

Qualitative portrayal of Esomeprazole Magnesium by exploring diverse analytical and investigative approaches

ABSTRACT

Purpose: The present study was intended to qualitatively analyze and derive meaningful statistics for Esomeprazole magnesium; to establish its inherent properties qualitatively & quantitatively.

Methods: Esomeprazole magnesium was analyzed using various traditional & modern analytical and investigative tools viz FTIR, HPLC, UV spectroscopy, X-ray diffraction, zetasizer.

Result: The absorption maxima were found at 301 nm with UV Spectrophotometric and HPLC analysis. The particle size of the drug was analyzed through Malvern zetasizer employing water as diluent and found to be 11.818 μm . The quantitative solubility of Esomeprazole magnesium was predicted in various solvents at 25 $^{\circ}\text{C}$ and found that it was most soluble in methanol and was least soluble in distilled water. Solubility was also found to be pH-dependent. Solubility increases with an increase in pH. The order of solubility determined was; Methanol (1.214 mg/ml) > Phosphate buffer pH- 7.4 (0.521 mg/ml) > Simulated Intestinal fluid pH-6.8 (0.475 mg/ml) > Phosphate buffer pH- 6.8 (0.466 mg/ml) > Simulated Gastric fluid pH-1.2 (0.147 mg/ml) > 0.1 N HCl (0.131 mg/ml) > Distilled water (0.017 mg/ml). The predicted log P value was found to be 2.39.

Conclusion: Esomeprazole magnesium was qualitatively analyzed using various analytical techniques to establish its inherent properties quantitatively. It is an off-white amorphous powder with a characteristic obnoxious odor and slightly bitter taste. The drug was identified through FTIR, using the classification of characteristic peaks of functional groups in the spectrum and also additionally through analytical methods viz HPLC, UV spectroscopy and found the absorption maxima at 301 nm. The linear regression analyses of a standard plot from UV spectrophotometric analysis demonstrate an unswerving relationship in the concentration range of 5-30 $\mu\text{g/ml}$. The log P value indicates that the drug is significantly lipophilic. Various Micromeritic parameters like the Angle of repose, compressibility index, and Hausner's ratio predict the inherent flow properties of Esomeprazole magnesium as Fair (aid not needed). The present study will be an asset in the development of a modified released quick action formulation (Tablet-in-Tablet), to offer quick and protracted relief in gastritis and allied disorders.

Key words: PPI, Esomeprazole Magnesium, HPLC, FTIR spectroscopy, UV Spectrophotometric analysis, Malvern Zetasizer.

INTRODUCTION

Gastric acid is secreted by oxyntic cells. These are one of the types of epithelial cells present and projected towards the periphery, in the gastric glands hence commonly termed as parietal cells. Acid secretion in these cells is regulated by endocrine, paracrine, and neurocrine pathways; that is activated by acetylcholine, gastrin, and histamine by binding to respective receptors [1]. The signal to secrete acid is conveyed to parietal cells through receptors that are present on the basolateral membrane of the parietal cell. These receptors are the histamine H₂ receptor, cholinergic receptor, muscarinic M₃ receptor, and gastrin CCK-B (cholecystokinin-B) receptor [2]. During stimulation of parietal cell, there is an initial augmentation of intracellular calcium and cAMP (Cyclic adenosine monophosphate) which is followed by activation of the protein-kinase pathway. The activation of the protein-kinase pathway translocates and brings in the H⁺/K⁺ Adenosine Tri Phosphatase enzyme into the apical plasma membrane of the parietal cell [1]. The gastric H⁺/K⁺ ATPase is a P₂ type of membrane protein that is characteristically α, β-heterodimeric enzyme that swaps cytoplasmic hydronium ions with extracellular potassium. During stimulation of parietal cell, H⁺/K⁺ ATPase enzyme relocates to canaliculi and secretes hydrochloric acid by electro neural exchange of intracellular hydrogen with extracellular potassium ions [3].

Persistent production of acid reduces the pH in the stomach which in turn stimulates the inhibitory mechanism to cease the production of gastric acid. The inhibitory paracrine stimulates the release of peptide, somatostatin from gastric antral D cells thus inhibits the production of gastrin from G cells and histamine from enterochromaffin-like cells [4]. Moreover, duodenal acidification causes the release of secretin (a peptide hormone) from S cells that are located in glands present in the intestine which sources the release of bicarbonate from pancreases leading to elevation in duodenal pH. The acid restraining effect of endogenous prostaglandins and somatostatin is attributed by EP₃ & SST₂ receptors respectively and adenylate cyclase activity by guanine nucleotide-binding proteins. All acid stimulatory & inhibitory pathways congregate and attune the H⁺/K⁺ ATPase enzyme [5].

The secretion of hydrochloric acid in the stomach must be adequate. Insufficient or overproduction of HCl in the stomach leads to pathological conditions like hypochlorhydria and hyperchlorhydria respectively. Hypochlorhydria manifest digestive issues, GI infections, nutritional deficiencies (Vitamin B-12, Iron deficiency). Gastric hyperacidity can manifest from acute to chronic medical complications (dyspepsia, heartburn, indigestion to hypergastrinemic syndromes like gastric ulcers, gastroesophageal reflux disease (GERD), Zollinger-Ellison syndrome, etc).

Antacids, Histamine₂-receptor antagonists (H₂RAs), anticholinergics, synthetic prostaglandin analogs, and Proton pump inhibitors are recommended to cure complications associated with hyperchlorhydria. PPIs are the drug of choice in treating various disorders associated with gastric acid hypersecretion as they have demonstrated superior acid suppression, safety & patient acceptance. All PPI's are benzimidazole derivatives; heterocyclic organic molecules that include both pyridine and benzimidazole moiety linked by a methylsulfonyl group. They are acid-labile weak bases hence imperatively enteric-coated to retard drug degradation in the stomach [6]. They are usually formulated with sodium bicarbonate to provide momentary elevation in pH to prevent drug degradation in a lower pH environmental condition. Furthermore, Sodium bicarbonate encourages the release of gastrin that in turn activates the H⁺/K⁺ +ATPase pump, permitting swift inhibition of acid production [7]. They are prodrugs that get triggered to active sulfenic acid and sulfonamide moiety after accumulating in canaliculi of the stimulated parietal cell. Here they bind irreversibly and covalently to cysteine residues on the alpha subunit of the H⁺/K⁺ +ATPase through disulfide linkage and restrain acid production, up to 36 hours [8]. All proton pump inhibitors are extensively metabolized in the liver through cytochrome P450 isozyme CYP2C19 & CYP3A4 [9] [10].

Esomeprazole is the S (Levo) - enantiomer of racemic omeprazole [11], chemically it is bis (5-methoxy-2-[(S)-[(4-methoxy-3, 5-dimethyl-2-pyridinyl) methyl] sulfinyl]-1H-benzimidazole-1-yl) magnesium, trihydrate comprising molecular formula (C₁₇H₁₈N₃O₃S) Mg 3H₂O (Fig 1) and molecular weight of 767.2 g/mol. The consistency of Esomeprazole magnesium is pH reliant; it swiftly degrades at lower pH and has reasonable stability exceeding pH- 6.8. It's a highly protein-bound drug with plasma protein binding up to

97% [12]. In contrast to racemic Omeprazole and other proton pump inhibitors, Esomeprazole has a superior pharmacokinetic profile, with reference to metabolizing isozyme CYP2C19. It has sluggish hepatic clearance which consequences in higher systemic exposure and bioavailability that invariably tenders superior efficacy in the management of acid peptic disorders [13]. The biotransformation of Esomeprazole magnesium is accomplished by hepatic enzymes cytochrome P450 involving two different isoforms CYP2C19 and CYP3A4. The Esomeprazole magnesium is metabolized by CYP2C19; predominantly to 5-hydroxy Omeprazole and inconsequentially to 5-O-desmethyl Omeprazole. CYP3A4 also plays a pivotal role in transforming Esomeprazole magnesium; predominantly to Omeprazole Sulfone & Omeprazole Sulfide and inconsequentially to 3-Hydroxy Omeprazole. These metabolites additionally get biotransformed by isozymes CYP2C19 and CYP3A4 i.e. 5-hydroxy Omeprazole further transformed by CYP3A4 to 5-Hydroxy Omeprazole Sulfone, Omeprazole Sulfone further get biotransformed by isozymes CYP2C19 and CYP3A4 to 5-Hydroxy Omeprazole Sulfone & Pyridine N-oxide Omeprazole Sulfone respectively [14]. 80% of Esomeprazole magnesium is excreted in the urine in, inactive form and rest as an inactive metabolite in feces. Esomeprazole magnesium is soluble in methanol, ethanol, acetone [12] & 1-butanol [15]. It is slightly soluble in water and insoluble in heptane [12]. It is primarily recommended for the treatment of gastric ulcer, gastroesophageal reflux disorder (GERD), non-erosive reflux disease (NERD), Zollinger-Ellison syndrome, and co-prescribed along with antibiotics for eradication of H.pylori infection [13]. The current investigation was aimed to qualitatively analyze Esomeprazole magnesium to establish its inherent properties which will be an asset in the development of modified release formulation, to offer quick and protracted relief in gastritis and allied disorders.

