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Spectrophotometric Methods for the Estimation of Esomeprazole magnesium trihydrate in Pharmaceutical Formulations Using Indigo Carmine Reagent

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Abstract: The aim of the present work is to develop a simple ,accurate, precise and cost effective UV-Vis Spectrophotometric method for the estimation of Esomeprazole in bulk and pharmaceutical dosage form.The solvent used was methanol and chloroform (80:20) using Indigo Carmine reageants and the λ max or the absorption of the drug was found to be 577 and 617 nm. A linear response was observed in the range of 5-35 µg/ml with as regression coefficient of 0.9997 and 0.9989. The method was then validated for different parameters as per the I.C.H guidelines.
Keywords: Esomeprazole, Simultaneous equation method, Indigo Carmine.

Introduction

Esomeprazole magnesium trihydrate [1] (ESO) is chemically bis(5-methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl - 2 -pyridinyl)methyl]sulfinyl] - 1-H - enzimidazole - 1 -yl) magnesium trihydrate , a compound that inhibits gastric acid secretion . Esomeprazole is cost effective in the treatment of gastric oesophageal reflux diseases. It is S-isomer of omeprazole and is the first single optical isomer proton pump inhibitor. It provides better acid control than current racemic proton pump inhibitors and has a favourable pharmacokinetic profile relative to omeprazole [2]. A detailed survey of literature revealed the estimation of omeprazole by gas chromatographic method [3], UV Spectrophotometric method [4, 5], TLC [6]and several HPLC [7-10] methods. Author of the article and his research team has developed a UV Method development different pharmaceutical dosage form using ferric chloride[9-11] and Indigo Carmine, Methyl orange[12].Hence an attempt has been made to develop new UV method for its estimation in bulk and pharmaceutical formulations with good accuracy, simplicity, precision and economy. The aim of this work is to develop and validate an analytical method by using UV Spectrophotometry for the estimation of Esomeprazole magnesium trihydrate in bulk and pharmaceutical dosage forms and also perform degradation studies on the drug as per ICH guidelines using the proposed method.

Materials and method

UV-visible double beam spectrophotometer, Shimadzu model 1700 with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 10 mm matched quartz cells was used. The commercially available tablets, Esomeprazole (ESO) was procured from local market. Acetonitrile, Ammonium acetate and Methanol (HPLC) grade, S.D. Fine Tablet formulation containing 50 mg of ESO.

UV method development

Solubility test

Solubility test for the drug Esomeprazole was performed by using various solvents. The solvents include Water, Methanol, Ethanol, Acetonitrile, Hydrochloric Acid (HCl), Sodium Hydroxide (NaOH) and Chloroform. All chemicals used in this study were analytical grade and used without further purification. Chloroform (s.d. finechem, Bombay, India), Indigo Carmine (s.d. finechem, Bombay, India).

Determination of λ max

Preparation of stock solution

Standard stock solution of Esomeprazole was prepared by dissolving 10mg of Esomeprazole in 10 ml of methanol and distilled water (50:50) which gives 1000 µg/ml. One ml of this stock solution was taken and was diluted up to 10ml by using methanol and distilled water (50:50) to produce a concentration of 100 µg/ml solution.

Preparation of working solution

From the above stock solution 2 ml was transferred into 10ml volumetric flask and volume was made up to the mark with methanol to make 50 µg/ml.

Then th sample was scanned with UV Spectrophotometer in the range 200-400 nm against methanol and distilled water (50:50) as blank and the wavelength corresponding to maximum absorbance was noted which is its λ max i.e. at 264nm (fig. 1).

