Original Research Article

Determination of Omeprazole, Esomeprazole and Pantoprazole by Quenching the fluorescence of Eosin Y

ABSTRACT

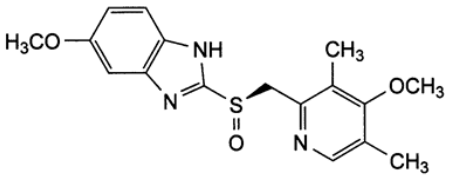
A non-extractive, simple and sensitive spectrofluorimetric method with good selectivity has been described for the determination of the proton pump inhibitors namely; Omeprazole, Esomeprazole magnesium and Pantoprazole sodium that are used for the treatment of peptic ulcer disease. The method is based on the quenching the fluorescence intensity of the eosin Y dye, as the result of the ion-pair complex formation of the studied drugs with the dye in the presence of acetate buffer solution of pH 3.5. The quenching of the eosin Y fluorescence intensity was measured spectrofluorimetrically at 540 nm after excitation at 352 nm. At the optimum reaction conditions, the quenching values of fluorescence (ΔF) and concentrations were rectilinear over the concentration ranges of 0.5–13.0, 0.7-15 and 0.3-15 µg/mL for Omeprazole, Esomeprazole magnesium and Pantoprazole sodium respectively. The recovery % values were in the range 99.92-100.56% and relative standard deviation values range was 0.430-2.521 for all the studied drugs. The method was applied successfully for determination of above drugs in their pharmaceutical formulations as capsule, tablet and injectables. The method was free from interferences of common excipients.

Keywords: Fluorometry; eosin Y; Omeprazole, Esomeprazole; Pantoprazole

1. INTRODUCTION

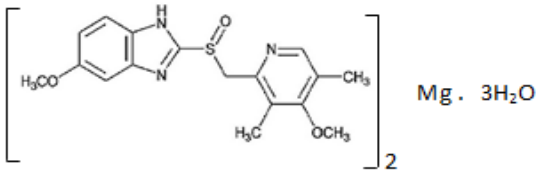
Omeprazole (OMZ), Esomeprazole magnesium trihydrate (ESO) and Pantoprazole (PAZ) are drugs classified as proton pump inhibitors, These are drugs that reduce the amount of stomach acid secreted by the glands on the inner wall of the stomach [1] and decrease the clopidogrel anti-platelet effect [2].

OMZ, chemically name as sodium 5-Methoxy-2-(((4-methoxy-3,5-dimethyl-2-pyridinyl)methyl)sulfinyl)-1H-benzimidazole, ESO is the S-isomer of omeprazole and its chemically name is magnesium bis[5-methoxy-2-[(*S*)-[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfinyl]-1*H*-benzimidazol-1-ide] trihydrate and PAZ, chemically is sodium, 5-(difluoromethoxy)-2-[(3,4-dimethoxy-2-pyridyl)methylsulfinyl]-1H-benzimidazole [3]. They have the following chemical structures:



OMZ

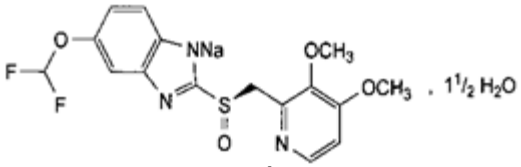
$C_{17}H_{19}N_3O_3S$ 345.4



ESO

$C_{34}H_{36}MgN_6O_6S_2,3H_2O$

767.2



PAZ

$C_{16}H_{14}F_2N_3NaO_4S,1½\ H_2O$ 432.4

Different analytical techniques and methods have been described to determine OMZ, ESO and PAZ concentrations including spectrophotometry [5-13], chromatography [14-16] and voltammetry [17-20]. Few spectrofluorimetric method, for determination of Proton-pump inhibitors, have been reported in the literature. These method were depended on the quenching effect on the fluorescence of Tb^{3+}-1,10-phenanthroline complex in the presence of bis(2-ethylhexyl) sulfosuccinate sodium and Tb^{3+}-1,10-phenantroline-silver nanoparticles fluorescence in the determination of OMZ and PAZ respectively [21,22] or the enhanced fluorescence effect of N^1-methylnicotinamide chloride for determination of OMZ and PAZ [23]. But no fluorometric method has been described for the determination of ESO in the literature. In this study, a simple, easy and accurate spectrofluorimetric method is described for the estimation of OMZ, ESO and PAZ concentrations from the fluorescence intensity of eosin Y.