MATERIALS & METHODS

MATERIAL

Esomeprazole Magnesium was received as a gift sample from Sun Pharmaceutical Industries Ltd. Dewas MP. India. Acetonitrile (HPLC grade) was purchased from Central Drug House, New Delhi; HPLC Grade Methanol was procured from S.D Fine Chemical Ltd. Mumbai, India. HPLC-grade water was purchased from Rankem India. All other chemical & Reagents used for analysis were of either analytical or laboratory grade.

METHODS

Analytical Evaluation:

1. Identification of Esomeprazole Magnesium:

1.1 Identification of Esomeprazole Magnesium by FTIR:

The identification of the pure Esomeprazole Magnesium was established through FT-IR spectrophotometric technique (Bruker-Alpha), through identification of different functional groups present in drug sample (Table 1). 2 mg of Esomeprazole Magnesium was triturated in glass mortar along with 200 mg of potassium bromide [16]. The mixture was compressed in hydraulic KBr pellet press at a pressure of 6000 kg/cm² to obtain a transparent pellet [17]. This pellet was analyzed under the FTIR spectrophotometer under a scanning range between 4000 cm⁻¹ - 400 cm⁻¹ at a resolution of 4 cm⁻¹ [16]. The peaks obtained in the spectrum are characteristic of a particular functional group, which were used to establish the identity of Esomeprazole Magnesium (Fig 2). The spectrum obtained through experimental work was also compare with the spectrum of Esomeprazole Magnesium available in official compendia.

1.2 Identification of Esomeprazole Magnesium by HPLC:

Reverse-phase HPLC (Waters with UV detector and Empower software) was used to carry out analytical determination of Esomeprazole Magnesium. For stationary phase C-18 column was used (ODS column Phenomenex -C18; column length 250 mm, 4.6 mm internal diameter & 5.0 μm particle size of silica) and for isocratic mobile phase solution of acetonitrile & methanol in ratio 50:50 (v/v) were considered [18]. They were filtered through a 0.22 μm Millipore membrane filter and degassed for 15 minutes on a bath sonicator [19]. The standard stock solution was prepared by introducing 10 mg of Esomeprazole Magnesium in a 10 ml volumetric flask. The Methanol was added; Q.S to make 10ml. At this stage, the concentration of the solution was 1000 $\mu\text{g/ml}$. This solution was termed; stock solution-A. 5 ml of this stock solution-A was diluted to 50 ml with methanol to make a working dilution of concentration 100 $\mu\text{g/ml}$; in a 50 ml volumetric flask. This solution was termed; stock solution-B. 1ml from working stock solution-B was then taken in a 10 ml volumetric flask. The rest of the volume was made using methanol, which constitutes the concentration of this solution to 10 $\mu\text{g/ml}$ [18]. This solution (concentration 10 $\mu\text{g/ml}$) was used to analyze and identify Esomeprazole magnesium through HPLC. The volume of solution injected; was 20 μL . The flow rate of the mobile phase was kept at 1 ml/min. Column temperature was 25 $^{\circ}\text{C}$ [20]. Run time was kept 10 min. The sample was scanned through a UV detector between 200 nm – 380 nm (Fig 3) to determine λ max [21].

1.3 Identification of Esomeprazole Magnesium by X-ray Powder Diffraction (XRD):

X-ray diffraction (Panalytical Empyrean DY-1769) exploration was used to predict bonding relationships between Esomeprazole heteroatom and magnesium [15]. Esomeprazole Magnesium was evaluated by Powder X-ray diffraction technique in which scan range was kept amid 10.00700 - 79.99380 at Position $^{\circ}2\theta$. The measurement temperature was 25 $^{\circ}\text{C}$. The Anode material was copper with K- α 1 wavelength at 1.5406 $^{\circ}\text{A}$, K- α 2 at 1.54443 $^{\circ}\text{A}$, and K- β at 1.39225 $^{\circ}\text{A}$ [22]. The ratio of K-A2/K-A1 was 0.5. The X-ray diffraction pattern was composed of 45 kV of tube voltage and 40 mA of tube current with step size at $^{\circ}2\theta$ was 0.008 and scan step time was 10.16 sec. The X-Ray diffractogram (Fig 4) was found to be concordant with available literature [16].

1.4 Identification of Esomeprazole Magnesium through Melting point:

All pure drug compounds melt isothermally at constant pressure. As per pharmacopoeial test protocol, the melting point was determined at the inception and completion of melt [23]. It was determined as per protocol given in USP25-NF20 US Pharmacopeia. 10 cm long glass capillary tube with internal diameter 0.8 - 1.2 mm and wall thickness 0.2 - 0.3 mm was used for experimental determination of the melting point of Esomeprazole Magnesium [24]. The drug was kept in desiccators for 6-8 hours using silica gel as a desiccant, to render it moisture free. The glass capillary was fused from one end by introducing it into a medium flame. Finely grounded Esomeprazole Magnesium was charged into fused glass capillary up to the level of 10 mm from the closed bottom end. This capillary containing Esomeprazole Magnesium was properly tied with the thermometer using cotton strand and placed in a Thiele tube holding liquid paraffin; clamped on a stand. The Thiele tube was heated at the sidearm with back and forth movement of the Bunsen burner. This sets in natural convection currents leading to the circulation of liquid paraffin in the Thiele tube. The Thiele tube was heated at the rate; 0.5 $^{\circ}\text{C}$ / minute. The Temperature was recorded when Esomeprazole Magnesium in the capillary tube begins to melt and the temperature at which the drug was completely molten and become transparent [25]. The average, triplicate readings were recorded.

2. Preparation of Calibration curve of Esomeprazole Magnesium.

2.1 Preparation of Stock solution:

A standard stock solution of Esomeprazole Magnesium was prepared by placing 10 mg of Esomeprazole Magnesium in a 100 ml volumetric flask. 10 ml Methanol and 40 ml of phosphate buffer with pH 6.8 were added to a volumetric flask. This solution was sonicated for 5 minutes. The volume was made up to 100 ml with phosphate buffer (pH-6.8) to get the concentration of working stock solution 100 µg/ml. [26] [27]

2.2 Determination of λ Max:

3 ml of stock solution was pipette out and transferred into 10 ml volumetric flask. The residual volume was made up to 10 ml with phosphate buffer (pH 6.8) to get the concentration of test solution 30 µg/ml. The test solution was analyzed for maximum absorbance in the range of 200-400 nm using phosphate buffer (pH 6.8) as blank (Fig 5). The absorption maxima was use to resolve λ Max of Esomeprazole Magnesium which corresponds to the literature value [28].

2.3 Preparation of working solution:

From stock solution (100 µg/ml) various dilutions were made corresponding to concentration 5µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, 25 µg/ml and 30 µg/ml. To prepare 5 µg/ml drug solution, 0.5 ml was pipetted out from stock solution and transferred into 10 ml volumetric flask; it was made up to 10 ml with phosphate buffer (pH 6.8). Similarly 1 ml, 1.5 ml, 2 ml, 2.5 ml, 3 ml were precisely pipetted out from stock solution to prepare solution with drug concentration 10 µg/ml, 15 µg/ml, 20 µg/ml, 25 µg/ml, 30 µg/ml respectively.

