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# Indirect spectroscopic determination of esomeprazole using malachite green dye in the presence of N-bromosuccinimide

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***Abstract*---**A simple, fast and sensitive spectrophotometric method has been developed for the determination of esomeprazole in its pure form and also in the form of a pharmaceutical preparation. The highest absorption was found at a wavelength of 618 nm and the limits of Beer were (2_24) µg/ml. The molar absorbance ($2.1070 \times 10^4$) $L.mol^{-1} .cm^{-1}$, Sandell sensitivity 0016. µg.cm-2, and limits of detection LOD 0.0153 µg/ml, LOQ 0.0510 µg/ml were determined the method was also applied to pharmaceutical preparations, and the validity of the method was confirmed by standard addition.

***Keywords*---**esomeprazole, malachite green dye, pharmaceutical preparation.

## Introduction

Esomeprazole (*S*-isomer of omeprazole). Chemically, it is (S)-5-methoxy-2-[[[4-methoxy-3,5-dimethyl-2- pyridinyl]methyl]sulfiny]-1H-benzimidazole as show in fig(1). [(1)]. Esomeprazole (ESO) is a proton pump inhibitor (PPI) (1) because it inhibits gastric acid secretion [(3,2)]., including Zollinger-Ellison syndrome, as well as the treatment and prevention of gastric and duodenal ulcers and erosive esophagitis [(4,5)] and also works to eliminate stomach germs (helicobacter pylori) and heartburn [(6)].

Esomeprazole is used in the treatment of inflammation resulting from the work of gastric and esophageal endoscopy [(7)], and esomeprazole helps to prevent esophageal cancer [(8)]. Esomeprazole also inhibits placental autophagy because it relieves symptoms of pre-eclampsia (PE), which plays a major role in causing preeclampsia in pregnant women [(9)]. As for the side effects of esomeprazole, studies have shown that it is simple for people who do not suffer from

cardiovascular diseases and includes simple headaches, diarrhea and abdominal pain that disappear when treatment is stopped [10] On the other hand, studies have warned against giving it to patients who suffer from cardiovascular diseases. Blood and high level of cholesterol in the blood, because of the weakness in the metabolism of fats, even if its effect is small [11] Regarding the effect of this drug on the liver, studies have shown that the effect of esomeprazole is limited and the study did not confirm its toxicity to the liver [12].

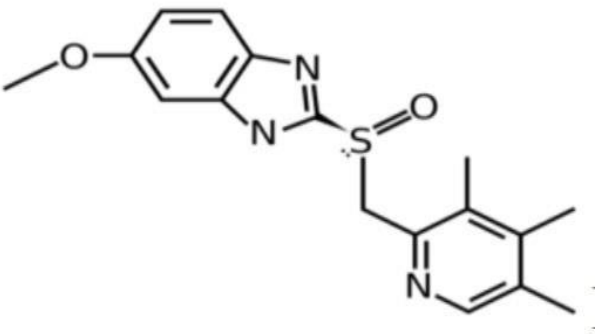



Figure 1: Esomeprazole

**Practical part Equipment and tools "used":**

1. Shimadzu( UV/Vis) 1800 spectrophotometer, Japan
2. ( BL 210S- sensitive) Scientific balance.
3. Quartz cell of (1.0) cm for absorbance measurements.

**Reagents and chemical materials used**

All chemicals were of( high purity) and from( known sources) and their solutions It was prepared as follows:

**1. Standard Esomeprazole solution, (200 ppm)**

The solution was prepared by dissolving 0.0200 g) of pure Esomeprazole (prepared by the General Company for Pharmaceuticals and Medical Appliances - SDI Samarra - Iraq) in 5 ml of ethanol, then the volume was completed with distilled water to Limit the mark to a 100-mL volumetric vial .

**2 .malachite green dye solution ,(100 ppm).**

The solution was prepared by dissolving 0.0100 g of malachite green salt in distilled water using a 100 ml volumetric flask.

**3. N_bromosuccinimde solution,(300 ppm).**

0.03 g of the substance is dissolved in distilled water in a volumetric flack of 100 ml

**4. hydrochloric acid solution, (1M).**

The acid was prepared by diluting 8.5 ml of concentrated acid using a volumetric bottle of 100 ml and completing the volume with distilled water.

