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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ESOMEPRAZOLE IN BULK AND TABLET DOSAGE FORM BY RP-HPLC

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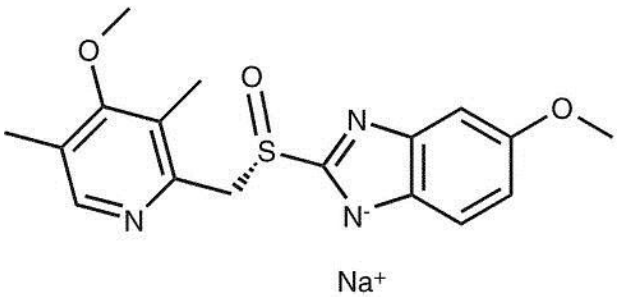
ABSTRACT
A simple, rapid, accurate, precise and reproducible Reverse Phase HPLC method was developed for the quantitative estimation of Esomeprazole in bulk and tablet dosage form. Esomeprazole is used as antiulcerative. The chromatographic separation was achieved with 250 x 4.6 mm, i.d. 5 µm C-18 column using methanol: water (80:20 v/v) at the flow rate of 0.8 ml/min at 302 nm. The linearity range was found to be 20-60 µg/ml for Esomeprazole. The coefficient of correlation for Esomeprazole was found to be 0.9962. The percent recovery obtained for Esomeprazole was found to be 98.64% the method was validated for linearity, range, precision, accuracy, specificity, selectivity, intermediate precision, ruggedness, robustness, stability and suitability.

KETWORDS: coefficient of correlation, Esomeprazole, intermediate precision.

INTRODUCTION (1-4)
Esomeprazole magnesium trihydrate (Esomeprazole) is used as antiulcerative in treatment of zollinger- Ellison syndrome. Chemically, Esomeprazole (ESO) is chemically bis (5-methoxy-2[(S)-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1-H-benzimidazole-1-yl)magnesium trihydrate, a compound that inhibits gastric acid secretion. Esomeprazole is the isomer of omeprazole, the first single optical isomer proton pump inhibitor. It is cost effective in the treatment of gastric oesophageal reflux diseases. Esomeprazole is official in The Merck Index, Martindale, The Extra Pharmacopoeia and I.P. Literature survey reveals that many analytical methods such as UV spectrophotometric, TLC, GC and HPLC methods are reported for determination of Esomeprazole individually from pharmaceutical dosage form. This paper represents simple, rapid, accurate, precise, reproducible and economic RP-HPLC method for estimation of ESO in bulk and tablet dosage form.



Esomeprazole

MATERIALS AND METHOD (2-5)
Reagents and Materials
Chemicals and reagents: Standard gift samples of Esomeprazole were procured from Glenmark Pharma Ltd., Methanol (HPLC grade) and Water (HPLC grade) was obtained from Merck Laboratories Pvt. Ltd., Mumbai.

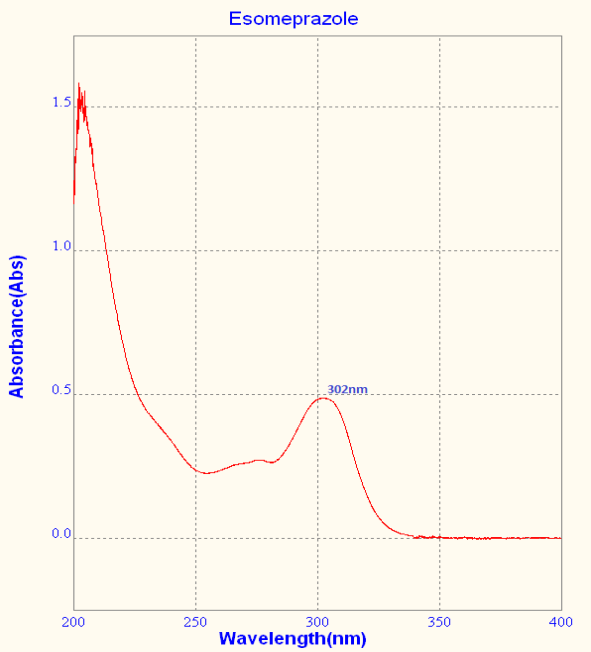
Instrument
HPLC 3000 Analytical Technologies Ltd. binary gradient pump and UV detector is used. The HPLC system consists of an. The system was controlled through HPLC Workstation software using Hexon C18 (4.6 x 250 mm, 5µm) column