2.4 Preparation of Calibration curve:

The calibration curve for Esomeprazole Magnesium was prepared by observing the absorbance of drug concentration solutions (5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, 25 µg/ml and 30 µg/ml) at 301 nm (Fig 6). The average of triplicate readings was taken and tabulated (Table-2). The calibration curve was plotted by taking concentration on the x-axis and absorbance on the y-axis. The slope, intercept, and the regression equation were determined. (Fig 7)

3. Solubility analysis of Esomeprazole Magnesium:

3.1 Preparation of solutions & buffers for Solubility analysis of Esomeprazole Magnesium:

3.1.1 Preparation of 0.1 N HCl acid solutions:

8.5 ml of HCl was added to a 1000 ml volumetric flask; distilled water was used to make up the volume. [29]

3.1.2 Phosphate buffer pH-6.8:

50.0 ml of 0.2 M potassium dihydrogen phosphate was take in a 200 ml volumetric flask, to this solution, 22.4 ml of 0.2 M sodium hydroxide was added then distilled water was added to make up the volume. [29]

3.1.3 Phosphate buffer pH -7.4:

50.0 ml of 0.2 M potassium dihydrogen phosphate was take in a 200 ml volumetric flask, to this solution 39.1 ml of 0.2 M sodium hydroxide was added then distilled water was added to make up the volume. [29]

3.1.4 Simulated Gastric fluid pH-1.2:

2 grams of sodium chloride was added to 3.2 grams of pepsin in 7 ml of hydrochloric acid. Volume was adjusted to 1000 ml with distilled water. [30]

3.1.5 Simulated intestinal fluid pH-6.8:

6.8 grams potassium dihydrogen orthophosphate was dissolved into 250 ml of distilled water and transferred to a 1000 ml volumetric flask. To this solution, 77 ml of 0.2 N sodium hydroxide and 500 ml distilled water were added. To this resultant solution, 10 grams of pancreatin was added and pH was adjusted to 6.8 with either 0.2 N NaOH or 0.2 N HCl. Volume was adjusted to 1000 ml with distilled water. [30]

3.2 Solubility Analysis:

Solubility of Esomeprazole Magnesium was determined by UV spectrophotometric analysis (Shimadzu UV-1800). The medium chosen were distilled water, methanol, 0.1 N HCl, phosphate buffer pH-6.8 & pH-7.4, and simulated gastric & intestinal fluids (pH-1.2 & pH-6.8 respectively). The standard curve of Esomeprazole Magnesium was prepared in experimental solvents at 301 nm. The saturated solution of Esomeprazole Magnesium in experimental solvents was prepared (fig-8 to fig-14) by adding an excess amount of Esomeprazole Magnesium in 10 ml volumetric flasks with occasional shaking for 2 hours at ambient temperature. Solubility of Esomeprazole Magnesium was determined and tabulated (Fig 15).

4. The Partition coefficient of Esomeprazole Magnesium:

The Partition coefficient symbolizes, the equilibrium partitioning of a neutral solute between two immiscible phases [31]. The Partition coefficient of Esomeprazole Magnesium was determined; to predict the affinity of the drug towards hydrophilic & lipophilic phases, at ambient temperature. An equal volume of n-octanol (10 ml) and distilled water (10 ml) were taken in a separating funnel. To these immiscible solvents in a separating funnel, 10 mg of Esomeprazole Magnesium was added and shaken for 20 minutes and kept aside undisturbed for 40 minutes. This process was repeated for the next 6 hours. Then the solutions in the separating funnel were kept undisturbed for 6-8 hours. Both phases containing the drug were separated and centrifuged at 2000 rpm for 10 minutes [27]. The concentration of Esomeprazole Magnesium in distilled water and n-octanol was determined by observing the separated aqueous and nonaqueous aliquot under UV Spectrophotometer at 301 nm.

$$\text{Log } P \text{ (o/w)} = \text{Log} \left\{ \frac{\text{Concentration of Esomeprazole Magnesium in n-octanol}}{\text{Concentration of Esomeprazole Magnesium in distilled water}} \right\}$$

5. Micromeritics of Esomeprazole Magnesium:

5.1 Assessment of particle size

5.1.1 Estimation of particle size; by optical microscopy:

The Particle size of Esomeprazole Magnesium was determined by optical microscopy. After the calibration of the eyepiece micrometer with a stage micrometer, the drop of suspension containing Esomeprazole Magnesium in liquid paraffin was placed on a glass slide and observed under a 45X lens of the microscope. A total of 625 particles were observed in terms of eyepiece divisions & frequency. The observations were tabulated and classified in terms of the mean size range (Table-3). These observations were statistically exploited to determine Mean length number diameter (d_n), Mean Surface number

diameter (d_{sn}), Mean volume number diameter (d_{vn}), Mean surface length diameter (d_{sl}), Mean surface volume diameter (d_{sv}), and Geometric mean ($\log d_g$). [32]

5.1.2 Estimation of particle size; by Malvern Zetasizer:

The particle size of Esomeprazole Magnesium was also determined through Malvern Zetasizer (Malvern Panalytical Zetasizer-AT). The diluent used for the assessment was water. The drug particle suspension was kept at 25.1 °C for 5 minutes to ensure temperature consistency in the test sample. Refractive Index was 1.3328 and viscosity was 0.8858 cP with scattering intensity 8951cps [33]. These values were used to predict intensity distribution (fig-16), volume distribution, and number distribution.

5.2 The angle of repose (θ°):

Factors like particle size, particle shape, surface texture, moisture content, density, porosity, storage conditions, internal cohesive forces (Vander Waals forces, hydrogen bonding, frictional forces), influences the flow properties of powder material. The inherent flow property of Esomeprazole magnesium was determined by Static angle of repose through fixed funnel method. Glass funnel with flat-edged stem (internal diameter of stem -15 mm) was clamped on a stand at a certain height, beneath it, a laboratory scissor jack was placed in such a way that its top platform can be ascended or descended, as and when required. A graph paper was placed on the top platform of a laboratory jack. The top platform of a laboratory jack was raised till it touches the edge stem of the funnel. The opening of a funnel now was blocked at this position. Now the appropriate amount of Esomeprazole magnesium was placed in a funnel and the laboratory jack was descended slowly to get a conical stack of the drug on the graph paper [32][34]. The height and diameter of a conical stack were determined to calculate the angle of repose using, the following equation (Table-4):

$$\theta = \text{Tan}^{-1} (h/r)$$

θ = Angle of repose

h = Height of heap (cm)

r = Radius of the base of a heap (cm)

5.3 Density (Bulk density & Tapped density):

5.3.1 Bulk density (ρ_b):

Bulk density is the ratio of mass (grams) of drug substance to bulk volume (ml) of drug substance comprising of void volume without consolidation of powder sample. Bulk density (gm/ml) depends upon the density of powder material and the arrangement of particles in the powder bed [35]. The bulk density of Esomeprazole magnesium was computed and reported (Table-4).

$$\text{Bulk density } (\rho_b) = m/V_0$$

m = Mass of Drug or experimental powder substance

V_0 = Bulk volume (unsettled apparent volume)

5.3.2 Tapped Density (ρ_t):

Tapped Density is an increase in bulk density achieved after mechanical tapping of powder mass in a measuring cylinder [35]. Tapped density of Esomeprazole magnesium was computed and reported after 1250 taps (Table-4).

$$\text{Tapped density } (\rho_t) = m/V_f$$

m = Mass of Drug or experimental powder substance

V_f = Final volume attained after tapping.

6. Compressibility characteristics:

The compressibility characteristic of the drug substance depends upon inter particulate interactions which not only influence bulkiness but also interfere with powder flow characteristics. Drug substances with significant inter particulate interaction and greater difference in bulk density and tapped density possess poor flow properties and vice-versa [36]. The compressibility characteristic of the drug substance can be estimated through Carr's compressibility Index & Hausner's ratio (Table-4).

6.1 Carr's Compressibility Index :

Compressibility index can be calculated through bulk volume & tapped volume; of powder drug substance:

$$\text{Compressibility Index} = 100(V_0 - V_f / V_0)$$

V_0 = Bulk volume (unsettled apparent volume)

V_f = Final volume attained after tapping

Compressibility index can also be calculated through bulk density & tapped density; of powder drug substance:

$$\text{Compressibility Index} = 100(\rho_{\text{tapped}} - \rho_{\text{bulk}} / \rho_{\text{tapped}})$$

ρ = Density

6.2 Hausner's ratio

Hausner's ratio can be calculated through bulk volume & tapped volume; of powder drug substance:

$$\text{Hausner's ratio} = V_0 / V_f$$

Hausner's ratio can also be calculated through bulk density & tapped density; of powder drug substance:

$$\text{Hausner's ratio} = \text{Tapped Density} / \text{Bulk Density}$$

RESULT AND DISCUSSION:

Esomeprazole magnesium was an off-white amorphous powder with a characteristic obnoxious odor with a slightly bitter taste.

