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Determination of Antihistamine in Tablets Preparation by Fluorescence Quenching Method

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Abstract

The fluorescence properties of complexes of dexchlorpheniramine maleate (DXC) and erythrosine B (ERB) under buffer conditions have been observed through experiments. After the excitation was maintained at 504 nm, the emission wavelength was measured at 556 nm. The outcome demonstrated that the amount of DXC added to the mixture causes the fluorescence of erythrosine to be correspondingly quenched. This system functions best at a pH of 3.6. The research demonstrates the potential of ERB as a complexing agent for DXC quantitative analysis through complex ion formation.

1. Introduction

Dexchlorpheniramine is pharmacologically active as a dextrorotatory enantiomer of chlorpheniramine typically used in maleate salt form [1]. An antihistamine group affects H1 receptors to decrease allergic reactions [2]. DXC significantly impaired driving performance after both past and daily administration due to the ability to cross the blood-brain barrier, which may be causing sedation [3]. The development of medication formulations containing active Dexchlorpheniramine maleate (DXC) and excipients is critical for correct DXC activity. Some methods were successfully established to determine DXC [4, 5, 6]. It is commonly acknowledged that chromatographic methods need vast volumes of hazardous organic solvents, laborious operations, sometimes requiring expensive detectors, and time-consuming processes. Unlike GC and HPLC, the spectrofluorometer is easy and economical to use. Our purpose in developing a novel method for the assay of DXC comes from its importance and wide availability for treating different allergy symptoms [1] and its sedative impact, which can impair cognitive functions such as driving performance.

Erythrosine B (ERB) (Figure 1a) was primarily used for food coloring and successfully used for spectrofluorimetric determination of some non-sedating antihistamines [7] and the colorimetric method for proteins determination [8].

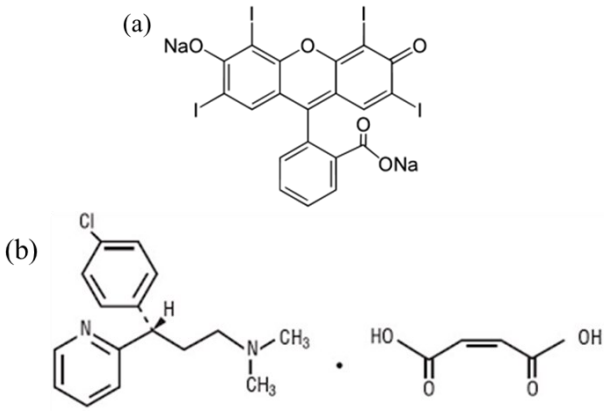

Figure 1. Structure of (a) Erythrosine (b) Dexchlorpheniramine maleate

The present study observed that DXC has a quenching effect on the fluorescence intensity of ERB through the formation of ion-pair complexes with ERB, which is measured spectrofluorimetrically without solvent extraction. The linear ranges of calibration curves for determining DXC were 0.3–8.0 µg/mL. The proposed method provides a fast and sensitive procedure for analyzing the DXC. The featured method is a low-cost technique for assessing the examined substances in quality control laboratories, particularly useful when the

cost is a significant consideration. Erythrosine B, chosen as a reagent, is more commercially accessible than most ordinary analytical laboratories can purchase.

2. Materials and Methods

Fluorescence emission was measured using Spectrofluorometer Shimadzu RF-6000 (Kyoto, Japan) with 1 cm quartz cell, pH meter Senz (Singapore). Erythrosine B (Sigma), distilled water, sodium hydroxide, acetic acid, and phosphoric acid were analytical reagents grade. Dexchlorpheniramine maleate was purchased from the National Agency of Drug and Food Control of Indonesia (Jakarta, Indonesia).

Britton-Robinson (BR) buffer solution was prepared by dissolving 1.143 g NaOH and 0.884 g H_3BO_3 in 300 mL of distilled water, a 0.830 mL of 85% CH_3COOH, and 0.990 mL phosphoric acid mixed thoroughly to the mixture, and the volume was fixed to 500 mL of distilled water. The pH (3.6–5.7) was adjusted by adding hydrochloric acid (37%).

ERB 175 µg/mL and DXC 100 µg/mL were used as stock solutions. A 2.0 mL ERB was pipetted into a volumetric flask, then added 1.5 mL BR buffer solution and DXC (0.3; 1.0; 2.0; 3.0; 5.0; 8.0 µg/mL). The mixture was then diluted to 10 mL with water, mixed evenly, and set aside for 10 min. The fluorescence spectra were recorded at 200–800 nm after excitation occurred at 504 nm. The study set a 5 nm slit width for excitation and emission.

