard solution.	7269	7273	NLT 2000
% RSD for the peak areas of Esomeprazole peak areas from six replicate injections of standard solution.	0.6	1.4	NMT 5.0

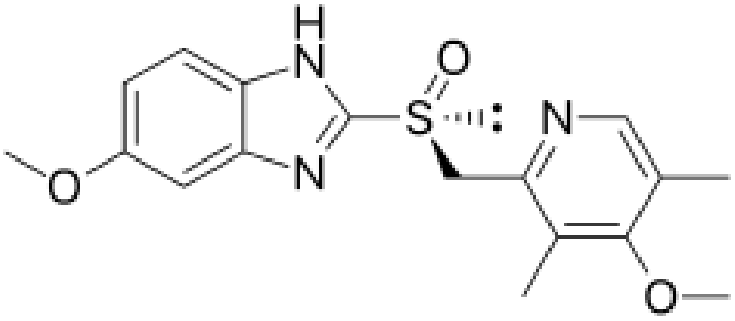

Fig. 1: Chemical structure of Esomeprazole

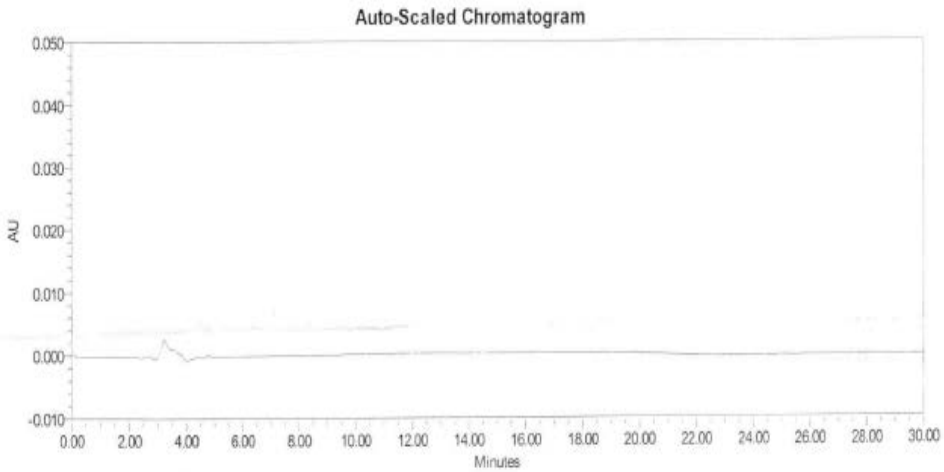

Fig. 2: Representative chromatogram for Blank

