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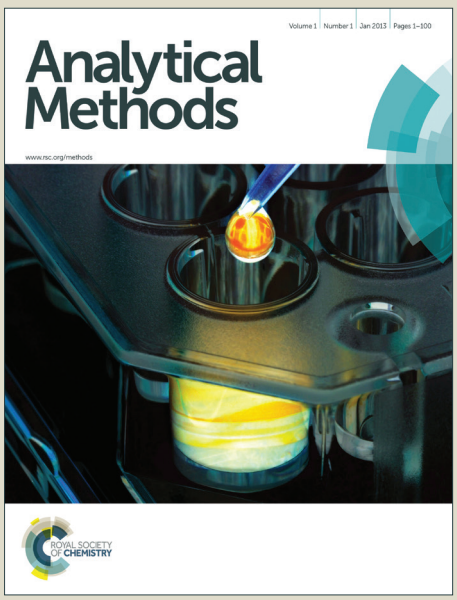

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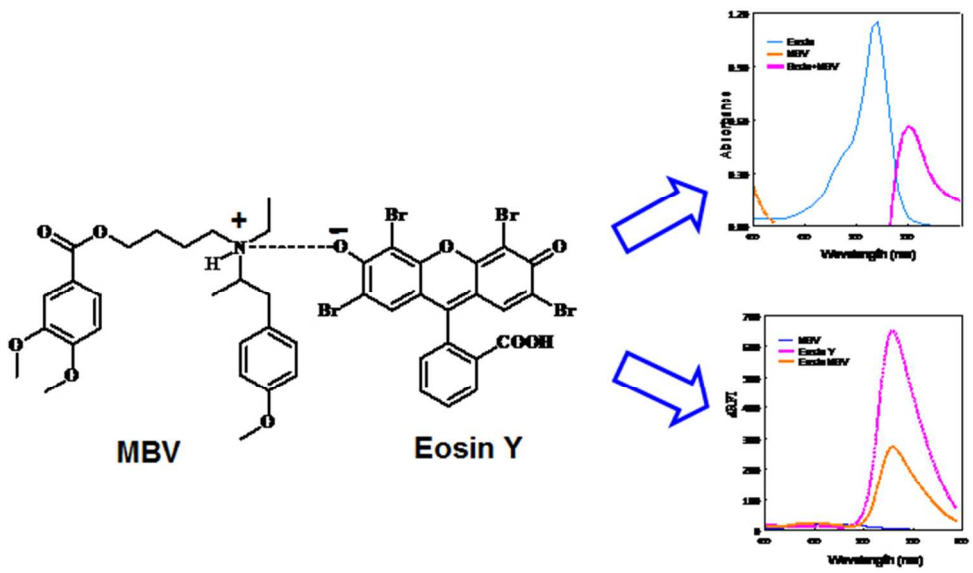

The formation of binary complex between eosin Y and mebeverine result in a blue shift in the UV spectra and decrease in the emission of fluorescence spectra of Eosin. Both effects could be used for the quantitative analysis of the drug.
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Application of Eosin Y for the selective Spectrophotometric and Spectrofluorimetric Determination of Mebeverine Hydrochloride

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Running title: spectrophotometric and spectrofluorimetric determination of mebeverine

ABSTRACT

Two rapid and simple methods were developed and validated for the selective determination of mebeverine hydrochloride based on binary complex formation with eosin Y. In the spectrophotometric method, the absorbance of the formed complex was measured at 551 nm. Beer's law is obeyed in the range of 1 - 12 µg ml^{-1}, The calculated formation constant was 3.95 x 10^5 and the Gibb's free energy change was -3.1 x 10^3 J mol^{-1}. The spectrofluorimetric depends on measuring the quenching effect of the drug on the native fluorescence of eosin Y at 540 nm after excitation at 390 nm. At optimum reaction conditions, the rectilinear calibration graph between the fluorescence quenching values (ΔF) and the drug concentration was obtained in the drug concentration range of 0.2–3.5 µg ml^{-1}. The analytical performance of both methods was fully validated, and the results were satisfactory. The selectivity of the methods was evaluated by studying the interference liability of the co-formulated drugs such as sulpiride, metronidazole, dimethicone and diloxanide. None of them did interfere. The methods were applied successfully for the assay of mebeverine hydrochloride in commercial tablets containing the drug alone or in combination with other drugs.

