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Physico-chemical characterization, UV spectrophotometric method development and validation studies of Esomeprazole Magnesium Trihydrate

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ABSTRACT

*Esomeprazole magnesium trihydrate (EMT) is a proton pump inhibitor used against peptic ulcer disease to suppress excess acid secretion in the stomach. Physico chemical characterization studies showed that EMT has showed a melting point of 177.33^0 C. The solubility of drug esomeprazole followed the order methanol>ethanol> acetone> buufer pH 9.0 > distilled water. The analytical method developed for the estimation of esomeprazole magnesium trihydrate in bulk fluids showed maximum absorbance λ_{max} of 203.5 nm in methanol between 200 nm and 400 nm. Linearity studies indicated that estimation of esomeprazole magnesium trihydrate between 2.00 µg /ml to 10.00 µg /ml was found to be linear with regression equation of y = 0.1546*X - 0.00414; (r^2 = 0.999). The method developed was validated for inter and intra day variation, limit of quantitation studies. The SD values of Inter day and Intra day variation studies indicated that the variation is minimum. Limit of Quantitation of esomeprazole was found to be of 1.00 µg /ml. The above analytical parameters indicated that the developed UV Spectrophotometric method of esomeprazole was simple, accurate and reproducible.*

Key words Esomeprazole magnesium trihydrate (EMT), UV Spectrophotometric Method.

INTRODUCTION

Proton pump inhibitors (PPIs) are the most potent inhibitors of gastric acid secretion and are effective for treating all gastric acid-related disorders. Esomeprazole is indicated for the

treatment of gastroesophageal reflux disease in adults and children, risk reduction of NSAIDs-associated gastric ulcer, Helicobacter pylori eradication and control of pathological hypersecretory conditions associated with Zollinger-Ellison syndrome [1]. Esomeprazole is bis(5methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole-1-yl) magnesium trihydrate. Its molecular formula is $(C_{17}H_{18}N_3O_3S)_2$ Mg x 3 H_2O with molecular weight of 767.2 as a trihydrate and 713.1 on an anhydrous basis. The stability of esomeprazole magnesium is a function of pH, it rapidly degrades in acidic media, but it has acceptable stability under alkaline conditions. At pH 6.8 (buffer), the half-life of the magnesium salt is about 19 hours at 25° C and about 8 hours at 37° C [2]. Esomeprazole has a half life of 1.25 ± 0.25 h and has a bioavailability of 48% when administered orally [3,4]. Esomeprazole, the S-isomer of omeprazole, inhibits the gastric parietal H^+/K ATPase irreversibly which involved in hydrochloric acid production in the stomach. It acts as proton pump inhibitor, used to treat gastroesophageal reflux disease (GERD), erosive esophagitis and gastric ulcer [5]. Esomeprazole is combined with antibiotics clarithromycin and amoxicillin or metronidazole in 7-14 days eradication triple therapy of *Helicobacter pylori* infection where majority of peptic and duodenal ulcers were caused by *H. pylori* [6].

Review of literature given in sight that very few spectrocolorimetric and high performance liquid chromatographic method for the analysis of esomeprazole magnesium trihydrate [7-9]. Hence the present investigation was undertaken for drug candidate physico chemical characterization, to develop a simple and robust UV Spectrophotometric Method and validation for the quantitation of Esomeprazole Magnesium Trihydrate in bulk fluids and tablets.

EXPERIMENTAL SECTION

Instruments and Apparatus:

Theil's melting point apparatus was used for drug melting point determination. Dhona 200 D electronic balance (Mumbai) was used for weighing of all samples. Shimadzu UV 1700 double beam spectrophotometer (Japan) was used for all the spectrophotometric measurements. The absorption spectra of the reference and test solutions were carried out in a 1 cm quartz cells over the range of 200 - 400 nm. All laboratory glassware like volumetric flasks and pipettes were calibrated and used for experimentation.

