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A VALIDATED RP HPLC METHOD FOR THE ESTIMATION OF ESOMEPRAZOLE SODIUM INJECTION

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ABSTRACT

Esomeprazole is chemically bis (5methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl]sulfinyl]-1Hbenzimidazole-1-yl). It is a gastric proton-pump inhibitor (PPI) used in treatment of gastric-acid related diseases. A Simple, sensitive, selective and accurate reverse phase high performance liquid chromatographic (RP-HPLC) methods were developed, estimated and validated for the analysis of Esomeprazole sodium in bulk and injection forms. The chromatographic separation was performed by the using C8, column having 250 x 4.6mm 5µm. Using mobile phase containing Acetonitrile and phosphate buffer (58:42v/v) adjusted to PH 7.6 with phosphoric acid. The analysis was run at a flow rate of 1.5ml/min and injection volume was 20 µL. The detection was monitored at 280nm. The retention time of Esomeprazole was 2.93 min. The developed method was validated for precision, intermediate precision (ruggedness), linearity, specificity, accuracy, and stability. Recovery of Esomeprazole in formulations was found to be in the range of 99.08%, 100.86%, and 101.52% respectively. And the correlation coefficient was 0.999. Hence, it was concluded that the developed method is suitable for routine analysis due to its less analysis time.

Keywords: Esomeprazole, RP HPLC, Method development, Relative standard deviation and Validation.

INTRODUCTION

Esomeprazole sodium belongs to the group of proton pump inhibitors (PPI). It is the enantiomer of omeprazole. Chemically it is bis (5methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl]sulfinyl]-1Hbenzimidazole-1-yl). Sodium molecular formula $C_{34}H_{36}MgN_6O_6S_2$• $3H_2O$ $C_{17}H_{18}N_3O_3S$•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. Esomeprazole is the S-isomer of omeprazole, the first single optical isomer proton pump inhibitor, generally provides better acid control than current racemic proton pump inhibitors and has a favorable pharmacokinetic profile relative to omeprazole[1]. Several methods have been employed for the estimation of esomeprazole alone and combination with other drugs such as UV and RP-HPLC method[2-5]. Keeping in view the importance of RP-HPLC method for estimation of drugs the present work was directed toward the development of a new, rapid and sensitive RPHPLC method for the simultaneous determination[6, 7]. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines (Code Q2A, Code Q2B) which are mandatory also[8,9]. Literature survey revealed that numerous methods have been reported for estimation of Esomeprazole in pharmaceutical formulations with high run time and solvent consuming[10]. Therefore the

present study was aimed to develop and validate an efficient RP-HPLC method for the determination of Esomeprazole injection. Validation was developed according to ICH guidelines.

MATERIALS AND METHODS

Chemicals and reagents: All reagents used were of analytical reagent grade. Monobasic sodium phosphate anhydrous dibasic sodium phosphate, phosphoric acid, Acetonitrile and Tribasic sodium phosphate were purchased from Merck, SDFCL and Rankem. Mumbai, India. HPLC grade water was obtained by double distillation and purification through mille-Q water purification system.

Instrumentation (specifications of HPLC): Analysis was performed using Agilent liquid chromatography with a pump series of 1260 and 1290, Agilent variable wave length programmable UV/visible detector set at 280nm A reverse phase system was used consisting of C8 column (250mmx 4.6 mmx5 µm)

Preparation of Mobile phase and Standard Stock Solution

Solution A preparation: Weigh 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 300 mL of water, dilute with water to 1000 mL, and mix. Dilute 250 mL of this solution with water to 1000 mL; adjust the pH with phosphoric acid to 7.6.

Solution B preparation:Mix 11 mL of 0.25 M tribasic sodium phosphate with 22 mL of 0.5 M dibasic sodium phosphate, and dilute with water to 100 mL.

Preparation of Mobile phase: Acetonitrile and Solution A were mixed in the ratio (58: 42 V/V) and degassed

Preparation of Standard stock solution: Standard stock solution was prepared by accurately weighing 40mg of drug, in 25ml of volumetric flask, dissolve 5 ml of solution B make up the volume with water.5ml of the solution is transferred in to 10ml of volumetric flask which was dissolved and the volume was made up by mobile phase to obtain final concentration of 1000 µg/ml of Esomeprazole sodium

Preparation of Sample Solution: Take 10 numbers of injection vials, each vial is reconstituted with 5mL of 0.9% w/v solution of sodium chloride and mixed properly, pipette 10 mL of the pooled solution in to 25mL volumetric flask, dilute in 5mL of solution B and make up the volume with water. 10mL of the solution is transferred into 50mL volumetric flask, dilute with water and make up the volume to 50 ml.