KEY WORDS: Mebeverine hydrochloride; eosin; spectroflurimetry; spectrophotometry; combined dosage forms

INTRODUCTION

Mebeverine hydrochloride (MBV, (RS)-4-(ethyl[1-(4-methoxyphenyl)propan-2-yl]amino)butyl 3,4-dimethoxybenzoate) is a musculotropic antispasmodic with a direct action on the smooth muscle of the gastrointestinal tract, relieving spasm without affecting normal gut motility. MBV is used in a variety of conditions affecting the vascular system and the gastro-intestinal and genito-urinary tracts. It is mainly used as gastro-intestinal antispasmodic in conditions such as irritable bowel syndrome [1]

Several analytical methods were reported for the determination of MBV in its bulk, pharmaceutical formulations or biological fluids. Among the reported analytical methods are spectrophotometric [2-5], spectrofluorometric [6], electrochemical [7, 8] and chromatographic [9-16].

Eosin Y (2-(2,4,5,7-tetrabromo-6-oxido-3-oxo-3H-xanthen-9-yl)benzoate) is an acidic dye was applied for the determination of several basic drugs through formation of binary [17-21] or ternary [22, 23] complexes.

In this work, simple, sensitive, and accurate extraction- free spectrophotometric and spectrofluorimetric methods were described for the determination MBV. Both methods are based on the reaction of the amino group in MBV with acidic dye, eosin Y, to form ion pair associate in the presence of acetate buffer. The water solubility of the formed ion pair is enhanced by the addition of non ionic surfactant, methyl cellulose. As a result the extraction step was omitted and the measurements are carried out directly in the aqueous solution. Therefore, the proposed procedure is considered environmentally safe compared with those methods utilizing volatile solvents. In addition, the method could selectively determine MBV in presence of other co-formulated drugs present in the commercial dosage forms without prior separation.

The methods, after their full optimization and validation, were adopted for the determination of MBV in its pharmaceutical tablets containing MBV alone or in combination with other active ingredients.

EXPERIMENTAL

Apparatus

The spectrofluorimetric measurements were performed on Perkin Elmer LS 45 Luminescence Spectrometer equipped with 150 Watt Xenon arc lamp and 1 cm quartz cell. Slit width for both monochromators were set at 10 nm. The spectrophotometric measurements were carried out using Spectronic™ genesys™ ultraviolet-visible spectrophotometer (Milton Roy Co, West haven, USA) with matched 1 cm quartz cells was used.

Materials

Mebeverine hydrochloride dimethicone and diloxanide were kindly supplied by Egyptian International Pharmaceutical Industries Company (EIPICO, Cairo, Egypt). Metronidazole was purchased from El-Nasr chemical Co. Cairo Egypt. Sulpiride were kindly supplied by Memphis Pharmaceutical Industries Company, Cairo, Egypt.

Reagents

Eosin Y (Merck, Darmstadt, Germany) 3×10^{-3} M aqueous solution for spectrophotometric method and 0.5×10^{-4} M aqueous solution for spectrofluorimetric method were freshly prepared in distilled water. Acetate buffer solution was prepared by mixing appropriate volumes of 0.2 M sodium acetate (El-Nasr chemical company, Cairo, Egypt) and 0.2 M acetic acid (El-Nasr chemical Co., Cairo, Egypt) and adjusting the pH to 3.5 using pH Meter. Methyl cellulose (Prolabo, France, 1500 CPS) was prepared as 0.25 % w/v by dissolving the appropriate amount in hot water (80°C) with stirring for 10 min, then chilling to 5°C for 30 min. All materials and reagents were of analytical grade and all solutions were prepared with distilled water.

Pharmaceutical formulations

Duspataline 135 mg tablet (Pharco, Alexandera, Egypt) is labeled to contain 135 mg mebeverine hydrochloride. Coloverine 135 mg tablet (Chemipharm, 6th October city, Cairo, Egypt) is labeled to contain 135 mg mebeverine hydrochloride. Colospasmine fort tablet (EIPICO, Cairo, Egypt.) is labeled to contain 135 mg mebeverine hydrochloride. Coloverine D tablet (Chemipharm, 6th October city, Cairo, Egypt) is labeled to contain 135 mg mebeverine hydrochloride and 50 mg dimethicone. Colona

tablet (Rameda, 6th October city, Cairo, Egypt) is labeled to contain 100 mg mebeverine hydrochloride and 25 mg sulipride. Dimetrol tablets (EVA Pharma, Cairo, Egypt) is labeled to contain 200 mg diloxanide fumarate, 50 mg mebeverine hydrochloride and 375 mg metronidazole..

Preparation of MBV HCl standard solutions

A stock standard solution of mebeverine hydrochloride was prepared by dissolving an accurately weighed 25 mg of the drug using about 15 ml of distilled water in a 25-ml volumetric flask. The contents of the flask were sonicated for 5 min, then completed to 25 ml with distilled water. The working standard solutions were prepared by further dilution of the stock solution with the same solvent to obtain concentrations covering the required range.

