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Spectrophotometric estimation of esomeprazole using diazotization reaction with meta- amino phenol reagent and application in pharmaceutical preparations

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***Abstract*---**The aim of the following study is to develop a sensitive, high-accuracy and fast spectrophotometric method for the determination of esomeprazole (ESO) in pharmaceutical preparations.The proposed method is based on the formation of dyzonium salt from the reaction of meta-aminophenol reagent with nitrite in an acidic medium and then coupling it with esomeprazole in an alkaline medium to form a yellow dye that gives the highest absorption at a wavelength of 450 nm. Obtaining a calibration curve within 1_12 with a correlation coefficient of 0. 999. The molar absorbance 3.3954 × 10^4 $L.mol^{-1}$ $.cm^{-1}$, Sandell sensitivity 0.0101 $\mu g.cm^{-2}$, and limits of detection LOD 0.048 µg/ml, LOQ 0.161 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, diazotization and coupling reactions Meta-aminophenol

Introduction

The chemical formula for esomeprazole is 6-methoxy-2-[(4-methoxy3,5-dimethylpyridine-2-yl) methylsulfinyl]-1H-benzimidazole (Fig. 1). [1] And its molecular weight is M.Wt =345.417 g/mol[2] . Slightly soluble in water [3].

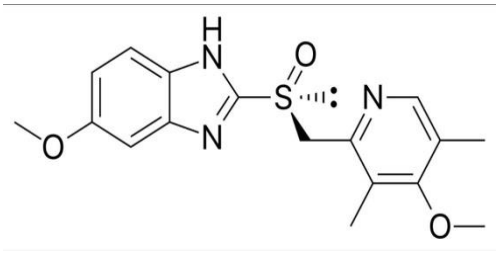


Figure 1: Esomeprazole

Esomiprazole inhibits the action of the proton pump PPI, which leads to disruption of the process of formation and secretion of hydrochloric acid in the stomach, Esomeprazole comes in the form of capsules and ampoules for injection in pharmaceutical preparations [4] Several methods have been developed for the determination of esomeprazole. Spectrophotometric methods are the most common [1.2.3.5.6]. liquid chromatography [7]. RP_HPlC using diclofenac sodium and mass chromatography supercritical liquid chromatography HPLC using oxytetracycline, tindazole and micro-extraction liquid chromatography [2]. Also, a method was developed for the determination of esomeprazole by solving the instantaneous equations for measuring the absorbance at two wavelengths [8]. Esomeprazole is of great importance in the treatment of Stomach diseases such as heartburn and esophageal irritation. It is also used to prevent hemorrhagic stomach ulcers and esophageal cancer [9] Most of the information regarding the safety of esomeprazole use during pregnancy is derived from research on omeprazole, since esomeprazole is an S-isomer of omeprazole, and information characterizing transplacental transport of esomeprazole and its excretion in breast milk is limited [10]. Esomeprazole is taken as a treatment in the form of sodium and magnesium salts [11]

Practical part

Equipment and tools "used":

1. Shimadzu UV/Vis 1800 spectrophotometer, Japan
2. Sartorius 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, (100 ppm)

The solution was prepared by dissolving 0.0100 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. Meta-aminophenol reagent solution (0.01%):

A vial of solution was prepared by dissolving 0.0100 g) of meta-aminophenol in 5 ml of hydrochloric acid (5 N), and completed the volume in Volumetric 100ml up to the mark with distilled water.