Chromatographic Conditions: The mobile phase acetonitrile and solution A (pH 7.6 adjusted with phosphoric acid) was mixed in a ratio (58:42 v/v) and filtered through 0.45µ Ultipor N66 Nylon 6,6 membrane solvent filter, degassed and the solvent was pumped into the column. The mobile phase was eluted at a flow rate of 1.5ml/min with an injection volume of 20µl and the effluent was monitored at a wavelength of 280 nm with a run time of 10min.

METHOD VALIDATION

The proposed method was validated with respect to precision, Intermediate precision (ruggedness), linearity, specificity, accuracy, and stability.

Precision

System Precision: For system precision six replicate of standard solution were injected. The percentage relative standard deviation of the assay result and column efficiency is calculated.

Method Precision: Method precision was performed by injecting separately 20 µL of blank, standard preparation in six replicate and six sample preparations in duplicate. The chromatogram where recorded and the peak response for the major peak is measured.

Intermediate Precision (Ruggedness): Intermediate precision was done by different analyst in different instruments, different day with different column. 20 µL of blank standard preparation in six replicate and six sample preparations were injected separately and the chromatogram where recorded and the peak response of the major peak was measured.

Linearity: The linearity response of Esomeprazole was determined by constructing calibration curve standard solution of Esomeprazole of different concentration (50%, 80%, 100%, 120%, and 150%). Each measurement was carried out in six replicates and the peak area of the chromatogram where plotted against the concentration to obtain the calibration curve and correlation coefficient.

Specificity: The specificity of the method is the ability to measure the analyte without any interference in the presence of excipients. Solution A preparation, Mobile phase, Standard solution, Placebo, and sample solution where prepared as per method of analysis. Inject 20 µL of blank, placebo standard solution in six replicates and sample

solution in duplicate. Chromatograms were recorded and measure the peak response of the major peak.

Accuracy: Accuracy was found by Recovery studies using standard addition method. A known amount of standard Esomeprazole solution at a level of 50%, 80%, 100%, 120%, and 150% (Three replicates of test concentration) was subjected to the proposed HPLC method. Percentage recovery was measured.

Robustness : Robustness is done in order to check the reliability by small deliberate variations in method parameters such as change in wave length ± 2nm, change in mobile phase composition ± 20%, change in flow rate ± 10%, change in column temperature ± 5^0 C and change in pH ± 0.2 units.

Stability: The solution used in the analytical method are analysed for its stability. Inject 20 µL of blank standard preparation and sample preparation in duplicate and chromatograms where recorded and the peak was measured at 0, 4,8,12,16,20,24 hours intervals.

RESUT AND DISCUSSION

The UV spectra of Esomeprazole showed that the drug absorbs appreciably at 280nm was selected as the detection wave length in liquid chromatography. Optimization of mobile phase was performed based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase solution A and acetonitrile (58:42 v/v). The retention time of Esomeprazole standard was found to be 2.937 min with peak area 25461821, which indicates a good baseline (Figure1). The retention time of sample Esomeprazole is 2.937 with peak area 25440407 (Figure-2).Precision of the method are shown in Table-1.Column efficiency determined from system suitability solution is 11464 and the relative standard deviation for replicate injection was found to be 0.053.The method precision, percentage relative standard deviation for the assay of six sample preparation is 0.076. In Intermediate precision studies (ruggedness) percentage relative standard deviation for the assay of six sample preparations is 0.19.The combined percentage relative standard deviation for method precision and intermediate precision was found to be 0.23.The results were found to be within the acceptance criteria. Hence the method was precise. The linearity of the method is shown in (Table-2 and Figure-3) the correlation coefficient R^2 is 0.999 which is found within the acceptance criteria hence the method is linear. There was no interference in the blank, placebo and the peak purity passes the standard and sample the results were found within the acceptance criteria hence the method is specific free from interference. The accuracy of the method is shown in (Table -3) the percentage recovery of all levels were between 98% and 102%.Hence the method was found to be accurate. Robustness of the method was done to find the deliberate variations in the method which is shown in (Table-4). The combined percentage relative standard deviation for method precision and change in robustness parameters was found to be less than 2% hence the method is robust.Table-5 shows that percentage difference of peak areas of standard and sample preparations at different time intervals is within ± 2.0%.Hence the standard and sample were stable for 24 hours.

CONCLUSION

The analytical parameters validated by HPLC for Esomeprazole injection was successfully developed which meets the acceptance criteria for specificity, Linearity, precision, accuracy, stability, ruggedness and robustness. Therefore the proposed method can be used for routine analysis of estimation of Esomeprazole in its injection formulation.