3. Results and Discussion

3.1. Characteristics of the spectra

Erythrosine is highly fluorescent and provides fluorescence spectra. The optimum excitation and emission wavelengths were obtained from 3D fluorescence spectra of freshly prepared erythrosine B solution. The stability of erythrosine B solution in distilled water was first checked by measuring the fluorescence spectrum at zero time after dilution and then observed every 15 minutes. Erythrosine B provided an emission wavelength at 556 nm and an excitation wavelength at 504 nm (Figure 2). The emission intensity of the erythrosine B solution was stable after preparation, but there was a minor decrease in emission intensity at 2 hours (Figure 4). Therefore, using erythrosine solution as a probe at a maximum of 2 hours after dilution is recommended.

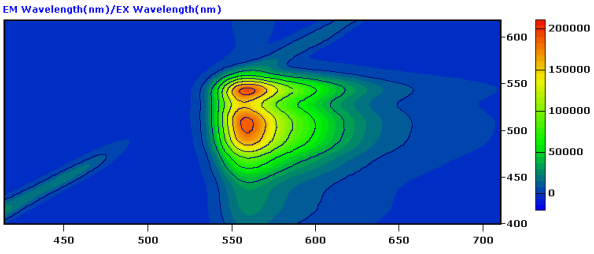

Figure 2. Three-dimensional intensity scan of Erythrosine B diluted in water

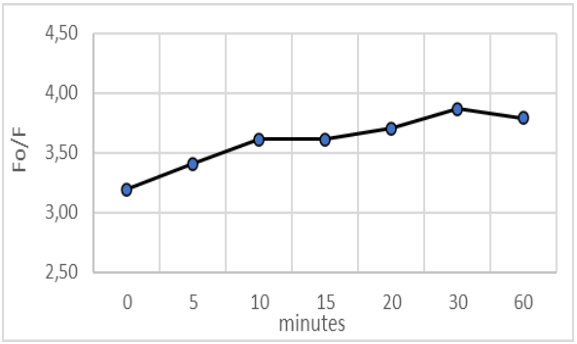

Figure 3. The emission intensity of a mixture of 5 ppm DXC and 11.25 ppm ERB in water with pH 3.6 BR buffer from 0 to 60 minutes after mixing

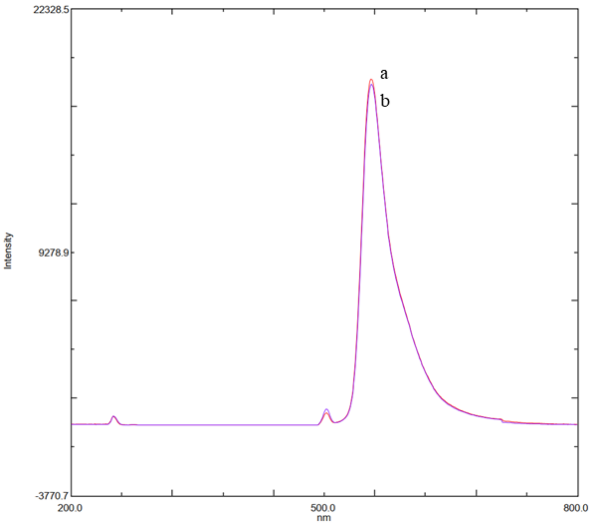

Figure 4. The fluorescence emission intensity of Erythrosine 128×10⁻⁷ mol/L in water solution (a) freshly made (b) at 2 hours

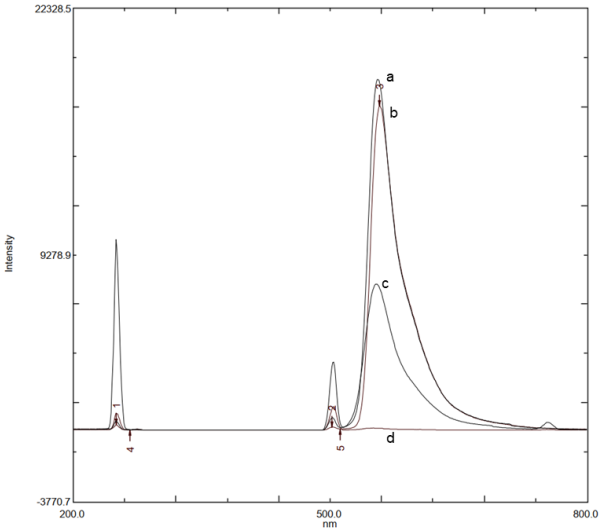

Figure 5. Fluorescence spectra of (a) ERB 128×10⁻⁷ mol/L (b) ERB-DXC (128×10⁻⁷ mol/L-128×10⁻⁷ mol/L) in water without buffer addition (c) ERB 128×10⁻⁷ (d) DXC 128×10⁻⁷ mol/L in the presence of 1.5 mL Britton-Robinson buffer of pH 3.6

