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Article

# Spectrophotometric Method for Estimation of Esomeprazole Magnesium Trihydrate Using Reagents – 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone, Chloranilic Acid, and 1-Chloro-2,4-dinitrobenzene with Charge–Transfer Complex Reaction

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**ABSTRACT**

Three simple, sensitive, and rapid spectrophotometric methods were developed for the estimation of esomeprazole magnesium (ESM) trihydrate in pure and its commercial dosage forms. These methods were based on the formation of charge–transfer reaction between the drug, an n-electron donor and π-acceptors, 2,3-dichloro-5, 6-dicyano-p-benzoquinone, chloranilic acid, and 1-chloro-2,4-dinitrobenzene. The absorbance of the formed charge–transfer complexes was measured and utilized for the determination of ESM in its pure and commercial dosage forms. The developed methods were evaluated in terms of standard deviation, relative standard deviation, correlation coefficient, limit of detection, and limit of quantitation. Molar absorptivity and Sandell's sensitivity were calculated at the optimum experimental conditions. The validity of the proposed methods was ascertained by recovery studies which indicated that the present methods can be successfully applied for the determination of ESM in pure and commercial dosage forms.

**Key words:** Spectrophotometric methods, Esomeprazole magnesium trihydrate, Charge–transfer reaction, 2,3-dichloro-5,6-dicyano-p-benzoquinone, Chloranilic acid, 1-Chloro-2, 4-dinitrobenzene.

## 1. INTRODUCTION

Esomeprazole magnesium (ESM) trihydrate is chemically bis(5-methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl] sulfinyl]-1-H-benzimidazole-1-yl) magnesium trihydrate (Figure 1). This is the first proton-pump inhibitor developed as a single optical isomer for the treatment of acid-related diseases. It inhibits gastric acid secretion. It is used for patients with gastroesophageal reflux disease, erosive reflux esophagitis, and peptic ulcer. ESM is cost effective in the treatment of gastric esophageal disorders, ESM is the S-isomer of omeprazole and generally provides better acid control than current racemic proton-pump inhibitors. A literature survey reveals that several methods have been reported for the estimation of ESM, such as high-performance liquid chromatography [1-8], voltammetry [9], high-performance thin-layer chromatography [10], and spectrophotometry [11-15]. Spectrophotometry is the technique of choice even today in the laboratories of research, hospitals, and pharmaceutical industries due to its low cost and inherent simplicity in operation. Visible spectrophotometry, because of its simplicity, cost effectiveness, sensitivity, selectivity, fair accuracy, and precision, has remained competitive in an era chromatographic techniques for pharmaceutical analysis.

There is no spectrophotometric method with a reagent of 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), chloranilic acid (CAA), and 1-chloro-2,4-dinitrobenzene (CDNB) reported in the literature so far. Hence, the current method was developed for the estimation of ESM with a reagent in bulk and pharmaceutical formulations.

Hence, in the present work, three methods were developed, which were based on the formation of a charge–transfer complex between ESM and DDQ and CAA and CDNB (Figure 1).

## 2. EXPERIMENTAL PROCEDURE

### *2.1. Instrumentation*

All measurements were carried out using a Shimadzu ultraviolet (UV)-visible spectrophotometer (UV-160A) with a matched pair of 10 mm quartz cells. Mettler Toledo analytical balance (accuracy 0.1 mg) was used for weighing all the samples.

### *2.2. Materials and Reagents*

ESM trihydrate from M/s. Hetero Drugs Limited, India, as a gift sample, (ESM trihydrate tablets IP), IZRA and ESOFAG were purchased in the local market, Tirupati. All the chemicals used were of analytical reagent grade. Double distilled water is used throughout the experiment.

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*2.2.1. Preparation of standard solutions*

A stock solution of ESM was prepared by dissolving accurately weighed 100 mg of pure drug in 100 ml of water and sonicated to get required concentration of 1 mg/ml. Further, it was diluted with double distilled water as required for the present study.

*2.2.2. Preparation of reagents*

About 2.5% (w/v) of DDQ solution was prepared by dissolving 2.5 g of compound in 100 ml of acetonitrile for Method A, 1.0% (w/v) of CAA solution was prepared by dissolving 1 g of compound in 100 ml of ethanol for Method B, and 3.0% (w/v) of CDNB solution was prepared by dissolving 3 g of compound in 100 ml of dimethyl sulfoxide for Method C.