2. Experimental part

2.1 Apparatus

Fluorometric measurements were carried out using a Shimadzu-RF-5301-PC-Spectrofluorometer equipped with a xenon lamp, using transparent quartz cells on all sides with a thickness of 1 cm. A Jenway 3510 pH Meter connected to an electrode supplied by the same company was used for pH measurements. The dissolutions were carried out using a Power Sonic 405 Ultrasonic Cleaner supplied by Lab Tech-Korea. All calculations in the computing process were performed in Microsoft Excel for Windows.

2.2 Reagents

All analytical reagents used are of high purity from Fluka and BDH companies.

Eosin Y solution was prepared at a concentration of 100 µg/mL by dissolving 0.01 g of dye in 100 mL of distilled water. To ensure complete dissolution of the dye, the volumetric vial was placed in an ultrasonic shaker for 5 minutes. The solution was kept in an opaque vial in the refrigerator and remained stable for one week. Acetate buffer solution of pH 3.5 was prepared by mixing 6.0 mL of 0.2 M sodium acetate with 44.0 mL of 0.2 M acetic acid and the volume was completed to 100 mL in a calibrated flask with distilled water.

2.3 Stock solutions of drugs

OMZ, ESO and PAZ were kindly provided by state company for Drug Industries and Medical appliance-(SDI) Sammara-Iraq with purity of 99, 98 and 99% respectively. The standard solutions of above drugs were prepared at a concentration of 100 µg/mL by dissolving 0.01g the pure form of the above medicinal compounds separately in 2 mL of ethanol in a beaker with 25 mL of distilled water, stirring until dissolved, then transferring the solution to a 100 mL volumetric flask and diluting to the mark with distilled water. To ensure completion of dissolution, volumetric flasks are placed in the ultrasonic shaker for 5 minutes, and the solutions are kept in the refrigerator and remain stable for a week.

2.4 Recommended procedure

To a set of 10 mL volumetric flasks, increasing volumes (mL) of standard solutions (100 µg/mL) of the medicinal compounds under study were added to obtain concentrations of 13.0, 0.7-15 and 0.3-15 µg/mL of OMZ, ESO and PAZ in the final volume separately and respectively, then 1.0 mL of eosin Y dye solution at a concentration of 100 µg/mL (10 µg/mL) was added, followed by the addition of 0.5 mL of acetate buffer solution (pH3.5), and diluted to the mark with distilled water. After setting for 5 min at room temperature and the fluorescence intensity (F) was measured at 540 nm after excitation at 352 nm. The fluorescence intensity (F°) of 100 µg/mL eosin Y dye solution in the presence of 0.5 mL of acetate buffer

was measured as the blank and the difference in the fluorescence intensity ($\Delta F = F° - F$) in the presence and absence of the drug was plotted versus the final drugs concentration.

2.5 Analysis of pharmaceutical formulations

2.5.1 Capsule

The content of 5 Gasec capsules, (each capsule containing 40 mg of OMZ), was carefully weighed, then crushed and mixed well, the weight of the powder equivalent to one capsule (40 mg of OMZ) was dissolved in 5 ml ethanol and 20 ml distilled water in a beaker and heating in a water bath, adjusted at 60°C, for five min, leaving the solution to cool, then filtered and completed to a 100 mL by distilled water in a volumetric flask to obtain 400 µg/mL. Suitable dilution was made to obtain 100 µg/mL concentration. Then aliquots of working solution of the drug was analyzed according to the recommended procedure.

2.5.2 Tablet

Five tablets of each commercial medicines, (each tablet contains 40 mg of ESO or PAZ), were carefully weighed, then crushed and mixed well, and the weight of the powder equivalent to one tablet was dissolved in 5 mL ethanol and 20 mL distilled water in a beaker and heating in a water bath for five min, leaving the solution to cool, then filtered and completed to a 100 mL by distilled water in a volumetric flask to obtain 400 µg/mL. Suitable dilution was made to obtain 100 µg/mL concentration. Then aliquots of working solution of the drug was analyzed according to the recommended procedure .

2.5.3 Injection

Mix the contents of three vials (each vial contains 40 mg PAZ) then weigh precisely the equivalent of one vial. Dissolve and complete the volume to the mark with distilled water in a 100 mL volumetric flask to obtain 400 µg/mL. Suitable dilution was made to obtain 100 µg/mL concentration. Then aliquots of working solution of the drug was analyzed according to the recommended procedure.

