

Research Article

Spectrophotometric Determination of Some Non-Sedating Antihistamines Using Erythrosine B

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A simple and sensitive spectrophotometric method has been developed for the determination of cetirizine (I), ebastine (II), fexofenadine (III), ketotifen (IV), and loratadine (V) based on ion-pair complex formation with erythrosine B. The pink color of the produced complex was measured at 550 nm without solvent extraction. Appropriate conditions were established by studying the color reaction between erythrosine B and the studied drugs to obtain the maximum sensitivity. Beer-Lambert's law is obeyed in the concentration ranges 1–7, 1–8, and 1–6 $\mu\text{g}/\text{mL}$ for (I, IV), (II, III), and (V), respectively. The method was validated according to ICH guidelines. The suggested method is applicable for the determination of the five investigated drugs in bulk and pharmaceutical dosage forms with excellent recoveries.

1. Introduction

In 1937, the first H1 antihistamine (thymo-ethyl-diethylamine) was synthesized. However, because of its weak activity and high toxicity, this compound was not used. Clinically useful H1 antihistamines such as phenbenzamine, pyrilamine, and diphenhydramine were introduced in the 1940s. Currently, H1 antihistamines constitute the second most commonly used class of medications after antibiotics. The older first-generation antihistamines are associated with troublesome sedative and antimuscarinic effects and are often termed “sedating antihistamines.” The newer generations of antihistamines, which are essentially devoid of these effects, are correspondingly termed “non-sedating antihistamines.”

Non-sedating antihistamines are of potential value in the management of allergic rhinitis in which they relieve nasal and conjunctival itching, sneezing, and rhinorrhoea. They are also useful in the treatment of acute and chronic urticaria [1].

Non-sedating antihistamines down regulate allergic inflammation directly through the H1-receptor by interfering with histamine action at H1-receptors on sensory neurons and small blood vessels. They also decrease the antigen presentation, expression of pro-inflammatory cytokines and

cell adhesion molecules, and chemotaxis. In a concentration-dependent manner, they inhibit mast cell activation and histamine release [2].

Second-generation non-sedating antihistamines investigated are cetirizine (CTZ), ebastine (EBS), ketotifen (KET), and loratadine (LOR), while Fexofenadine (FXD) is one of the third-generation drugs. Their chemical structures are given in Figure 1.

The studied drugs, CTZ, EBS, FXD, KET, and LOR are available in cheap generic forms, and they are considered over-the-counter (OTC) drugs and used without prescription with very low or no sedating effect.

Many procedures were described for quantitative determination of CTZ, EBS, FEX, KET, and LOR in the literature. Among these methods are liquid chromatography (HPLC) [3–8], gas chromatography (GC) [9–12], electrochemical [13–17], spectrophotometric (derivative-spectrophotometry, formation of ion-pair complexes with methyl orange, bromocresol blue and eosin, and charge transfer with DDQ) [18–23], and fluorimetric methods [19, 22, 24–27].

In the present study, CTZ, EBS, FXD, KET, and LOR were determined through formation of ion-pair complex with erythrosine B (Figure 2), as acidic dye without extraction.