***2.3. Method Development***

*2.3.1. Method A (DDQ method)*

Different aliquots of ESM solution were prepared in the range of 4–24 µg/ml and transferred into a series of clean and dry volumetric flasks. 1.4 ml of 0.2% DDQ solution into each flask was added and the pale reddish color was observed in solution and maximum absorbance was measured at 472 nm against the reagent blank.

*2.3.2. Method B (CAA method)*

The standard ESM solution was transferred into volumetric flasks in the concentration from 6 to 30 µg/ml. For this solution, added 2.2 ml of 1% CAA solution into the series of flasks, kept the solution for about 5 min and observed the solution color as yellowish-green. Maximum absorbance was measured at 435 nm against the reagent blank.

*2.3.3. Method C (CDNB method)*

Prepared different aliquots of ESM solution using a stock solution with ranging from 6 to 30 µg/ml were transferred into a series of clean and dry volumetric flasks and 3.6 ml of 3% CDNB solution was added into this flask. The total contents were heated to 98±2°C and the contents were kept at room temperature for few minutes, and the yellowish-pink color was observed. Maximum absorbance was measured for this solution at 420 nm against the reagent blank.

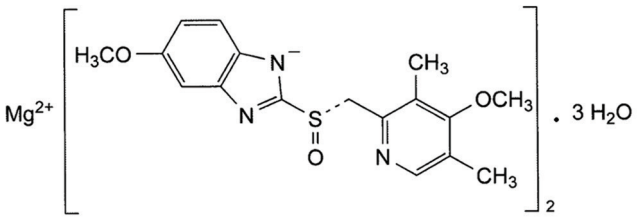


**Figure 1:** Structure of esomeprazole magnesium trihydrate.

*2.3.4. Procedure for analysis of pure drug*

An accurately weighed amount of ESM was transferred into a clean and dry volumetric flask, subsequently diluted with water to get the required concentration and analyzed by above mentioned the procedure.

*2.3.5. Procedure for commercial dosage forms*

The dosages of IZRA and ESOFAG containing ESM trihydrate were purchased from the local market and analyzed by the developed methods. Ten tablets of each formulation were weighed and grounded to make a fine powder. A quantity of grounded powder equivalent to 100 mg was taken into a volumetric flask and analyzed as described above.

## 3. RESULTS AND DISCUSSION

In the present work, DDQ, CAA, and CDNB were π-acceptors and ESM as n-donor. The charge–transfer complex was formed by an electron which is transferred from the donor to acceptor, which produces high intense color in the visible region of the electromagnetic spectrum.

***3.1. Spectral Characteristics***

*3.1.1. Absorption spectrum*

The reaction of DDQ, CAA, and CDNB with ESM results in the formation of pale reddish, greenish-brown, and pale yellowish-green complexes, respectively, which exhibits maximum absorbance at 472 nm, 435 nm, and 420 nm, respectively (Figures 2-4).

*3.1.2. The effect of reagent concentration*

To measure the effect of concentration of the reagent on the formation of colored products at the chosen wavelength, various volumes of reagents were added to a fixed concentration of drug solution and absorbance was measured. It was found that 1.4 ml of 0.2% DDQ (Method A), 2.2 ml of 1.0% CAA (Method B), and 3.6 ml of 3% CDNB solutions (Method C) were optimum for the production of high-intensity color and no change was observed after addition of few more milliliter of respective reagents.

*3.1.3. Effect of the concentration drug*

To study, the effect of concentration of drug solution on the absorbance maximum, fixed volume of reagent, i.e., DDQ, CAA, and CDNB, was added to each volumetric flask containing different aliquots of drug solution which was measured the absorbance at 472 nm, 435 nm, and 420 nm, respectively, against reagent blank. It was found that ESM obeyed Beer's law in the range of 4 –24 µg/ml, 6–30 µg/ml, and 6–30 µg/ml with DDQ, CAA, and CDNB, respectively.