Active pharmaceutical ingredient and Reagents:

Esomeprazole magnesium trihydrate was kindly supplied by Aurobindo pharma limited, Hyderabad, A.P, India) the drug was used without further purification. All the solvents and chemicals like n-octanol (INR Chemicals, Mumbai). Methanol (Pampasara distillaries). Potassium dihydrogen ortho phosphate and Sodium hydroxide pellets from S.d. Fine chemicals limited, Mumbai used in Spectrophotometric analysis were of LR grade.

Methods used for Physico-chemical characterization of the drug:

Melting point determination: Melting point of the drugs was determined by taking a small amount of drug in a capillary tube closed at one end and was placed in theil's melting point apparatus and the temperature at which the drug melts was noted. Average of triplicate readings was noted.

Solubility studies: The solubility of esomeprazole magnesium trihydrate was determined in distilled water, different buffers, viz., pH 1.2, pH 4.0, pH 9.0 and in acetone, ethanol and methanol according to the method proposed by Diez et. al [10]. Triplicate readings were taken and average was calculated.

Partition coefficient [11]**:** The partition coefficient of the drugs was determined by taking equal volumes of n-octanol and aqueous phases in a separating funnel. A drug solution was prepared and 1ml of the solution was added to octanol: water (50:50) was taken in a separating funnel and shaken for 10 minutes and allowed to stand for 1 h and is continued for 24 h. Then aqueous phase and octanol phase was separated, centrifuged for 10 min at 2000 rpm. The aqueous phase and octanol phase were assayed before and after partitioning using UV Spectrophotometer at their respective λ max to get partition coefficient. Triplicate readings (n=3) were taken and average was calculated.

Analytical method developed for the estimation of esomeprazole magnesium trihydrate in bulk fluids:

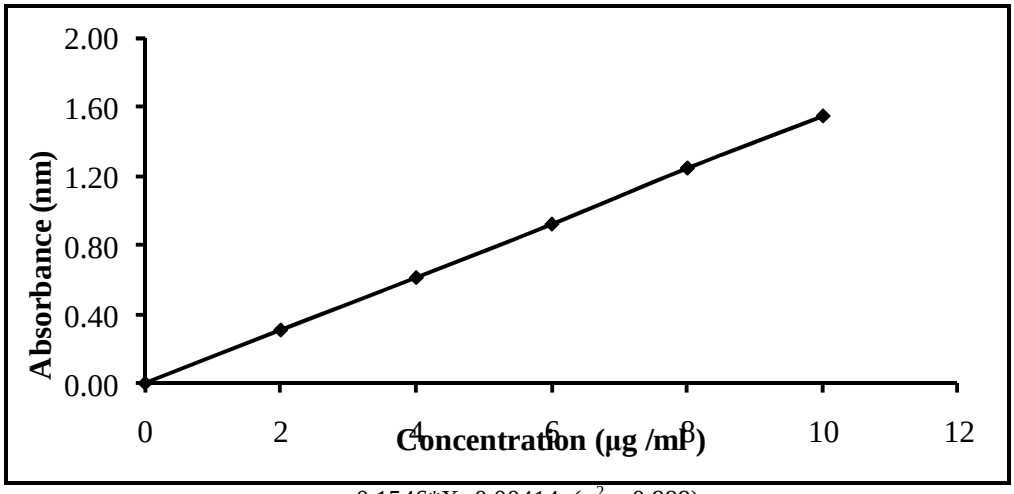
Preparation of stock solution of esomeprazole magnesium trihydrate: Stock solution of Esomeprazole magnesium trihydrate was prepared by dissolving 100 mg of accurately weighed amount of Esomeprazole magnesium trihydrate in 10 ml of methanol and then the volume was adjusted to 100 ml with the same solution to get 1 mg / ml solution.

Procedure: The above stock solution of drug was subsequently diluted with methanol to get 2 µg, 4 µg, 6 µg, 8 µg and 10 µg, of drug per ml. Later, 5 ml of 10 µg / ml solution was pipetted out into quartz cuvettes of UV spectrophotometer (against blank of methanol), and scanned for maximum absorbance between 200 nm and 400 nm in a UV-VIS 2000 Spectrophotometer, (double beam) Hitachi. Japan. Average of triplicate readings was taken. The peaks (n=3) with their respective absorbance's were noted and from the maximum absorbance, a λ_{max} of 203.5 nm was obtained. This value corroborates to literature value 205 nm [12].