Preparation of mobile phase
The mobile phase was composed of methanol: water (80:20 v/v). and pH3 adjusted using O-Phosphoric acid

Chromatographic conditions
The HPLC system consists of an. The system was controlled through HPLC Workstation software using Hexon C18 (4.6 x 250 mm, 5µm) column maintained at 30°C temperature and at flow rate 1.0 ml/min. The measurements were done with UV detection at 302 nm. The mobile phase was composed of methanol: water (80:20 v/v). The mobile phase was kept in ultrasonicator for 30 min. and filtered through a 0.45 µm nylon membrane filter with 8 min run time.

Determination of wavelength of maximum absorbance

The standard solutions of Esomeprazole were scanned in the range of 200 -400 nm against mobile phase as a blank. Esomeprazole showed maximum absorbance at 302 nm. So the wavelength selected for the determination of Esomeprazole was 302 nm.



UV Absorbance Spectra of Esomeprazole at 302nm

Standard Stock Solutions

For HPLC analysis 10 mg of Esomeprazole powder was weighed accurately using wencer high precision balance (readability 0.01 mg) and transferred in to 10 mL volumetric flask, dissolved and diluted to 10 mL with mobile phase to produce stock solution containing 1000 µg/ mL of Esomeprazole. Form this stock solution further dilutions prepared 0.2, 0.3, 0.4, 0.5, 0.6 ml stock solution take and make up the volume up to 10 ml gives 20,30,40,50,60, µg/ mL concentration respectively

Calibration curve of Esomeprazole

Appropriate aliquots of standard stock solutions of ESO were diluted with mobile phase to obtain concentrations in the range of 20,30,40,50 and 60 µg/ml of ESO. The linearity of ESO was found to be in the concentration ranges of 20-60 µg/ml, (Table 1). The coefficient of correlation was found to be 0.9994 for ESO (Table 1). The mixed standard solution containing10 µg/ml of ESO was prepared from standard stock solution and injected into HPLC system.

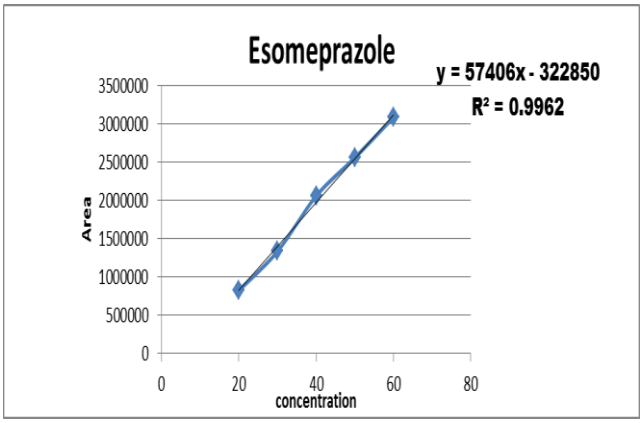


Figure 1: Linearity plot of Esomeprazole

Table 1: Linearity Concentration range and Area

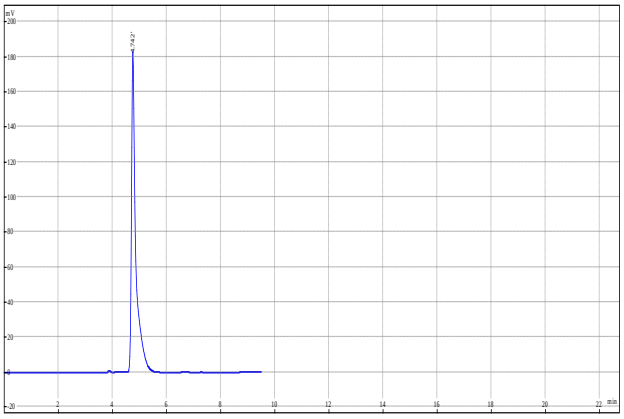
Concentration	Area
20	826351
30	1337042
40	2059506
50	2557828
60	3086270

System Suitability

In order to determine the adequate resolution and reproducibility of the proposed methodology, suitability parameters including retention time, asymmetry factor, %RSD of retention time and peak areas were investigated. The results are summarized in Table 2.

