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ORIGINAL PAPER

Validated Spectroflurimetric Determination of Some H_1 Receptor Antagonist Drugs in Pharmaceutical Preparations Through Charge Transfer Complexation

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Abstract A validated simple, rapid, and selective spectrofluorimetric method was developed for the determination of some antihistaminic H_1 receptor antagonist drugs namely ebastine (EBS), cetirizine dihydrochloride (CTZ), and fexofenadine hydrochloride (FXD). The method is based on the reaction of the cited drugs with some Π acceptors namely p-chloranilic acid (CLA), tetracyanoethylene (TCNE), and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) to give highly fluorescent derivatives. The fluorescence intensity—concentration plots were rectilinear over the concentration ranges of 0.2–3.0, 0.2–2.5 and 0.15–2.0 μg/ml for EBS with CLA, DDQ, and TCNE respectively; 0.5–7.0, 0.5–6.0, and 0.2–4.0 μg/ml for CTZ with the previously mentioned reagents, and 0.2–3.5, 0.5–6.0, and 0.2–3.5 μg/ml for FXD. The factors affecting the formation of the reaction products were carefully studied and optimized. The method was applied for the determination of the studied drugs in their dosage forms. The results obtained were in good agreement with those obtained by the comparison methods. Reactions Stoichiometries of the complexes formed between the studied drugs and Π acceptors were defined by the Job's method of the continuous variation and found in 1:1 in all cases.

Keywords Ebastine · Cetirizine · Fexofenadine · Charge transfer · Spectrofluorimetry

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Introduction

Ebastine; (4′-*tert*.-butyl-4-[4-(diphenylmethoxy)- piperidino] butyrophenone (Fig. 1), cetirizine dihydrochloride; (±)-[2-[4-[(4-chlorophenyl)phenylmethyl]-1- piperazinyl]ethoxy]acetic acid (Fig. 2), and Fexofenadine hydrochloride; α, α -dimethyl-4-[1-hydroxy-4-[4-(hydroxydiphenyl-methyl)-1 piperidinyl]butyl]-benzene acetic acid (Fig. 3) are potent long acting antihistaminic H_1 receptor antagonist drugs [1].

Literature survey reveals several methods for the determination of FXD in pharmaceutical preparations and biological fluids including: spectrophotometry [2–4], spectrofluorimetry [4], and High Performance Liquid Chromatography (HPLC) [5–9]. Several analytical methods were reported for the determination of CTZ, either in pure form or pharmaceutical preparations and biological fluids. These methods included spectrophotometry [3, 4, 10], spectrofluorimetry [4], High Performance Liquid Chromatography (HPLC) [5, 10–13], and capillary zone electrophoresis [14]. High performance liquid chromatography was used for the estimation of ebastine either alone in its dosage forms [15–17] or in presence of its metabolites [18, 19].

Although chromatographic methods offer a high degree of specificity, yet they require large amount of high purity organic solvents and generate high amount of waste. Therefore, there is a need for an alternative substitute to these techniques for the routine quality control analysis of the concerned drugs. Spectrofluorimetry has many advantages over HPLC methods such as: rapidity (measurements of fluorescence are nearly instantaneous), good analytical selectivity, higher capacity against blank interference and it can also improve the limit of detection when compared with spectrophotometric methods.

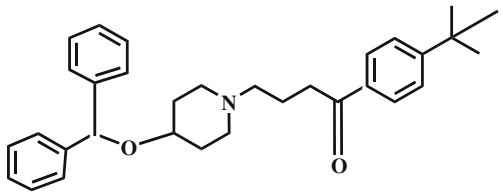


Fig. 1 Structural formula of ebastine

In spite that numerous methods were reported in the literature concerning the use of charge transfer complex agents for the spectrophotometric determination of many pharmaceutical compounds [20–27], few reports were recorded for their use as fluorigenic agents for the determination of drugs as exemplified by the determination of some quinolones [28, 29].

Since few spectrofluorimetric methods were applied for the determination of the studied drugs [4], we tried to introduce some simple spectrofluorimetric methods in our laboratory to simplify the analysis of the concerned drugs in quality control processes [30, 31]. Our interest to develop new methods for the assay of H_1 receptor antagonist drugs is a result of their high consumption in the local market in our community for treating various symptoms of allergy [1] caused by the remarkable air pollution in our country, which is now being under consideration by the government.

