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Application of the Cloud Point Extraction Method in Spectrophotometric Estim Esomeprazole using Diazotised p-Nitroanline and Triton X -114

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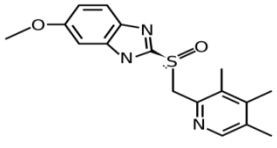
Abstract

A simple, sensitive, and precise spectrophotometric method has been developed for the quantification of esomeprazole as pure and in its dosage form (injection). The developed method is based on the diazo-coupling reaction of esomeprazole with a diazotized p-nitroaniline to produce a yellow azo dye soluble in an aqueous medium and gave a maximum absorption at 440 nm. Then using cloud point extraction method by adding Triton X-114 to azo dye solution and heating in water-bath at 50 ^{0}C for 25 minutes. The cloudy solution cooled in ice bath, then centrifuged for 20 minutes at 4000 rpm, separation the filtrate by decantation, and finally adding 1ml ethanol. The color of the product changed to pink color (redshift) and the pink-colored solution gave a maximum absorption at 585 nm. The optimization of the method conditions was investigated. Under the optimal conditions, Beer's law obeyed from 1 -20 µg/ml with determination coefficients (R^2) equal to 0.9989 and an excellent value of molar absorptivity 1.9929 x 10^4 with a limit of detection (LOD) of 0.045µg/ml and limit of quantitation (LOQ) of 0.147 µg/ml. The developed method was successfully applied to the assay of esomeprazole in injection dosage with accepted analytical results.

Keywords: Cloud point extraction; diazo- coupling; diazotized p-nitroaniline; esomeprazole

1. Introduction:

Esomeprazole is the greatest act of the proton-pump inhibitors for the acid-related diseases. Esomeprazole (ESOPL) is commonly used in combination with naproxen to avoid the danger of gastrointestinal toxicity [1,2]. Chemically name of ESOPL is ((S)-5-methoxy-2-(((4-methoxy-3,5-dimethylpyridin2-yl) methyl) sulphinyl)-1H-benzo[d]imidazole), and the chemical structure as shown in Scheme 1[3].



M.Wt =345.4 g/mol

Scheme 1. The chemical structure of ESOPRL.

The review of literature exposed the various methods used in the estimation of ESOPL such as RP-HPLC [4-6], RP-HPLC in presence of diclofenac sodium [7],HPLC in presence of oxytetracycline, tinidazole [8], supercritical fluid chromatography-tandem mass [9], micro-extraction-liquid chromatography-tandem mass [10], RP-HPLC, dual-wavelength and derivative spectrophotometry in presence of diclofenac [11], simultaneous electrochemical assay of ESOPL and diclofenac sodium [12], electrochemical assay using Au electrode [13], rare recent spectrophotometric assay of ESOPRL: UV-Spectroscopy [14], using 5-sulfosalicylic acid and NBS reagents [15], charge transfer reaction using chloranilic acid (π-acceptor) [16], oxidation-reduction of the ESOPL in the presence of ciprofloxacin using potassium permanganate [17], and simultaneous determination of esomeprazole magnesium trihydrate and naproxen via spectrophotometric method based on absorbance subtraction and ratio difference [18].

To our knowledge, there is no spectrophotometric method using cloud point extraction for the estimation of ESOPRL, the present developed method is based on a diazo-coupling reaction of ESOPRL with a diazotized p-nitroaniline in alkaline medium, then cloudy colored product formed by adding Triton X-114 as a surface-active area. The cloudy colored product is extracted by using ethanol and can be spectrally followed and used in determination ESOPRL.

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2. Experimental

2.1. Apparatus used

Spectrum was performed by a JASCOV-630 spectrophotometric apparatus (Japan) and used glass and quartz cells with an optical path of 1 cm. The pH of the solutions was measured by using a HANNA pH 211 pH meter. The heating process was carried out in a BS11 Lab water bath, using a type centrifuge (Minor centrifuge/MSE/England)

2.2. Chemical materials and solutions used

All chemicals used of analytical grade.

2.2.1. Esomeprazole solution (100 µg/ ml) prepare by dissolving 0.010 g of the pure substance in a 1ml of ethanol, then transfer to a 100 ml volumetric flask and complete the volume to the mark with distilled water.