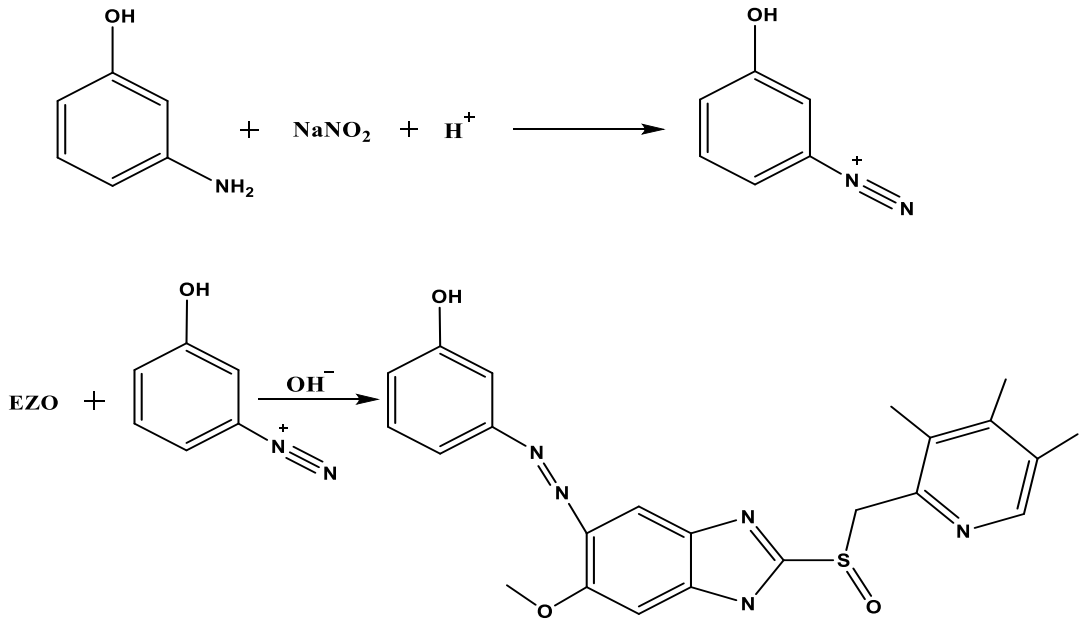
3. Sodium nitrite solution (0.5%):

Prepare by dissolving (0.5g) of sodium nitrite in distilled water, then complete the volume in a 100 mL volumetric vial to the mark with distilled water.

4. Sulfamic acid solution (1%):
Dissolve (1 g) of sulfamic acid in distilled water, then fill the volume in a 100-capacity bottle milliliters to the mark with distilled water.
5. Sodium hydroxide solution (1M):
Dissolve (4g) of NaOH in distilled water and fill the volume in a volumetric vial 100 milliliters to the mark with distilled water.
6. Pharmaceutical solutions

Esomeprazole capsules solution, 100µg /ml
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 0.0100 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 ml.

The principle of the method
The basic principle of this method depends on the reaction of meta-aminophenol reagent with an amount of sodium nitrate in an acidic medium to form the diazonium salt, then the excess nitrite is removed by the addition of sulfamic acid, then the diazonium salt is reacted with esomeprazole in an alkaline medium to form a yellow dye.



Yellow azo day

Preliminary study
1.5 ml of meta-aminophenol reagent prepared in acidic medium was reacted with 1 ml of sodium nitrite, then waited for two minutes, then 1 ml of sulfamic acid was added and waited for two minutes to get rid of the excess nitrite, then 1 ml of esomeprazole was added and the medium was made basic by adding 1 ml of solution As a rule, it was observed that a solution with a dark yellow color gave

the highest absorption at a wavelength of 450 nm compared to the blank solution, and all this was done using a volumetric flask of 25 ml

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.

Effect of sodium nitrite amount and time on dye absorption
The effect was studied by taking increasing volumes of (0.1-2.0) ml of sodium nitrite (1%) and for different time periods. It was noted that 1 ml of the solution for 5 minutes gave the highest absorption of the formed dye.