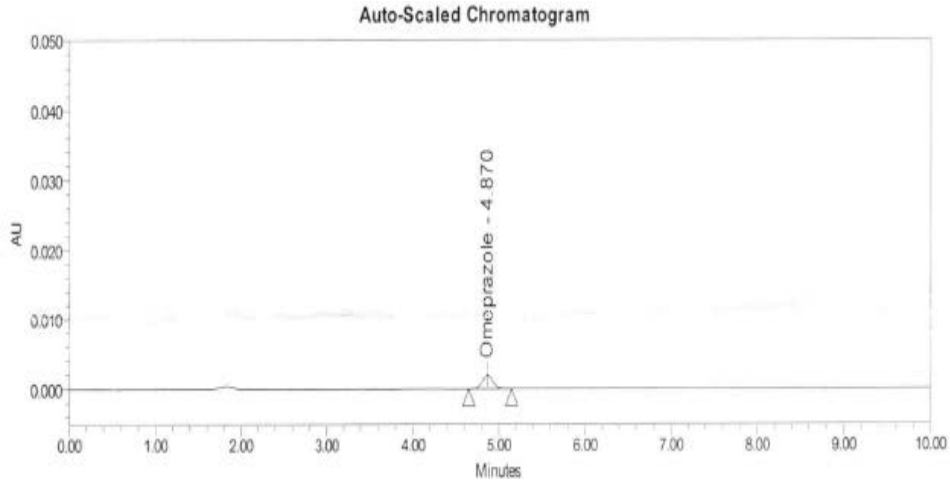

Fig. 3: Representative chromatogram for Sensitivity Solution

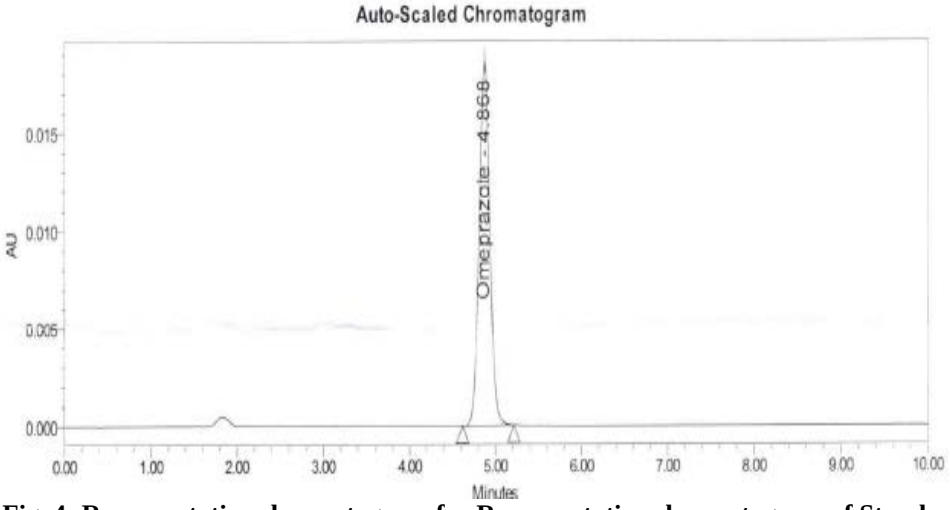

Fig. 4: Representative chromatogram for Representative chromatogram of Standard

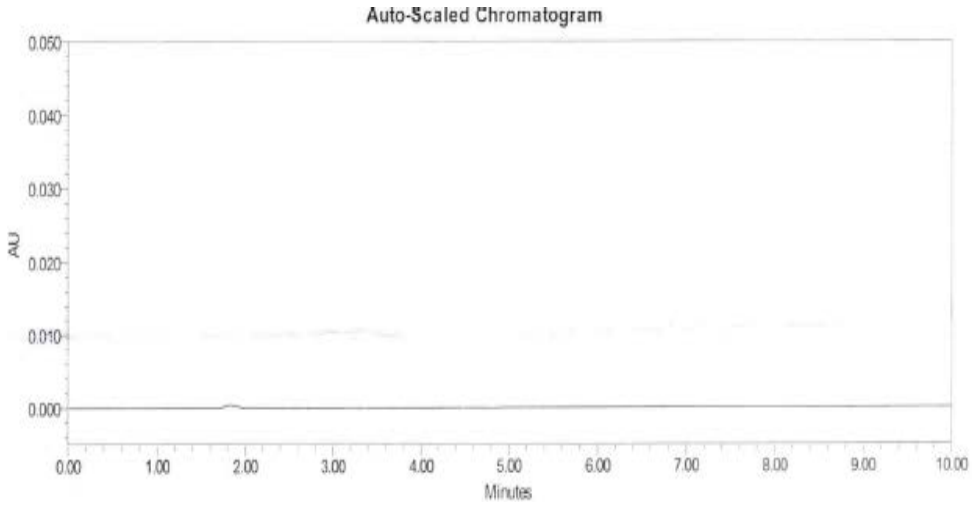

Fig. 5: Representative chromatogram for Representative chromatogram for Swab Blank