**5. Pharmaceutical solutions Esomeprazole capsules solution, (200 ppm)**

A mixture of powder of five esomeprazole in capsules was prepared, each containing 40 mg of esomeprazole, the weight of each one was 0.044, then 0.0200 of it was weighed and dissolved in 5 ml of ethanol, and the volume was completed with distilled water in a volumetric flask of 100 m

**The principle of the method**

The principle of the method depends on the oxidation of the drug (ESO) by the oxidizing agent in an acidic medium, after which the dye solution is added, so that the resulting solution is green, indicating that the oxidizing agent has run out.

**Preliminary study**

1ml of (ESO) was added, then 1 ml of the oxidizing agent N_bromosuccinimde was added, then 1 ml of hydrochloric acid was added, and finally 1 ml of malachite green dye was added, All of this was done using volumetric bottles of 25ml capacity. It was noticed that the color of the dye appeared as an indication of the depletion of the oxidizing agent The highest absorption was at a wavelength of 618 nm.

**Study the optimal conditions**

For the reaction In order to obtain a colored product with high absorption and sufficient stability to complete the measurements, the optimal conditions were studied.

**Study of the effect of the oxidizing agent N_bromosuccinimide (NBS)**

The effect was studied by adding increasing volumes of (0.25_ 2.0) ml of (NBS) solution and it was found that 1.0 ml is the appropriate volume.

Table No. (1) Effect of the oxidizing agent N_bromosuccinimide (NBS)

| Volume of (NBS) (0.03%)(ml) | Absorbance |
|---|---|
| 0.25 | 0.102 |
| 0.5 | 0.186 |
| 1.0 | 0.293 |
| 1.25 | 0.221 |
| 1.5 | 0.175 |
| 2.0 | 0.116 |

**Study of the effect of the amount of green dye malachite**

The effect of the dye was studied by adding different volumes of (0.25 - 2.5) ml at a concentration of (0.01%) of the dye, as shown by the results in Table (2)

Table No. (2) Effect of the amount of dye

| Volume of dye (0.01%) (ml) | Absorbance |
|---|---|
| 0.25 | 0.141 |
| 0.5 | 0.198 |
| 1.0 | 0.292 |
| 1.25 | 0.378 |
| 1.5 | 0.409 |
| 2.0 | 0.275 |
| 2.25 | 0.265 |
| 2.50 | 0.194 |

**Study the type of acid**

Preliminary experiments showed that the process is carried out in an acidic medium to form a colored product, so the effect of several strong and weak acids was studied in the estimation of esomeprazole by adding fixed amounts of 1 ml of (1M) to each of them separately. It is clear from the table below that hydrochloric acid is the best

Table No. (3) Study the type of acid

| Type of acid (1M) (ml) | $H_2SO_4$ | HCl | $CH_3COOH$ | $HNO_3$ |
|---|---|---|---|---|
| Absorbance | 0.293 | 0.405 | 0.113 | 0.201 |

**Study the effect of the amount of HCl**

The effect was studied by adding different volumes of HCl with a concentration of 1M to the reaction mixture, then the absorbance of these solutions was measured against the blank solution and the following table shows the results.

Table (4) Studying the effect of the amount of HCl

| Volume of HCl (1M) | 0.25 | 0.5 | 0.75 | 1.0 | 1.5 | 2.0 |
|---|---|---|---|---|---|---|
| Absorbance | 0.190 | 0.245 | 0.301 | 0.490 | 0.401 | 0.220 |

**Oxidation time study**

The effect of oxidation time on the intensity of absorption was studied by adding the calculated amount of the oxidizing agent to (ESO) in an acidic medium and leaving it for different periods of time (0_30) minutes, then adding a calculated amount of dye and completing the volume with distilled water, then the absorbance was measured.

Table (5) studying the effect of oxidation time

| Time of oxidation (min) | Absorbance |
|---|---|
| 0 | 0.321 |
| 5 | 0.491 |
| 10 | 0.475 |
| 15 | 0.405 |
| 20 | 0.322 |
| 25 | 0.312 |
| 30 | 0.278 |

**Study the effect of surfactants**

The effect of surfactants was studied, where different volumes (0.5_2.0) ml of each of the surfactants were added to the reaction, and then the absorbance was measured at a wavelength of 618 nm, and the results showed that it had a negative effect on the reaction.