General recommended procedures

Spectrophotometric method (method I) : One ml of sample or standard MBV solution (10-150 µg ml^{-1}) was transferred into a 10-ml calibrated volumetric flask, followed by 1.0 ml of eosin solution (3 x 10^{-3} M), 1.0 ml of 0.25 % methylcellulose solution and 1.0 ml of acetate buffer (pH 3.5). The volume was completed to the mark with distilled water. The absorbance of the solution at was measure at 551 nm against a reagent blank treated similarly

Spectrofluorimetric method (method II) : One ml of sample or standard MBV solution (0.2-40 µg ml^{-1}) was transferred into a 10-ml calibrated volumetric flask, followed by 1.0 ml of eosin solution (0.5 x 10^{-4} M), 1.0 ml of 0.25 % methylcellulose solution and 1.0 ml of acetate buffer (pH 3.5). The volume was completed to the mark with distilled water. The emission of the solution was measure at 540 nm after excitation at 390 nm. The difference in the fluorescence intensity (ΔF) was plotted *vs* the final drug concentration.

Procedures for validation of the proposed methods

1. Construction of the calibration curves

The general analytical procedures were applied for a series of MBV HCl standard solutions having different concentrations (10-150 or 0.2-40 µg ml^{-1} for

spectrophotometric or Spectrofluorimetric methods respectively). The absorbance or ΔF were measured for each concentration and the calibration graphs were constructed between concentration and the measured absorbance or ΔF. Analytical parameters such as correlation coefficient, slope and intercept were calculated by applying linear regression equation. The limit of detection was calculated using the formula: LOD = 3 σ / S while LOQ= 10 σ / S, where σ is the standard deviation of intercept. S is the slope of calibration curve

2. Precision

Repeatability of the proposed method was investigated by applying the general recommended procedures using 10 and 3 μg ml^{-1} for method I and II, respectively. Intra-day precision was performed by carrying the analysis at three successive times within the same day. Intermediate (inter-day) precision was calculated using three replicates at three successive days.

3. Inference study and method selectivity:

Stock solutions were prepared by dissolving 50 mg of MBV and one of its co-formulated drugs (100 mg of dimethicone, 250 mg diloxanide, 200 mg sulpiride or 375 mg metronidazole) in 50 ml distilled water. Using the suitable dilution, solutions containing 10 or 3 μg ml^{-1} of MBV were analyzed by the general recommended procedures for method I or II, respectively. The mean and standard deviation of three replicates were calculated.

Procedures for analysis of MBV HCl tablet

Twenty tablets were accurately weighed and finely powdered. A quantity of the powdered tablets equivalent to 50.0 mg of MBV HCl was transferred into a 50-ml volumetric flask`, about 30 ml of distilled water was added and the content of the flask was sonicated for 15 min. The volume was completed to the mark with distilled water. The solution was filtered and the first portion of the filtrate was discarded. A portion of the filtrate was diluted quantitatively with distilled water to obtain solutions containing 10 or 3 μg ml^{-1} of MBV HCl. The dug content of final solutions were analyzed with general recommended procedures using five times using.

Determination of molar ratio of the reactions between MBV and eosin Y

Job's method of continuous variation was employed under the working condition to estimate the stiochiometry of the reaction. Master solutions of equimolar solution (1.5×10^{-4} M) of both eosin Y and MBV HCl were prepared. Series of 1.0 ml portions of the master solutions of eosin Y and drug were made up comprising different complimentary proportions (0:1.0, 0.1:0.9, …, 0.9:0.1, 1.0:0) in 10-ml volumetric flasks. 1.0 ml Methyl cellulose surfactant (0.25%) and 0.5 ml of acetate buffer (pH 3.5) was added. The volume was completed to 10 ml with distilled water. In the spectrophotometric method the absorbance was measured at 549 nm against reagent blank treated similarly, omitting the drugs. The same procedure was applied for the spectrofluorimetric method, but the molar concentration of both drug and dye were 0.5×10^{-4} M and the emission of the solution was measure at 540 nm after excitation at 390 nm.

RESULTS AND DISCUSSION

Binary complexe between eosin and basic compounds was previously applied in spectrophotometric analysis of some drugs [17-20]. Because of the low water solubility of the formed ion pair associate, it was usually extracted with organic solvent such as dichloroethane [21]. This extraction step complicates the method. Alternatively, the addition of a surfactant greatly enhanced the water solubility of the formed complex. This enables the direct measurements in the aqueous solution without the need for the extraction step[22, 23]. In the present work methylcellulose was used as surfactant to improve the solubility of the formed ion pair complex..