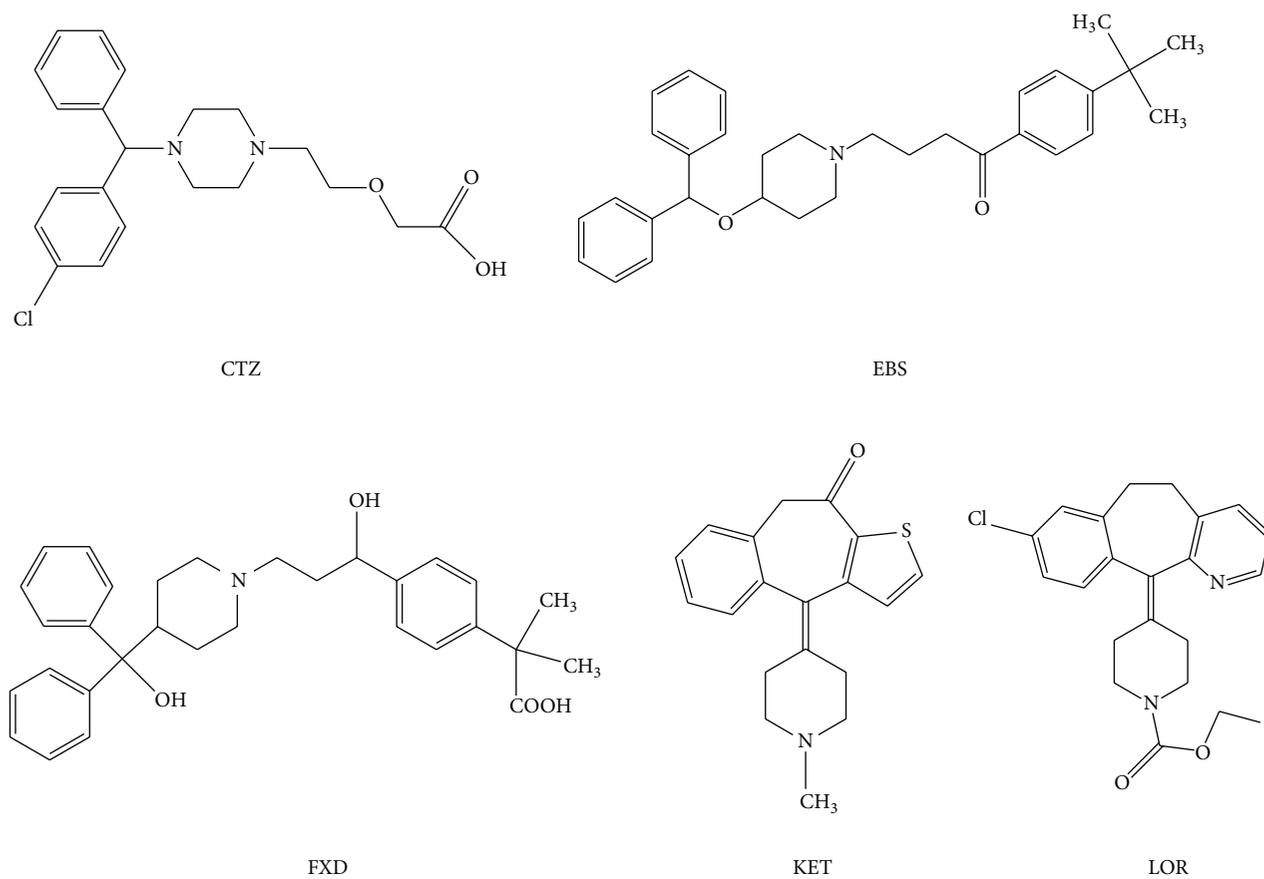


FIGURE 1: Chemical structures of the investigated drugs.

Erythrosine B is the disodium salt of 2,4,5,7-tetraiodofluorescein, primarily used for food coloring and was used for spectrofluorimetric determination of imipramine [28].

The proposed method provides a rapid and sensitive procedure for the analysis of the studied drugs in pure and in pharmaceutical formulations. The proposed method provides an inexpensive tool for determination of the studied drugs in quality control laboratories especially in developing countries where the cost is the main concern.

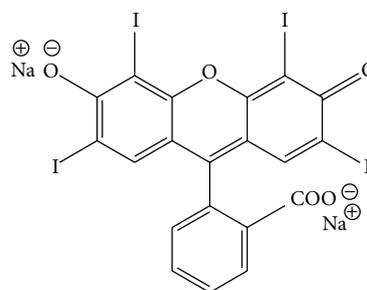


FIGURE 2: Chemical structure of erythrosine B.

2. Experimental

2.1. Apparatus

- (i) Spectrophotometric measurements were carried out using a Shimadzu UV-1601 PC UV-Visible spectrophotometer (Tokyo, Japan) with 1 cm glass cells.
- (ii) Jenway 3505 pH meter (UK) was set to check pH values of acetate buffer solutions (pH 3.3; 0.2 M).

2.2. Materials and Reagents

- (i) CTZ HCl was kindly supplied by Egyptian International Pharmaceutical Industries Company E.I.P.I.Co. (10th of Ramadan city, Egypt).