Table 2: System Suitability

Parameter	Esomeprazole
Linearity Range (ug/ml)	20-60
Correlation of coefficient	0.9962
Limit Of Detection	2.65 µg/ml
Limit of quantitation (ug/ml)	6.2 µg/ml
Retention Time (min)	4.75
Tailing Factor	1.33
Therotical Plates	6688



Time	Conc.	Area	Resolut.	T.Plate Num	Asymmetry
4.742	100	2059506	0.00	6205	1.27

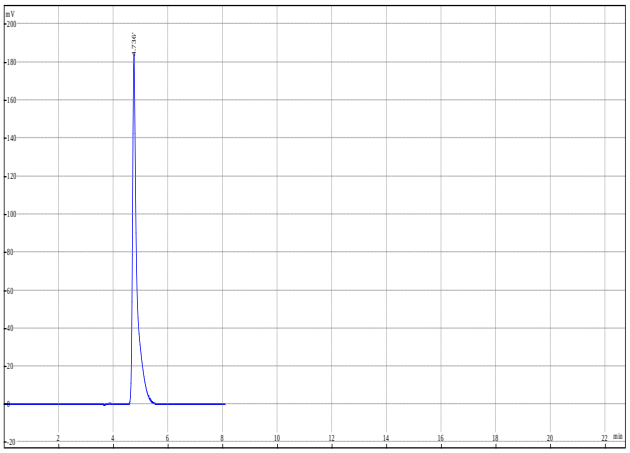
Figure 2: Typical Chromatogram of Standard ESO

Analysis of tablet formulation

Each tablet strength contains 20 mg of ESO. Twenty tablets were weighed and crushed into the glass mortar to obtain fine powder. The powder sample equivalent to 10 mg of ESO was weighed and transferred into a 100 ml volumetric flask and dissolved in 50 ml methanol HPLC grade. The flask was kept in an ultrasonic bath for 20 min. The volume was adjusted to 100 ml with methanol. HPLC grade. The solution was filtered through 0.2 µ nylon membrane filter. From this stock solution, 4 ml solution was pipetted out and transferred to 40 ml and made volume up to the mark with mobile phase to get the concentration 40 µg/ml of ESO. The solution was injected into HPLC system (Fig.3). The results of the assay of tablet formulation and its statistical validation data are given in Table 3.

Table 3: Assay of tablet formulation

Sr. NO.	% Composition	Area of Standard	Area of Sample	% Assay
1	**% Assay**	2059506	2056223	99.84059



Time	Conc.	Area	Resolut.	T.Plate	Num Asymmetry
4.736	100	2056223	0.00	4278	1.3 8

Figure 3: Chromatogram of Esomeprazole In Tablet Formulation

Accuracy

Accuracy of the method was performed at three levels 20, 40, 60% the results are shown in table 4.

Table 4: Accuracy Study for method

			Standard Deviation		Accuracy	Precision
Conc.	**Conc.**	**Area**	**Mean**	**SD**	**%SD**	**%RSD**
	20	826351				
1	20	836058	830729.3333	4922.785018	0.592585915	0.592585915
	20	829779				
	40	2059506				
2	40	2059894	2057180.333	4368.500467	0.212353793	0.212353793
	40	2052141				
	60	3086270				
3	60	3073369	3061419	32516.82874	1.062148916	1.062148916
	60	3024618				

Recovery Study

The Percent Recovery was found within limit.

Table 5: Recovery Study:

Sr. NO.	% Composition	Area of Standard	Area of Sample	% Recovery
1	**50% Recovery**	1337042	1321142	98.81080774
2	**100% Recovery**	2059506	2047344	99.40947004
3	**150% Recovery**	2557828	2499891	97.73491415

Precision (3, 8, 9)

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.

Interday

Day 1			Day 2			Mean	% RSD
2059506	2059894	2052141	2086362	2021805	2039525	2039525	**1.06%**

Intraday

Table 6: Inter day and intraday precision of the method

Day 1			Day 2			Mean	% RSD
2059506	2059894	2052141	2086362	2021805	2039525	2039525	**1.06%**

Robustness

The ability of method to remain unaffected by small changes in parameters. Here in this study wavelength change.

