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RP- HPLC METHOD FOR THE ESTIMATION OF ESOMEPRAZOLE MAGNESIUM IN BULK AND ITS PHARMACEUTICAL DOSAGE FORMS

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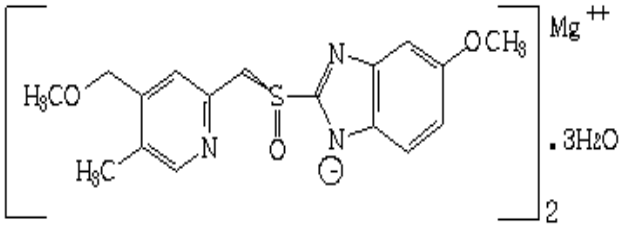
ABSTRACT

A simple reverse phase HPLC method was developed for the determination of Esomeprazole magnesium present in bulk and pharmaceutical dosage forms. Efficient chromatographic separation was achieved on Kromasil100-C18 stationary phase (250 X 4.6 mm i.d., 5µ particle size) with simple mobile phase combination of acetonitrile: phosphate buffer 55: 45 (V/V) in an isocratic mode at a flow rate of 1.0 mLmin-1 at 301 nm. The retention time was 4.09 min (±0.5). The proposed method has been applied successfully for the analysis of Esomeprazole magnesium either in bulk or pharmaceutical dosage form with good accuracy and precision. The method herein described can be employed for quality control and routine analysis of Esomeprazole magnesium in pharmaceutical dosage form.

INTRODUCTION: Esomeprazole belongs to a class of medications known as proton pump inhibitors (PPIs). PPIs, via the inhibition of H^+/K^+ ATPase enzyme pumps located in the gastric lumen, decrease the amount of gastric acid produced. Esomeprazole consists only of the active isomer (S-isomer), whereas its counterpart, Omeprazole, contains both active and inactive isomers (R- and S-isomers).

Structure:



Bis 5- Methoxy- 2- [(S- [(4- methoxy 3, 5 - dimethyl- 2- pyridinyl) - methyl] sulfinyl] - 1H- benzimidazole magnesium tri- hydrate

Molecular Formulae: $C_{34}H_{36}MgN_6O_6S_2.3H_2O$

Molecular Weight: 767.17

Description: The magnesium salt is a white to slightly colored crystalline powder.

Solubility: Soluble in methanol, slightly soluble in water, insoluble in heptanes.

Esomeprazole, like other PPIs, is a prodrug that is activated in an acidic environment to its active form (sulfenamide). The active form of the prodrug creates a covalent bond to H^+/K^+ ATPase pumps located on parietal cells in the gastric lumen. H^+/K^+ ATPase pumps are involved in the final step of the acid secretion pathway. This bond irreversibly inhibits the subsequent release of hydrogen ions. Inhibition of acid production is maintained until new H^+/K^+ ATPase pumps are regenerated (~18 hours). Esomeprazole, intended for the oral route of administration, is formulated with an enteric coating to prevent rapid dissolution in the acidic environment of the gastric cavity. Intravenous forms of esomeprazole have a slightly different pharmacokinetic profile than the oral forms. Esomeprazole metabolism is mediated via the cytochrome P-450 enzyme system (CYP450). The hydroxyl and desmethyl metabolites are formed by the CYP2C19 isoenzyme, while the sulphone metabolite is formed via the CYP3A4 enzyme. All metabolites formed are inactive.

A survey of literature reveals that good analytical methods are not available for the drug like Esomeprazole magnesium. Even though very few methods of estimation of above drug are available, many of them suffer from one disadvantage or the other, such as low sensitivity, lack of selectivity and simplicity etc. The existing physicochemical methods are inadequate to meet the requirements; hence it is proposed to improve the existing methods and to develop new RP-HPLC method for the assay of Esomeprazole magnesium in pharmaceutical dosage forms.

EXPERIMENTAL:

Instrumentation: Quantitative HPLC was performed on Shimadzu HPLC with LC 10AT VP series pumps besides SPD 10AVP UV-Visible detector. Win chrome software is used along with C_{18} stationary phase (250 X 4.6 mm i.d., 5µ particle size) column for the separation. Universal injector 7725 I (Rheodyne) with 20 µL loop is used.