- (ii) EBS was kindly supplied by Global Napi Pharmaceutical Company (Giza, Egypt).
- (iii) FXD HCl and LOR were kindly supplied by Amoun pharmaceutical company (Obour, Egypt).
- (iv) KET fumarate was kindly supplied by Novartis pharmaceutical company (Cairo, Egypt).
- (v) Erythrosine B (Market Harborough Leicestershire, UK), 0.15% solution in distilled water, was used.
- (vi) Acetate buffer (pH 3.3; 0.2 M) was prepared from acetic acid and sodium hydroxide [29].
- (vii) All other chemicals and reagents used were of analytical grade.

2.3. *Pharmaceutical Formulations.* All pharmaceutical formulations were obtained from the Egyptian market as follows:

- (i) Epirizine tablets (labeled to contain 10 mg CTZ HCl per tablet);
- (ii) Evastine tablets (labeled to contain 10 mg EBS per tablet);
- (iii) Allerfen tablets (labeled to contain 60 mg FXD HCl per tablet);
- (iv) Zaditen tablets (labeled to contain 1 mg KET fumarate per tablet);
- (v) Mosedine tablets (labeled to contain 10 mg LOR per tablet).

2.4. *Preparation of Standard Solutions.* Stock standard solutions were prepared to contain 1 mg/mL by dissolving 50.0 mg of each drug in 25 mL methanol, and then the volumes were completed to 50 mL with the same solvent.

The working solutions were prepared by appropriate dilution of the stock solutions with methanol.

2.5. *General Procedure.* One milliliter of the working standard or sample solution of CTZ, EBS, FXD, KET, or LOR was transferred to 10 mL volumetric flask. Also, 0.45 mL of erythrosine B (0.15% w/v) was added, then 1 mL of acetate buffer. The contents were left for 9 minutes at room temperature. Then, the mixture was diluted with distilled water, and the absorbance was measured at 550 nm for all drugs against a reagent blank prepared in the same manner.

2.6. *Construction of Calibration Curves.* Transfer different aliquots of standard solutions equivalent to 0.01–0.07, 0.01–0.08, and 0.01–0.06 mg of (I, IV), (II, III), and (V), respectively, to 10 mL volumetric flasks. Then, the assay was completed as under general procedure.

3. Procedure for Tablets

Accurately weighed powder samples obtained from 30 tablets, equivalent to 25 mg of each drug, were suspended in methanol, and then the volumes were completed to the mark with the same solvent in 25 mL calibrated flasks, filtered and the first portion of the filtrate was rejected. Then, the assay was completed as under general procedure.

4. Method Validation

Typical analytical performance characteristics for the validation of procedures according to ICH Q2 guidelines were described in this study, including specificity, linearity, limit of detection, limit of quantification, precision, accuracy, recovery, and robustness [30].

4.1. *Specificity.* To assess the specificity of the method, the effect of diluents, excipients, and additives commonly used in pharmaceutical formulations such as lactose, titanium dioxide, starch, cellulose, and microcrystalline cellulose was studied.

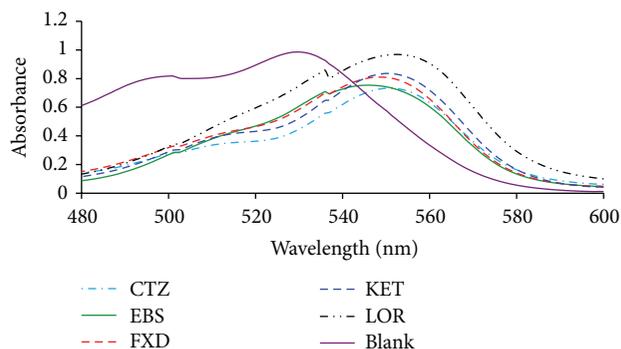


FIGURE 3: Absorption spectra of ion-pair complexes of CTZ, EBS, FXD, KET, and LOR (6 $\mu\text{g/mL}$) with erythrosine B.

4.2. *Linearity.* Linearity was determined either by plotting absorbance versus concentration or from linear regression equation $A = bC + a$, where A is the absorbance of the ion-pair complex solution, b is the slope, a is the intercept, and C is the concentration of the drug.

4.3. *Limits of Detection (LOD) and Quantification (LOQ).* LOD and LOQ were calculated as $3.3r/S$ and $10r/S$, respectively, where r is the standard deviation of y -intercept of the regression equation and S is the slope of the calibration curve.