Table No. (1) Effect of sodium nitrite amount and time on dye absorption

Volume of $NaNO_2$ (0.5%) (ml)	Absorbance/min. standing time			
	0	3	5	7
0.1	0.075	0.135	0.152	0.098
0.5	0.221	0.254	0.282	0.201
1.0	0.422	0.532	0.581	0.501
1.5	0.371	0.382	0.392	0.356
2.0	0.142	0.163	0.191	0.151

Effect of Sulfamic acid amount and time
This effect was study by adding increasing volumes (0.1 - 2.0) of 1% sulfamic acid for a different period of time in order to get rid of the residual excess of sodium nitrite that affects the absorption value of the solution and causes side reactions

Table No. (2) Effect of Sulfamic acid amount and time

Volume of 1% sulfamic acid (1%) (ml)	Absorbance/min. standing time		
	1	3	5
0.1	0.092	0.107	0.113
0.5	0.201	0.214	0.210
1.0	0.326	0.354	0.341
1.5	0.525	0.585	0.545
2.0	0.385	0.398	0.375

Studying the effect of the amount of meta-aminophenol (reagent)
The optimal amount has been studied by adding different volumes of the reagent to a fixed amount of the diazotized drug after making the medium basic and diluting it to the mark, the absorbance of the solutions was measured, and the following table shows the results.

Table No. (3) Studying the effect of the amount of (reagent)

Volume of reagent (0.01%)(ml)	Absorbance
0.1	0.082
0.5	0.321
1.0	0.583
1.5	0.642
2.0	0.810
2.5	0.544
3.0	0.501

Studying the effect of the base type

From the preliminary study, it is clear that the dye is formed in an alkaline medium, and for the purpose of knowing the effect of the type of base, the effect was studied on different types of strong and weak bases by adding a fixed amount of the base at a concentration of 1M to each of them individually. Table No. 4 shows the results

Table No. 4 Studying the effect of the base type

Type of base (1M)	absorbance
NaOH	0.811
KOH	0.624
Na_2CO_3	0.221
$NaHCO_3$	0.106

Study the effect of the amount of sodium hydroxide

The effect was studied by adding different volumes of base 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 No. 4 Studying the effect of the amount of NaOH

Volume of NaOH (1 M)(ml)	absorbance
0.1	0.201
0.5	0.554
1.0	0.810
1.5	0.802
2.0	0.611
2.5	0.426

Effect of surfactants

Many surfactants have been studied, and the study showed that these materials have a negative effect on the absorption of the colored solution the following table illustrates this.

Table No. 4 Studying the effect of the amount of surfactants

Surfactant	Absorbance/ml of surfactant used			
	0.5	1.0	1.5	2.0
CTAB 0.1%	0.615	0.633	0.651	0.623
CPC0.1%	0.523	0.544	0.592	0.581
SDS 0.1%	0.736	0.749	0.771	0.721
Triton X-100 0.1%	0.781	0.789	0.749	0.752
Without Surfactant	0.810			

Studying the effect of stability time

When studying the resulting stability time with different time periods, the results showed that the dye is immediate and stabilizes after 5 minutes as shown in the following table

Table No. 6 Studying the effect of the stability time

Esomeprazole 8μgml^{-1} Room temp.	Absorbance/time (min)
After addition	0.811
5	0.817
10	0.817
15	0.816
20	0.816
25	0.816
30	0.815
35	0.815
40	0.814
45	0.812
50	0.812
55	0.812
60	0.812
90	0.811
120	0.809
Over night	0.801

Table No.7 Summary of optimal conditions

Material solution	Concentration	Optimum amount (ml)
m-aminophenol	0.01%	2
$NaNO_2$	0.5%	1
Sulphamic acid	1%	1.5
NaOH	1M	1
$\lambda_{max\ (nm)}$	450	
Colour	Dark yellow	
Temp.(C)	Room temperature 22	
Stability period	60 Min.	

Final absorption spectrum

After preparing, the diazonium salt depending on the optimal conditions, which were applied to 2 ml of the drug, equivalent to 8 micrograms, a yellow dye was formed that gave the highest absorption at a wavelength of 450 nm compared to the mock solution, which gives very weak absorption.