Table - 1: Precision of Esomeprazole

	System precision:	Method precision		Intermediate Precision	
Injection No	Area of Esomeprazole	Esomeprazole 20mg/vial	(Esomeprazole %)	Esomeprazole 20mg/vial	(Esomeprazole %)
1	25405522	19.884	99.42	19.840	99.20
2	25374711	19.883	99.41	19.855	99.27
3	25409349	19.884	99.42	19.795	98.97
4	25407346	19.883	99.41	19.745	98.72
5	25409686	19.884	99.42	19.812	99.06

6	25399768	19.885	99.23	19.792	98.96
Mean	25401064		99.39		99.03
Std Dev	13405.0559		0.07609		0.19596
% RSD	0.053		0.076		0.19
Theoretical Plate	11464				

Table - 2: Linearity of Esomeprazole

Concentration (µg/mL)	Injection1	Injection2	Injection3	%RSD
81.06	12894009	12888607	12876013	0.07
129.70	20007384	20014833	20023177	0.04
162.13	24869708	24863388	24865330	0.01
194.55	30104817	30120523	30991582	1.67
243.19	37774870	37774406	37733789	0.06

Table - 3: Accuracy of Esomeprazole

Accuracy Level %	Effective Amount Added in mg	Net Amount Recovered in mg	%Recovery	%RSD
50	21.82	21.62	99.08	1.09
	22.11	22.40	100.84	
	21.96	21.71	98.84	
80	34.64	34.29	98.99	1.41
	34.76	34.10	98.11	
	35.02	35.25	100.86	
100	44.03	43.77	99.41	1.22
	44.32	44.82	101.12	
	44.28	43.73	98.76	
120	51.92	52.03	100.20	0.17
	52.22	52.36	100.27	
	52.11	52.08	99.94	
150	66.19	65.57	99.06	1.23

Table - 4: Robustness of Esomeprazole

S.No	Parameter Name	%RSD	Theoretical Plate
01	Change in wavelength 282nm	0.03	5976
02	Change in wavelength 278nm	0.07	6947
03	Change in Mobile phase -20% relative to organic phase	0.03	7295
04	Change in Mobile phase +20% relative to organic phase	0.02	5363
05	Change in Mobile phase pH +0.2 unit	0.21	3796
06	Change in Mobile phase pH -0.2 unit	0.06	6375
07	Change in Flow rate 1.35mL	0.04	6522
08	Change in Flow rate 1.65mL	0.07	6081
09	Change in column temperature 25°C	0.05	6735
10	Change in column temperature 35°C	0.07	6771

Table - 5: Stability of Standard and sample solution of Esomeprazole

S.No	Time point	STABILITY OF STANDARD SOLUTION		STABILITY OF SAMPLE SOLUTION	
		Area	Difference in Area%	Area	Difference in Area%
01	0 hour	25178401	0.00	24455124	0.00
02	5th hour	25181080	0.00	24663531	0.85
03	8th hour	25179740	0.00	24559328	0.41
04	12th hour	25180410	0.00	24611429	-0.02
05	16th hour	25180075	-0.01	24585378	-0.55
06	20th hour	25180242	0.00	24598404	0.59
07	24th hour	25180159	0.00	24591891	0.56