4.4. *Accuracy.* To determine the accuracy of the proposed method, three levels of drug concentrations (low, medium, and high) were prepared from stock solutions and analyzed ($n = 6$).

4.5. *Precision.* Intra-day precision of the proposed method was tested by replicate analysis of three separate solutions of the working standard of CTZ, EBS, FXD, KET, and LOR at three different concentration levels: low (2 $\mu\text{g/mL}$), middle (4 $\mu\text{g/mL}$), and high (6 $\mu\text{g/mL}$). This study was repeated for three days to determine the inter-day precision ($n = 6$).

4.6. *Recovery.* The recovery of the proposed method was determined using the standard addition technique, by adding a known amount of standard at three different levels to the pre-analyzed sample.

4.7. *Robustness.* The robustness of the proposed method was determined by studying the effect of minor changes on the absorbance of the formed complex: the pH of the media, buffer concentration, reaction time, and volume and concentration of the reagent on the method performance.

5. Results and Discussion

5.1. *Absorption Spectra.* The five studied drugs react with erythrosine B through an ion-pair salt formation, forming a pink chromophore with λ_{max} at 550 nm (Figure 3).

5.2. *Optimum Reaction Conditions for Complex Formation.* To optimize the assay parameters, the effects of pH, reaction time, effect of temperature, erythrosine B concentration and

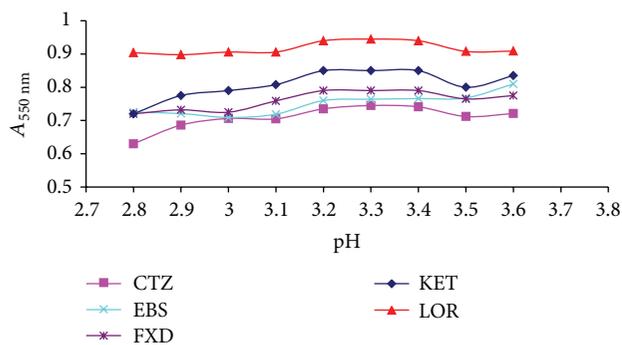


FIGURE 4: Effect of pH on the reaction of the studied drugs ($6 \mu\text{g/mL}$) with erythrosine B.

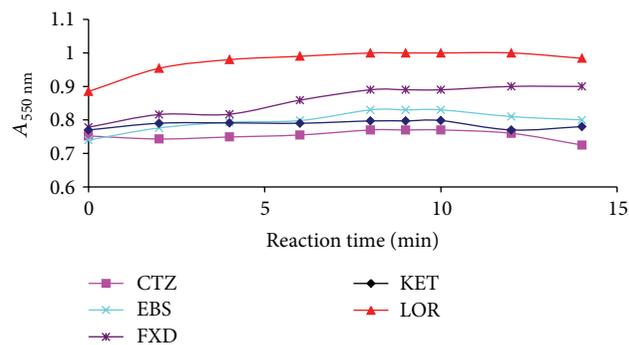


FIGURE 6: Effect of reaction time on the ion-pair complexation of the studied drugs ($6 \mu\text{g/mL}$) with erythrosine B.

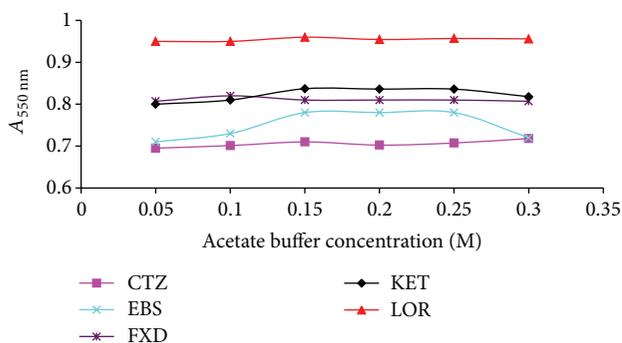


FIGURE 5: Effect of buffer concentration on the reaction of the studied drugs ($6 \mu\text{g/mL}$) with erythrosine B.