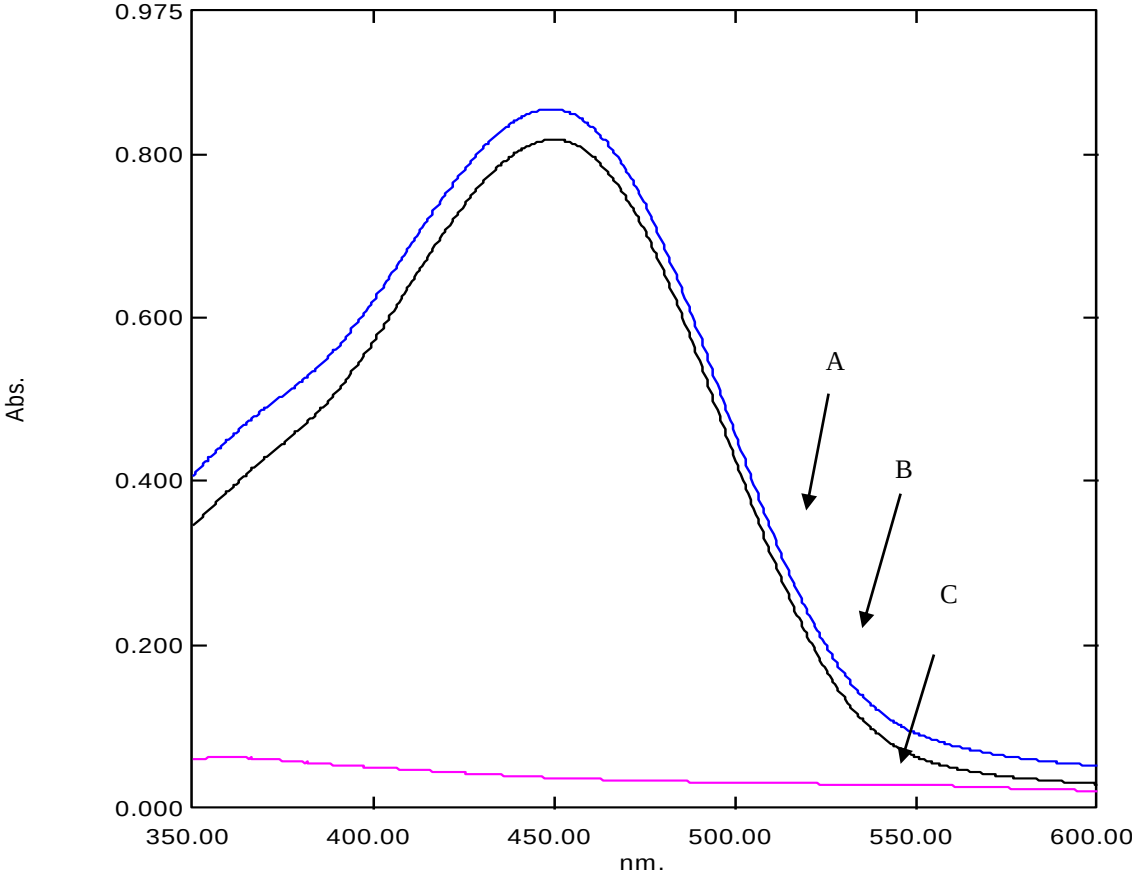


Fig.2 final absorption spectrum of (8ppm) Esomeprazole solution: A- versus blank, B- versus distilled water, C-blank versus distilled water.

Calibration curve

After stabilizing the optimal conditions, increasing amounts of the drug are added to the solution containing the reagent, sodium nitrite and sulfamic acid, where increasing amounts are added in several volumetric bottles, then the base is added and the volume is completed with water. After waiting for five minutes, the measurement is made against the blank solution at a wavelength of 450 nm.