Dexchlorpheniramine maleate itself has no fluorescence, but erythrosine has. After DXC was mixed with ERB, the spectra of ERB and ERB-DXC were scanned with a spectrofluorometer. As shown in Figure 5, the

emission peak intensity of ERB (λ_{em} = 554 nm) was decreased but not shifted. Thus, the result showed that ERB and DXC reacted and formed a new complex.

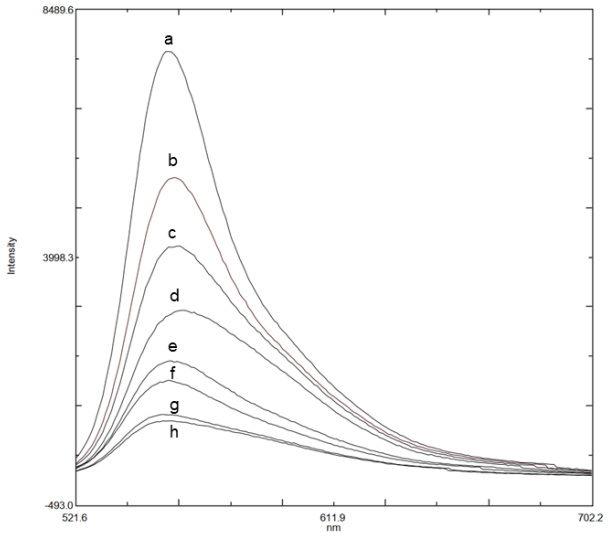

Figure 6. Fluorescence spectra of (a) ERB 35 µg mL^{-1} (b) 0.03 mL (c) 0.075 mL (d) 0.1 mL (e) 0.2 mL (f) 0.3 mL (g) 0.5 mL (h) 0.8 mL of 100 µg mL^{-1} of DXC. A 1.5 mL Britton Robinson buffer of pH 3.6 was added to every single solution and made up to 10 mL with water

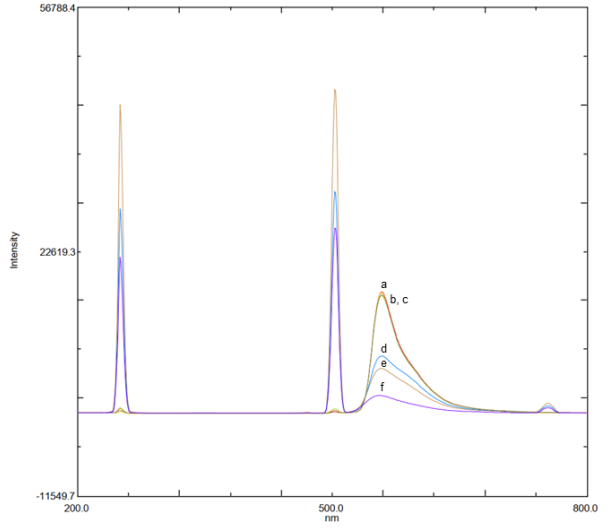

Figure 7. Fluorescence spectra of ERB 35 µg mL^{-1} and 3 µg mL^{-1} of DXC in the (a) absence (b) presence of 1.5 mL Britton Robinson buffer of pH 5.0; (c) 4.5; (d) 4.3; (e) 4.1; (f) 3.6. Each solution was diluted to 10 mL with water

3.2. Reasons for fluorescence quenching

Erythrosine B provides emission without adding a buffer or studied drug (Figure 2). The fluorescence of ERB (λ_{em} = 556 nm) was decreased when the Dexchlorpheniramine maleate was added to the ERB solution. The fluorescence emission peak did not shift, which showed that the ion complex was formed. Fluorescence quenching has resulted from ion-complexes forming, leading to decreased free ERB concentration. While in an acidic solution, the quenching effect is more visible due to the preferred ionization of ERB.