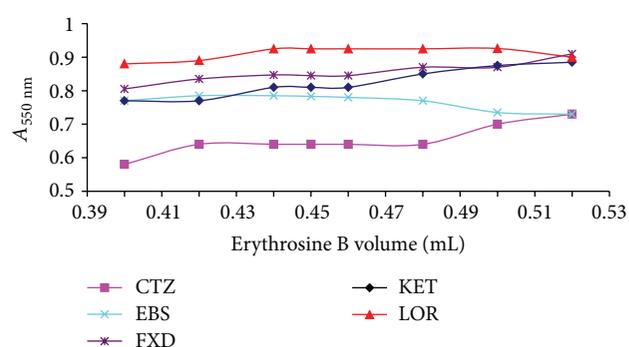


FIGURE 7: Effect of erythrosine B volume on the ion-pair complexation of the studied drugs ($6 \mu\text{g/mL}$) with erythrosine B.

volume, order of addition of reactants, and the effect of solvent were studied.

5.2.1. Effect of pH. The procedure was carried out in the presence of various buffers such as phosphate, acetate, borate, and potassium hydrogen phthalate-HCl buffers. Acetate buffer was the best one giving the highest absorbance. Various concentrations of acetate buffer (0.05–0.3 M) were also tried. It was found that the ion-pair formation was optimized using 0.2 M for all drugs. At pH 3.7 precipitation occurred; therefore acetate buffer in the pH range (2.8–3.6) was studied; the maximum color intensity was observed by using acetate buffer of pH 3.3, 0.2 M (Figures 4 and 5).

5.2.2. Effect of Reaction Time. The effect of time (Figure 6) and temperature on the developed reaction was judged based on the observation of complete color development which remained stable for one day. The optimum reaction time was studied from 0.0 to 14.0 min. High temperature caused turbidity of the solution. It was observed that absorbance reached the maximum in 9 min at 25°C (room temperature).

5.2.3. Effect of Erythrosine B Concentration and Volume. Different volumes (0.4–0.52 mL) and various concentrations of erythrosine B (0.05%–0.225% w/v) were added to the studied drugs. Higher dye concentrations caused turbidity of the solution. It was found that the ion-pair formation was

TABLE 1: The studied orders of addition of reactants.

Order of addition	λ_{max} (nm)	Absorbance at λ_{max}
Drug—buffer—dye	546	0.647
Drug—dye—buffer	550	0.776
Dye—drug—buffer	544	0.637
Dye—buffer—drug	543	0.602

EBS ($6 \mu\text{g/mL}$) was used as a representative example.

optimized using 0.45 mL of 0.15% w/v erythrosine B for all drugs (Figures 7 and 8).

5.2.4. Effect of Solvent. *n*-Butanol, ethanol, methanol, chloroform, dichloroethane, acetone, and distilled water were studied as diluting solvents. The maximum color intensity and highest absorbance values were observed with distilled water.

5.2.5. Effect of Order of Addition of Reactants. The studied orders of addition of reactants are summarized as shown in Table 1.

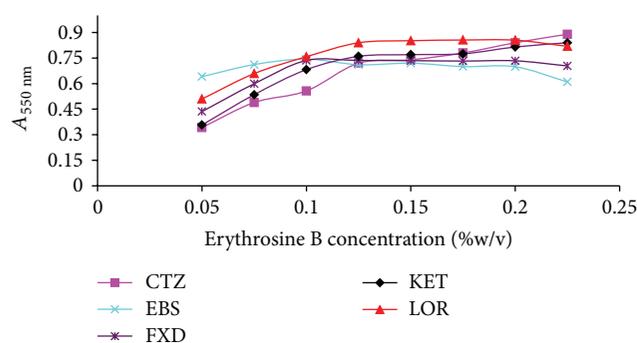
It was found that the most favorable sequence is drug—dye—buffer for all the studied drugs to attain the highest color intensity and stability.

TABLE 2: Spectral characteristics for the reaction of the studied drugs with erythrosine B^a.

