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Stability indicating method validation for the estimation of Esomeprazole Magnesium Capsules and determination of Potential Impurities by RP-HPLC

K. S. Nataraj*

M. Sushma Divya Tejaswini

K. Chaitanya

N. Sayeed

N. Roshna

I. Manasa

Shri Vishnu College of Pharmacy, Bhimavaram, West Godavari District, Andhra Pradesh, India, Pin -534202.

Corresponding Authors:
K. S. Nataraj
Professor
Shri Vishnu College of Pharmacy
Bhimavaram -534202
West Godavari District
Andhra Pradesh, India
Email: kalakondan@yahoo.com

Abstract:

A novel reverse phase high performance liquid chromatographic method was developed and validated for the determination of Esomeprazole capsules. The method was found to be simple, precise and accurate. The method involved a mobile phase comprising of Glycine(0.05M) buffer (PH 9+0.05) and Acetonitrile (70:30v/v) and a X-BRIDGE- 250*4.6, 5µ, C8 (4.6 X 250, 5µ) column. The flow rate was maintained at 1.8 ml/min and the detection was done at 305 nm. The retention time was found to be 3.9 mins. The method was found to be linear in the concentration range of 30-180ppm. The analytical method was validated according to ICH guidelines (ICH Q2b). The correlation coefficient (r^2) was found to be 0.9996, % recovery was 98.7-100.8% and %RSD for precision on replicate injection was 0.6 and intermediate precision for intraday precision at condition-I and II was 0.1, 0.34 and interday precision was 0.13% respectively. The precision study was precise, robust, and repeatable. LOD value was and LOQ value was. The developed method was validated by performing validation parameters like linearity, accuracy, precision, specificity and robustness. The method was found to be reliable for the determination of Esomeprazole in pharmaceutical dosage forms.

Keywords: Esomeprazole, Capsules, determination, RP-HPLC, Validation.

Introduction:

Esomeprazole bis(5-methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole-1-yl) magnesium trihydrate. It is freely soluble in water methanol, and Acetonitrile soluble. Soluble in glycine buffer at pH 9, and practically nsoluble in n-hexane.

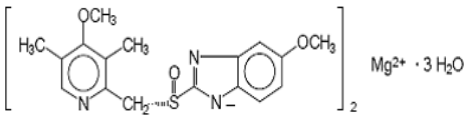


Fig1: Structure of Esomeprazole magnesium

The primary uses of esomeprazole are gastro esophageal reflux disease, treatment of duodenal ulcers caused by H. pylori, preventing of gastric ulcers in those on chronic NSAID therapy, and treatment of gastrointestinal ulcers associated with Crohn's disease, esomeprazole is a proton pump inhibitor (brand name Nexium) which reduces acid secretion through inhibition of the H+ / K+ ATPase in gastric parietal cells. By inhibiting the functioning of this transporter, the drug prevents formation of gastric acid. It is used in the treatment of dyspepsia, peptic ulcer disease (PUD), gastro esophageal reflux disease (GORD/GERD) and syndrome. Esomeprazole is a competitive inhibitor of the enzymes CYP2C19 and CYP2C9, and may therefore interact with drugs that depend on them for metabolism, such as diazepam and warfarin. The concentrations of these drugs may increase if they are used

concomitantly with esomeprazole.[1] Conversely, Clopidogrel (Plavix) is an inactive prodrug that partially depends on CYP2C19 for conversion to its active form; inhibition of CYP2C19 blocks the activation of clopidogrel, thus reducing its effects Single 20–40 mg oral doses generally give rise to peak plasma esomeprazole concentrations of 0.5-1.0 mg/L within 1–4 hours, but after several days of once-daily administration these levels may increase by about 50%. A 30 minute intravenous infusion of a similar dose usually produces peak plasma levels on the order of 1–3 mg/L. The drug is rapidly cleared from the body, largely by urinary excretion of pharmacologically-inactive metabolites such as 5-hydroxymethylesomeprazole and 5-carboxyesomeprazole.

Literature survey reveals that very few colorimetric methods[1-3] RP- HPLC method[4-7], methods are available for the determination of Esomeprazole in capsule dosage form.. The aim of the present study is to develop a simple, precise, rapid and accurate RP-HPLC method for the determination of Esomeprazole in capsules.