3. Results and discussion

The aqueous solution of eosin Y showed a fluorescence activity at 540 nm (λ_{em}) after excitation at 350 nm(λ_{ex}). When the drugs solutions were added to the eosin Y solution, a decrease in the fluorescence intensity was observed (Figure 1). The reason for the decrease in the fluorescence intensity of the reagent is due to the electrostatic attraction between the drugs and the anionic functional group of Eosin Y forming a non-fluorescent ion-pair complexes at pH3.5. However; the difference in the fluorescence intensity (ΔF) of Eosin Y, in absence and presence of the studied drugs was the basis for the spectrofluorimetric measurement at 540 nm after excitation at 352 nm.

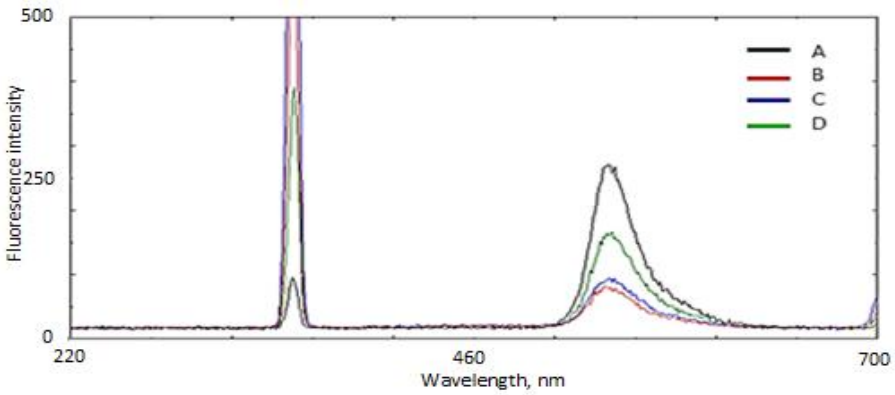


Figure (1): excitation and emission spectra of 10 µg/mL eosin Y dye in acetate buffer medium (pH3.5) (A), and in the presence of 10 µg/mL of OMZ (B), ESO (C) and PAZ (D).

3.1 optimization of reaction conditions

Different parameters affecting the reaction products on the fluorescence intensity are studied, where the parameter under study was changed while keeping the others. These parameters including concentration of reagent, type of buffer solution and its volume, temperature, reaction time and effect of solvent.

3.1.1 Effect of eosin Y reagent concentration

To select the optimum concentration of eosin Y dye for the determination of the intended drugs, aliquots of 100 µg/mL of dye transferred into a series of 10 mL volumetric flasks, then diluted to the mark with distilled water. The emission of fluorescence intensity was measured after 5 min at 540 nm after excitation at 352 nm. A calibration graph was constructed by plotting fluorescence intensity versus concentration of the dye and found a rectilinear range 0.5-10 µg/mL (Fig. 2). However; higher concentration of eosin Y was chosen for subsequent experiments.

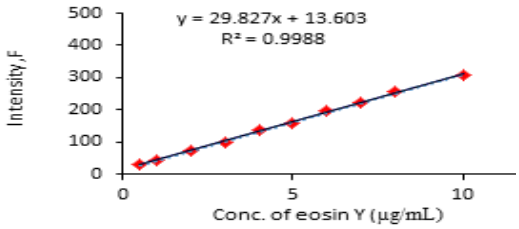


Figure 2: Calibration graph of eosin Y

3.1.2 Effect of buffer solution and pH

It was found in the preliminary study that the eosin Y dye is affected by addition of HCl and quenching its florescence intensity even in the absent of the drugs. In addition, it was found that HCl does not help to form the ion-pair complexes between the dye and the studied drugs. But was found that the acidic buffer solutions did not significantly affect the fluorescence intensity of the eosin Y dye alone, and helped the formation of the ion-pair complexes. Therefore, the effect of different types of acidic buffer solutions, with pH ranged between 2.5 and 5, on the fluorescence quenching intensity (ΔF) of eosin Y in the presence of fixed amount of the intended drug compounds was studied. The fluorescence intensity was measured at 540 nm with an excitation wavelength of 352 nm. It indicates that the buffer acetate solution is the best at the pH of 3.5 (Table 1), and accordingly it was adopted in the subsequent studies.

Table 1: Effect of buffer solution and pH on the intensity (ΔF) of 10µg/mL eosin Y in the presence of 10 µg/mL of each of the studied drugs.