2.2.2. Diazotized p-nitroaniline solution, **0.005 M**

Prepared by dissolving 0.0690 g of the p-nitroanilinein 85 ml of distilled water with 8 ml of concentrated hydrochloric acid at a standard of (11.8), which is carried out first by the heating process and then transferred to an ice bath at a temperature of (0-5 ^{0}C), after that 3.44 ml of sodium nitrite is added at a concentration of (1%) shake for 5 minutes, then the volume is completed with distilled water in a 100 ml volumetric flask [19].

2.2.3. Triton X-114 surface active agent solution, 10%

It was prepared by diluting 10 ml of Tritonx-114 surfactant in a volume of distilled water and then completing the volume with distilled water to the point of the mark using a 100 ml volumetric flask.

2.2.4. Other aqueous solutions

Sodium nitrite (1%), sodium hydroxide and hydrochloric acid(1M) solutions were prepared.

2.2.5. Esomeprazole injection solution, 100µg /ml

The preparation based on mixing the powder of three esomeprazole injection containers (each one contains 40 mg ESOPRL and the average weight =0.044 g). 0.0112 g of the powder was weighing and dissolved in 1 ml ethanol and complete the volume with distilled water in the 100-ml volumetric flask.

3. Procedure and calibration curve

The procedure and standard curve for determination of esomeprazole obtained by adding increasing volumes of ESOPRL solution (100 µg / ml) to 10 ml volumetric flasks to cover the concentration of ESOPRL from 1 to 20 µg / ml, followed by adding 1.25 ml of each of the diazotized p-nitroaniline(D-PNAN) and1ml of sodium hydroxide, then adding 0.5 ml of Triton X-114 surfactant solution (10%), then complete the volume with distilled water. The solutions were placed in the waterbath at a temperature of 50°C for 25 minutes, a cloudy solution was formed, then the cloudy particles were separated from the sediment in a centrifuge, cooling the components of the solution using an ice bath for the purpose of increasing the viscosity, after which the filtrate was separated from the sediment by pouring, then 1 ml of ethanol was added to dissolving the sediment. The spectrum of the solution gave a maximum absorption at the wavelength 585 nm. The absorbance of all solutions was measured at the wavelength of 585 nmagainst the blank solution, the standard curve was obtained following Beer's law within the range from 1to 20 µg / ml (Figure 1) and there is a negative deviation from Beer's law above 20 µg/ml.

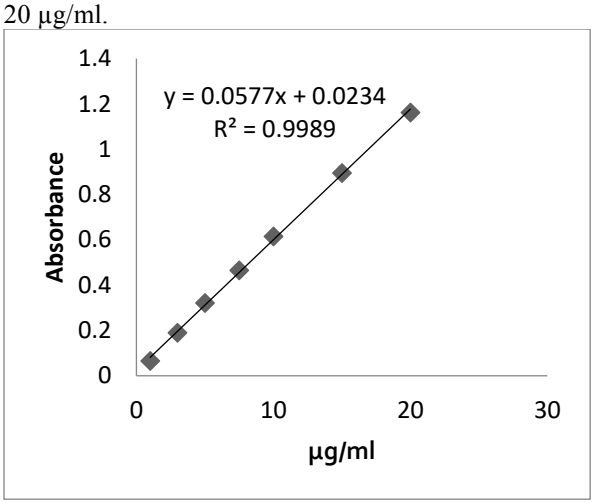
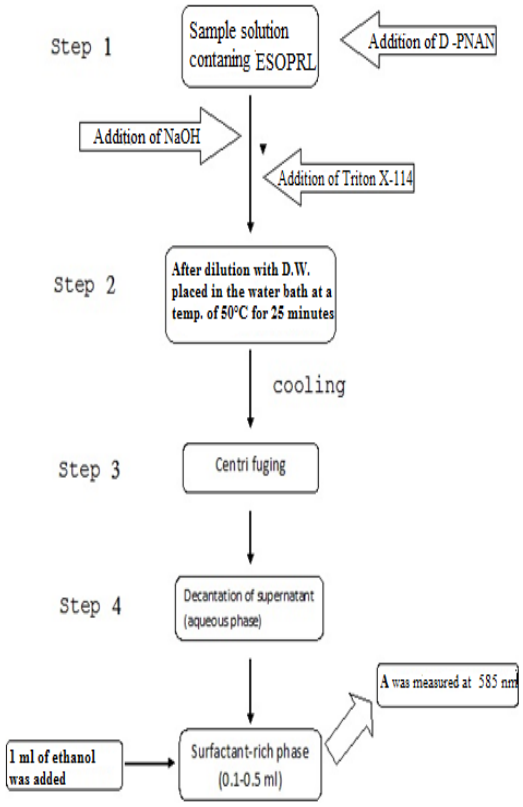