EXPERIMENTAL

Instrument and Reagents:

The instrument used was WATERS (2695) HPLC with PDA (2996) and Dual wavelength UV detector (2487). A Shimadzu UV-visible spectrophotometer – 1601 with empower-2 software and X-BRIDGE-250*4.6, 5µ, C8) column were used. A 20 µl Hamilton injection syringe was used for sample injection. HPLC grade reagents were used for the preparation of buffer (Ph 9+0.5). Reagents were obtained from E Merck (India). Milli Q Water (Millipore (USA)) was used throughout the procedure. A freshly prepared Glycine buffer (0.05M)solution (pH9-9..5) and solvent mixture (Buffer, Acetonitrile) in 70:30/v was used as a mobile phase. The solvents were filtered through 0.45 µ membrane filter and sonicated before use. The flow rate of mobile phase was maintained at 1.8ml/min. The column temperature was maintained at 25°C and the detection was carried out at 305 nm.

Preparation of Mobile phase:

Mix 700 volume of pH 9.0 buffer and 300 volume of Acetonitrile and degas.

Preparation of pH 9.0 Glycine buffer:

Dissolve 3.75g of Glycine (C2H5NH2) in 1000mL Water, mix and adjust pH to 9 ± 0.05 with Tri ethyl amine.

Selection of column:

Column trials were performed using Xtera C8 (150 x 4.6mm) 5µ Agilent Xtend C 18, 150*4.6mm, 5µ XBRIDGE C8, 250*4.6, 5µ column. Better peak resolution with less tailing was observed with. XBRIDGE C8, 250*4.6, 5µ

Selection of mobile phase:

The method development for the determination of Esomeprazole capsules was tried with different solvent systems. Different mobile phases containing NaOH, Acetonitrile, water and Glycine buffer (pH 9.05) in various compositions were tried and finally Glycine buffer (pH9.05.) and solvent mixture of Buffer: Acetonitrile of 70:30v/v was selected as good chromatograms were obtained with that composition. 100% Purified Milli Q Water was selected as solvent.

Preparation of Standard Stock solution:

Weigh accurately and transfer about 56 mg of Esomeprazole magnesium working standard / reference standard into a 250 mL volumetric flask,

add 150 ml quantity of solvent, sonicate to dissolve and dilute to volume with solvent.

Preparation of Impurity stock solution:

Weigh and transfer about 1.0 mg of the following impurities into a 10 mL volumetric flask, dissolve and dilute to volume with solvent..

Preparation of standard solution:

Pipette 5 ml of the standard stock solution into a 25 mL volumetric flask and dilute to volume with solvent and Filter through 0.45 µm Nylon or PVDF membrane filter. The solution was sonicated and cooled to room temperature and was further diluted with solvent mixture up to the mark.

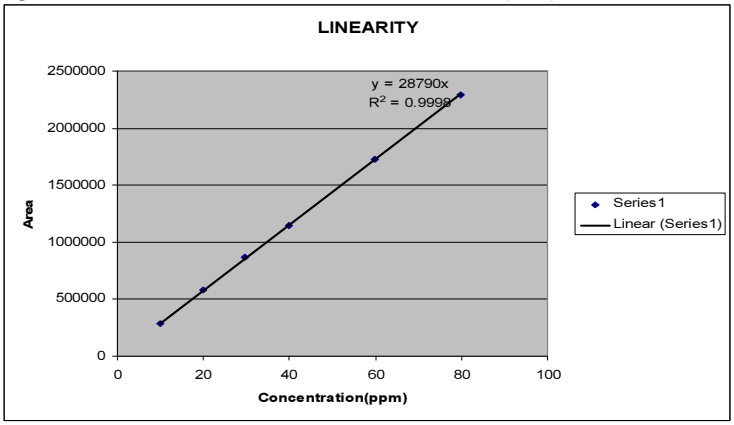
Linearity and construction of calibration curve:

Aliquots of 30-180ppm solutions were prepared from standard solution to determine the linearity range. Each of these drug solutions (10 µl) was injected 5 times into the column by maintaining a flow rate of 1.8 ml/min. The detection was carried out at 305 nm. Chromatograms were recorded and peak area was recorded for all injections. A calibration plot of concentration over the peak area was constructed and shown in Fig 2. Linearity studies are shown in table 1.