Parameter	CTZ	EBS	FXD	KET	LOR
λ_{\max} (nm)	550	550	550	550	550
Linearity range ($\mu\text{g}/\text{mL}$)	1-7	1-8	1-8	1-7	1-6
$(r) \pm \text{SD}^*$	$0.9996 \pm 6.3 \times 10^{-4}$	$0.9997 \pm 1.4 \times 10^{-4}$	$0.9998 \pm 2.2 \times 10^{-4}$	$0.9996 \pm 2.3 \times 10^{-4}$	$0.9998 \pm 1.7 \times 10^{-4}$
$R^2 \pm \text{SD}^*$	$0.9992 \pm 1.2 \times 10^{-3}$	$0.9994 \pm 2.8 \times 10^{-4}$	$0.9996 \pm 2.5 \times 10^{-5}$	$0.9992 \pm 4.3 \times 10^{-4}$	$0.9996 \pm 3.9 \times 10^{-4}$
Intercept (a) $\pm \text{SD}^*$	$-0.084 \pm 5.1 \times 10^{-3}$	$-0.118 \pm 1.1 \times 10^{-2}$	$-0.093 \pm 6.2 \times 10^{-3}$	$-0.098 \pm 1.0 \times 10^{-3}$	$-0.119 \pm 1.1 \times 10^{-2}$
Slope (b) $\pm \text{SD}^*$	$0.131 \pm 1.1 \times 10^{-3}$	$0.143 \pm 3.7 \times 10^{-3}$	$0.150 \pm 1.4 \times 10^{-3}$	$0.157 \pm 1.7 \times 10^{-3}$	$0.188 \pm 6.4 \times 10^{-3}$
LOD ^b ($\mu\text{g}/\text{mL}$)	0.13	0.25	0.14	0.21	0.19
LOQ ^c ($\mu\text{g}/\text{mL}$)	0.39	0.77	0.41	0.64	0.58

^a Average of six replicates.^b Limit of detection.^c Limit of quantitation.

* Standard deviation.

FIGURE 8: Effect of erythrosine B concentration on ion-pair complexation of the studied drugs (6 $\mu\text{g}/\text{mL}$) with erythrosine B.

5.2.6. *Stability Time.* Absorbances of all formed complexes are stable for at least 2.5 h after final dilution for all the studied drugs.

5.2.7. *Stoichiometric Ratio.* Job's method of continuous variations [31] was employed using equimolar (3×10^{-4} M) standard solutions of CTZ, FXD, KET, LOR, and (2×10^{-4} M) of EBS with erythrosine B (3×10^{-4} and 2×10^{-4} M, resp.). A series of solutions were prepared in which the total volume of drugs and erythrosine B was kept at 5 mL then diluted to volume in 10 mL calibrated flask with distilled water following the general procedure. The absorbance was measured at the optimum wavelength (550 nm).

The molar ratio of drug to dye in the ion-pair complex was found to be 1 : 2 (Figure 9).

5.3. Validation of the Developed Method

5.3.1. *Linearity, Detection and Quantitation Limits.* Under the described experimental conditions, standard calibration curves for CTZ, EBS, FXD, KET, and LOR with erythrosine B were constructed by plotting absorbance against concentration (Figure 10).

Conformity with Beer-Lambert's law was evident in the concentration range of the final solution cited in Table 2. The

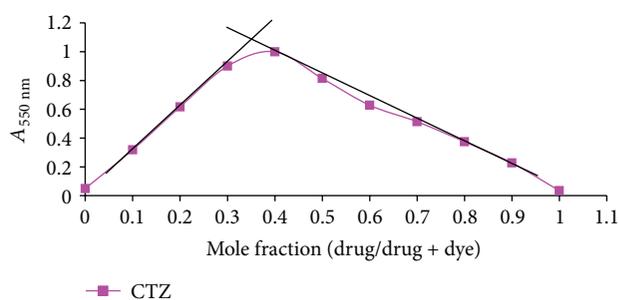


FIGURE 9: Continuous variation plot for CTZ as a representative example with erythrosine B.