Fig. 1. The calibration curve of determination of ESOPRL via suggested method.

Scheme 2 illustrates the steps of the proposed working method in a simple manner.



Scheme 2. Main steps of the proposed working method.

Various analytical parameters constructed from procedure and calibration curve illustrated in Table 1.

Table 1
The more important analytical parameters for suggested method.

Parameter	Value
Maximum wavelength, nm	585
Beer's law, µg / ml	1-20
Relation equation	y = 0.٠٥٧٧ x + 0.0234 Y = Absorbance x= concentration of ESOPL in µg / ml Intercept = 0.0234
ε, l/mol.cm	1.9929×10^4
Determination coefficient	0.9989
Sandell's index,µg / cm^2	0.01733
LOD, µg / ml	0.045
LOQ, µg / ml	0.147

4. Results and discussion

Variousexperiments have been conducted to study the effect of the reaction components on absorbance and the conditions that give the highest absorbance have been chosen.

4.1. Preliminary study

The developed spectrophotometric method for the determination of ESOPRL using the D-PNAN reagent, an initial reaction was performed between the ESOPRL. and D-PNAN in alkaline medium of sodium hydroxide, a yellow color azo dye resulted from the aboveadditions. Then the addition of the Triton X-114 agent, and then the mixture was heated in a water bath at 50 ºC for 25 minutes, a cloudy solution was formed, then separated via centrifuge, cooling the solution using an ice bath, and pouring the filtrate. The extraction of the product by adding 1 ml of ethanol, then the absorption spectrum of the solution has been done and the highest wavelength at 585 nm was confirmed by subsequent experiments

4.2. Optimum conditions

The optimum conditions for the proposed method were obtained by studying all the components of the reaction by changing the factor understudyand fixing the other factors.

4.2.1. The effect of the amount of D-PNAN and sodium hydroxide

The effect of increasing volumes of the D-PNAN and the sodium hydroxide was studied, where equal volumes of both were added gave high intensity, and the optimum volume for each of them was 1.25 ml, which was the best, it gave the highest absorbance and highest value of determination coefficient(see Table 2).

Table 2
The optimal amounts of D-PNAN and sodium hydroxide.

D-p-NAN/ NaOH solutions(ml/ml)	Absorbance/ µg ESOPRL in10 ml			R^2
	5	10	15	
0.5/0.5	0.209	0.373	0.449	0.957
1/1	0.217	0.464	0.693	0.998
1.25/1.25	0.225	0.540	0.889	0.999
1.5/1.5	0.238	0.497	0.767	0.985

4.2.2. Study of the base type

After the optimal volume of both the reagent and the base was confirmed, the type of base was studied by using 1.25 ml of various base(1M) with 1.25 ml of D-PNAN(Table 3).

Table 3.
Selection of the base type.

Base type, 1M	Absorbance
NaOH	0.543
KOH	0.432
Na_2CO_3	0.379
$NaHCO_3$	0.301

From the results in Table 3 and according to the values of absorbance indicated that the coupling needs strong alkaline medium and sodium hydroxide

is the best base, so its use was retained for subsequent experiments.

4.2.3. The effect of the amount of Triton X-114 solution (10%)

The effect of increasing volumes of Triton X-114 solution at a concentration (10%) was studied after adding 10 µg / ml of the ESOPRL and adding the optimal amount of D-PNAN and NaOH, then 0.3-1.0 ml ofTriton X-114 solution was added. After completing the volume to 10 ml with distilled water, the samples were placed in a water bath at 60°C for a period of 25 minutes, the cloud point method mentioned previously was applied and the absorbance was measured at 585 nm(see Table 4)

Table 4
The optimal amount ofTriton X-114.