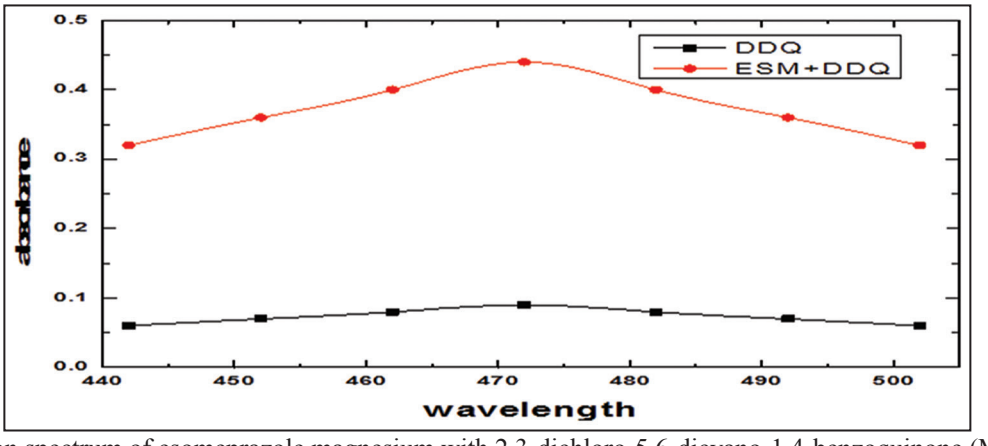


**Figure 2:** Absorption spectrum of esomeprazole magnesium with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (Method A).