In this work, the authors continue their effort to present a new simple fluorimetric method to assay the concerned antihistaminic drugs depending on the few spectrofluorimetric reports mentioned about such drugs in the literature and on the unpopular use of Π acceptors as fluorigenic agents. TCNE, CLA, and DDQ were selected for this study to present Π acceptors carrying diverse functional groups responsible for the electron deficiency; (cyano groups in TCNE, chloride ions for CLA, and a combination of chloride and cyano groups in DDQ). CLA was used only once for the fluorimetric determination of fluoroquinolones [29], while TCNE and DDQ were not used before for fluorimetric assay of drugs, which encouraged us to use these reagents to extend their application beyond the spectrophotometric measurements. In addition; their availability and relatively affordable prices helped us to carry out this work.

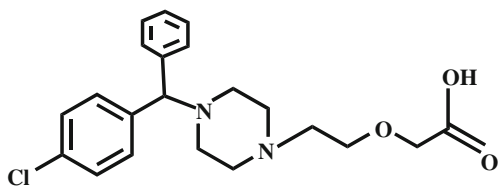


Fig. 2 Structural formula of cetirizine

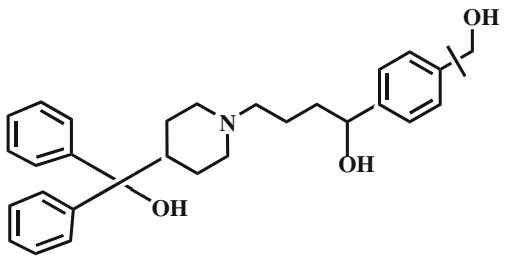


Fig. 3 Structural formula of fexofenadine

Experimental Procedures

Apparatus

The fluorescence spectra and measurements were recorded using a Perkin Elmer LS 45 Luminescence Spectrometer equipped with a 150 W Xenon arc lamp. A 1 cm quartz cell was used.

Materials and Reagents

All reagents and solvents were of Analytical Reagent grade.

a) Ebastine (EBS); of purity 99.94% was kindly provided by Meivo Pharmaceutical Company, Cairo, Egypt.
b) Cetirizine dihydrochloride (CTZ); of purity 99.87% was kindly provided by Pharco Pharmaceutical Company, Alexandria, Egypt.
c) Fexofenadine hydrochloride (FXD); of purity 99.68%; was kindly provided by El-Obour Modern Pharmaceutical Industries Company, Cairo, Egypt.
d) Pharmaceutical preparations:

*Bastab® tablets (BN#112038), labeled to contain 20 mg ebastine/tablet, Meivo Pharmaceutical Company, Cairo, Egypt.
*Evastine® syrup (BN# 94634), labeled to contain 5 mg ebastine 5 cm^{-3}, Marcyrl Pharmaceutical Industries, El Obour City, Egypt
* Zyrtec® oral solution (Batch # 092639A), labeled to contain 1 mg/ml Cetirizine dihydrochloride, and Zyrtec® oral drops (Batch # 093858A), labeled to contain 10 mg/ml Cetirizine dihydrochloride, Glaxomithkline, El-Salam city, Cairo, Egypt-under license from UCB, Belgium.
*Cetrak® syrup (Batch # 145), labeled to contain 5 mg/5 ml Cetirizine dihydrochloride, and Cetrak® tablet (Batch # 149), labeled to contain 10 mg Cetirizine dihydrochloride/tablet, Pharco Pharmaceuticals, Alexandria, Egypt.
*Clearest® capsules (Batch # 90380A), labeled to contain 5 mg Cetirizine dihydrochloride and 120 mg

pseudoephedrine hydrochloride/capsule, Chemi-Pharm Pharmaceutical Industries, S.A.E. 6th Ocober, Egypt.
*Allercet cold® capsules (Batch # 820834), labeled to contain 10 mg Cetirizine dihydrochloride, 30 mg pseudoephedrine hydrochloride and 400 mg paracetamol/capsule, Global Napi Pharmaceuticals, 6th October city, Giza, Egypt.
*Fastofen® tablets (Batch # 7065), labeled to contain 60 mg fexofenadine hydrochloride/tablet, El-Obour Modern Pharmaceutical Industries Company, Cairo, Egypt.
*Fastofen® tablets (Batch # 109108), labeled to contain 120 mg fexofenadine hydrochloride/tablet, El-Obour Modern Pharmaceutical Industries Company, Cairo, Egypt.
*Fexodine® capsules (Batch # 308134), labeled to contain 180 mg fexofenadine hydrochloride/capsule, Memphis Company for Pharmaceutical and Chemical Industries, Cairo, Egypt.

e. CLA, DDQ, and TCNE (Aldrich); were all prepared as 3×10^{-3} M solutions in acetone
f. Methanol and acetone (Aldrich)

All were obtained from commercial sources in the local market.