Table (6) Effect of surfactants

| Surfactant | Absorbance /ml of surfactant used | | | |
|---|---|---|---|---|
| | 0.5 | 1.0 | 1.5 | 2.0 |
| CTAB 0.1% | 0.324 | 0.355 | 0.316 | 0.301 |
| CPC 0.1% | 0.275 | 0.294 | 0.319 | 0.271 |
| SDS 0.1% | 0.241 | 0.276 | 0.232 | 0.211 |
| TritonX-100 0.1% | 0.401 | 0.422 | 0.418 | 0.391 |
| Without | 0.490 | | | |

**Study of the effect of dye stability time**

The effect of time on the stability of the resulting dye was studied by using the approved method and the optimal conditions obtained from previous experiments by using a volumetric bottle of 25 ml and completing the volume with distilled water after that, then measuring the absorbance of the colored solution with different time periods at a wavelength of 618 nm.

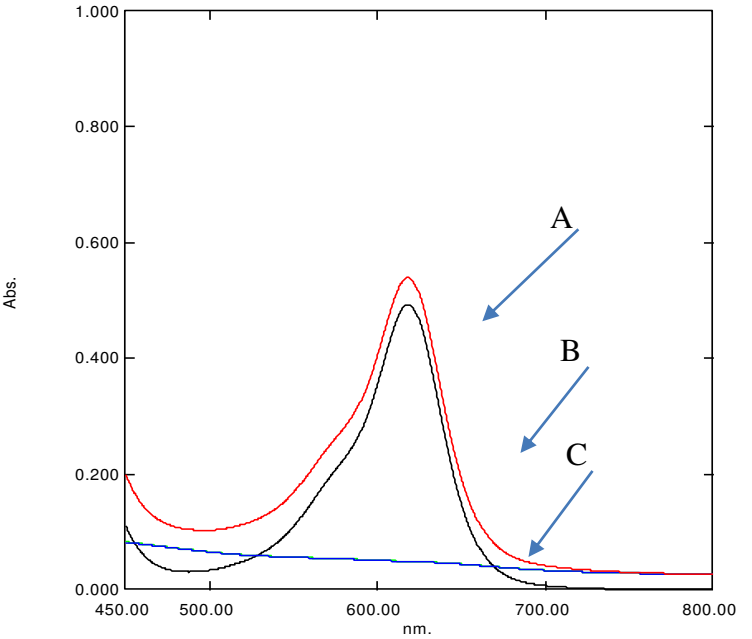
Table (7) Effect of time on dye stability

| Esomeprazole 8µgml$^{-1}$ Room Temp. | Absorbance/time (min) |
|---|---|
| After addition | 0.491 |
| 5 | 0.493 |
| 10 | 0.493 |
| 15 | 0.493 |
| 20 | 0.491 |
| 25 | 0.491 |
| 30 | 0.491 |
| 35 | 0.491 |
| 40 | 0.490 |
| 45 | 0.490 |
| 50 | 0.489 |
| 55 | 0.489 |
| 60 | 0.489 |
| 90 | 0.489 |
| Over night | 0.482 |

Table No.8 Summary of optimal conditions

| Material solution | Concentration | Optimum amount (ml) |
|---|---|---|
| Malachite Green | %0.01 | 1.5 |
| NBS | %0.03 | 1 |
| HCL | 1 M | 1 |
| $\lambda_{max}$ ( nm ) | 618 | |
| Temp. | 35 C° | |
| Stability period | min)60 ( | |

**Final absorption spectrum:**
Esomeprazole is oxidized by a known amount of oxidizing agent (NBS) in an acidic medium, and the excess of the oxidizing agent leads to shortening the dye at a wavelength of 618 nm.




Fig(.2) final absorption spectrum of (8ppm) Esomeprazole solution: A-( versus blank), B( versus distilled water), C-(blank versus distilled water).