The proposed methods described in the present study are based on the interaction of MBV and eosin Y at pH 3.5. The formed complex is mainly due to the electrostatic interaction between the tertiary amino groups in MBV and anionic functional group of eosin under acidic pH. The formed ion pair associate has a pink color and exhibits absorption maximum at 551 nm while, neither the drug nor eosin Y has such peak at 551 nm as shown in Fig 1. Both MBV itself and the formed ion pair complex are not fluorescent. Therefore, the formation of complex is accompanied by decrease in the native fluorescence intensity of eosin Y at 540 nm (excitation at 390 nm). The fluorescence quenching is due to conversion of the fluorescent free eosin Y into the complexed non fluorescent form (Fig 2)

Optimization of reaction conditions

All factors affecting the formation and stability of binary complex between MBV and eosin Y, have been studied and optimized for the development of the assay procedures. The studied factors included; concentrations of eosin Y, pH and the diluting solvent.

The effect of eosin Y concentration on the absorbance and fluorescence quenching (ΔF) of the reaction product was studied. It was found that by increasing the reagent concentration, the absorbance and ΔF were gradually increased. Maximum values were obtained when eosin Y was between $1.0 \times 10^{-3} - 4 \times 10^{-3}$ M for absorbance and $0.1 \times 10^{-4} - 1.0 \times 10^{-4}$ M for ΔF. Higher or lower concentration decrease the obtained results. Therefore, 3×10^{-3} M and 0.5×10^{-4} M eosin Y were chosen for the spectrophotometric and spectrofluorimetric methods respectively.

The influence of pH on the absorbance and fluorescence quenching of the binary complex was studied over a pH range of 2.6 to 6 using acetate buffer. As shown in Fig. 3, the complex formation was greatly affected by pH of the medium. Maximum absorbance and ΔF values were obtained when the pH of reaction mixtures were 3.5.

At room temperature, the reaction between the drug and dye was completed immediately after mixing of the solution as the absorbance and ΔF remain constant. The formed complex was found to be stable for at least 24 hr. after dilution.

The formed chromogen was diluted with different solvents such as acetone, methanol, ethanol, 1-propanol. Water was found to be the most appropriate solvent for dilution. Other studied solvents gave lower results.

Constitution of the complex

The nature of the binary complex between eosin Y and MBV has been determined by applying Job's method of continuous variation. As shown in Fig 4, the plots reached a maximum value at a mole fraction of about 0.5 which indicated the formation of a 1:1 drug–dye complex.

Depending on the pH of the solution, eosin can exist in any of the following forms:

$$H_3R^+ \leftrightarrows H_2R \leftrightarrows HR^- \leftrightarrows R^{2-}$$

where R denotes the anionic part of eosin Y. The pK_{a1}, pK_{a2}, pK_{a3}, in the presence of MC were 2.10, 2.85 and 4.95, respectively. In pH 3.5, weak acidic medium, eosin Y exist mainly in the monovalent anionic form (HR^-). There are two possibilities for the ionization of eosin, by dissociation of the hydroxyl or carboxylic groups. In fluorescene (eosin analogue without bromo substitutions), carboxylic group is first ionized then followed by hydroxyl group. However, in eosin the presence of two strong electron withdrawing bromo group close to the hydroxyl group reduce the charge density at the oxygen atom of the hydroxyl. Consequently, the hydroxyl group tends to dissociate more easily than the carboxylic group. Therefore, eosin monovalent anion is formed by the ionization of the hydroxyl group.

Mebeverine have a tertiary amino group that is easily protonated in acidic medium to form a positively charged cation. The ion associate complex is formed by the interaction of the protonated tertiary amino group of MBV with the ionized hydroxyl group of the eosin mono anion through electrostatic attraction and the hydrophobic forces[24]. The suggested structure of the formed complex is shown in figure 5.

The results of Job's method was utilized for the calculation of the formation constant (K_f) of the ion pair complex between MBV and eosin according to the following equation[25]

$$K_f = \frac{(A / A_{ex})}{(1 - A / A_{ex}) C^n n^n}$$

where; A and $A_{ex,}$ are observed maximum absorbance and the absorbance obtained from extrapolation of the two tangent line from Job's continuous variation method., n is the number of mole of MBV involved in the complex formation (n=1) and C is the drug molar concentration used in Job's method

Using the previously mentioned equation, the formation constant for the complex between eosin Y and MBV was found to be 3.95×10^5. This indicates that the formed complex have high stability. The Gibb's free energy change for the complex formation reaction can be calculated using the following formula: $\Delta G^o = - RT \ln K$, where, R is the gas constant (8.314 joule K^{-1} mol^{-1}) and T is the temperature (K), The calculated ΔG^o was -3.1×10^3 J mol^{-1}. The negative sign of the value proof that the reaction is spontaneous one.