Table 7: Robustness study for method

Conc.	Conc.	Area	Mean	SD	%SD
	30	1343566			
1	30	1332301	1343736	11520.44	0.857343997
	30	1355340			

Forced degradation Studies (5-10)

Drug product and placebo were subjected to forced degradation at various stressed conditions like acid, base, hydrolysis, peroxide, heat, photo light, U.V light and Humidity. All the samples were analyzed for peak purity of Esomeprazole peak. In all the samples, Peak purity meet the acceptance limits.

Degradation with Peroxide

Esomeprazole 40ppm Treated with 3%H_2O_2 at RT for 24Hrs. At Wavelength 304nm, Mobile Phase was Methanol: Water pH 3 (80:20) Sample volume was 20µl and Flow rate 0.8 ml/min. Pressure was 9-10 MPa And Run time 9.84min.

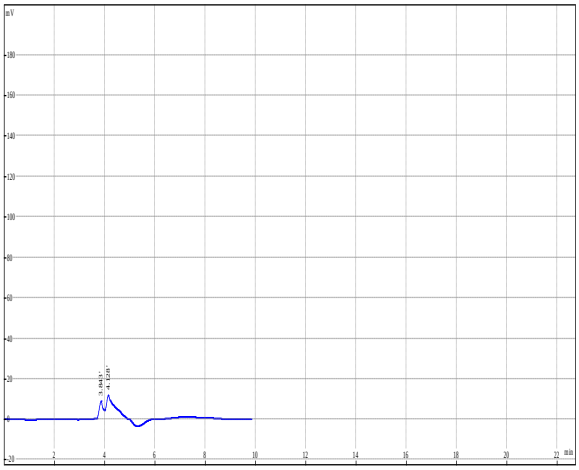


Figure 4: Degradation of ESO with Peroxide

Degradation with Acid

Esomeprazole 40ppm Treated with 0.1N HCl at RT for 24Hrs. At Wavelength 304nm, Mobile Phase was Methanol: Water pH 3 (80:20) Sample volume was 20µl and Flow rate 0.8 ml/min. Pressure was 9-10 MPa And Run time 8.12 min.

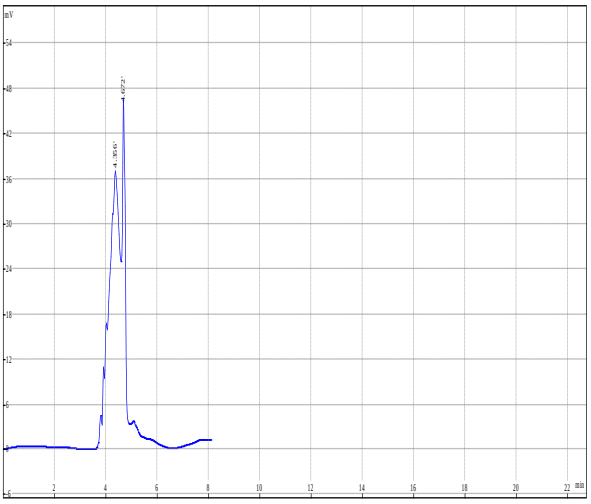


Figure 5: Degradation of ESO with Acid

Degradation with Alkali

Esomeprazole 40ppm Treated with 0.1N NaOH at RT for 24Hrs. At Wavelength 304nm, Mobile Phase was Methanol: Water pH 3 (80:20) Sample volume was 20µl and Flow rate 0.8 ml/min. Pressure was 9-10 MPa And Run time 8.02 min.