Amount of Triton X-114(10%), ml	Absorbance
0.3	0.523
0.5	0.592
0.7	0.577
1.0	0.546

It was noted from the results in Table 4 that the optimal volume is 0.5 ml, which gave high intensity of cloud point extraction product.

4.2.4. The effect of temperature

The effect of temperature on the absorbance of the colored product was studied. The results listed in Table 5 illustrate the effect of different temperatures from 30 to70° C on the formation of the cloudy solution in the presence of a fixed amount of the ESOPRLand the optimum quantities of the D-PNAN / the base, and the Triton X-114, and by following the aforementioned work steps (Scheme 2) the absorbance was measured at the specified wavelength(585nm).

Table 5
The effect of temperature.

Temperature, C^0	Absorbance
30	0.534
40	0.552
50	0.612
60	0.595
70	0.402

The cloudy solution is formed with the highest sensitivity at a temperature of 50 ° C. While it was found that the absorbance decreases with increasing the temperature more than 60°C according to decomposition of the product so the temperature 50°C was adopted in the subsequent studies, with a heating time of 25 minutes (see Table 6).

Table 6
The effect of time on absorbance.

Time, minutes	Absorbance
20	0.520
25	0.614
30	0.508
35	0.489
40	0.436

4.2.5.The effect of separation time in the centrifuge

The effect of the separation time on the absorbance of the colored solution has been studied. 10-30 minutes was studied using a centrifuge with a number of cycles of 3000 and of 4000 revolutions/minute, it was found that the best separation of the two layers is at a centrifuge rotation speed 4000 rpm, and it takes 20 minutes to complete separation, so this time was adoptedin subsequent experiments (see Table 7).

Table 7
The effect of separation time in the centrifuge.

Time(minutes)	Absorbance / Speed, rpm	
	3000	4000
10	0.415	0.454
20	0.467	0.618
30	0.487	0.551

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20	0.467	0.618
30	0.487	0.551

4.2.6.The optimum conditions

Table 8 contains the optimal conditions of the proposed method used in the determination ESOPRL.

Table 8
The optimal conditions of the method.

Parameter	Optimal
Amount of (0.005 M)D-p-NAN, ml	1.25
Amount of 1M sodium hydroxide solution, ml	1.25
Amount of Triton X-114 ,ml	0.5
The temperature,^{0}C	50
The time of heating, minutes	25
Speed of revolutions, rpm	4000
Maximum wavelength, nm	585

5. The final absorption spectrum

After fixing the optimal conditions for ESOPRL determination as mentioned in Table 8, the final absorption spectrum of 10 µg ESOPRL/ ml was taken, as it gave the highest absorbance at the wavelength of 585 nm as in t figure (2).

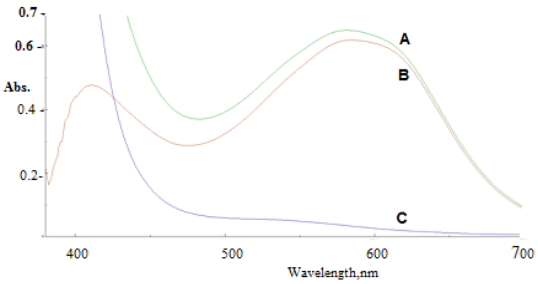

Fig. 2. The final spectrum of 10 µg ESOPRL / ml determination via applying, the proposed method: (A)cloudy product versus distilled water, (B) cloudy product versus the blank solution, and (C)the blank solution against distilled water.

6. Accuracy and precision of the method

The accuracy and precision of the method were checked by calculating the relative error and the relative standard deviation using five replicates for three different concentrations of the ESOPRL within the linear relationship (see Table 9).

Table 9

The accuracy and precision of the method.

Concentration of Esomeprazole (µg/ml)		Rec.* (%)	RE%	RSD %
Present	Found			
5	5.22	104.4	+ 4.4	0.37
10	10.38	103.8	+3.8	0.21
15	15.29	101.93	+1.9	0.08

*Average of five determinations.

The results above in Table 9 indicated that the method has good accuracy and precision according to the values of RE% and RSD% whose values fall within the acceptable analytical errors(≤±5%).