Further calibration curve of Esomeprazole magnesium trihydrate was plotted by measuring absorbances of 2 µg /ml, 4 µg/ml, 6 µg/ml, 8 µg/ml and 10 µg /ml solutions at a λ_{max} of 203.5 nm. Average of triplicate readings was taken and tabulated. Regression equation was derived from the slope of the curve ($y = 0.1546*X - 0.00414$; $r^2 = 0.999$). Also the analytical method so developed was validated for linearity. Further the analytical method will be validated for other parameters like accuracy, precision and etc in prospective work.

Table.1. Spectrophotometric data for the estimation of esomeprazole magnesium trihydrate at 203.5 nm:

Sl. No	Concentration (µg/ml)	Absorbance
1	0. 00	0.00
2	2. 00	0.307
3	4. 00	0.616
4	6. 00	0.900
5	8. 00	1.247
6	10. 00	1.544



y = 0.1546*X -0.00414; (r^2 = 0.999)

Fig 1. Calibration curve of esomeprazole magnesium trihydrate

Table 2. Linearity studies of esomeprazole magnesium trihydrate

Sl. No	Concentration (µg/ml)	Absorbance (nm)	Regression Data		
1	2	0.307	m^* = 0.1546	c^* = -0.00414	r^2 =0.999766
2	4	0.616			
3	6	0.900			
4	8	1.247			
5	10	1.544			

**m = slope; c = intercept; r = regression.*

Validation of analytical method of esomeprazole magnesium trihydrate developed in the laboratory :

The analytical method so developed in the laboratory was validated for accuracy, precision and linearity. Inter day and Intra day variation was also studied.

Table 3. Inter day and Intra day variation studies of esomeprazole magnesium trihydrate

Concentration (µg /ml)	Absorbances			
MORNING	**DAY 1 AVG**	**DAY 2 AVG**	**DAY 3 AVG**	**AVG±SD**
0	0.000	0.000	0.000	0.000
2	0.314	0.310	0.318	0.314±0.004
4	0.610	0.612	0.610	0.611±0.001
6	0.917	0.925	0.917	0.920±0.005
8	1.213	1.238	1.225	1.225±0.013
10	1.506	1.549	1.524	1.526±0.022
AFTERNOON	**DAY 1**	**DAY 2**	**DAY 3**	**AVG±SD**
0	0.000	0.000	0.000	0.000
2	0.327	0.320	0.315	0.321±0.006
4	0.646	0.623	0.628	0.632±0.012
6	0.952	0.935	0.939	0.942±0.009
8	1.258	1.242	1.250	1.250±0.008
10	1.577	1.551	1.565	1.564±0.013
EVENING	**DAY 1**	**DAY 2**	**DAY 3**	**AVG±SD**

0	0.000	0.000	0.000	0.000
2	0.318	0.314	0.313	0.315±0.003
4	0.621	0.627	0.611	0.620±0.008
6	0.920	0.933	0.922	0.925±0.007
8	1.224	1.236	1.229	1.230±0.006
10	1.514	1.539	1.535	1.529±0.013

Table 4. Average readings of Inter day and Intra day variation studies of esomeprazole magnesium trihydrate

Concentration (µg /ml)	Absorbances			
	DAY 1 M* AVG	DAY 2 A* AVG	DAY 3 E* AVG	AVG±SD
0	0.000	0.000	0.000	0.000
2	0.314	0.321	0.315	0.317±0.004
4	0.611	0.632	0.62	0.621±0.011
6	0.92	0.942	0.925	0.929±0.012
8	1.225	1.250	1.230	1.235±0.013
10	1.526	1.546	1.529	1.534±0.011

** M → Morning; A → Afternoon E → Evening*

Limit Of Quantitation studies (LOQ) of Esomeprazole magnesium trihydrate: LOQ studies were conducted by taking concentrations of 0.25 µg /ml, 0.50 µg/ml, 0.75 µg/ml, 1.00 µg/ml and 1.50 µg /ml for lower limit and 15.00 µg /ml, 18.00 µg/ml, 20.00 µg/ml, 25.00 µg/ml and 30.00 µg /ml for upper limit to find out the deviation from beers lamberts law. The esomeprazole magnesium trihydrate obeyed beers law in the concentration range of 2.00 µg /ml to 10.00 µg /ml and the LOQ was found to be 1 µg/ml in UV spectrophotometer (UV 1700, Shimadzu, Japan).