The drug was analyzed to recognize its distinctiveness through various modern and traditional analytical approaches. The identification of Esomeprazole magnesium was established with FTIR, HPLC, UV Spectrophotometry, and X-ray powder diffraction.

The elucidation of the obtained spectrum (fig-2) from FTIR was done to identify Esomeprazole magnesium through the classification of characteristic peaks of functional groups. IR absorption band (Table-1) obtained at 3192.25 indicates the presence of N-H group; peaks correspond to 2995.4 & 2831.39 specifies C-H stretching in Aromatics; peak corresponds to 2949.75 denotes C-H stretching in Aliphatics; the presence of absorption band at 1612.93 indicates C=N stretching (pyridine); absorption band at 1569.55 & 1271.28 signifies C=C and C-N stretching respectively; peaks obtained at 1226.37 &

1199.64 corresponds to C-O stretching (Methoxy); peak corresponds to 1076.93 indicates the presence of S=O group.

The identification of Esomeprazole Magnesium was established through HPLC (fig-3), in which 20 μL of Esomeprazole Magnesium (concentration 10 $\mu\text{g}/\text{ml}$) was injected and analyzed amid 200 nm – 380 nm through UV detector. The λ max was found at 301 nm with retention time 6.432 minutes, peak area 27 283 691 & peak height 7 87 862; which were comparable with the available literature assessment [21]. A single sharp peak was observed which indicates the purity of the drug.

The X-ray diffraction spectrum (fig-4) was also found to be concordant with available literature [16]. The average melting point from triplicate recordings for Esomeprazole Magnesium was found to be 184.1-188.9 $^{\circ}\text{C}$; which also corroborates with the literature value [38] [39]. UV Spectrophotometric analysis was done to establish absorption maxima. The absorption Maxima of Esomeprazole Magnesium (fig-5) was found at 301nm in phosphate buffer (pH-6.8) [28].

The linearity spectra (fig-6) & standard plot of Esomeprazole Magnesium (fig-7) were prepared at 301nm in phosphate buffer (pH-6.8).

The solubility of Esomeprazole Magnesium was predicted in various solvents. The standard plot was prepared for establishing drug solubility in experimental solvents viz Distilled water (fig-8), Methanol (fig-9), 0.1 N HCl (fig-10), Simulated Gastric fluid pH-1.2 (fig-11), Phosphate buffer pH- 6.8 (fig-12), Phosphate buffer pH- 7.4 (fig-13), and Simulated Intestinal fluid pH-6.8 (fig-14). The solubility was most in methanol and was least in distilled water. Solubility was also found to be pH dependent. Solubility increases with increase in pH. Order of solubility was; Methanol (1.214 mg/ml) > Phosphate buffer pH-7.4 (0.521 mg/ml) > Simulated Intestinal fluid pH-6.8 (0.475 mg/ml) > Phosphate buffer pH- 6.8 (0.466 mg/ml) > Simulated Gastric fluid pH-1.2 (0.147 mg/ml) > 0.1 N HCl (0.131 mg/ml) > Distilled water (0.017 mg/ml).

The Partition coefficient of Esomeprazole Magnesium was determined and the predicted log P value was 2.39; which corresponds with the literature value [37]. The drug concentration in the organic phase and in the aqueous phase was found to be 0.996 (mg/ml) and 0.004 (mg/ml) respectively.

The Micromeritics of Esomeprazole Magnesium were investigated and found the mean particle size in different dimensions (Table-3) viz the Mean length number diameter d_{ln} was 13.49 μm ; Mean Surface number diameter d_{sn} was 14.47 μm ; Mean volume number diameter d_{vn} was 16.49 μm ; Mean surface length diameter d_{sl} was 15.53 μm ; Mean surface volume diameter d_{sv} was 17.50 μm and Geometric mean $\log d_g$ was found to be 12.316 μm . The drug was also analyzed for its particle size through Malvern zetasizer and particle diameter was found to be 11.818 μm (fig- 16). The Bulk density and Tapped density was found to be $0.231 \pm 0.001 \text{ g}/\text{cm}^3$ & $0.287 \pm 0.001 \text{ g}/\text{cm}^3$ respectively. The Angle of repose (θ) was $38.86^{\circ} \pm 0.21$; the compressibility Index was 19.44 % and Hausner's ratio was found to be 1.24, which predicts the inherent flow properties of Esomeprazole magnesium (Table-4) as Fair (aid not needed).

DISCUSSION

The focus of the present study was to qualitatively and quantitatively portray Esomeprazole magnesium by exploring diverse analytical & investigative approaches and tools. Esomeprazole magnesium was found to be an off-white amorphous powder with a characteristic obnoxious odor and slightly bitter taste. The Identification of Esomeprazole Magnesium was done by employing various analytical approaches viz FTIR, HPLC, X-Ray diffraction, Melting point. The absorption maxima were found at 301 nm with UV Spectrophotometric & HPLC analysis. The drug was also identified through FTIR, through the classification of characteristic peaks of functional groups in IR spectra. X-ray diffractogram and melting

point corresponds to literature statistics. The particle size of the drug was analyzed through Malvern zetasizer and found to be 11.818 μm . The quantitative solubility of Esomeprazole magnesium was predicted in various solvents and found that it was most soluble in methanol and was least soluble in distilled water. Solubility was also found to be pH-dependent. Solubility increases with an increase in pH. The log P value indicates that the drug is significantly lipophilic. Various Micromeritic parameters like the Angle of repose, compressibility index and Hausner's ratio predict the inherent flow properties of Esomeprazole magnesium as Fair (aid not needed).

CONCLUSION

Hyperchlorhydria can manifest acute to chronic medical complications viz dyspepsia, heartburn, indigestion to Hypergastrinemic Syndromes like gastric ulcers, gastroesophageal reflux disease (GERD), Zollinger-Ellison syndrome. Esomeprazole is the drug of choice amongst PPI & available therapeutic alternatives but it is acid-labile hence it is predominantly enteric coated to prevent its release in the gastric environment. This causes a delay in the onset of action with a prolonging lag phase. The drug was qualitatively analyzed using various analytical techniques to establish its inherent properties quantitatively, which will prove the most valuable asset in the development of modified release formulation, to offer quick and protracted relief in gastritis and allied gastric disorders.

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

REFERENCES

1. Yao X, Forte JG. Cell biology of acid secretion by the parietal cell. *Annu Rev Physiol.* 2003;65:103-31. doi: 10.1146/annurev.physiol.65.072302.114200. Epub 2002 Aug 5. PMID: 12500969.
2. Forte JG, Zhu L. Apical recycling of the gastric parietal cell H,K-ATPase. *Annu Rev Physiol.* 2010;72:273-96. doi: 10.1146/annurev-physiol-021909-135744. PMID: 20148676.
3. Shin JM, Munson K, Vagin O, Sachs G. The gastric HK-ATPase: structure, function, and inhibition. *Pflugers Arch.* 2009 Jan;457(3):609-22. doi: 10.1007/s00424-008-0495-4. Epub 2008 Jun 6. Erratum in: *Pflugers Arch.* 2011 Mar;461(3):399. PMID: 18536934; PMCID: PMC3079481.
4. Savarino V, Marabotto E, Zentilin P, Furnari M, Bodini G, De Maria C, Pellegatta G, Coppo C, Savarino E. Proton pump inhibitors: use and misuse in the clinical setting. *Expert Rev Clin Pharmacol.* 2018 Nov;11(11):1123-1134. doi: 10.1080/17512433.2018.1531703. Epub 2018 Oct 10. PMID: 30295105.
5. Savarino V, Di Mario F, Scarpignato C. Proton pump inhibitors in GORD An overview of their pharmacology, efficacy and safety. *Pharmacol Res.* 2009 Mar;59(3):135-53. doi: 10.1016/j.phrs.2008.09.016. Epub 2008 Oct 8. PMID: 18977444.