Reagent Preparation

5.0 gm of Indigo Carmine was weighed and transferred into a 100 ml standard flask and the volume was made up to the mark to get the required concentration (0.5%w/v).Varying aliquots (0.5-2.0 mL) of standard 200 µg mL-1 Esomeprazole solutions were measured accurately and delivered into a series of 10 mL calibrated flasks and the total volume was brought to 5.0 mL with ether. To each flask were added 1 mL of 2.0 M acetic acid and 5.0 mL of bromate-bromide mixture (200 µg mL-1 in $KBrO_3$) by means of micro burette; the flasks were let stand for 15 min with occasional shaking. Then, 5 mL of 50 µg mL^{-1} indigo carmine solution was added to each flask, the volume was adjusted to the mark with water and mixed well. The absorbance of each solution was measured at 678.0 nm against a reagent blank after 30 min. In either method, the concentration of the unknown was read from the calibration graph or computed from the regression equation derived from the Beer's law data.

Preparation of calibration curve

One ml of this 50 µg/ml solution was further diluted and the volume was made up to 50 ml by using method to produce 50µg/ml solution. 1ml, 5ml, 8ml and 11 ml of 100µg/ml solution were diluted and the volume was made up to 10ml using methanol to produce 10µg/ml, 50µg/ml, 110µg/ml, 1000µg/ml solutions respectively. Then the construction of calibration curve was done by taking the above prepared solutions of different concentrations ranging from 5-35 µg/ml. Then taking the absorbance, calibration curve was plotted taking concentration on x-axis and absorbance on y-axis which showed a straignt line. The straight line obeyed linearity in the concentration range of 5-35 µg/ml.

Method-I (Simultaneous equation method):

Simultaneous equation method of analysis is based on the absorption of drugs Esomeprazole at the wavelength maximum of the each other. Two wavelengths were selected for the development of the simultaneous equations were 577 nm and 613 nm, λmax of Esomeprazole respectively. The absorptivity values E (1%, 1cm) determined for Esomeprazole at 577nm and 613 nm were. These values were means of six estimations. The absorbances and absorptivity at these wavelengths were substituted in following equations to obtain the concentration of both drug.

$$C_{ESO} = \frac{(A_2 \times 577 - A_1 \times 466)}{316543} \quad \text{.....Eqn.1}$$

$$C_{ESO} = \frac{(A_1 \times 613 - A_2 \times 466)}{316543} \quad \text{.....Eqn.2}$$

Method validation

Accuracy:

To check the accuracy of the proposed methods, recovery studies were carried out at 80,100, and 120% of the test concentration as per ICH guidelines. The recovery study was performed three times at each level. The results of the recovery studies are given in Table 2.

Precision

Repeatability:

To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Repeatability was performed for six times with tablet formulation. The standard deviation, coefficient of variation and standard error were calculated. The result of statistical evaluation is given in Table 2.

Intermediate Precision (Interday and Intraday precision):
The interday and intraday precision was determined by assay of the sample solution on the same day and on different days at different time intervals respectively. The results of the same are presented in Table 3.

Linearity:
For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. For method I and II, the Beer- Lambert's concentration range was found to be 5-35 µg/ml for Esomeprazole. The linearity data for both methods are presented in Table 3.

Limit of Detection (LOD) and Limit of Quantitation (LOQ):
The LOD and LOQ of Esomeprazole by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3σ/S and 10σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of response. The results of the same are shown in Table 3.

Robustness
Robustness of the method was determined by carrying out the analysis under different temperature condition i.e. at room temperature and at 18^0c. The respective absorbances of 40µg/ml were noted and the result was indicated as %RSD.

Ruggedness
Ruggedness of the method was determined by carrying out the analysis by different analyst and the respective absorbance of 50 µg/ml was noted. The result was indicated as %RSD .

Degradation studies
The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Esomeprazole using the proposed method 4.