Table 1: Results of the linearity studies

% Spike level	Average "mg" Added (API)	Average "mg" Found (API)	% recovered	% RSD
30	15.64	15.64	100.8	1.7
50	25.08	25.03	99.8	0.5
75	37.57	38.03	101.2	0.5
100	50.01	49.33	98.6	0.2
150	74.91	74.3	99.2	0.4
180	95.04	93.82	98.7	0.1
Coefficient of Correlation		0.999		

Fig 2: Standard calibration curve of the proposed method



Precision:

Intra-day and inter-day precision studies were performed by injecting each concentration for six times and the percent RSD was calculated and it was found to be within limits. The results of precision studies were shown in table-2

Table 2: Precision of Esomeprazole

S. No	Rt	Peak Area	USP Tailing	Plate count	Acceptance Criteria
1	3.921	1047879	1.11	10553	NMT 2
2	3.813	1139529	1.11	10123	NMT 2
3	3.806	1135067	1.11	10418	NMT 2
4	3.827	1145178	1.11	10414	NMT 2
5	3.832	1142240	1.11	10127	NMT 2
6	3.831	1144482	1.11	10105	NMT 2
7	3.834	1143825	1.11	10149	NMT 2
Avg	3.838	1128313	1.11	10269	NMT 2
SD		3866			
%RSD		0.34			

Accuracy:

The percentage recovery studies were carried out at three different concentration levels. The percentage recovery and the percentage RSD values were found within the limits. The results of accuracy studies were shown in table-3.

Table 3: Results of Accuracy studies

Sample No.	% Spike level	"mg" added	"mg" found	% Recovery	Mean % Recovery	% RSD
1	30	15.29	15.73	101.9	102.7	0.3
1	50	25.14	24.99	99.5	99.8	0.5
1	75	37.8	38.05	101.8	101.2	0.5
1	100	50.1	49.31	99.9	98.6	0.2
1	150	75.06	74.3	99.2	99.2	0.4
1	180	95.02	93.88	98.8	98.7	0.1

Robustness:

The robustness studies were performed by changing the organic phase proportion of the mobile phase and buffer pH. The results of robustness were shown in table-4.

Table 4: Results of robustness studies

Parameter	Mobile phase composition		Variation in temperature(^{0}C)		Variation in flow rate(ml)		Variation in pH of Buffer		Acceptance criteria
	+3%	-3%	20	30	2	1.6	8.8	9.2	
Tailing Factor	1.2	1.1	1.1	1.1	1.1	1.1	1.14	1.15	NMT 2
%RSD	0.2	0.6	0.3	0.3	0.2	0.2	0.2	0.3	NMT 2

Specificity:

Chromatogram of blank did not show any peak at the retention time of analyte peak. There is no interference due to blank at the retention time of analyte. No interference was observed from the excipients and degradation products of degradation studies. Hence the method was found to be specific and stable.

System suitability:

Inject 20µl of blank, Resolution solution and standard solution (five times) record the chromatograms and measure the peaks response. The tailing factor for the Esomeprazole peak should be not more than 2.0 from the chromatogram of standard solution. The Relative standard deviation of Esomeprazole peak area from five replicate injections of standard solution should be not more than 2.0%.results were shown in table 5.

Table 5: System suitability parameters

Drug	RT (min)	Peak area	Theoretical plates	Tailing factor
Esomeprazole	6.4 min	792091	7610	1.24

Impurities interference

The impurities interference was also evaluated by performing assay on test preparation spiked with known impurities at 1.0 % level in triplicate as per test method. The difference in % average assay between known impurities spiked test preparation and unspiked test preparation is found to be within the limit. The results of peak purity of esomeprazole were shown in table 6.

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Table 6: Results of peak purity for Esomeprazole in the presence of know impurities.

S. No	Impurity name	Retention Time	Purity1 Angle	Purity1 Threshold	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
1	N-Oxide	2.289	2.048	2.504	12072	0.97	3.10	0.99	4990.60
2	C-789	2.443	1.477	1.595	44432	3.57	1.70	1.08	6136.71
3	Sulphone	2.783	3.373	4.366	11311	0.91	2.71	1.47	6503.90
4	Desmethoxy	3.711	3.773	4.492	10608	0.85	5.20	1.11	8232.27
5	Esomeprazole	4.035	0.060	0.277	1140930	91.74	1.91	1.10	8658.92
6	N-methyl	6.237	6.322	6.462	12627	1.02	11.06	1.13	10064.48
7	Sulphide	10.665	8.876	10.064	8005	0.64	14.25	1.06	16037.98

Interference from Degradation products:

Separate portions of Drug product were exposed to the stress conditions to induce degradation to effect partial degradation of the drug. Forced degradation studies were performed to show the method is suitable for the degraded products. Moreover, the studies provide information about the conditions in which the drug is unstable so that measures can be taken during formulation to avoid potential instabilities and results are shown in table.7