6. Strand DS, Kim D, Peura DA. 25 Years of Proton Pump Inhibitors: A Comprehensive Review. *Gut Liver*. 2017 Jan 15;11(1):27-37. doi: 10.5009/gnl15502. PMID: 27840364; PMCID: PMC5221858.
7. Benetti C, Flammini L, Vivo V, Colombo P, Colombo G, Elviri L, Scarpignato C, Buttini F, Bettini R, Barocelli E, Rossi A. Esomeprazole immediate release tablets: Gastric mucosa ex vivo permeation, absorption and antisecretory activity in conscious rats. *J Control Release*. 2016 Oct 10;239:203-10. doi: 10.1016/j.jconrel.2016.08.032. Epub 2016 Aug 26. PMID: 27574989.
8. Shi S, Klotz U. Proton pump inhibitors: an update of their clinical use and pharmacokinetics. *Eur J Clin Pharmacol*. 2008 Oct;64(10):935-51. doi: 10.1007/s00228-008-0538-y. Epub 2008 Aug 5. PMID: 18679668.
9. Hsu WH, Kuo FC, Hu HM, Hsu PI, Wu DC, Kuo CH. Genetic polymorphisms of CYP2C19 and IL1B have no influence on esomeprazole treatment for mild erosive esophagitis. *Kaohsiung J Med Sci*. 2015 May;31(5):255-9. doi: 10.1016/j.kjms.2015.01.006. Epub 2015 Mar 10. PMID: 25910560.
10. Worden JC, Hanna KS. Optimizing proton pump inhibitor therapy for treatment of nonvariceal upper gastrointestinal bleeding. *Am J Health Syst Pharm*. 2017 Feb 1;74(3):109-116. doi: 10.2146/ajhp151032. PMID: 28122752.
11. Yeomans ND. Reducing the risk of gastroduodenal ulcers with a fixed combination of esomeprazole and low-dose acetyl salicylic acid. *Expert Rev Gastroenterol Hepatol*. 2011 Aug;5(4):447-55. doi: 10.1586/egh.11.42. PMID: 21780891.
12. Joshi AA, Nerkar PP. Determination of Proton Pump Inhibitors by Spectrophotometric, Chromatographic and by Hyphenated Techniques: A Review. *Crit Rev Anal Chem*. 2020 Apr 20:1-22. doi: 10.1080/10408347.2020.1750339. Epub ahead of print. PMID: 32312104.
13. Sugimoto M, Furuta T. Efficacy of esomeprazole in treating acid-related diseases in Japanese populations. *Clin Exp Gastroenterol*. 2012;5:49-59. doi: 10.2147/CEG.S23926. Epub 2012 May 14. PMID: 22649281; PMCID: PMC3359912.
14. Ward RM, Kearns GL. Proton pump inhibitors in pediatrics : mechanism of action, pharmacokinetics, pharmacogenetics, and pharmacodynamics. *Paediatr Drugs*. 2013 Apr;15(2):119-31. doi: 10.1007/s40272-013-0012-x. PMID: 23512128; PMCID: PMC3616221.
15. Skieneh J, Khalili Najafabadi B, Horne S, Rohani S. Crystallization of Esomeprazole Magnesium Water/Butanol Solvate. *Molecules*. 2016 Apr 23;21(4):544. doi: 10.3390/molecules21040544. PMID: 27120591; PMCID: PMC6273358.
16. Balakrishna T. Formulation and Evaluation of Lansoprazole Fast Dissolving Buccal Films. *Asian Journal of Pharmaceutics (AJP): Free full text articles from Asian J Pharm*. 2018 Aug 19;12(02).
17. Jain A, Teja MR, Pariyani L, Balamuralidhara V, Gupta NV. Formulation and evaluation of spray-dried esomeprazole magnesium microspheres. *Tropical Journal of Pharmaceutical Research*. 2013 Jun 25;12(3):299-304.
18. Jain V, Shah VK, Jain PK. HPLC Method Development and Validation for the Estimation of Esomeprazole in Bulk and Pharmaceutical Dosage Form. *Journal of Drug Delivery and Therapeutics*. 2019 Jul 15;9(4):292-5.
19. Kayesh R, Sultan MZ. A novel ion-pair RP-HPLC method for simultaneous quantification of naproxen and esomeprazole in pharmaceutical formulations. *J Chromatogr Sci*. 2015 May-Jun;53(5):687-93. doi: 10.1093/chromsci/bmu103. Epub 2014 Sep 2. PMID: 25182005.
20. Jain DK, Jain N, Charde R, Jain N. The RP-HPLC method for simultaneous estimation of esomeprazole and naproxen in binary combination. *Pharm Methods*. 2011 Jul;2(3):167-72. doi: 10.4103/2229-4708.90356. PMID: 23781450; PMCID: PMC3658060.
21. Rathi GG, Singh RK, Patel P, Singh R, Kumar B. RP-HPLC method for the estimation of esomeprazole magnesium in bulk and its pharmaceutical dosage forms. *Int. J. Pharm. Sci. Res*. 2010;1.
22. Agarwal, V., Rathore, D.S. and Bajpai, M., 2016. Investigation of effect of non-ionic stabilizers on the physical stability of drug nanosuspension prepared by bottom up approach. *International Journal of Pharmaceutical Sciences and Drug Research*, 8(4), pp.189-198.

23. Young JC. True melting point determination. Chem. Educator. 2013;18:203-8.
24. Pharmacopoeia US. National Formulary, 2002, USP25/NF20, United States Pharmacopoeial Convention.
25. Nichols L. Organic chemistry laboratory techniques. Independent; 2016.
26. Sharma MC, Sharma S. Spectrophotometric methods for the estimation of esomeprazole magnesium trihydrate in pharmaceutical formulations using indigo carmine reagent. International Journal of Pharm Tech Research. 2011 Apr;3(2).
27. Kumar PR, Shyale S, Gouda MM, Kumar SS. Physico-chemical characterization, UV spectrophotometric method development and validation studies of Esomeprazole Magnesium Trihydrate. J. Chem. Pharm. Res. 2010;2:484-90.
28. Prabu SL, Shirwaikar A, Shirwaikar A, Kumar CD, Joseph A, Kumar R. Simultaneous Estimation of Esomeprazole and Domperidone by UV Spectrophotometric Method. Indian J Pharm Sci. 2008 Jan;70(1):128-31. doi: 10.4103/0250-474X.40351. PMID: 20390100; PMCID: PMC2852053.
29. Indian Pharmacopoeia Commission. Indian Pharmacopoeia 2007.
30. Pharmacopoeia US. USP 29–NF 24. Rockville, MD: USP. 2005.
31. Işık M, Levorse D, Mobley DL, Rhodes T, Chodera JD. Octanol-water partition coefficient measurements for the SAMPL6 blind prediction challenge. J Comput Aided Mol Des. 2020 Apr;34(4):405-420. doi: 10.1007/s10822-019-00271-3. Epub 2019 Dec 19. PMID: 31858363; PMCID: PMC7301889.
32. Subhramanyam CV, Setty JT. Laboratory manual of physical pharmaceutics. Vallabh prakashan. 2002; 21.
33. Anderson W, Kozak D, Coleman VA, Jämting ÅK, Trau M. A comparative study of submicron particle sizing platforms: accuracy, precision and resolution analysis of polydisperse particle size distributions. J Colloid Interface Sci. 2013 Sep 1;405:322-30. doi: 10.1016/j.jcis.2013.02.030. Epub 2013 Mar 1. PMID: 23759321.
34. European Directorate for the Quality of Medicines (EDQM). The European Pharmacopoeia. 2008. Strasbourg: EDQM; 5.
35. European Pharmacopoeia Commission, European Directorate for the Quality of Medicines & Healthcare. European pharmacopoeia. Council of Europe; 2010.
36. European Directorate for the Quality of Medicines (EDQM). The European Pharmacopoeia. 2008. Strasbourg: EDQM; 5.
37. <https://www.drugbank.ca/salts/DBSALT001222>
38. https://www.chemsrc.com/en/cas/217087-09-7_1027301.html
39. https://www.chemicalbook.com/chemicalproductproperty_us_cb9740619.aspx

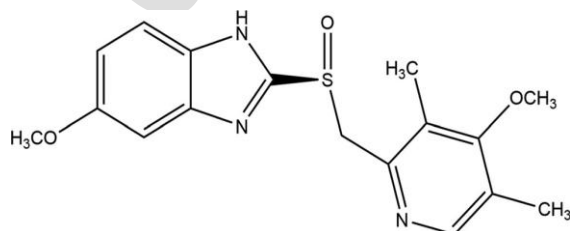


Fig 1: Chemical structure of Esomeprazole Magnesium [12].