Table 1. Optical Characteristics Data of Method-I

Parameters/ Working λ in max	**Esomeprazole**	
	577 nm	**613 nm**
Beer's law limit (µg/ml)	5-35	5-35
Absorptive E (1%,1cm)*	295	265
Molar absorptivity (l/mol.cm)*	34254	54664
Correlation coefficient*	0.9997	0.9989
Intercept*	0.276	0.115
Slope*	0.543	0.0654

Table 2: Analysis Data of Tablet Formulation, Statistical Validation and Recovery studies

Method	**Drug**	**Label claim mg/tab**	**Amount found* mg/tab**	**Label claim (%)**	**S.D.***	**% COV**	**S.E*.**	**Amount Added** at (%)	**% Recovery #**
I	ESO	50	50.12	100.05	0.432	0.365	0.265	80	100.08
								100	99.96
								120	101.37

Method I-, Simultaneous equation method, *Average of six estimations. Standard deviation, COV: Coefficient of variation, S.E.: Standard error# Average of three estimation at each level of recovery

Table 3: Validation Parameters

Method	Drug	LOD* µg/ml	LOQ* µg/ml	Precision (% COV)			
				Intraday	Interday*		
				n=6	First day	Second day	Third day
I	ESO	6.85	4.578	3.654	2.765	1.776	0.987

Method I-, Simultaneous equation method, , *Average of six estimations. Standard deviation, COV: Coefficient of variation, S.E.: Standard error,*

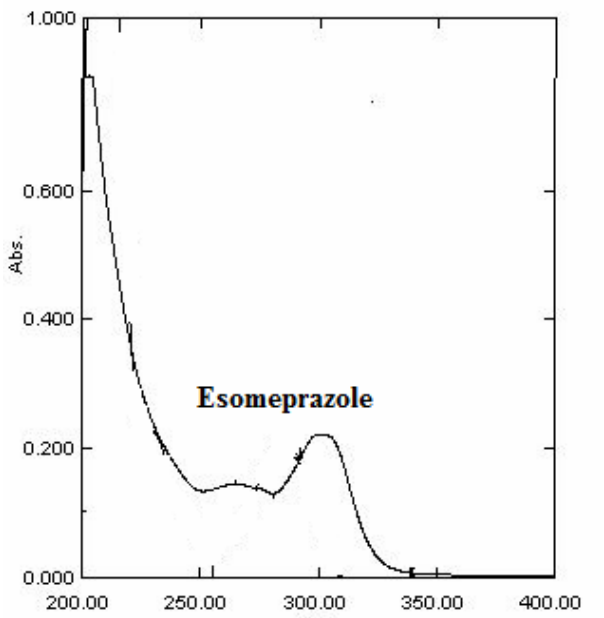

Fig. 1: Overlain spectra of Esomeprazole

Results and discussion

The validity and reliability of proposed methods were assessed by recovery studies. Sample recovery for both the methods are in good agreement with their respective label claims.The developed method was found to be precise as the % RSD values for the intraday and interday were found to be less than 2%.Good recoveries (100.05% to 101.03%) of the drug were obtained at each added concentration, indicating that the method was accurate.The method was also found to be specific indicated by the % recoveries ranging from 99.97%-100.32%.The LOD and LOQ were found to be in sub-microgram level indicating the sensitivity of the method.The method was also found to be robust and rugged as indicated by the % RSD values which are less than 2%.

The results of assay show that the amount of drug was in good agreement with the label claim of the formulation as indicated by % recovery (101.8 %). Summary of validation parameters of proposed spectrophotometric method is shown in table 1. Linearity range for Esomeprazole were found to be 5-35 µg/ml at respective selected wavelengths and coefficient of correlation were found 0.9997, 0.9989, for Esomeprazole at 311, 326, nm (Table 1). Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicates the precision under the same operating conditions over a short interval time and inter-assay precision. The standard deviation, coefficient of variance and standard error were calculated for Esomeprazole. Intermediate precision study expresses within laboratory variation in different days. In both intra and inter-day precision study for both the methods % COV were not more than 1.0% indicates good repeatability and intermediate precision.

Conclusion

The developed new three methods proved to be simple in procedure and it produced more accurate results. Hence methods effective for the routine analysis of Esomeprazole in bulk and tablet dosage form.

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