Validation of the proposed methods

Quantification, accuracy and precision: Under optimum reaction conditions, calibration curves were constructed and the analytical parameters were calculated. (Table I). The limit of quantitation (LOQ) and limit of detection (LOD) were calculated according to ICH Q2 Recommendation [26]. LOQ is the lowest concentration that can be measured, below which the calibration graph is non linear and was found to be 1.04 and 0.21 μg ml^{-1} for methods I and II respectively. LOD s the concentration of the analyte which can be reliably detected but not necessarily quantified, under the stated experimental conditions and was found to be 0.53 and 0.11 μg ml^{-1} for methods I and II respectively.

Specificity: The specificity of the method was investigated by observing any interference encountered from co-formulated drugs, such as dimethicane, diloxanide, sulpiride, and metronidazole. The stock solutions were prepared by dissolving 50 mg of MBV and the amount of the other drugs mentioned in table 2 in 50 ml distilled water. After suitable dilution, the obtained solution was analyzed by both methods. Results show that neither of these drugs produced any significant interfere with the proposed method, Table 2. The behaviors of dimethicane, diloxanide, and metronidazole was expected since neither of these drugs contain a basic center in their structure. As a result these drugs can not form ion pair complex with eosin. However, sulpiride contain a tertiary amino group but it fail to form an association complex. This may be due to its low lipophlicty of sulpiride (log p = 0.57)[27]. while, MBV has a higher lipophilicty as indicated by its high log p value (5.1)[27]. This may decrease the hydrophobic forces between the sulpiride and eosin Y. It should be mentioned that all drugs which were previously analyzed by binary complex formation with eosin were basic compounds having high hydrophobic properties as indicated by their log p values (2.1 – 6.1).

Precision: Precision was estimated at two level, repeatability and intermediate precision through replicate analysis of the drug (10 μg ml^{-1} for method I and 3 μg ml-1 for method II). The repeatability (intra-day precision) was performed by the analysis three successive times within the same day, Intermediate (inter-day) precision was performed at three successive days. The low values of standard deviations indicates high precision of the proposed methods (Table 3).

Application to commercial tablets:

At the optimum reaction conditions, the two proposed methods were applied for the determination of MBV hydrochloride content in different commercial tablets. These tablets either contain MBV alone such as Duspataline, Coloverine and Colospasmine fort tablets or in combination with other drugs such as Coloverin D tablets (MBV and dimethcone), Colona tablets (MBV and sulpiride) and Dimetrol tablets (MBV, metronidazole and diloxande).The results of the present methods were statistically compared with those of the reference method [16]. Table 4 shows that the calculated *t*-(paired) and *F*- values at 95% confidence level are less than the tabulated *t* value (2.31) and *F* value (6.39). This proves that there is no significant difference between the performance of the developed method and the reference method. In addition, this is an indication for the selectivity of the methods for the analysis of MBV in the presence of the mentioned co-formulated drugs.

CONCLUSION

Eosin Y as an ion-pairing was utilized for the spectrophotometric and spectrofluorimetric determination of MBV in pharmaceutical tablets containing the drug alone or with other co-formulated drugs. The proposed methods are sensitive, and environmentally safe since water is the reaction solvent which is the most important green solvent; volatile solvent is omitted in the present work; in addition, eosin Y is a less toxic regent. Beside the environmental safety, the most important advantage of the methods is their simplicity, as the sample is extracted with water and the formed ion pair was directly measured in the aqueous solution without the need of extraction with organic solvents. The satisfactory sensitivity and simplicity make the methods suitable for routine analysis of MBV in quality control laboratories

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Table 1: Analytical parameters for the analysis of the studied drug by the proposed methods

Parameters	Spectrophotomeric method	Spectrofluorimetric method
Concentration range (μg ml^{-1})	1-12	0.2 – 3.5
Intercept	-0.0814	21.4
Slope	0.064	90.6
Standard deviation of slope (S_b)	0.0013	1.54
Standard deviation of intercept (S_a)	0.005	1.30
Correlation coefficient (r^2)	0.9979	0.9988
Molar absorptivity (ε)	2.88×10^4	---
Limit of detection (LOD) μg ml^{-1}	0.53	0.11
Limit of quantitation (LOQ) μg ml^{-1}	1.04	0.21

Table 2 : Analysis of 50 mg MBV in the presence of co-formulated drugs.