Type of buffer solutions (1mL)	pH	OMZ		ESO		PAZ	
		ΔF	pH	ΔF	pH	ΔF	pH
Acetate buffer	2.5	90.1	2.7	84.7	2.4	56.4	2.4
	3.0	116.6	3.1	101.2	3.1	64.1	3.2
	3.5	132.0	3.6	121.1	3.5	74.0	3.4
	4.0	112.0	4.1	103.8	4.2	62.6	4.1
	4.5	85.5	4.5	80.3	4.4	56.3	4.4
	5.0	78.1	5.0	71.6	4.9	51.5	5.0
Phthalate buffer	2.5	82.2	2.4	71.3	2.3	55.4	2.3
	3.0	78.8	2.9	75.1	2.9	61.2	2.9
	3.5	90.1	3.7	82.2	3.5	65.7	3.5
	4.0	80.5	4.0	77.8	4.2	59.6	4.1
	4.5	75.9	4.4	70.4	4.6	54.2	4.5
	5.0	70.3	5.0	68.9	5.1	47.9	5.2
Citrate	2.5	67.8	2.5	60.3	2.3	48.0	2.3

buffer	3.0	73.1	2.9	63.9	3.1	52.2	2.8
	3.5	78.6	3.3	70.8	3.6	55.8	3.5
	4.0	71.5	4.1	66.0	4.0	50.1	4.0
	4.5	68.0	4.7	62.3	4.7	47.2	4.6
	5.0	65.7	5.2	60.8	5.1	42.6	5.1

3.1.3 Effect of the volume of acetate buffer solution

The effect of adding increasing amounts (0.1-2.0 mL) of acetate buffer solution (pH3.5) on the fluorescence quenching intensity of eosin-Y dye was studied, as it was shown in Figure (3) that using 0.5 mL gave the best value for ΔF, so recommended in this study.

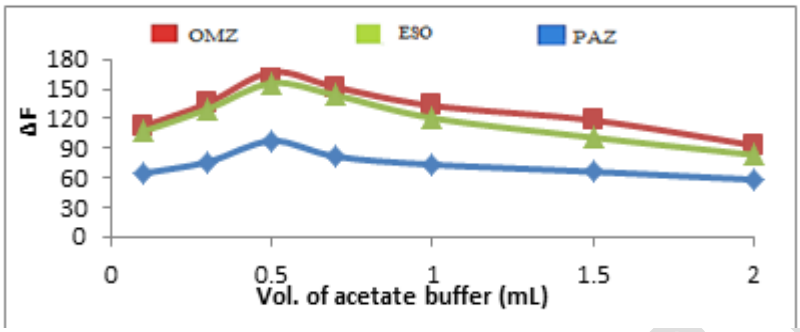


Figure 3: Effect of volume of acetate buffer solution

3.1.4 Effect of temperature on reaction time and stability of the ion-pair complexes

The effect of three temperatures, room temperature (22º), 30º and 40ºC, on the quenching of fluorescence intensity of the dye was studied in the presence of the optimal amounts of buffer solution and eosin Y dye. The results indicated that ion-pair complexes are formed directly between the dye and the drug compounds, and the maximum value of ΔF is obtained after 5 minutes from the start of the reaction at room temperature with a stability time of the complexes not less than 120 minutes (Table 2 and Figure 4), and accordingly, measurements were taken in the subsequent studies after 5 minutes of dilution to the mark.

Table 2: Effect of temperature on the fluorescence intensity (ΔF)

Temp (C°)	ΔF / min standing time								
	5	10	15	20	30	40	50	60	120
Omeprazole									
R.T*	163.5	162.4	163.4	163.1	163.4	162.9	163.8	162.9	161.7
30	113.5	112.1	111.5	111.0	109.8	100.1	100.1	100.2	109.4
40	88.7	88.9	87.9	87.8	86.9	86.7	86.8	86.2	86.0
Esomeprazole Magnesium									
R.T*	153.9	152.6	152.9	151.9	152.6	152.1	152.9	153.5	151.8
30	103.1	103.6	102.1	101.7	101.3	101.9	102.0	102.3	101.5
40	80.2	78.4	77.9	77.9	78.9	70.1	70.1	70.2	68.0
Pantoprazole Sodium									
R.T*	98.0	98.9	97.8	98.0	98.9	97.9	98.0	98.8	97.8
30	74.4	73.6	72.1	72.3	72.5	72.3	72.9	72.9	71.1
40	58.7	57.9	56.5	55.1	57.8	56.3	56.8	56.9	55.8