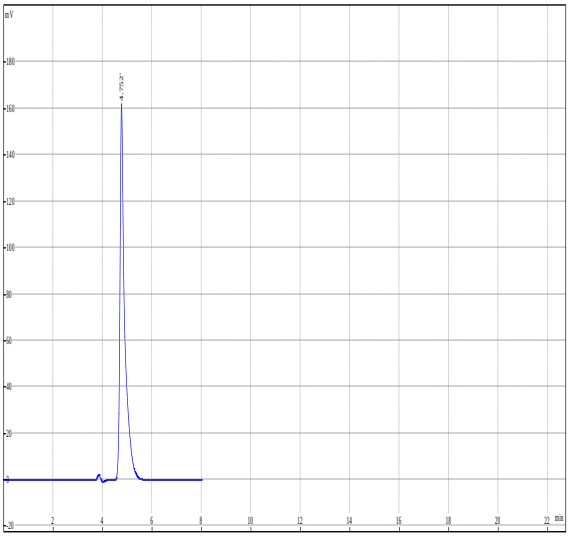


Figure 6: Degradation of ESO with Alkali

Thermal Degradation

Esomeprazole 40ppm Treated. Thermally at 60^{o}C for 24Hrs. At Wavelength 304nm, MobilePhase was Methanol: Water pH 3 (80:20) Sample volume was 20µl and Flow rate 0.8 ml/min. Pressure was 9-10 MPa And Run time 8.12 min.

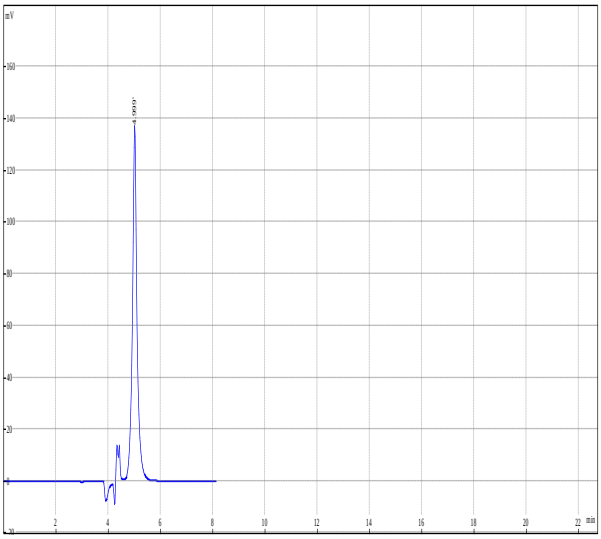


Figure 7: Thermal Degradation of ESO

Photolytic Degradation

Esomeprazole 60ppm Treated. Photolytically at RT for 24Hrs. At Wavelength 304nm, Mobile Phase was Methanol: Water pH 3 (80:20) Sample volume was 20µl and Flow rate 0.8 ml/min. Pressure was 9-10 MPa And Run time 8.01 min.

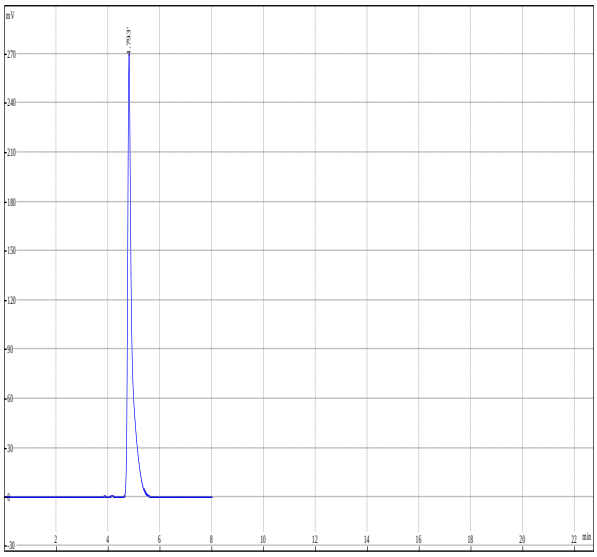


Figure 8: Photolytic Degradation

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CONCLUSION

The reversed phase-HPLC method developed for analysis of Esomeprazole in its pharmaceutical preparations is rapid, precise, accurate and reproducible and with short run time. The method was fully validated showing satisfactory data for all the method validation parameters tested. The retention time for Esomeprazole was found to be 3.560 min. The percent recoveries obtained for Esomeprazole was found to be 99.82. The developed method can be conveniently used by quality control department to determine the assay of pharmaceutical preparations. Drug product and placebo were subjected to forced degradation at various stressed conditions like acid, base, hydrolysis, peroxide, heat, photo light, U.V light and Humidity. All the samples were analyzed for peak purity of Esomeprazole peak. In all the samples, Peak purity meet the acceptance limits.

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