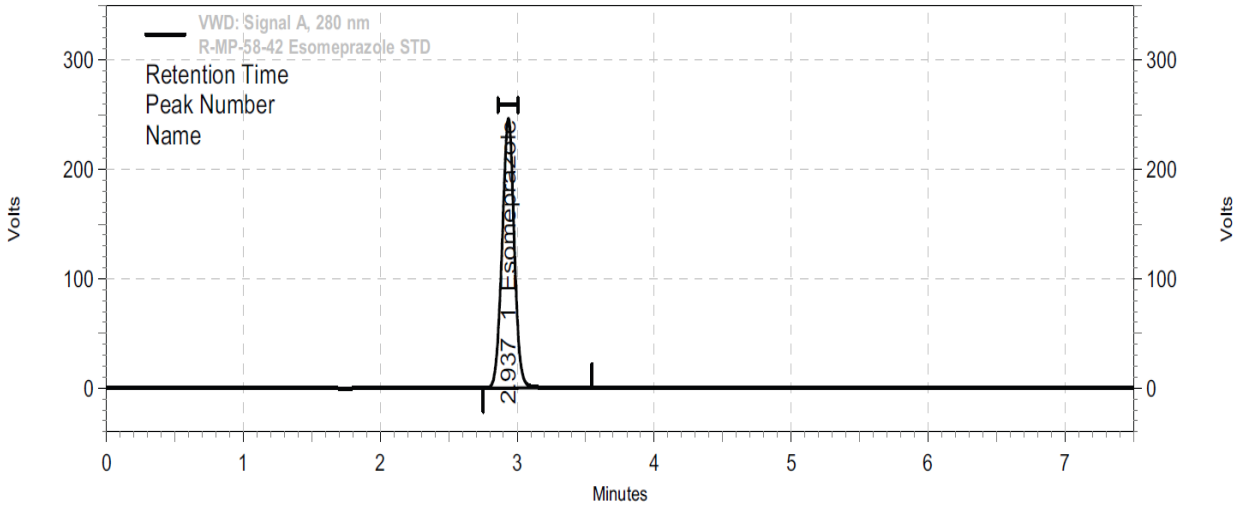

Fig. 1: Chromatogram of Standard Esomeprazole

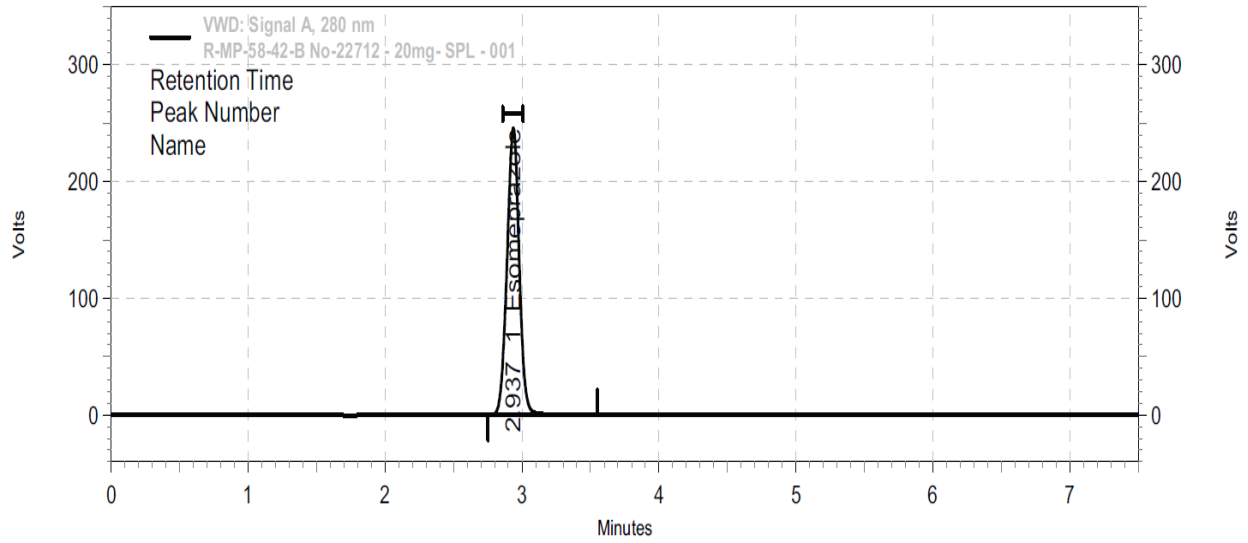

Fig. 2: Chromatogram of sample Esomeprazole

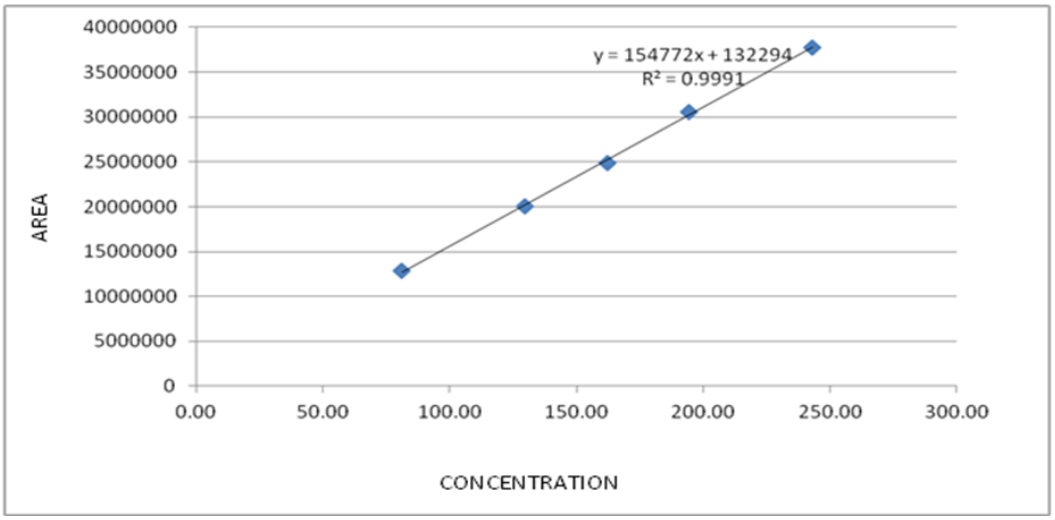

Fig. 3: Linearity of Esomeprazole

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