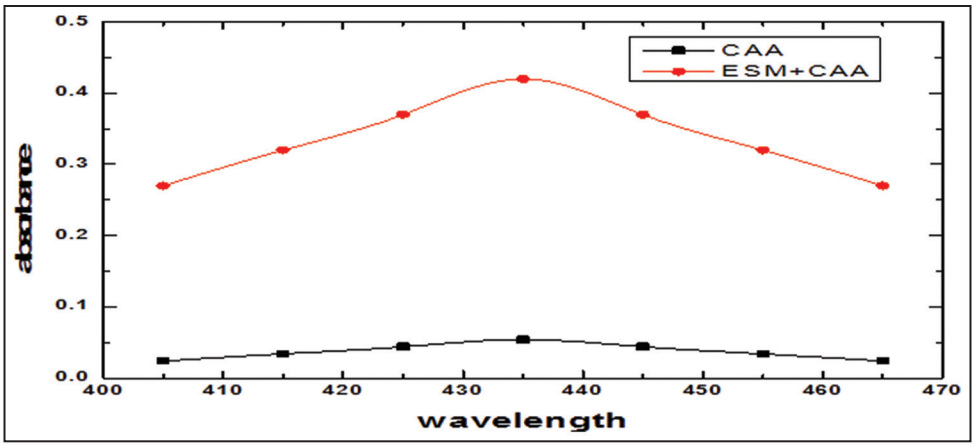


**Figure 3:** Absorption spectrum of esomeprazole magnesium with chloranilic acid (Method B).

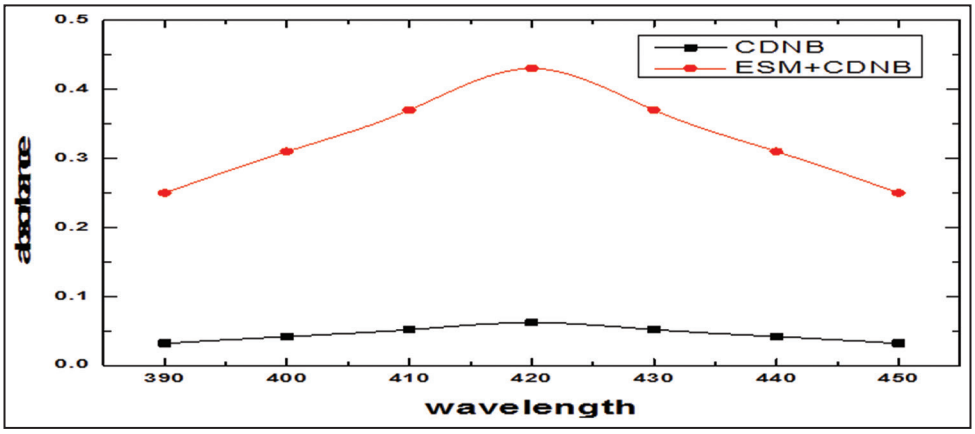


**Figure 4:** Absorption spectrum of esomeprazole magnesium with 1-chloro-2,4-dinitrobenzene (Method C).

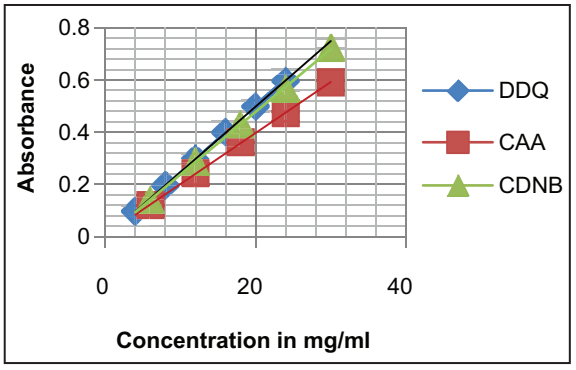


**Figure 5:** Calibration plot of esomeprazole magnesium trihydrate with analytical reagents.

### *3.2. Analytical Method Validation*

Validation is one of the important steps in analytical method evaluation [16]. The validation parameters, i.e., linearity, accuracy, precision, recovery, specificity, limit of detection (LOD), limit of quantification (LOQ), and robustness, were evaluated to assess the method suitability.

#### *3.2.1. Linearity*

The linearity of the concentration drug solution for the developed methods was studied, and calibration plots were constructed (Figure 5). From the calibration plots, a linear correlation was calculated between the absorbance and the concentration. Beer's Law limit, Sandell's sensitivity, and molar absorptivity are reported in Table 1.

**Table 1:** Spectral characteristics of the drug with a reagent.

| Parameter | Method A | Method B | Method C |
|---|---|---|---|
| $\lambda_{max}$ (nm) | 472 | 435 | 420 |
| Beer's law limit (µg/ml) | 4–24 | 6–30 | 6–30 |
| Molar absorbance ($L.mol^{-1}$ $cm^{-1}$) | 15599 | 6786 | 9144 |
| Sandell's sensitivity (µg.$cm^{-2}$/0.001 AU) | 0.0024 | 0.0023 | 0.0019 |
| Correlation coefficient ($r^2$) | 0.9999 | 0.9998 | 0.9997 |
| Slope (m) | 0.0219 | 0.0002 | 0.0223 |
| Intercept (c) | 0.0012 | 0.0183 | 0.0056 |
| % RSD | 0.2381 | 0.2272 | 0.2127 |
| Color | Pale reddish | Greenish-brown | Pale yellowish-green |
| LOD | 0.1367 | 0.1642 | 0.1342 |
| LOQ | 0.4555 | 0.5469 | 0.4469 |

LOD: Limit of detection, LOQ: Limit of quantification, RSD: Relative standard deviation

#### *3.2.2. Robustness and ruggedness*

For the evaluation of robustness, some parameters, such as the concentration of drug and reagent, wavelength range, and shaking

**Table 2:** Evaluation of accuracy and precision of the proposed method in bulk form.

| Method | Taken (mg/ml) | Intraday | | | | Interday | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *Found | Recovery (%) | ±SD | % RSD | *Found | Recovery (%) | ±SD | % RSD |
| A | 2 | 1.98 | 99.08 | 0.015 | 0.74 | 1.98 | 98.75 | 0.019 | 0.95 |
| | 4 | 3.98 | 99.54 | 0.012 | 0.29 | 3.97 | 99.25 | 0.018 | 0.45 |
| | 6 | 5.98 | 99.58 | 0.014 | 0.23 | 5.96 | 99.33 | 0.026 | 0.44 |
| B | 2 | 1.98 | 98.75 | 0.016 | 0.83 | 1.99 | 99.25 | 0.010 | 0.53 |
| | 4 | 3.97 | 99.29 | 0.017 | 0.43 | 3.97 | 99.13 | 0.023 | 0.57 |
| | 6 | 5.96 | 99.39 | 0.021 | 0.35 | 5.93 | 98.83 | 0.047 | 0.80 |
| C | 2 | 1.98 | 99.83 | 0.014 | 0.69 | 1.98 | 99.00 | 0.014 | 0.71 |
| | 4 | 3.97 | 99.17 | 0.018 | 0.44 | 3.97 | 99.25 | 0.018 | 0.45 |
| | 6 | 5.96 | 99.39 | 0.021 | 0.35 | 5.96 | 99.39 | 0.026 | 0.43 |