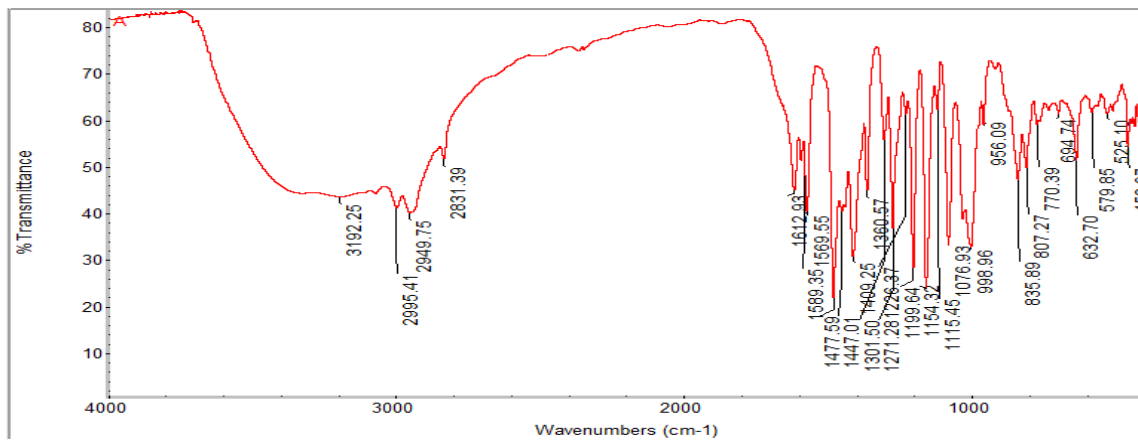


Fig 2: FTIR spectrum of Esomeprazole Magnesium

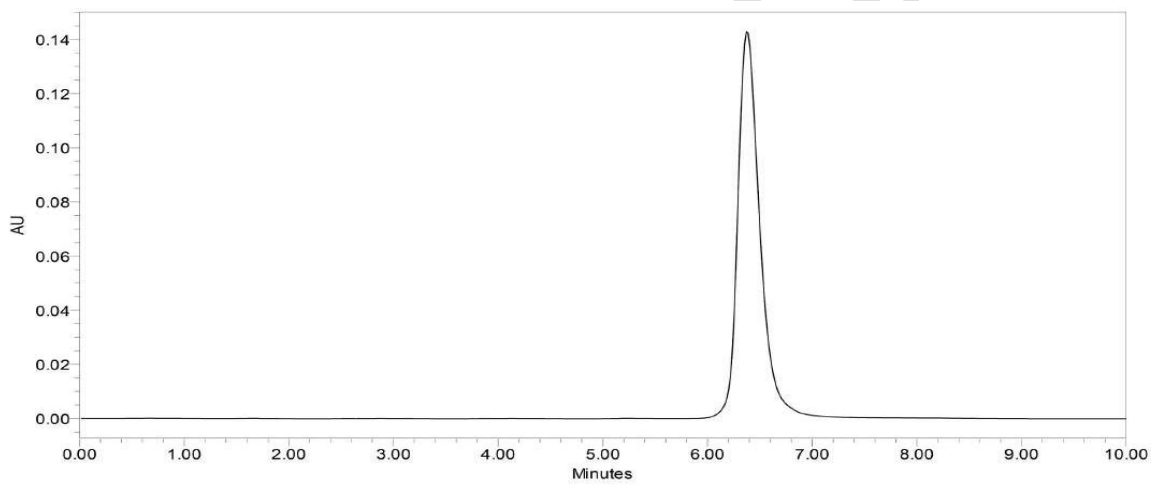


Fig 3: Chromatogram of Esomeprazole Magnesium

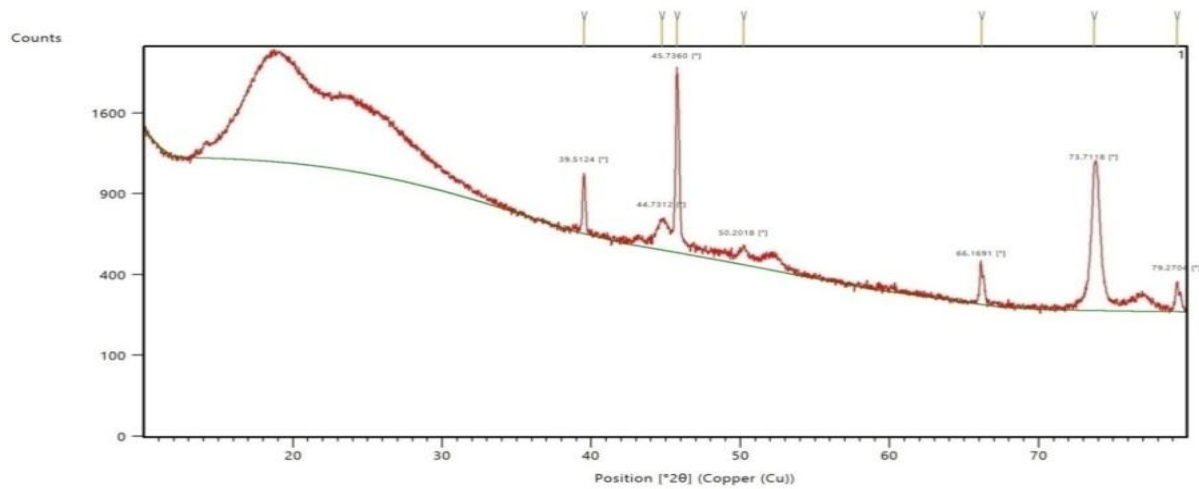


Fig 4: X-Ray powder diffraction Spectrum of Esomeprazole magnesium

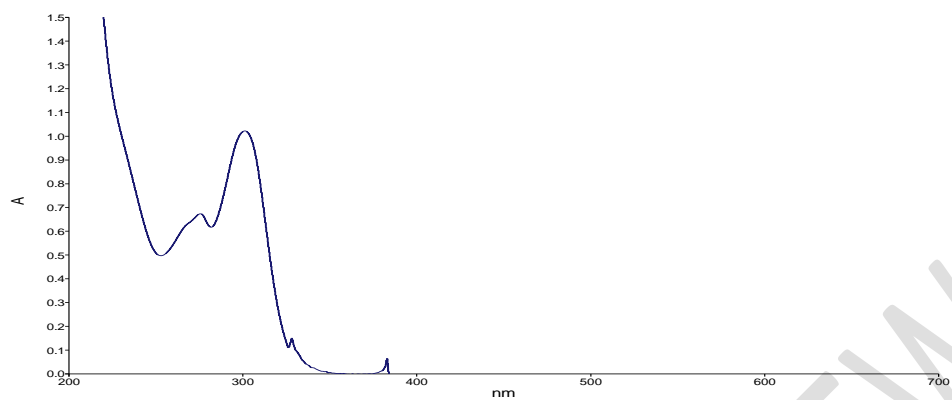


Fig 5: Absorption Maxima of Esomeprazole Magnesium was found at 301 nm in phosphate buffer (pH-6.8)

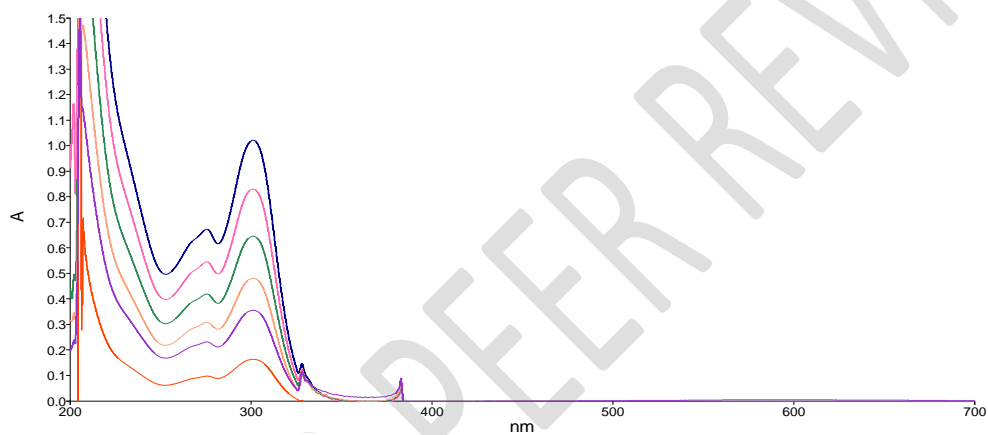


Fig 6: Linearity spectra of Esomeprazole Magnesium at 301 nm in phosphate buffer (pH-6.8)