Reagents used:

1. Water HPLC grade (Millipore water)
2. Acetonitrile HPLC grade (Rankem)

3. Potassium dihydrogen orthophosphate (SD fine chem.)

4. Di- potassium hydrogen orthophosphate (SD fine chem.)

5. Esomeprazole magnesium

Optimization: To ascertain the maximum wavelength (λ_{max}) of the proposed method, the drug solution (10 μgmL^{-1}) was scanned between the wavelength ranges of 200 – 380 nm. The λ_{max} was found to be 301 nm for Esomeprazole magnesium. To develop a suitable and robust HPLC method for the determination of Esomeprazole magnesium, different mobile phases acetonitrile : phosphate buffer, used in different compositions of the mobile phases (90:10, 50:50, 10:90) at different flow rates (1.5, 1.0, 0.8 $mLmin^{-1}$). The mobile phase acetonitrile: phosphate buffer 55:45 (V/V) at flow rate of 1.0 $mLmin^{-1}$ gave sharp peak with minimum tailing for Esomeprazole magnesium. Optimized chromatographic conditions are shown in table 1.

TABLE 1: OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Parameters	Conditions
Stationary phase (column)	Kromasil100-C_{18} column (250 X 4.6 mm i.d., 5µ particle size)
Mobile Phase	Acetonitrile : Phosphate buffer (55:45) V/V
Flow rate ($mL\ min^{-1}$)	1.0 mL
Run time (minutes)	10
Column temperature (°C)	Ambient
Volume of injection loop (µL)	20
Detection wavelength (nm)	301
Drug RT (min)	4.09 minute

METHODS: Standard solutions preparation: 50 mg of Esomeprazole magnesium was weighed accurately and transferred to a 100 mL volumetric flask. To this mobile phase was added and sonicated for 15 minutes. This yielded a working standard solution with concentration 500 μgmL^{-1} of Esomeprazole magnesium. This working standard solution was diluted to give solutions of 1-200 μgmL^{-1} and analyzed using the HPLC conditions mentioned above.

Sample solutions preparation: The sample solution was prepared by taking 20 tablets. The twenty tablets were powdered and powder equivalent to 10 mg of Esomeprazole magnesium was taken in 100 mL volumetric flask and by sonicating up to 15 minutes with mobile phase and the final volume was made to 100 mL to get a stock solution of 100 μgmL^{-1}. This solution was filtered through a 0.45µm membrane filter and further analyzed by using above mention HPLC conditions.

Procedure for calibration curve: Prior to injection of the drug solutions, the column was equilibrated for at least 60 min with the mobile phase flowing through the systems. Twenty micro liters of each of standard and sample solutions were injected into the HPLC system for three times to get the chromatograms. The retention time, average peak areas were recorded. A graph was plotted by taking conc. on X- axis and peak area on Y-axis. The linearity was found to be in between 0.781-200 μgmL^{-1} for Esomeprazole. The results are shown in table 2 and linearity graph is shown in fig (A).

TABLE 2: LINEARITY TABLE OF ESOMEPRAZOLE IN WORKING STANDARD

Conc. (μgmL^{-1})	Injection 1	Injection 2	Average
0.781	32438	32396	32417
1.562	75472	76618	76045
3.125	162851	162456	162653
6.25	334253	332566	333409
12.5	689818	681059	685438
25	1367809	1368731	1368270
50	2733714	2750139	2741926
100	5392155	5415340	5395056
200	10323817	10670668	10497242

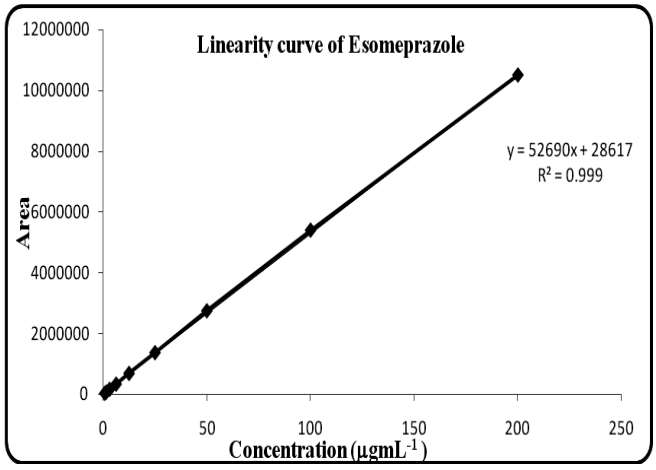


Fig (A) Linearity curve of Esomeprazole

Analysis of formulations: The amount of drugs present in each pharmaceutical formulation was calculated by using the standard calibration curve (concentration in μgmL^{-1}) was taken on X-axis and peak area on Y- axis. The results are shown in table 3. Typical chromatograms of blank and Esomeprazole in standard and formulation are shown in fig. B, C and D respectively.