*Average of six determinations (mg/ml), RSD: Relative standard deviation, SD: Standard deviation

**Table 3:** Evaluation of accuracy and precision of the proposed method in pharmaceutical dosage forms.

| Method | Pharmaceutical formulation | Taken (mg/ml) | Intraday | | | | Interday | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Found | Recovery (%) | ±SD | % RSD | *Found | Recovery (%) | ±SD | % RSD |
| A | IZRA | 4 | 3.93 | 98.33 | 0.039 | 0.99 | 3.98 | 99.38 | 0.014 | 0.35 |
| | ESOFAG | 6 | 5.96 | 99.39 | 0.026 | 0.43 | 5.95 | 99.19 | 0.053 | 0.89 |
| B | IZRA | 8 | 7.92 | 98.96 | 0.050 | 0.63 | 7.96 | 99.54 | 0.023 | 0.28 |
| | ESOFAG | 4 | 3.97 | 99.13 | 0.026 | 0.65 | 3.97 | 99.21 | 0.021 | 0.54 |
| C | IZRA | 6 | 5.96 | 99.39 | 0.023 | 0.38 | 5.97 | 99.44 | 0.022 | 0.36 |
| | ESOFAG | 8 | 7.95 | 99.31 | 0.027 | 0.34 | 7.97 | 99.65 | 0.017 | 0.22 |

*Average of six determinations (mg/ml), RSD: Relative standard deviation, SD: Standard deviation

**Table 4:** Determination of esomeprazole magnesium trihydrate in the presence of excipients.

| Excipients | Amount taken (mg/ml) | *Found (mg/ml) | Recovery (%) | ±SD | RSD % |
|---|---|---|---|---|---|
| Glucose | 5 | 4.96 | 99.13 | 0.023 | 0.45 |
| Sucrose | 10 | 9.94 | 99.37 | 0.045 | 0.45 |
| Lactose | 15 | 14.85 | 98.97 | 0.096 | 0.65 |
| Dextrose | 10 | 9.96 | 99.62 | 0.023 | 0.23 |
| Talc | 15 | 14.88 | 99.18 | 0.072 | 0.48 |
| Starch | 20 | 19.75 | 98.76 | 0.171 | 0.87 |

*Average of six determinations, RSD: Relative standard deviation, SD: Standard deviation

time, were interchanged. The capacity remained unaffected by small changes in these parameters. Method ruggedness was expressed as a relative standard deviation (RSD%) of the same procedure applied by two analysts and in two different instruments on different days. The results showed no statistical difference between different analysts and instruments suggesting that the developed methods were robust and rugged.

### *3.2.3. Accuracy, precision, and recovery*

Accuracy of the proposed methods was proved by recovery studies (Tables 2 and 3). The recovery studies were carried out using the developed methods by adding a known quantity of the pure drug. The obtained results proved that the recovery values in drugs and dosages were within the acceptance limit.

Repeatability is determined using different concentrations and studied the variances in intraday and interday using proposed analytical methods and found the %RSD <1.0, which indicated that the developed methods were precise.

### *3.2.4. Specificity and selectivity*

To assess the specificity and selectivity of the developed method, the effect excipients, such as starch, lactose, glucose, sugar, and talc, were studied. The results indicated (Table 4) that there was no effect of interference from the excipients on the developed methods.

### *3.2.5. LOD and LOQ*

LOD and LOQ were calculated for the proposed methods using the formula.

$$LOD=3.3\ s/S \text{ and } LOQ=10\ s/S$$

where s = standard deviation of the response, S = slope of the calibration curve.

## 4. CONCLUSIONS

The methods reported in this paper are simple, specific, accurate, and precise for the estimation of ESM in bulk and pharmaceutical formulation. Using this method, we can measure the reported concentration range with good precision and accuracy. The linearity of the calibration standards of the drug by the spectrophotometric method was good from the result of the correlation coefficient. LOD, LOQ, molar absorptivity, and Sandell's sensitivity values indicated that the proposed analytical method, i.e., spectrophotometric method is accurate, simple, and reproducible for the estimation of ESM in bulk and pharmaceutical formulations.

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### **Bibliographical Sketch*

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