*RT= 22 C°

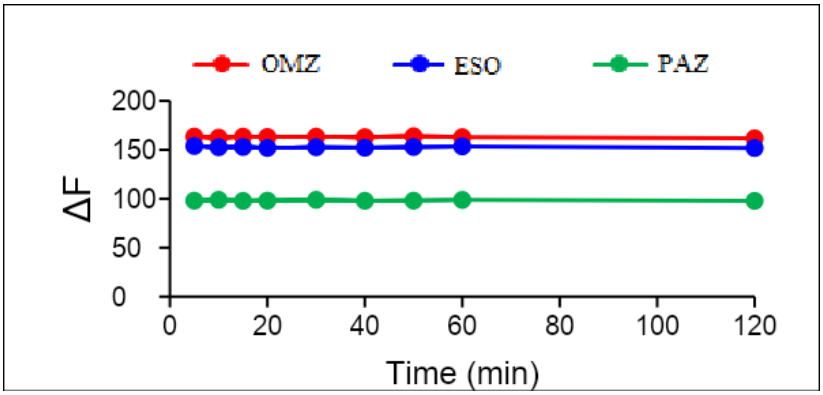


Figure 4: Effect of developing time on eosin Y-drugs complexes at room temperature

3.1.5 Effect of surfactant

In order to study the effect of surfactants on the sensitivity of the developed method for the determination of the drugs under study, several types of surfactants (positive, negative and neutral) were individually studied. Figure 5 indicated a negative effect of surfactants on the fluorescence intensity (ΔF), and accordingly they were excluded in subsequent studies.

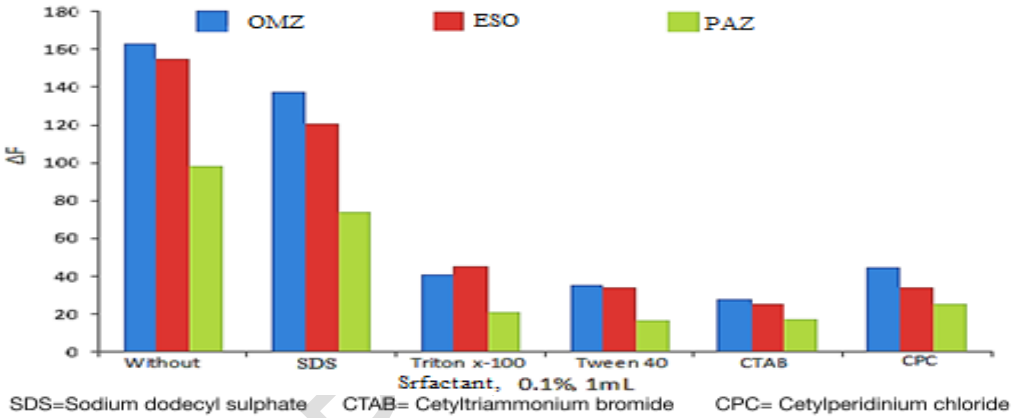


Figure 5: Effect of surfactant on the fluorescence intensity (ΔF) of eosin Y in the presence of drugs

3.1.6 Effect of solvent

In order to know the effect of solvents, with different polarity, on the quenching of fluorescence intensity (ΔF) of eosin Y in the presence of the intended drugs and the acetate buffer solution. as the results shown in the figure 6 that distilled water is a suitable medium for the reaction, as it gave the highest value for ΔF.

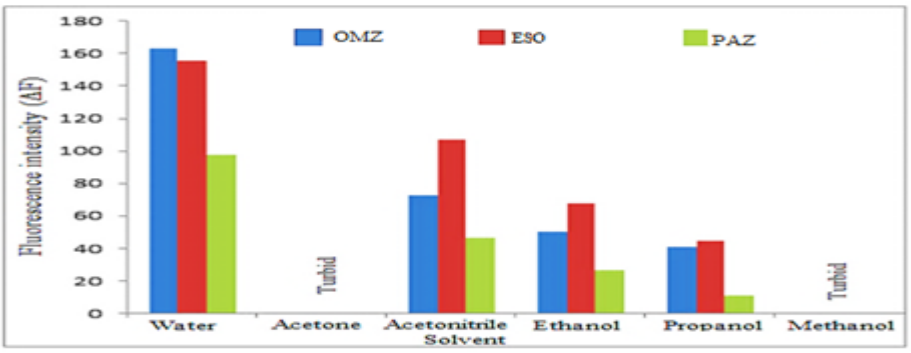


Figure 6: Effect of solvent on the fluorescence intensity

3.1.7 Addition sequence effect

In order to choose the best sequence for the addition of the reactants, the effect of three different sequences was studied. It was found from the results in figure 7 that sequence gave the maximum value for ΔF was addition of drug followed by eosin Y and acetate buffer solution, was chosen for the assay of drug compounds.