The stoichiometry of the ERB-DXC complex was studied by the mole ratio method. It was done by combining different volumes of 2.558×10^{-6} M dexchlorpheniramine maleate (0.3–0.8 mL) with 0.5 mL of 2.558×10^{-6} M erythrosine B in the presence of Britton-Robinson buffer solution (pH 3.6). The reduction in fluorescence intensity of erythrosine B was recorded at 556 nm after constant excitation (504 nm). The ratio of F_0/F was plotted (Figure 7) against to mole ratio of dexchlorpheniramine maleate and erythrosine B. The strongest quenching was reached with 0.5 mL of 2.558×10^{-6} M dexchlorpheniramine maleate reacted with 0.5 mL of 2.558×10^{-6} M erythrosine B, as shown in Figure 7. The stoichiometry provided by this reaction was 1 mol of dexchlorpheniramine maleate and 1 mol of erythrosine B.

Proper reaction time was determined by monitoring the emission intensity from 0 to 60 minutes after the mixture was made. As shown in Figure 3, the emission stability of the DXC-ERB mixture was studied from 0 to 60 minutes after the mixture was made. The result showed stable emission at 10 to 15 minutes after the mixture was made, chosen as the operating time for emission recording.

3.3. Linearity

The determination of linearity was done either by plotting the fluorescence ratio against a concentration or linear regression equation F_0/F = bC+ a, where F_0 stands for the fluorescence intensities of erythrosine B observed in existence DXC, and F is the emission intensity after DXC addition. While b is the slope, a is the intercept, and C is the dexchlorpheniramine maleate concentrations (µg/mL). Table 1 shows the linear regression equation of the proposed method, where the limit of detection and quantitation is derived from the generated equation. The Pearson correlation coefficients ranged from 0.995 to 0.999, confirming that the results were linear.

Table 1. Linear regression equation, LoD, and LoQ of the proposed method

Equation	Pearson correlation	Limit of Detection	Limit of Quantitation
F_0/F = 0.5817C + 0.8356	0.99804	0.4198 µg/mL	1.3992 µg/mL

3.4. Limit of Detection and Limit of Quantification

Respectively, LoD and LoQ were 3 and 10 σ/b; both are derived from the linear regression equation, where σ is the standard deviation of the F_0/F-intercept and b is the slope of the calibration curve. Under the defined experimental environments, standard calibration curves for DXC with erythrosine B were constructed by plotting DXC concentration against the fluorescence quenching value (Figure 5).

3.5. Precision

Replicated analysis of three different solutions of DXC at three different concentration levels: low (2.0 mg/L), middle (5.0 mg/L), and high (8.0 mg/L), were studied to determine the intra-day precision of the proposed method. This experiment (n = 6) was repeated

for three consecutive days to assess the inter-day accuracy. RSD% values for DXC for intra-day ranged from X to X, and inter-day precisions ranged from X to X, indicating good repeatability (Table 2).

Table 2. RSD of three different concentration levels of DXC added to ERB

Concentrations	2 ppm	5 ppm	8 ppm
N1	1.8991	3.6305	5.5347
N2	1.9316	3.5376	5.8155
N3	1.9831	3.4861	5.6346
N4	1.9385	3.5019	5.7211
N5	1.9233	3.5276	5.6944
N6	1.9219	3.5686	5.7721
average	1.9329	3.5421	5.6954
SD	0.027962	0.051944	0.100469
RSD (%)	1.45	1.47	1.76

3.6. Accuracy

Three different DXC concentrations (2, 5, and 8 µg mL^{-1}) were prepared from the stock solution and analyzed (n = 6) to determine the accuracy of the proposed method. Accuracy was determined by comparing measured concentrations of DXC with the actual values. The accuracy of the proposed method for the studied drugs ranged from 94.4% to 107.4% representing good accuracy of the proposed method as shown in Table 3. Either obtained accuracy and precision value were fit for measurements of quality control.

Table 3. Recovery of the proposed method at three different levels of DXC concentration. The value in % to the F_0/F

cons (ppm)	2	5	8
$(F_0/F)i$ 1	105.2610	103.1292	99.17756
$(F_0/F)i$ 2	103.4873	105.8358	94.38993
$(F_0/F)i$ 3	100.8016	107.3995	97.41903
$(F_0/F)i$ 4	103.1218	106.9160	95.94591
$(F_0/F)i$ 5	103.9332	106.1373	96.39705
$(F_0/F)i$ 6	104.0137	104.9190	95.09887
average±SD	103.44±1.48	105.72±1.53	96.41±1.71

4. Conclusion

Dexchlorpheniramine maleate can be determined using fluorescence spectrophotometry with erythrosine B as a probe. Dexchlorpheniramine maleate reacted with erythrosine B to form an ion complex, leading to fluorescence quenching of erythrosine B.

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