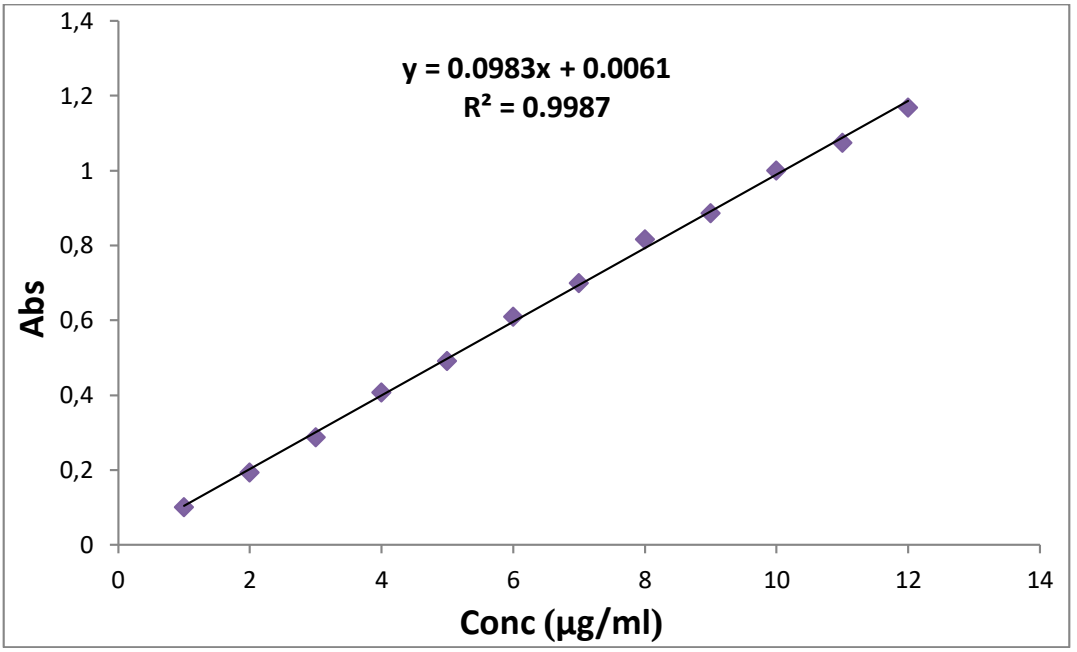


Fig. 2: The standard curve for determination of esomeprazole

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.8 εmax LOD, LOQ, Sandel's sensitivity and correlation coefficient

Beer's law range(ppm)	1-12
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	3.3954×10^4
Sandell's sensitivity ($\mu g.cm^{-2}$)	0.0101
LOD (µg/ml)	0.048
LOQ (µg/ml)	0.161
slope	0.0983

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.9 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,%
4	4.11	102.75	2.75	0.279
6	6.17	102.89	2.89	0.443
8	9.11	101.3	1.3	0.897

The molar ratios method

The Job's method (Delevie; 1997) was used to find the reaction ratio of dizonium salt with esomeprazole and the concentration of esomeprazole was 2.9 x 10^{-4} molarity and meta-aminophenol reagent was 2.9 x 10^{-4}, and the total final volume was 1.5 ml The figure confirms that the conjugation ratio is 1:1