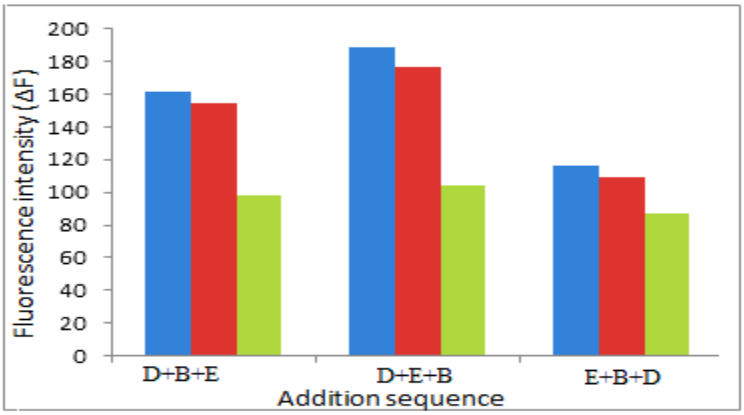


Figure 7: Effect of addition sequence, D=Drug, E= Eosin Y and B= Acetate buffer solution

3.2 Linearity

By following the recommended procedure for the determination of the drug compounds under study, the standard curves were prepared by plotting difference in the fluorescence intensity (ΔF) against the concentration of drugs (Figure 8). Linearity ranges were found 0.5-13, 0.7-15 and 0.3-15 µg/mL for OMZ, ESO and PAZ with excellent correlation coefficients respectively. The detection and quantification limits values LOD and LOQ) indicate the sensitivity of the method. Recovery% and relative standard deviation (RSD) values indicated the method is accurate and precise. The parameters of the suggested method are illustrated in Table 3.

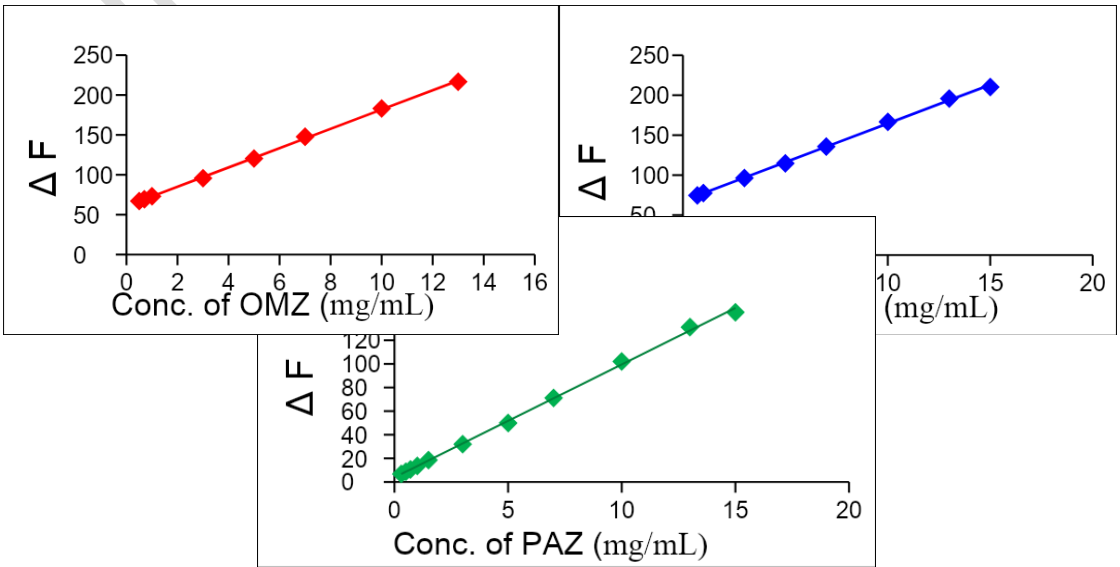


Figure 8: Calibration plots of the studied drugs

Table 3: Characteristic parameters of the suggested method

Parameters	Value		
	OMZ	ESO	PAZ
Linearity (µg/mL)	0.5-13	0.7-15	0.3-15
Intercept	60.807	67.778	3.9152
Slope	12.101	9.6851	9.5637
Correlation coefficient	0.9995	0.9990	0.9988
LOD (µg/mL)	0.028	0.043	0.098
LOQ (µg/mL)	0.093	0.145	0.327
Average recovery %	99.92	100.25	100.56
RSD	≤ 1.857	≤ 1.670	≤ 2.521