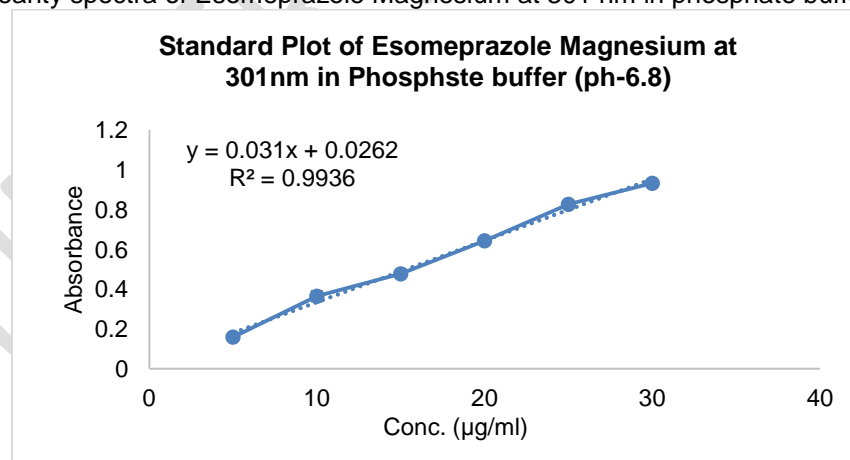


Fig 7: Standard Plot of Esomeprazole Magnesium at 301 nm in Phosphate buffer (pH-6.8)

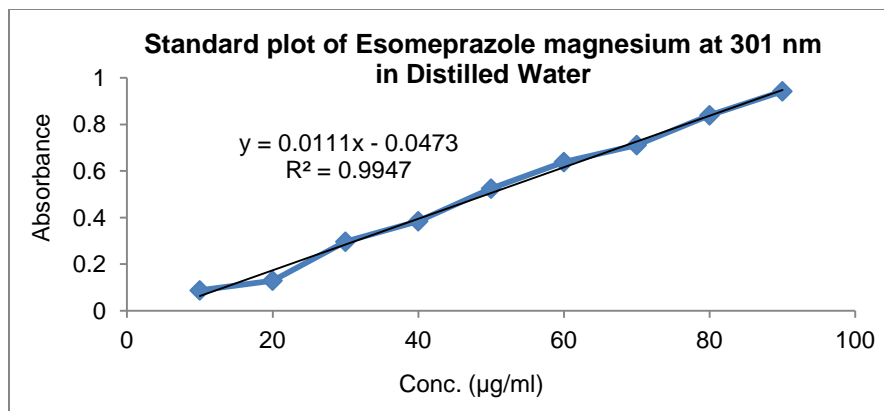


Fig 8: Standard Plot of Esomeprazole Magnesium in Distilled water at 301 nm.

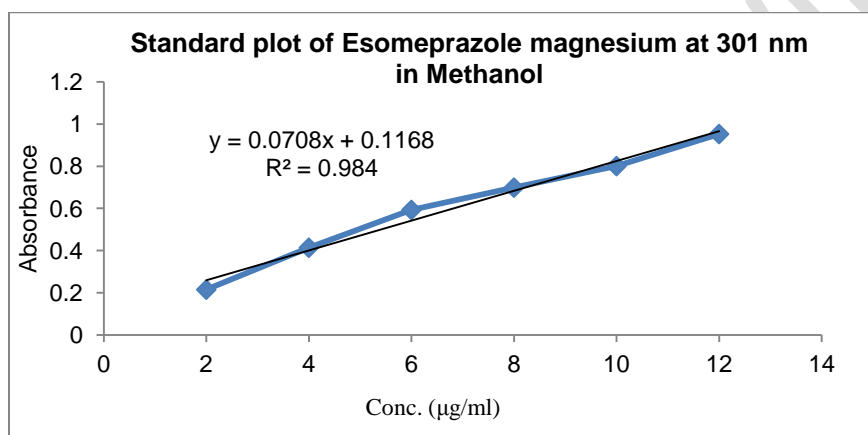


Fig 9: Standard Plot of Esomeprazole Magnesium in Methanol at 301 nm

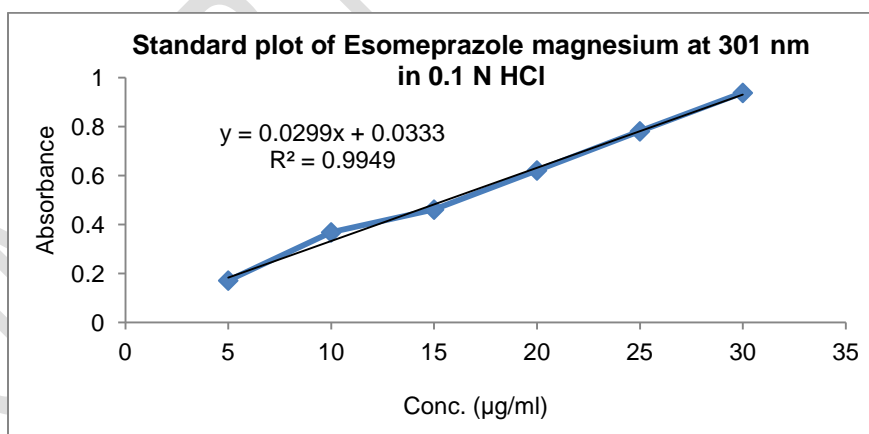


Fig 10: Standard Plot of Esomeprazole Magnesium in 0.1 N Hydrochloric acid solutions at 301 nm

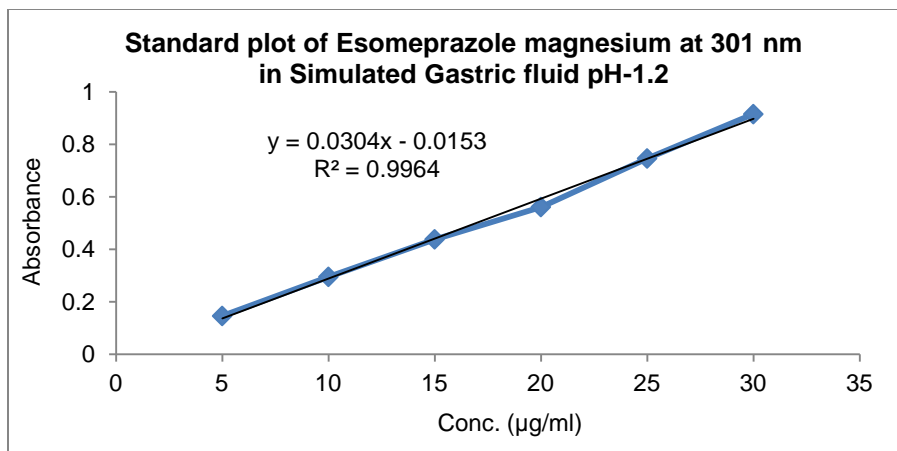


Fig 11: Standard Plot of Esomeprazole Magnesium in Simulated Gastric fluid (pH-1.2) at 301 nm

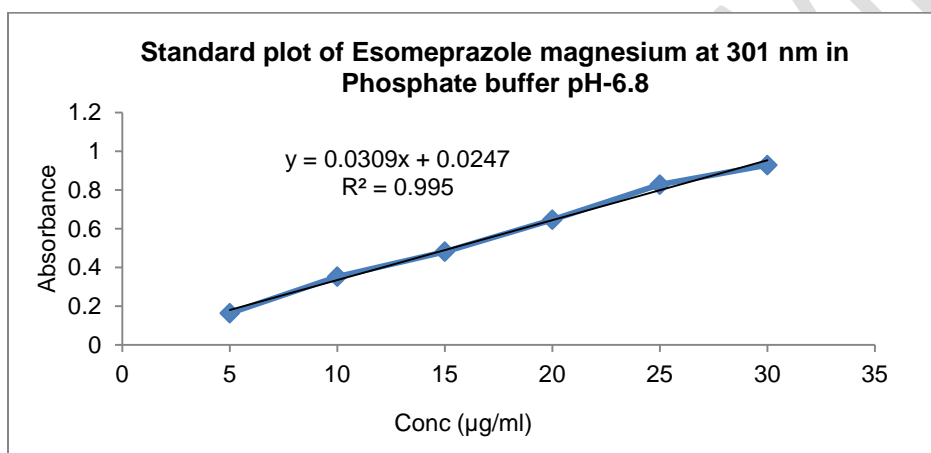


Fig 12: Standard Plot of Esomeprazole Magnesium in Phosphate buffer pH-6.8 at 301 nm