Interfering drugs	Amount added (mg)	% Recovery* ± SD	
		Method I	Method II
Dimethicone	100	100.67 ± 0.56	99.05 ± 1.21
Diloxanide	250	99.71 ± 1.61	98.75 ± 0.84
Sulpiride	200	99.80 ± 0.52	98.55 ± 1.32
Metronidazole	375	100.88 ± 0.46	99.71 ± 1.01

* The value is the average of three determinations.

Table 3 : Intra- and Inter- day assay of precision of the proposed methods for the determination of MBV

Precision		% Recovery* ± SD	
		Method I	**Method II**
Intraday precision	Mean ± SD	100.53 ± 0.46	99.85 ± 0.32
Inter-day precision	Day 1	99.64 ± 0.72	100.34 ± 0.41
	Day 2	100..93 ± 1.12	100.46 ± 1.21
	Day 3	100.75 ± 0.55	99.61 ± 0.85
	Mean ± SD	100.44 ± 0.70	100.14 ± 0.46

* The value is the mean of three determinations.

Table 4: Analysis of commercial tablets containing MBV HCl using the proposed and reported methods

Product	% Recovery [a] ± SD		
	Reported method	**Method I**	**Method II**
Duspataline 135 tablets	99.83 ± 1.09	100.51 ± 0.65 *(t=1.2 , F=2.81)*	100.64 ± 0.91 *(t=1.28 , F=1.43)*
Coloverine 135 tablets	99.73 ± 1.18	100.3 ± 0.82 *(t=0.89 , F=2.07)*	100.67 ± 0.95 *(t=1.39 , F=1.54)*
Colospasmine fort tablets	98.44 ± 0.89	97.51 ± 1.23 *(t=1.37 , F=1.91)*	98 ± 0.91 *(t=0.77 , F=1.05)*
Coloverin D tablets [b]	100.71 ± 0.86	100.59 ± 0.59 *(t=0.26 , F=0.47)*	100.55 ± 1.14 *(t=0.25 , F=1.76)*
Colona tablets [b]	98.12 ± 0.91	98.15 ± 1.04 *(t=0.05 , F= 1.31)*	98.34 ± 1.15 *(t=0.34 , F=1.6)*
Dimetrol tablets [b]	100.69 ± 61	100.43 ± 1.06 *(t=0.48 , F=3.02)*	101.13 ± 1.03 *(t=0.82 , F=2.85)*

[a] The value is the average of five determinations for both proposed and reported methods.

[b] Multi-components tablets

[c] Tabulated t-value = 2.31, F-value = 6.39 (DF = 8).

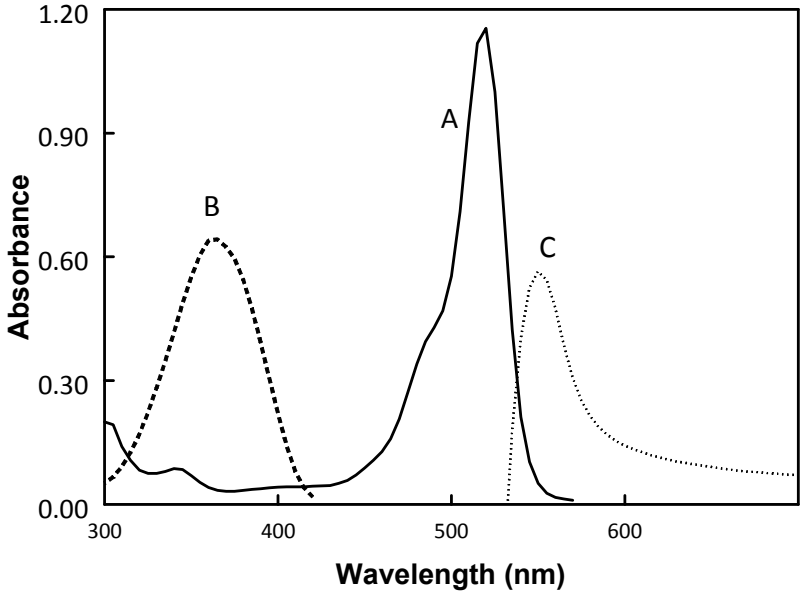

Fig. 1: Absorption spectra of.; (**A**) Eosin Y (**3 × 10^{-3} M**) in water (_ _ _). (**B**) MBV HCl (5 µg ml^{-1}) in water at pH 3.5 (–). (**C**) Eosin Y binary complex with MBV HCl (5 µg ml^{-1}) in water at pH 3.5 (.....)