3.3 Application to pharmaceutical preparations

The developed method was applied successfully for the determination of pharmaceutical preparations for the intended drugs, which were in the form of injection, capsule and tablet and manufactured from different sources. The results shown in Table 4 indicate the good accuracy of the suggested method for the determination of the intended drugs. The proposed method has the advantage of being satisfactorily selective and free from excipients interferences.

Table 4: Estimation of the studied drug compounds in their pharmaceutical preparations by the proposed method

Pharmaceutical preparation	Certified value	Amount present (µg/ml)	Drug content found* (mg)	Recovery* (%)	Average recovery (%)
OMZ					
Gasec capsules	40 mg	1 3 5 10	39.64 39.74 40.22 39.90	99.11 99.35 100.56 99.74	99.69
Omeprazole capsules	40mg	1 3 5 10	39.56 40.46 39.93 39.13	98.90 101.14 99.81 97.82	99.42
Lordin	40mg	1 3	39.64 40.18	99.11 100.45	99.95

vial		5 10	39.70 40.41	99.24 101.02	
ESO					
Pumpinox Tablets	40mg	1 3 5 10	40.46 39.85 39.54 39.51	101.16 99.63 98.86 98.78	99.61
Esofag Tablets	40mg	1 3 5 10	40.46 40.44 39.01 39.35	101.16 101.09 97.51 98.37	99.53
PAZ					
Panzol coted /tablets	40mg	1 3 5 10	40.19 40.86 39.91 40.37	100.48 102.16 99.77 100.91	100.83
Pantodar coted /tablets	40mg	1 3 5 10	40.30 39.43 39.49 39.68	100.74 98.58 98.73 99.19	99.31
Pantoprazole Vial	40mg	1 3 5 10	39.88 40.31 39.66 40.42	99.70 100.76 99.15 101.04	100.16

*Average of four determinations

4. Stoichiometry, stability constant and mechanism

Continuous variation method [24] was applied to find out the structural molar ratio of the ion-pair complexes formed between the studied drugs and eosin Y dye in the buffered acetate medium. So, solutions of the drugs and eosin Y dye were prepared with equal concentrations ($3x10^{-4}$M) and the recommended procedure has been applied in above method. It was found from the obtained results, as shown in figures 9, that the ion-pair complexes are formed in ratio of 1:1 for OMZ and PAZ and ESO with Eosin Y reagent. It is noted in the figure 8 that the molar ratio of the complex ESO: Eosin Y is 1:2, since the drug formulation consists of two molecules of OMZ.