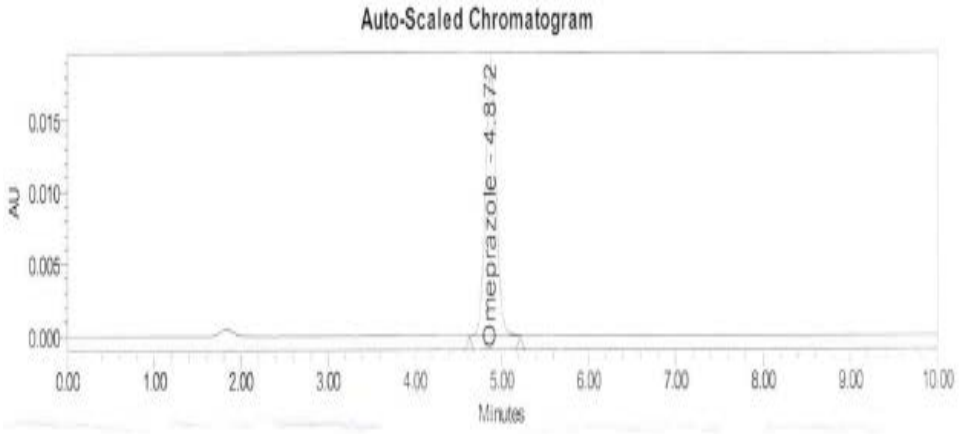

Fig. 6: Representative chromatogram for Linearity

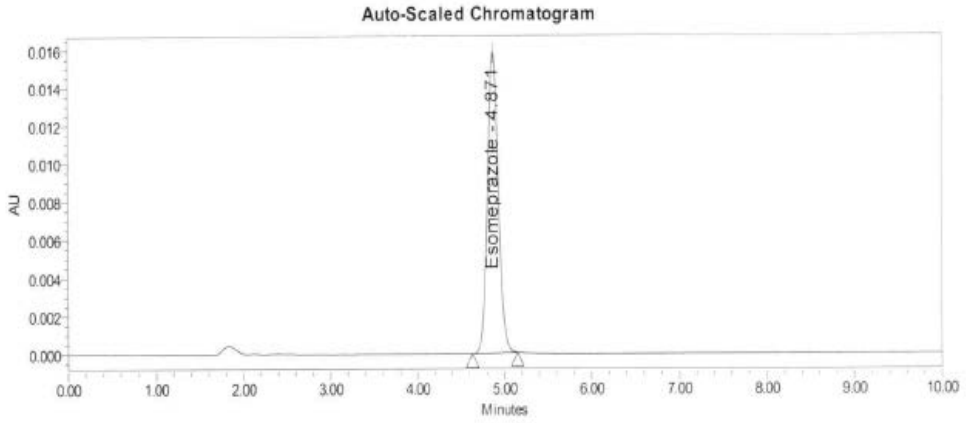

Fig. 7: Representative chromatogram for method precision

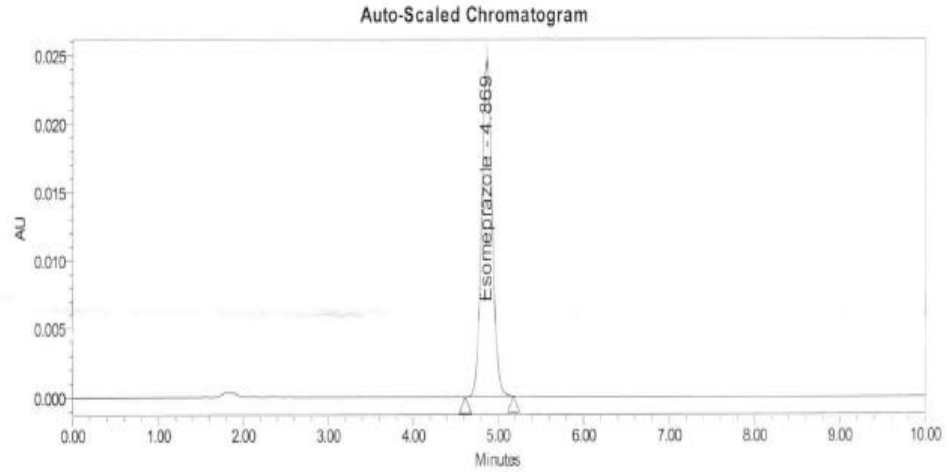

Fig. 8: Representative chromatogram for recovery at 150% level