7. Application part

The determination of ESOPRL in injection via two types of calculation of drug content:

7.1. Determination of ESOPRL in injection by using the linear relationships of the method (calibration curve).

The results of application the suggested method in assay of ESOPRL in injection were illustrated in Table 10**.**

Table 10

The results of application part by using the equation of relationship of linearity

Pharmaceutical preparation	Certified Value (mg)	Amount present (µg/ml)	Recovery* (%)	Drug content found* (mg)
Esomeprazole Injection	40 mg	3	40.44	101.11
		5	40.12	100.31
		10	39.93	99.83

^Average for three determinations.

The results listed in the above table confirm the validity of applying the suggested method for estimating ESOPRL in itspharmaceutical preparation (injection) based on the recovery% of ESOPRLin within allowable errors analytically.

7.2. Determination of ESOPRL in injection via the standard addition method

In order to prove the developed method, its success in the estimation of ESOPRL initsformulation(Esomeprazole injection), and its free from additive interference, the standard addition method (SAM) was applied to the estimation of ESOPRL in injection formulation, the results as shown in Figure3

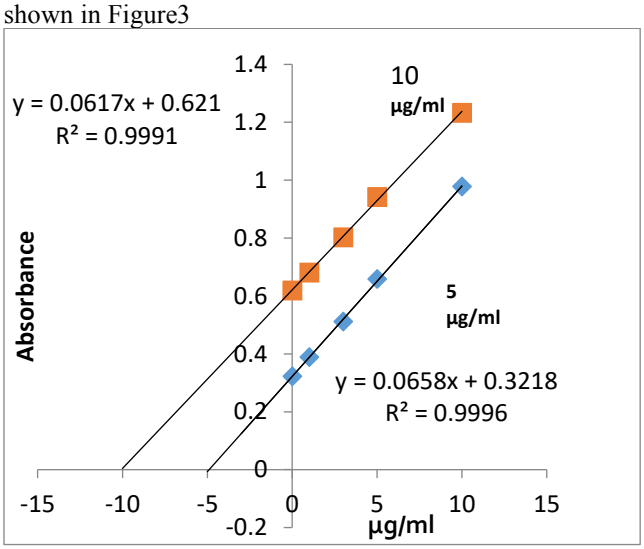

Fig. 3. The curve of standard addition method plot for estimation of ESOPRL in injection formulation**.**

From the equations of the straight lines for the two standard curves of concentration 5 and 10µg/ml in Fig. 3, the percentage of recovery and drug content were calculated, and the results in Table 1١ it is evidenced that the method has reliability in calculating ESOPRL in injection formulation.

Table 1١.

The results of application SAM in estimation of ESOPL in injection.

Pharma. preparation	Certified Value (mg)	Amount(µg/ml)		Rec. (%)	Drug content found* (mg)
		Present	Found		
ESOPLInjection	40 mg	5	4.890	97.80	39.120
		10	10.064	100.64	40.256

Average for three determinations.

8. Comparison of the methods

The comparison of some parameters of the developed method with the same as in other spectrophotometric methods (see Table 12).

Table 12.
The comparison of some variables of the developed method with the same of other methods.

Analytical parameter	Present method	Literature method[16]	Literature method [17]
Type of reaction	Cloud Point Extraction	Charge transfer complex	Oxidation with potassium permanganate
Beer's law rang,µg/ml	1-20	1.25–150.00	5-20
Maximum wavelength (nm)	585	521-525	525
ε, l/mol.cm	1.9929×10^4	4.932×10^3	1.5611×10^4
LOD µg.ml^{-1}	0.045	0.156	1.01
LOQ µg.ml^{-1}	0.147	0.473	3.062
Application, formulation	Injection	Capsule	Capsule

The results above indicated that the developed method was more sensitive than the other methods used in comparison and has good range of Beer's law rang.

9. Conclusion

A new spectrophotometric method was suggested for the determination of ESOPRL, the method depends on the formation of a colored azo dye between the ESOPRL and D-PNAN in the presence of sodium hydroxide, and by applying the cloud point extraction method in the presence of the surfactant agent Triton X-114, the maximum wavelength of determent was 585 nm. The method was successfullyapplied in the estimation of ESOPRL in the pharmaceutical product (injection).

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