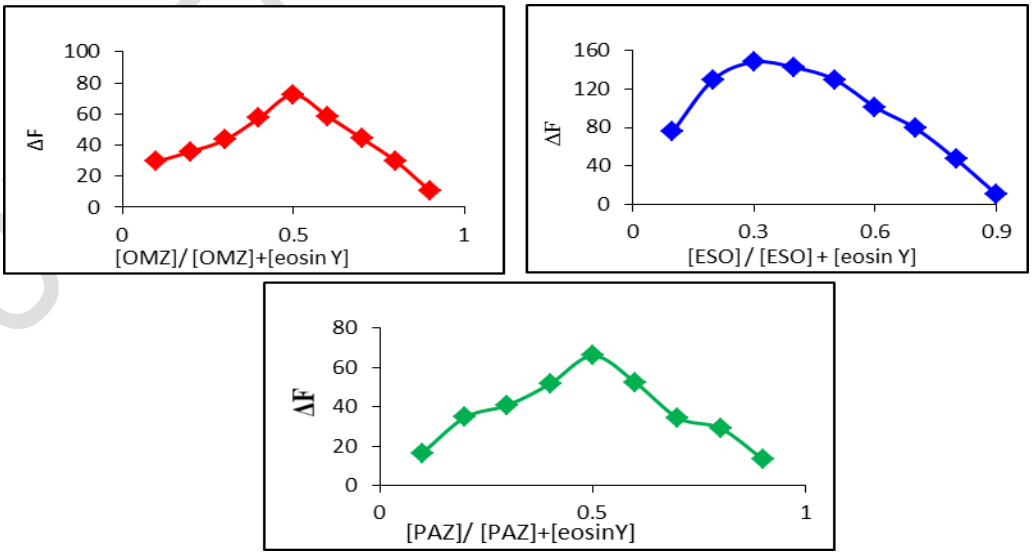
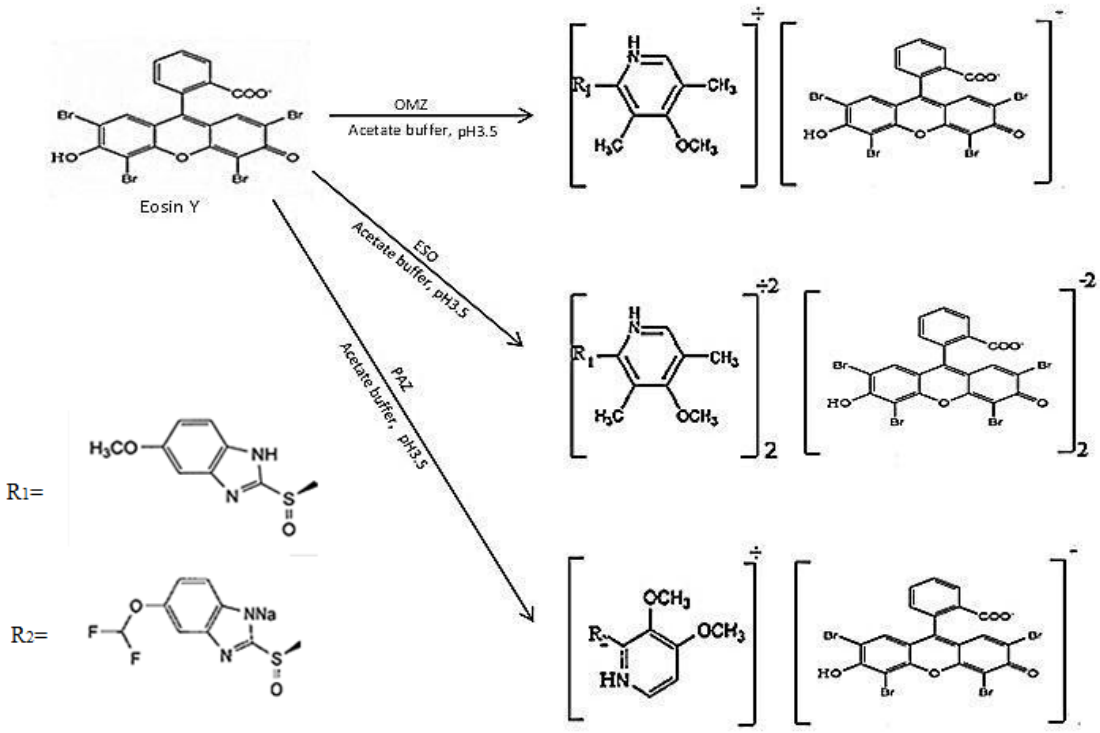


Figure 9: Continuous variation plots of drugs-eosin Y ion-pair complexes

The conditional stability constants of the complexes were calculated by preparing solutions containing the same molar ratios for Omeprazole, Pantoprazole and eosin Y, While the concentration of eosin Y was used twice that of the Esomeprazole. However, the values of stability constant for the complexes were 1.39×10^{6}, 4.58×10^{6} and 2.09×10^{6} $L.mol^{-1}cm^{-1}$. for Omeprazole, Pantoprazole and Esomeprazole, indicating the good stability of the complexes.

According to the results obtained from continuous variations, the mechanism has been suggested for ion-pair complexes formation between the studied drugs and eosin Y (Scheme 1).



Scheme 1: suggested mechanism for ion-pair formation reaction

Conclusion

A spectrofluorimetric method has been developed for determination of proton pump inhibitor drugs including OMZ, ESO and PAZ through quenching of the fluorescence intensity of eosin Y dye in the presence of acetate buffer solution of pH=3.5 in aqueous medium. The method is simple, rapid, accurate and precise, As well as being at room temperature. The method was successfully applied for the determination of the drugs in their pharmaceutical preparations, as tablet, capsule and injection, with good recoveries.

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