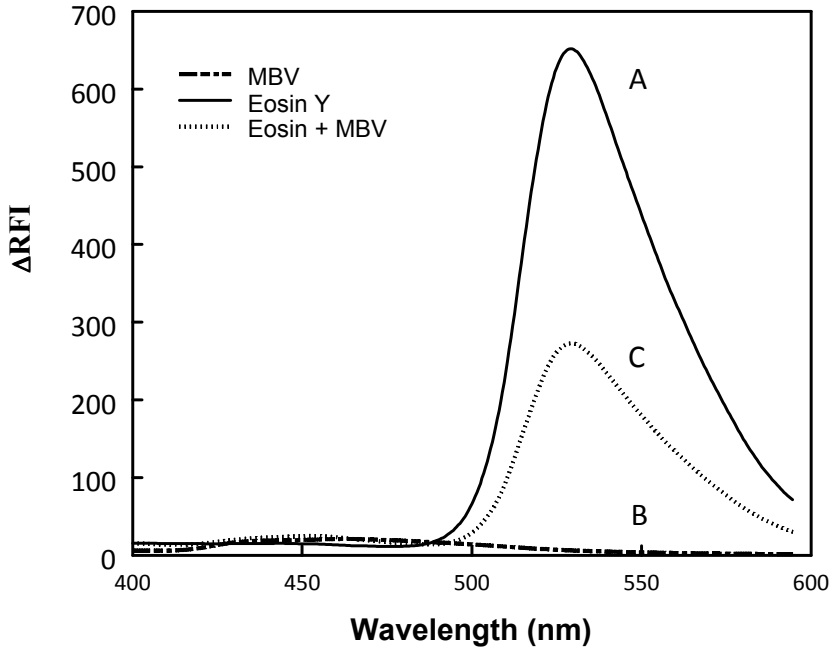

Fig. 2: Emissiion spectra (excitation at 390 nm in water at pH 3.5); (**A**) Eosin Y (**0,5 × 10^{-4} M**) (_ _ _). (**B**) MBV HCl (2 µg ml^{-1}) (–). (**C**) Binary complex of Eosin Y (**0,5 × 10^{-4} M**) with MBV HCl (3 µg ml^{-1}) (.....)

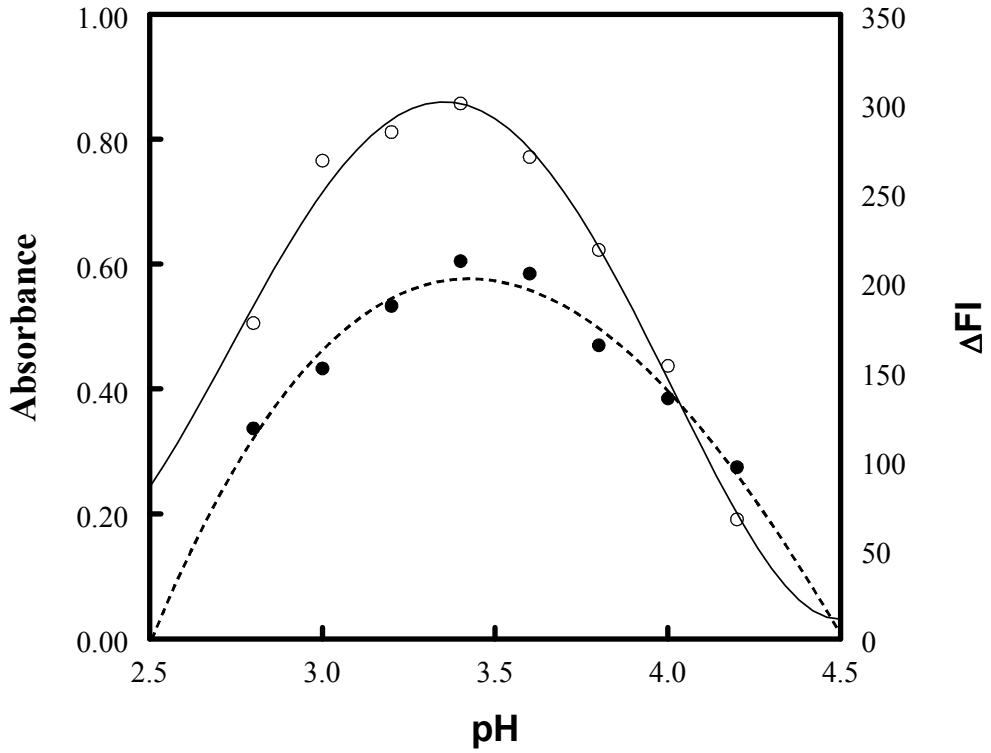

Fig. 3: Effect of pH on the absorbance (-●-) and relative fluorescence intensity (-○-) of the formed binary complex