Table 7: Results of Forced degradation studies

S. No	Stress conditions	% Degradation	Peak purity		Area	%Area
			Purity angle	Purity threshold		
1	Acid	4.55%	0.062	0.281	1100239	95.45
2	Base	2.88%	0.068	0.277	1114065	97.12
3	Heat	1.88%	0.067	0.277	1124308	98.18
4	Peroxide	6.55%	0.068	0.278	1047613	93.45

Results and discussion:

The proposed HPLC method was found to be simple, rapid, precise, accurate and sensitive for the determination of Esomeprazole in pharmaceutical dosage forms. Hence this method can be easily and conveniently adopt for routine analysis of Esomeprazole in pure and its pharmaceutical formulations. The dosage form was analyzed in symmetry C8 column (250mmx4.6mm) C8 of Glycine (0.05M) buffer (PH 9+0.05) and Acetonitrile (70:30v/v) in an isocratic programmed with flow rate1.8ml/min and UV detection was performed at 305nm. The retention times observed as 3.9min. The linearity for detector response was observed in the concentration range of 30-180ppm of the concentration and the correlation coefficient(r) for calibration curve was found to be0.9996. The results of the recovery studies between 30-180ppm were in the range of 98.7-100.8% indicating accuracy of the method. The %RSD for the capsule analysis is less than 2 which is indicating high degree of precision. The results of the robustness study indicates that the method is robust and is unaffected by small variations in the chromatographic conditions. It was found that the known impurities were not interfering with Esomeprazole peak, the peak purity of Esomeprazole in the chromatogram of known impurities spiked test preparation is found to be within the limit. Forced degradation studies were performed and degradation For all forced degradation samples there is no interference from degradants in quantification of the Esomeprazole .The developed method was

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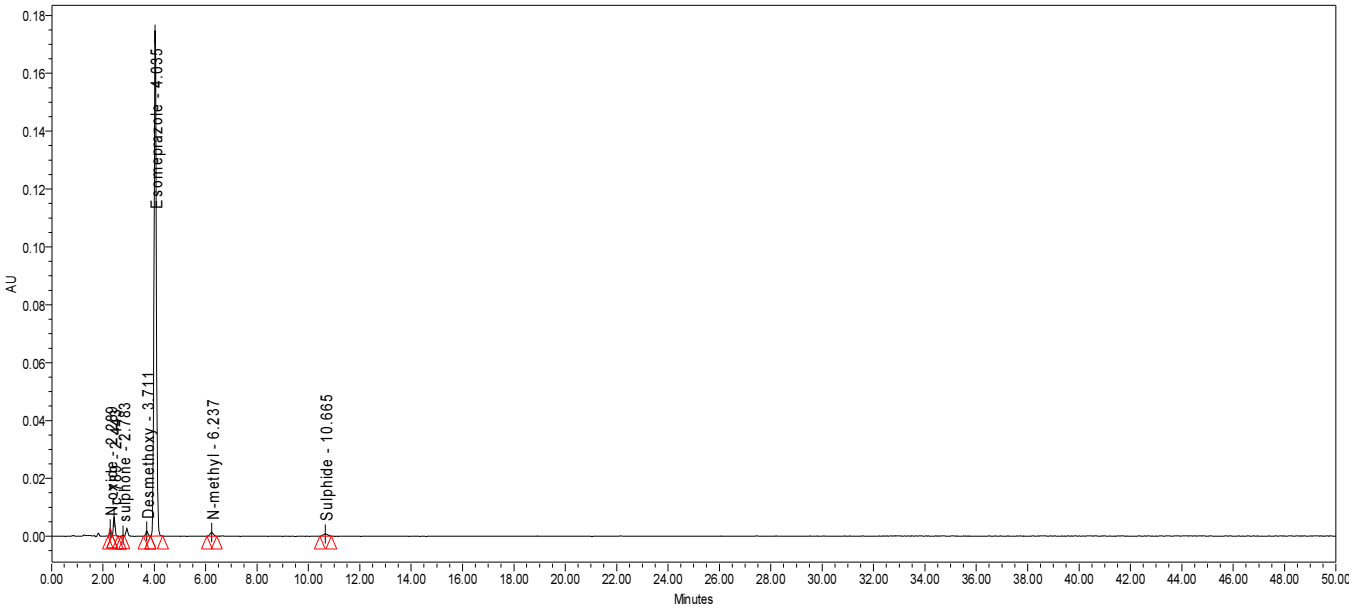
found to be appropriate for the determination of Esomeprazole in capsule dosage form. A typical chromatogram obtained by using the above parameters was shown in fig-3.

Fig-3: A typical chromatogram of Esomeprazole



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