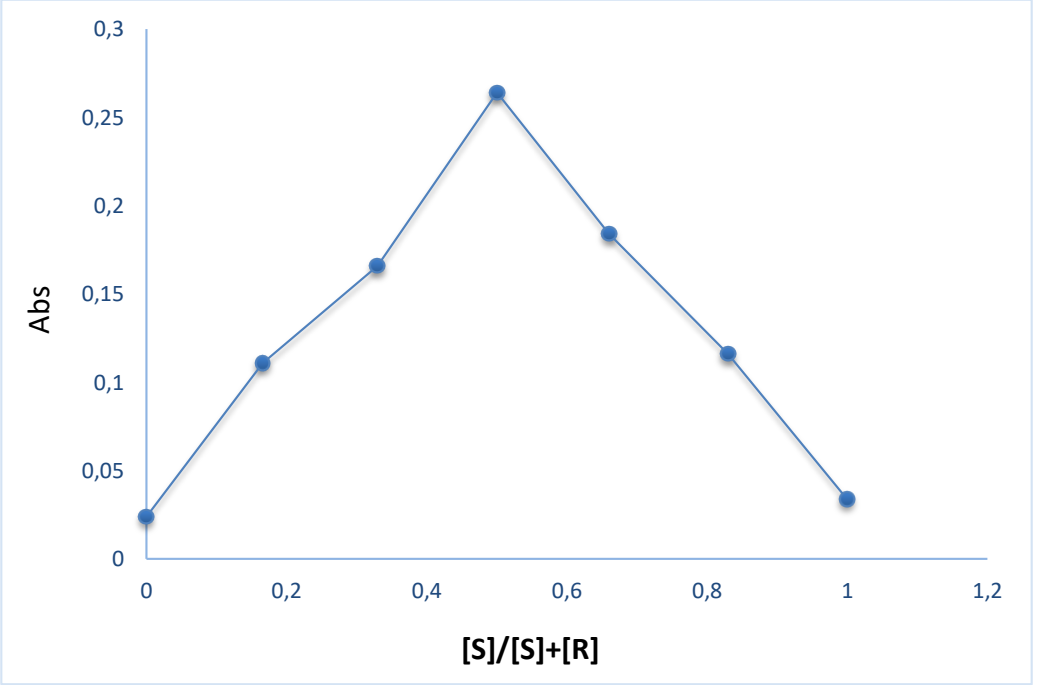


Fig.3 Continuous changes curve (Job's method) of dye resulting from the reaction of dizonium salt with esomeprazole

Method of molar proportions

The purpose of the molar ratio is to confirm the formation ratio, where 1 ml of esomeprazole was added and increasing volumes of 0.25_2 ml of meta-aminophenol were added with the same concentration of esomeprazole 2.9×10 and the absorbance was measured at wavelength 450. The figure confirms that the conjugation ratio is 1:1 as well**.**

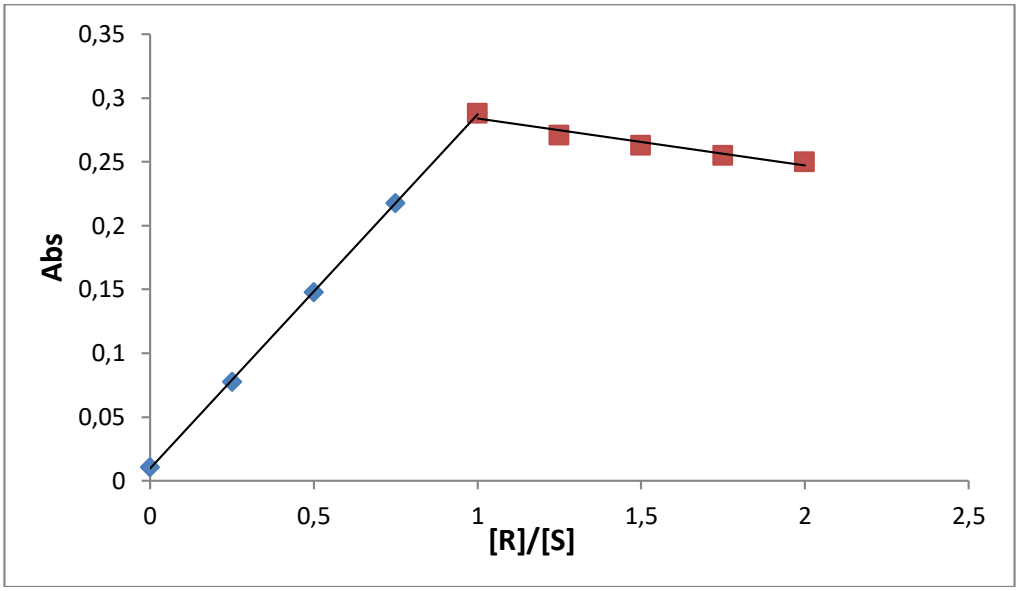


Fig 4. Curve of the molar ratio of the dye formed by coupling diazonium salt with esomeprazole