Table 5. LOQ studies of Esomeprazole magnesium trihydrate

Sl.no.	Concentration (µg /ml)	Absorbance			
		I	II	III	Average
1	0.00	0.000	0.000	0.000	0.000
2	0.25	0.085	0.087	0.082	0.085
3	0.50	0.144	0.141	0.139	0.141
4	0.75	0.169	0.176	0.171	0.172
5	1.00	0.212	0.214	0.211	0.212
6	1.50	0.247	0.253	0.249	0.250
7	2.00	0.308	0.306	0.309	0.307
8	4.00	0.615	0.616	0.617	0.616
9	6.00	0.899	0.901	0.90	0.900
10	8.00	1.247	1.248	1.247	1.247
11	10.00	1.548	1.543	1.542	1.544
12	15.00	1.995	2.011	2.016	2.007
13	18.00	2.223	2.334	2.157	2.238
14	20.00	2.451	2.612	2.436	2.500
15	25.00	2.571	2.683	2.567	2.607
16	30.00	2.709	2.682	2.573	2.655

RESULTS AND DISCUSSION

The melting point of esomeprazole magnesium trihydrate was 177.33^0 C which corroborates with the literature value 180.083^0 C [13]. The λ_{max} of esomeprazole magnesium trihydrate was 203.5 nm which corroborates to the reported literature value 205 nm [12]. Results of studies on melting point and UV absorption maxima of drug suggested the values to be corroborating with previously reported literature values. The slope (m) of calibration curve of esomeprazole magnesium trihydrate was 0.1546. Linearity studies indicated that estimation of esomeprazole magnesium trihydrate between 2.00 µg / ml to 10.00 µg /ml was found to be linear with slope (m) 0.1546, intercept (c) is -0.00414 (r^2 = 0.999). The SD values of Inter day and Intra day variation studies indicated that the variation is minimum. Limit of Quantitation of esomeprazole was found to be of 1.00 µg /ml in UV spectrophotometer (UV 1700, Shimadzu, Japan). The solubility of drug esomeprazole follows the order methanol > ethanol > acetone > buffer pH 9.0 > distilled water. The log p value of Esomeprazole magnesium trihydrate was found to be 0.649 which corroborates to the reported literature value 0.60 [14]. The physico-chemical characterization studies showed that the bulk sample obtained was pure and analytical work for the estimation of esomeprazole was found to obey beer's limit and the curve was found to be linear. Using the proposed analytical technique, further quantitation work of prospective *in vitro* studies of esomeprazole could be carried out.

CONCLUSION

The literature review encompasses the literature reports on various analytical methods of esomeprazole magnesium trihydrate estimation useful in the study. The corroborating experimental values suggest the bulk sample drug obtained is pure. The solubility studies of esomeprazole magnesium trihydrate suggests that the drug is highly soluble in polar solvents (Methanol> ethanol>acetone), moderately soluble in alkaline borate buffer pH 9.0, and least soluble in water. The analytical method developed using UV spectrophotometer is linear and LOQ is 1 µg /ml and the inter-day and intra-day variation is minimum. Although there are reports and publications of either colorimetric and HPLC methods for esomeprazole magnesium trihydrate estimation, but there is no report or publication corresponding to the intended investigation, development and validation of UV Spectrophotometric method of esomeprazole magnesium trihydrate. Therefore it could conclude that the proposed investigation is a novel work and the investigation would help in estimation of drug candidate spectrophotometrically in the bulk fluids.

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