**Calibration curve**
By following the previously mentioned method and under optimal conditions, increasing amounts of esomeprazole are taken in volumetric flask 25 ml to make the standard curve and measure the absorbance of the solutions against the blank solution at a wavelength (618 nm). The following figure shows the standard curve for the determination of esomeprazole, which follows Beer's law in the range Concentration (2_24) µg/ml, and the value of the correlation coefficient indicates that the linear specifications are good

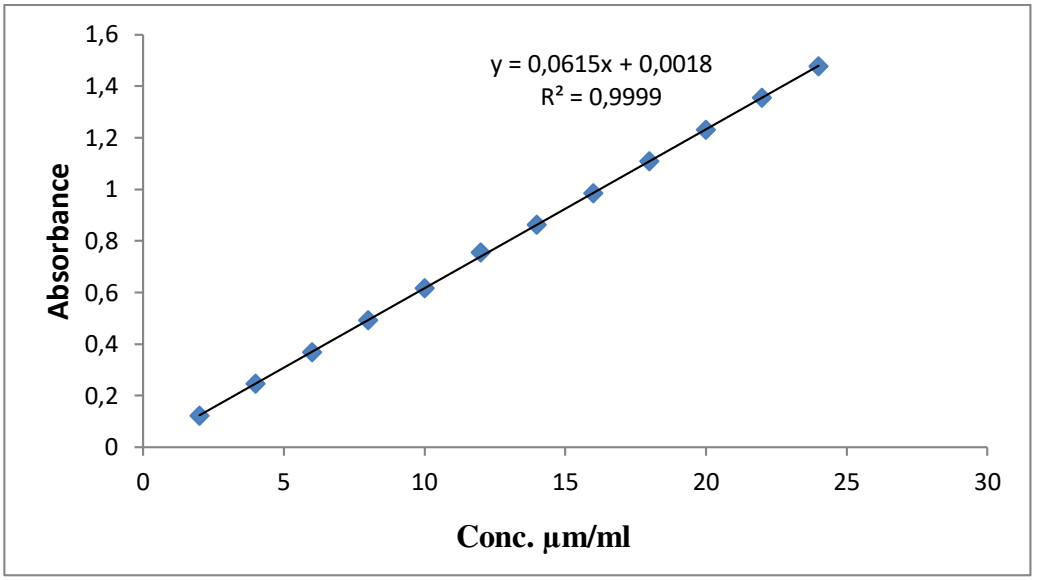



Fig.3: The standard curve for determination of esomeprazole

The values of εmax LOD, LOQ, Sandel's sensitivity and correlation coefficient were calculated as shown in the table. It is clear that the method has "good" sensitivity, and the correlation coefficient value shows the high linear specifications of the calibration curve.

Table No.9 εmax LOD, LOQ, Sandel's sensitivity and correlation coefficient

| | |
|---|---|
| Beer's law range(ppm) | (2-24) |
| Molar absorptivity ($l.mol^{-1}.cm^{-1}$) | $2.1070 \times 10^4$ |
| Sandell's sensitivity ($\mu g.cm^{-2}$) | 0.016 |
| LOD (µg/ml) | 0.0153 |
| LOQ (µg/ml) | 0.0510 |
| slope | 0.061 |

**The accuracy and compatibility of the method**

To calculate the accuracy of the calibration curve, several concentrations of esomeprazole were taken as shown in the following table

Table No.10 The accuracy and compatibility of the method

| Amount of ESO µg/ml present | Amount of ESO µg/ ml found | Recovery % | Rrelative error,% | Relative standard deviation,% |
|---|---|---|---|---|
| 2 | 1.986 | 99.3 | 0.7 | 0.967 |
| 4 | 3.938 | 98.45 | 1.55 | 0.900 |
| 6 | 6.019 | 100.3 | -0.3 | 0.190 |

**Application of the method to pharmaceutical preparations**

The proposed method was applied to the pharmaceutical preparations of esomeprazole, which were in the form of a capsule. Three different concentrations of the solution were taken. All the steps mentioned in the method were applied in the optimal conditions. The value of the recall ratio, relative error, and relative standard deviation was calculated. The following table shows that the proposed method is successful with an estimate Esomeprazole is in the capsule form and has good accuracy and compatibility.