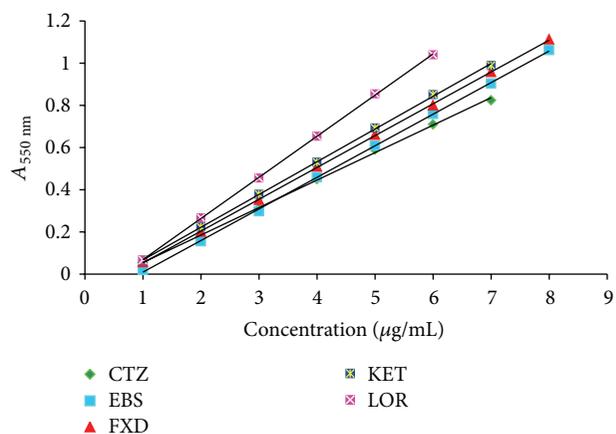


FIGURE 10: Calibration curves of the ion-pair complexes of the studied drugs with erythrosine B.

linear regression equation, limit of detection, and limit of quantitation for each drug are presented also in Table 2. The correlation coefficients were 0.9996–0.9998 indicating good linearity.

5.3.2. *Precision and Accuracy.* RSD% values for CTZ, EBS, FXD, KET, and LOR for intra-day and inter-day precisions were ranged from 0.22 to 1.64 indicating good repeatability. Accuracy was determined by comparing measured

TABLE 3: Accuracy and precision of the developed spectrophotometric method.

Authentic drug	Concentration ($\mu\text{g/mL}$)	RSD %		Accuracy* %
		Inter-day precision	Intra-day precision	
CTZ	2	0.69	0.85	98.17
	4	1.31	0.59	101.76
	6	0.85	0.85	100.99
EBS	2	1.22	1.15	99.20
	4	1.27	0.76	98.45
	6	0.26	0.38	99.46
FXD	2	1.59	1.07	99.80
	4	0.73	0.65	99.84
	6	0.53	0.51	101.43
KET	2	0.98	0.59	99.33
	4	0.73	0.71	100.24
	6	0.37	0.28	101.90
LOR	2	1.64	1.29	99.87
	4	0.62	0.45	99.66
	6	0.33	0.22	99.83

* Average of six replicates.

TABLE 4: Robustness of the developed spectrophotometric method.

Drug	% Recovery* \pm SD				
	CTZ	EBS	FXD	KET	LOR
No variation**	101.1 \pm 0.76	100.4 \pm 0.45	100.2 \pm 0.93	100.9 \pm 1.29	100.2 \pm 1.20
pH of acetate buffer					
3.2	101.7 \pm 0.82	100.7 \pm 1.16	100.6 \pm 1.07	100.9 \pm 0.61	100.3 \pm 0.52
3.4	100.9 \pm 0.49	100.9 \pm 0.27	101.3 \pm 0.72	102.3 \pm 1.22	97.4 \pm 0.01
Acetate buffer conc.					
0.15 M	100.8 \pm 0.57	101.0 \pm 0.73	100.2 \pm 0.63	99.6 \pm 1.12	99.4 \pm 0.84
0.25 M	101.7 \pm 0.08	101.2 \pm 0.56	101.4 \pm 0.02	101.8 \pm 0.80	98.7 \pm 0.91
Erythrosine B conc.					
0.14% w/v	100.1 \pm 1.14	99.3 \pm 0.97	100.2 \pm 1.11	98.1 \pm 0.44	99.4 \pm 0.67
0.16% w/v	102.1 \pm 0.77	98.2 \pm 0.63	100.8 \pm 0.39	100.7 \pm 0.36	100.6 \pm 1.02
Erythrosine B volume					
0.44 mL	101.9 \pm 0.43	98.5 \pm 0.89	100.3 \pm 0.55	98.6 \pm 0.32	102.6 \pm 1.11
0.46 mL	102.3 \pm 0.37	102.3 \pm 1.57	102.5 \pm 0.22	103.0 \pm 0.54	102.8 \pm 0.66
Reaction time					
8 min.	101.6 \pm 0.15	98.5 \pm 1.03	100.9 \pm 0.83	102.1 \pm 0.69	99.1 \pm 1.11
10 min.	98.8 \pm 1.8	102.9 \pm 0.42	99.6 \pm 0.19	98.9 \pm 1.29	98.3 \pm 0.82

* Average of six replicates.

** No variation in the assay condition of the proposed method.

concentrations of CTZ, EBS, FXD, KET, and LOR with the actual values and expressed as percentage in Table 3. The accuracy of the developed method for the studied drugs ranged from 98.17% to 101.76% indicating acceptable accuracy. The obtained accuracy and precision were satisfactory for quality control measurements.