TABLE 3: AMOUNT OF ESOMEPRAZOLE IN FORMULATION

Formulation	Labelled amount (mg)	Observed amount	% Drug recovered	%RSD
ZEPRAN (Mercury)	40	38.90±0.3040	97.25*	0.7778
SOMPRAZ (Sun)	40	39.19±0.1323	97.97*	0.7814

*Each value is average of three determinations

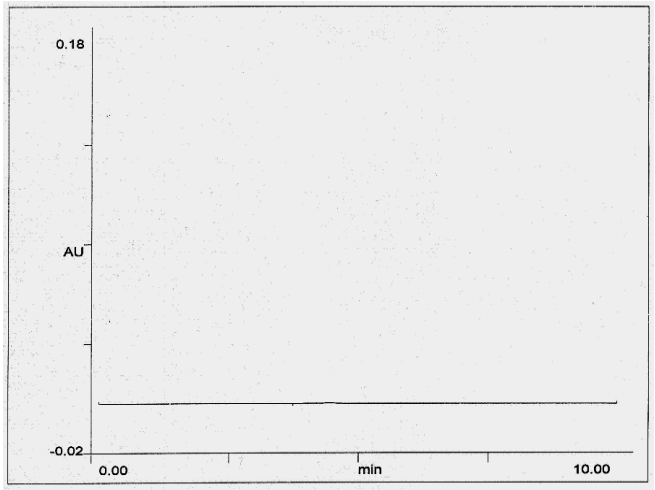


FIG. (B): CHROMATOGRAM OF BLANK

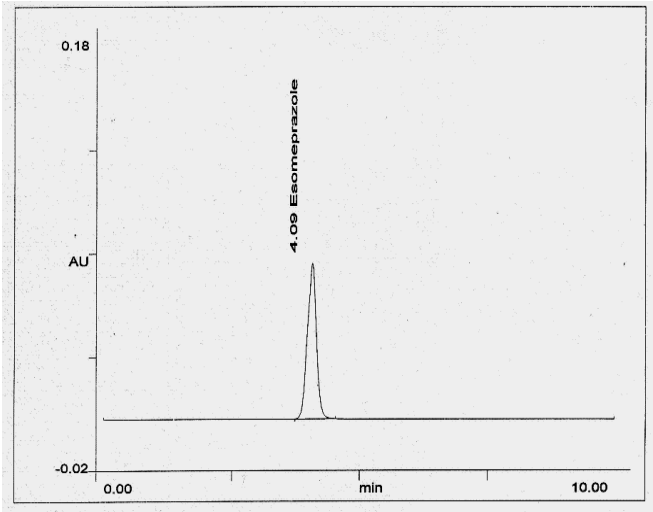


FIG. (C): CHROMATOGRAM OF ESOMEPRAZOLE (STANDARD) 100 μGML^{-1}

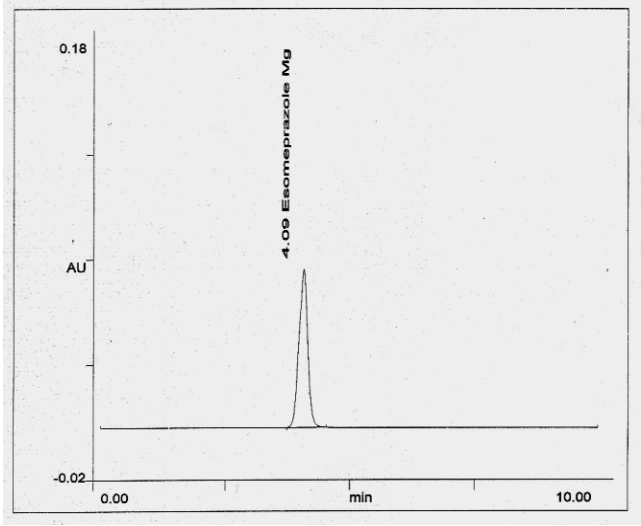


FIG. (D): CHROMATOGRAM OF ESOMEPRAZOLE (FORMULATION) 100 μGML^{-1}

METHOD VALIDATION PARAMETERS:

Linearity: The linear fit of the system was illustrated graphically. Least square regression analysis was carried out for the slope, intercept and correlation coefficient. The results are presented in table 2 and fig A respectively.