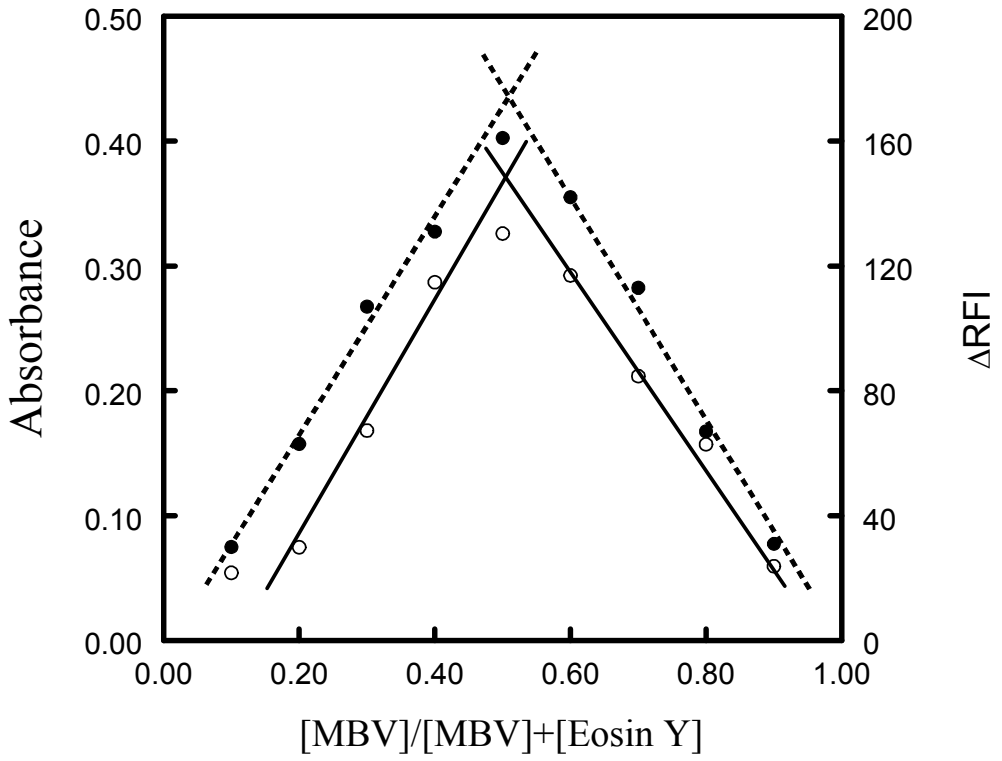

Fig. 4: Molar ratios obtained for the reaction of eosin Y with MBV using molar concentration of 1.5 x 10^{-3} M and 0.5 x 10^{-4} M for spectrophotometric (-○-) and spectrofluorimetric (-●-) methods respectively.

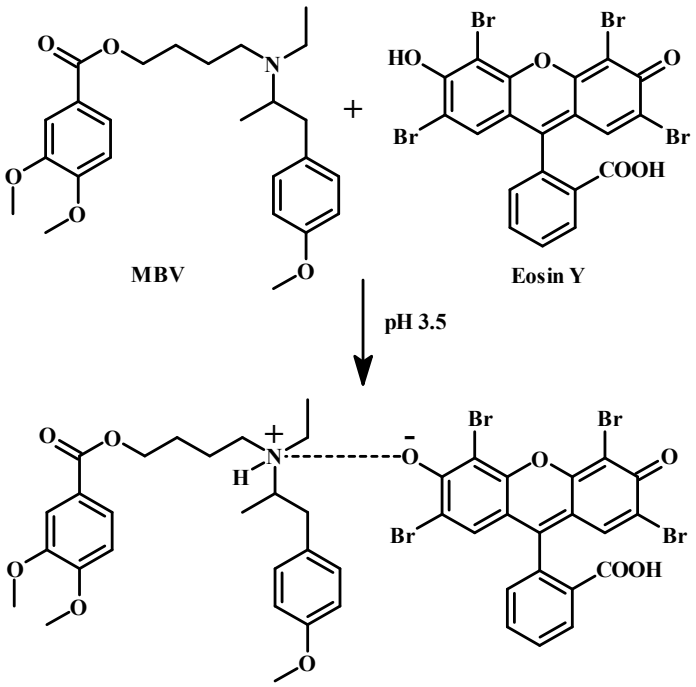

Fig 5: Suggested chemical reaction for the binary complex formation between eosin Y and MBV.