5.3.3. Robustness. The robustness of an analytical procedure refers to its capability to remain unaffected by small and deliberate variations in method parameters without changes

in quantitation. For the determination of the method's robustness, five factors were selected from the analytical procedure to be examined in the robustness testing: pH and concentration of the buffer, volume and concentration of the dye, and reaction time. Results are shown in Table 4. It was found that none of these variables had a significant effect on the determination of investigated drugs. This provides an indication of the reliability of the proposed method during normal usage, so the developed spectrophotometric method is considered robust.

TABLE 5: Assay of tablets of investigated non-sedating antihistamines by the developed method and reported methods.

Authentic drug	Dosage form	% Recovery ^a ± SD		<i>t</i> -value ^b	<i>F</i> -value ^b
		Proposed method	Reported method		
CTZ	Epirizine tablets	99.97 ± 0.65	99.74 ± 1.45*	0.36	4.94
EBS	Evastine tablets	98.24 ± 1.12	98.07 ± 1.49**	0.22	1.77
FXD	Allerfen tablets	99.39 ± 0.90	99.25 ± 1.38*	0.22	2.36
KET	Zaditen tablets	98.52 ± 1.07	98.18 ± 1.07***	0.55	1.00
LOR	Mosedine tablets	100.31 ± 1.25	99.20 ± 0.97*	1.72	1.66

^a Average of six determinations ± standard deviation.

^b Theoretical values at 95% confidence limit; *t* = 2.228, *F* = 5.053.

* Reference [22].

** Reference [24].

*** Reference [21].

TABLE 6: Assay of tablets by standard addition method.

Authentic drug	Dosage form	Claimed taken (μg/mL)	Authentic added (μg/mL)	Found concentration (μg/mL)	% Recovery ± SD*	C.V
CTZ	Epirizine tablets	2	2	1.996	99.82 ± 1.18	1.18
			3	2.995	99.83 ± 1.09	1.09
			4	3.999	99.97 ± 0.65	0.65
EBS	Evastine tablets	2	2	1.965	98.24 ± 1.12	1.14
			3	2.996	99.87 ± 1.53	1.53
			4	3.977	99.42 ± 1.57	1.58
FXD	Allerfen tablets	2	2	2.005	100.23 ± 1.31	1.31
			3	3.004	100.14 ± 1.18	1.18
			4	3.976	99.39 ± 0.90	0.90
KET	Zaditen tablets	2	2	1.967	98.36 ± 1.41	1.44
			3	2.956	98.52 ± 1.07	1.09
			4	3.988	99.69 ± 1.44	1.45
LOR	Mosedine tablets	2	2	2.026	101.28 ± 1.65	1.63
			3	3.009	100.31 ± 1.25	1.25
			4	4.038	100.96 ± 1.12	1.11

* Average of six replicates.

6. Application of the Developed Method to Pharmaceutical Preparations

Different commercial dosage forms of the studied drugs were successfully analyzed by the developed method, and results were compared with those obtained by reported methods [21, 22, 24] as shown in Table 5. It was observed that there was no significant difference between results obtained by the developed method and the reported methods as indicated by *t*- and *F*-tests. Additionally, recovery experiments were carried out for the studied drugs in their respective pharmaceutical formulations by standard addition method. The results in Table 6 indicate that the developed method is convenient for all investigated drugs with good recoveries, and there is no interference from either the coadministered drugs or frequently encountered excipients. The proposed method is sensitive, accurate, and precise. It is suitable for the determination of the studied drugs in their dosage forms and application in quality control laboratories.

7. Conclusion

A non-extractive spectrophotometric method was developed for the determination of some non-sedating antihistamines (cetirizine, ebastine, fexofenadine, ketotifen and loratadine) based on ion-pair complex formation with a simple and commercially available reagent (erythrosine B), which most ordinary analytical laboratories can afford. The method is sufficiently sensitive to permit determinations of as low as 1.0 μg/mL. Unlike GC and HPLC procedures, the spectrophotometer is relatively simple to handle and affordable. The proposed method is simple, precise, accurate, and convenient. Hence, the proposed method should be useful for routine quality control purposes.

Disclosure

All the authors of the paper do not have a direct financial relation with the commercial identity mentioned in the paper.

Conflict of Interests

All the authors declare that there is no conflict of interests in their submitted paper.

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