Precision: Precision of a method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings. It is measured by injecting a series of standards. The measured standard deviation can be subdivided into repeatability and reproducibility. Repeatability is obtained if the analysis is carried out in one lab by one operator using single equipment. Reproducibility is defined as long term variability of the measurement process which may be determined for a method run within a single laboratory but on different days. The results of injection precision and method precision are shown in table 4 and 5 respectively.

TABLE 4: INJECTION PRECISION

INJECTION PRECISION		
	RT	**AREA**
IP 1	4.09	5416986
IP 2	4.09	5355145
IP 3	4.08	5392155
IP 4	4.09	5397958
IP 5	4.09	5415340
IP 6	4.09	5398790
AVG	4.086	5396062
STDEV	0.007071	12866.51
%RSD	0.1730	0.2384

TABLE 5: METHOD PRECISION

METHOD PRECISION			
	AVERAGE	**INJ. 1**	**INJ. 2**
MP1	5414336	5417982	5410690
MP2	5398804	5398732	5398876
MP3	5396640	5398960	5394320
MP4	5406808	5401262	5412354
MP5	5402629	5404004	5401254
MP6	5394040	5399418	5388662
AVG	5402210		
STDEV	14351.43		
%RSD	0.2656		

Accuracy: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of bulk samples of Esomeprazole standard within the linearity range were taken and added to the pre-analyzed formulation of concentration of 50 μgmL^{-1}. From that percentage recovery values were calculated. The results are shown in table 6.

TABLE 6: ACCURACY RESULTS

SAMPLE ID	CONCENTRATION (μGML^{-1})		% RECOVERY OF PURE DRUG	STATISTICAL ANALYSIS	
	Pure drug	Formu -lation			
S_1 : 80 %	40	50	99.46	Mean	99.68
S_2 : 80 %	40	50	99.86	SD	0.1979
S_3 : 80 %	40	50	99.74	% RSD	0.1985
S_4 : 100 %	50	50	99.83	Mean	99.40
S_5 : 100 %	50	50	99.43	SD	0.6151
S_6 : 100 %	50	50	98.96	% RSD	0.6188
S_7 : 120 %	60	50	99.60	Mean	99.68
S_8 : 120 %	60	50	99.83	SD	0.01414
S_9 : 120 %	60	50	99.62	% RSD	0.01418

System suitability parameters: System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation have been completed. The USP (2000) defines parameters that can be used to determine system suitability prior to analysis. The system suitability parameters like Theoretical plates (N), Resolution (R), Tailing factor (T), LOD (μgmL^{-1}) and LOQ (μgmL^{-1}) were calculated and compared with the standard values to ascertain whether the proposed RP-HPLC method for the estimation of Esomeprazole in pharmaceutical formulations was validated or not. The results are shown in table 7, 8 and 9 respectively.

TABLE 7: SYSTEM SUITABILITY PARAMETERS

PARAMETERS	RESULTS
Theoretical plates (N)	2395
LOD (μgmL^{-1})	0.25
LOQ (μgmL^{-1})	0.781
Asymmetry	0.85
Height	17661

TABLE 8: PRECISION AT LOD

PRECISION AT LOD	
S. No.	RT
1	4.08
2	4.08
3	4.08
4	4.09
5	4.08
6	4.09
AVG	4.0833
SD	0.007071
%RSD	0.1731

TABLE 9: PRECISION AT LOQ

PRECISION AT LOQ		
S. No.	**RT**	**Area**
1	4.08	32438
2	4.08	32396
3	4.08	32446
4	4.09	32368
5	4.08	32429
6	4.09	32479
AVG	4.0833	32426
SD	0.007071	28.99
%RSD	0.1731	0.0894

RESULTS AND DISCUSSION: From the linearity table 2, it was found that the drug obeys linearity within the concentration range of 0.781-200 μgmL^{-1} for Esomeprazole. From the results shown in precision table 4 and 5, it was found that % RSD is less than 2%; which indicates that the proposed method has good reproducibility. From the results shown in accuracy table 6, it was found that the percentage recovery values of pure drug from the pre-analyzed solutions of the formulations were in between 98.96-99.86%, which indicates that the method is accurate and also reveals that the commonly used excipients and additives present in the pharmaceutical formulations were not interfered the proposed method. The system suitability parameters also reveal that the values were within the specified limits for the proposed method.

CONCLUSION: The proposed method was found to be simple, accurate, precise and rapid for determination of Esomeprazole from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non – interference of formulation excipients in the estimation. Hence, the method can be easily and conveniently adopted for routine analysis of Esomeprazole in dosage forms and can also be used for dissolution or similar studies.

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