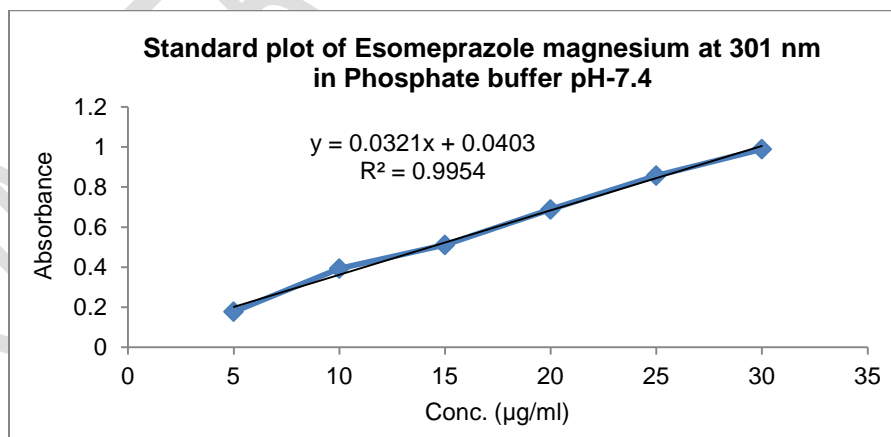


Fig 13: Standard Plot of Esomeprazole Magnesium in Phosphate buffer pH-7.4 at 301 nm

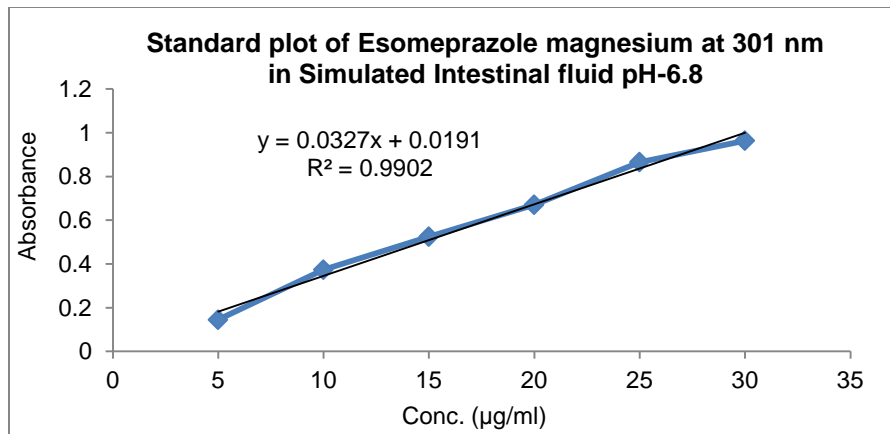


Fig 14: Standard Plot of Esomeprazole Magnesium in Simulated intestinal fluid (pH-6.8) at 301 nm

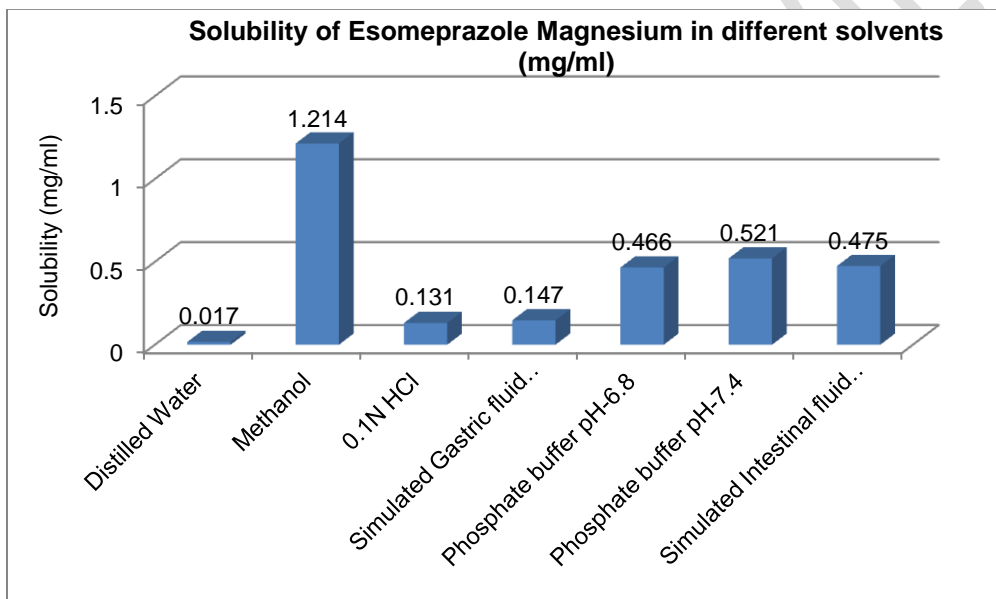


Fig 15: Column Graph showing Solubility of Esomeprazole Magnesium in Different Solvents

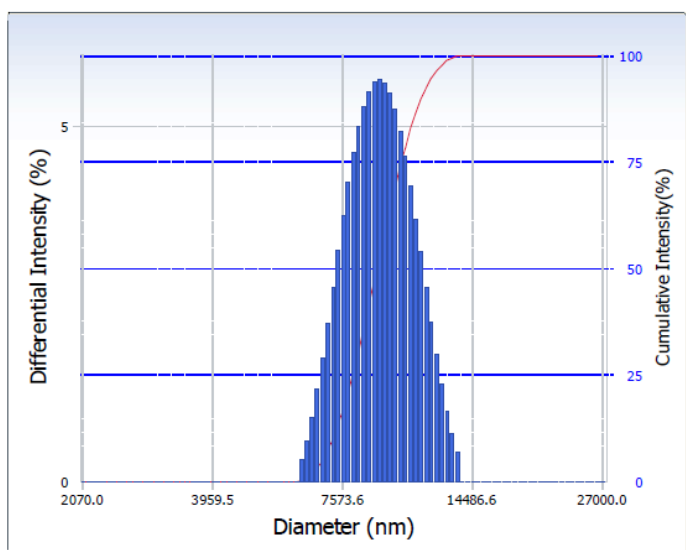


Fig 16: Intensity distribution of Esomeprazole Magnesium by Malvern Zetasizer

S.No	IR Absorption Band (cm ⁻¹) (Experimental)	Functional Groups
1	3192.25	N - H stretching
2	2995.4	C - H stretching in Aromatics
3	2949.75	C - H stretching in Aliphatics
4	2831.39	C - H stretching in Aromatics
5	1612.93	C = N stretching (pyridine)
6	1569.55	C = C stretching
7	1271.28	C - N stretching
8	1226.37	C - O stretching Methoxy
9	1199.64	C - O stretching Methoxy
10	1076.93	S = O stretching

Table 1: FTIR Spectrum of Esomeprazole Magnesium showing different wave number

S. No.	Conc. (µg/ml)	Absorbance 1	Absorbance 2	Absorbance 3	Average Absorbance	S.D
1	5	0.163	0.149	0.169	0.16	0.01
2	10	0.352	0.349	0.395	0.365	0.026
3	15	0.481	0.46	0.492	0.478	0.016
4	20	0.646	0.629	0.657	0.644	0.014
5	25	0.827	0.821	0.834	0.827	0.007
6	30	0.928	0.948	0.923	0.933	0.013

Values are expressed as mean± SD; (n=3)

Table 2: Linearity Table of Esomeprazole Magnesium in phosphate buffer (pH-6.8) at 301nm

S.No	Mean Diameter	Formula	Mean particle size
1	Mean length number diameter d_{ln}	$\Sigma n \cdot d / \Sigma n$	13.49 µm
2	Mean Surface number diameter d_{sn}	$\sqrt{(\Sigma n \cdot d^2 / \Sigma n)}$	14.47µm
3	Mean volume number diameter d_{vn}	$\sqrt{(\Sigma n d^3 / \Sigma n d)}$	16.49 µm
4	Mean surface length diameter d_{sl}	$\Sigma n \cdot d^2 / \Sigma n d$	15.53 µm
5	Mean surface volume diameter d_{sv}	$\Sigma n \cdot d^3 / \Sigma n d^2$	17.50 µm
6	Geometric mean log d_g	$\Sigma(n \log d) / \Sigma n$	12.316 µm

Table 3: Mean particle size of Esomeprazole Magnesium (µm)

Angle of repose (θ)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Compressibility Index (%)	Hausner's ratio
38.86 ± 0.21	0.231 ± 0.001	0.287 ± 0.001	19.44	1.24

Values are expressed as mean \pm SD; (n=3)

Table 4: Micromeritic properties of Esomeprazole Magnesium

UNDER PEER REVIEW