Table No.11 Application of the method

| AwaNex capsules 40 mg/capsule Iraq | Amount of ESO µg/ml present | Amount of ESO µg/ ml found | Recovery % | Relative error,% | Relative standard deviation,% |
|---|---|---|---|---|---|
| | 2 | 1.970 | 98.50 | 1.50 | 0.569 |
| | 4 | 3.970 | 99.25 | 0.75 | 0.574 |
| | 6 | 6.019 | 100.31 | -0.3 | 0.465 |

**Standard addition method**

Because of the difficulty in providing the necessary tools and chemicals for the standard method in the British Constitution, and in order to clarify the efficiency of the method and prove its accuracy, the standard addition method was applied to the pharmaceutical preparation of esomeprazole by adding fixed volumes (0.25_0.50) of the solution of the 200 µg/ ml preparation to two series of 25 ml volumetric flask and then adding Increasing volumes of the standard solution of esomeprazole at a concentration of 200 µg/ ml under all optimum conditions were applied and the absorbance was measured at a wavelength 618 nm The results shown in the figure and the table below showed that the method is compatible with the developed method and has an acceptable selectivity.

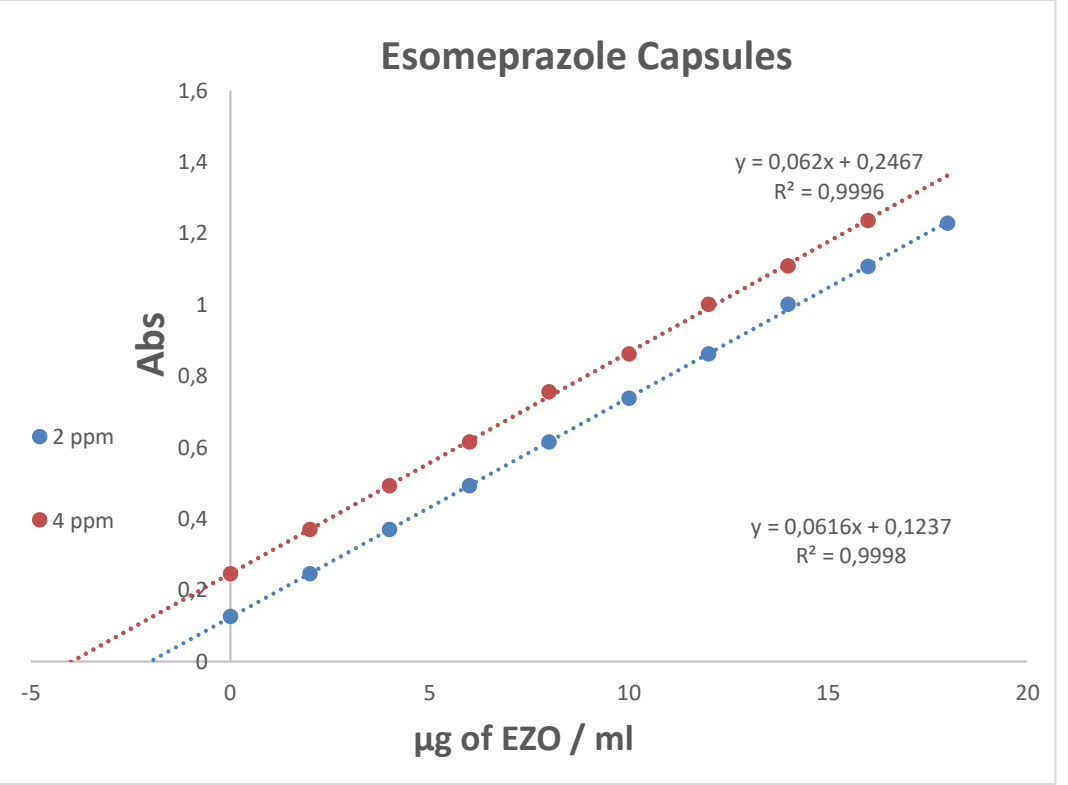



Fig - 4. Standard addition method curves for determination of esomeprazole in pharmaceutical preparation at concentration (2,4 µg/ ml).

Table No.12 The results of the standard addition method according to the proposed method of work

| pharmaceutical preparation | esomeprazole present µg/ml | esomeprazole measured µg/ml | Recovery % |
|---|---|---|---|
| esomeprazole AwaNex capsules 40 mg/capsule Iraq | 2 | 2.008 | 100.4 |
| | 4 | 3.979 | 99.47 |

**Conclusions**

A new developed spectrophotometric method has been proposed for the determination of esomeprazole using malachite green dye and the oxidizing agent N bromosuccinimde

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