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.9 Application of the method

	Amount of ESO µg/ml present	Amount of ESO µg/ ml found	Recovery %	Rrelative error,%	Relative standard deviation,%
AwaNex capsules 40 mg/capsule Iraq	1	1.01	101.02	1.02	0.97
	2	1.969	98.4	-1.6	0.507
	4	4.1	102.5	2.5	0.196

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 100 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 100 µg/ ml under all optimum conditions

were applied and the absorbance was measured at a wavelength 450 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.

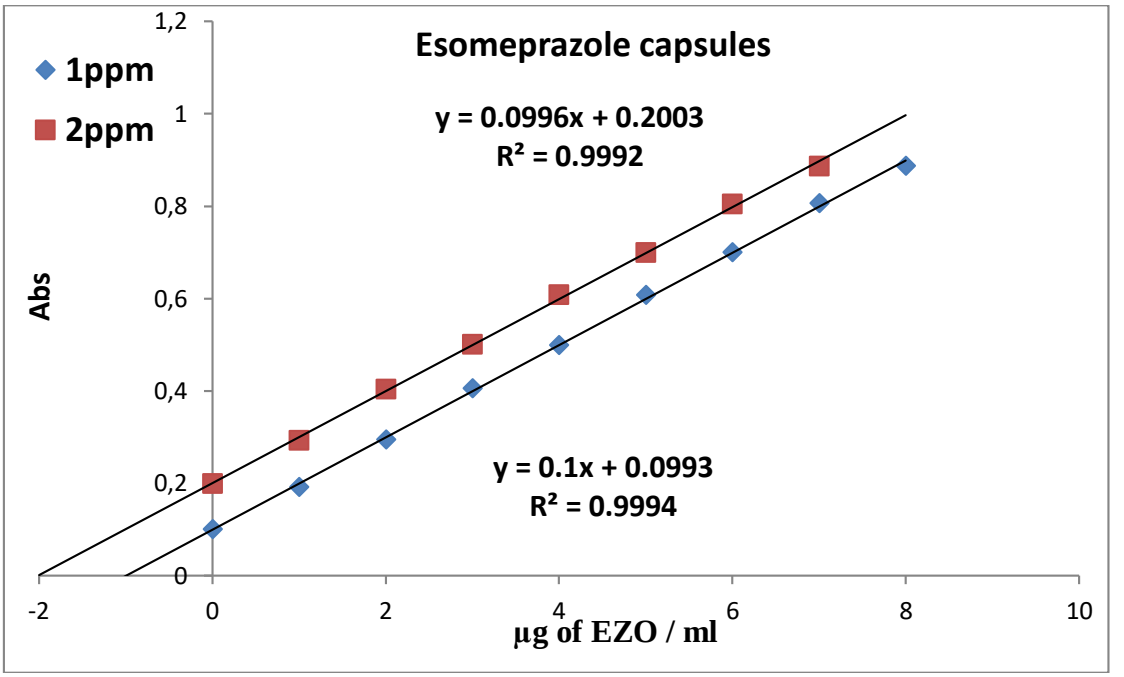


Fig -10. Standard addition method curves for determination of esomeprazole in pharmaceutical preparation at concentration (1,2 µg/ ml).

Table No.10 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	1	0.99	99
	2	2.02	101.01

Conclusions

A new developed spectrophotometric method has been proposed for the determination of esomeprazole using meta-aminophenol by diazotization and coupling reactions.

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