Standard Solutions

Stock solutions were prepared by dissolving 100.0 mg of the studied drugs in methanol and further diluted with the same solvent as appropriate. The standard solutions were stable for 7 days when kept in refrigerator.

General Procedure

Procedure for Construction of the Calibration Curve

Aliquots of standard solutions covering the working concentration ranges of the studied drugs (Table 1) were transferred into a series of 10 ml volumetric flasks; the specified volume (Table 1) of CLA, DDQ, or TCNE were added to react with the concerned drugs. The volume was completed to the mark with acetone except for the reaction between EBS and TCNE where methanol was used, and then the solutions were mixed well. The fluorescence intensity of the resulting solutions was measured at $\lambda_{em}/\lambda_{ex}$ mentioned in Table 1—against a blank experiment. The corrected relative fluorescence intensity was plotted *versus* the final concentration of the drug (μg/ml) to get the calibration graphs; alternatively, the corresponding regression equations were derived.

Procedure for Tablets and Capsules

Twenty tablets were weighed and pulverized. An accurately weighed quantity of the powdered tablets or the mixed capsular content equivalent to 100.0 mg of the studied drug was transferred into a small conical flask, extracted with methanol on three successive times each with 30 ml (3×10). The extract was filtered into 100 ml volumetric flask. The conical flask was washed with few mls of the solvent and the washings were passed into the same volumetric flask and the volume was completed with the same solvent. Aliquots covering the working concentration range were transferred into 10 ml volumetric flasks and the steps mentioned under "procedure for construction of calibration curve" were followed. The nominal content of the tablets or capsules was determined either from the corresponding calibration graphs or regression equations.

Procedure for Syrup, Oral Solution and Oral Drops

Aliquot volumes equivalent to 100 mg of the studied drug were quantitatively transferred into a 100 ml volumetric flask, serial dilution was performed with methanol to obtain the working concentration range and the steps described under "procedure for construction of calibration curve" were followed. The nominal content of the dosage form was determined either from the corresponding calibration graphs or regression equations.

Table 1 Experimental parameters for charge transfer reaction

Parameter	EBS			CTZ			FXD		
	CLA	DDQ	TCNE	CLA	DDQ	TCNE	CLA	DDQ	TCNE
Volume of ∏ acceptor (ml)	1.5	1.5	1.5	1	2	2	1.5	1	1
Concentration range (μg/ml)	0.2–3.0	0.2–2.5	0.15–2.0	0.5–7.0	0.5–6.0	0.2–4.0	0.2–3.5	0.5–6.0	0.2–3.5
Diluting solvent	acetone		methanol			acetone			
Working temperature	Room temperature								
$\lambda_{em}/\lambda_{ex}$	453/387	340/244	434/384	432/363	433/395	446/385		434/390	

Results and Discussion

The charge transfer complexes were formed by the interaction of the investigated drugs as electron donor and CLA, DDQ and TCNE reagents as electron acceptors (Fig. 4a–c). The spectrofluorimetric properties of the formed complexes as well as the optimal conditions for the assay procedure were extensively studied. The reaction was studied as a function of the volume of reagents, nature of solvent, reaction time, temperature and stoichiometric ratio between the drugs and the reagents.

Optimization of the Reaction Conditions

The spectrofluorimetric properties of the formed complexes as well as the different experimental parameters affecting development and stability of the reaction products were carefully studied and optimized. Such factors were changed individually while the others were kept constant.

Effect of Volume of Π Acceptors

Keeping all the variables constant, and using 3×10^{-3} M of the reagents it was found that increasing the volume of CLA resulted in a gradual increase in the relative fluorescence intensity of the complexes up to 0.5 ml in case of CTZ and 1 ml in case of EBS and FXD, after which it remained constant, therefore, 1±0.5 or 1.5±0.5 ml were chosen respectively. 1.5±0.5 ml of DDQ was used with EBS, while 2±0.5 ml and 1±0.5 ml were used with CTZ and FXD respectively. On the other hand, 1.5±0.5 ml, 2± 0.5 ml, and1±0.5 ml of TCNE were found to be sufficient for the reaction with EBS, CTZ and FXD respectively.

Effect of Different Diluting Solvents

Different diluting solvents were tested to choose the most suitable one for the complex formation, the investigated solvents included: methanol, acetonitrile, dimethylsulfoxide, dimethylformamide, and acetone. The highest fluorescence intensities in all of the reactions were achieved upon using acetone except for the reaction between EBS and TCNE where methanol was the solvent of choice. The results are abridged in Table 2.

Effect of Temperature

The reactions were carried out at different temperature settings (room temperature, 40, 60, 80, 100 °C) using a

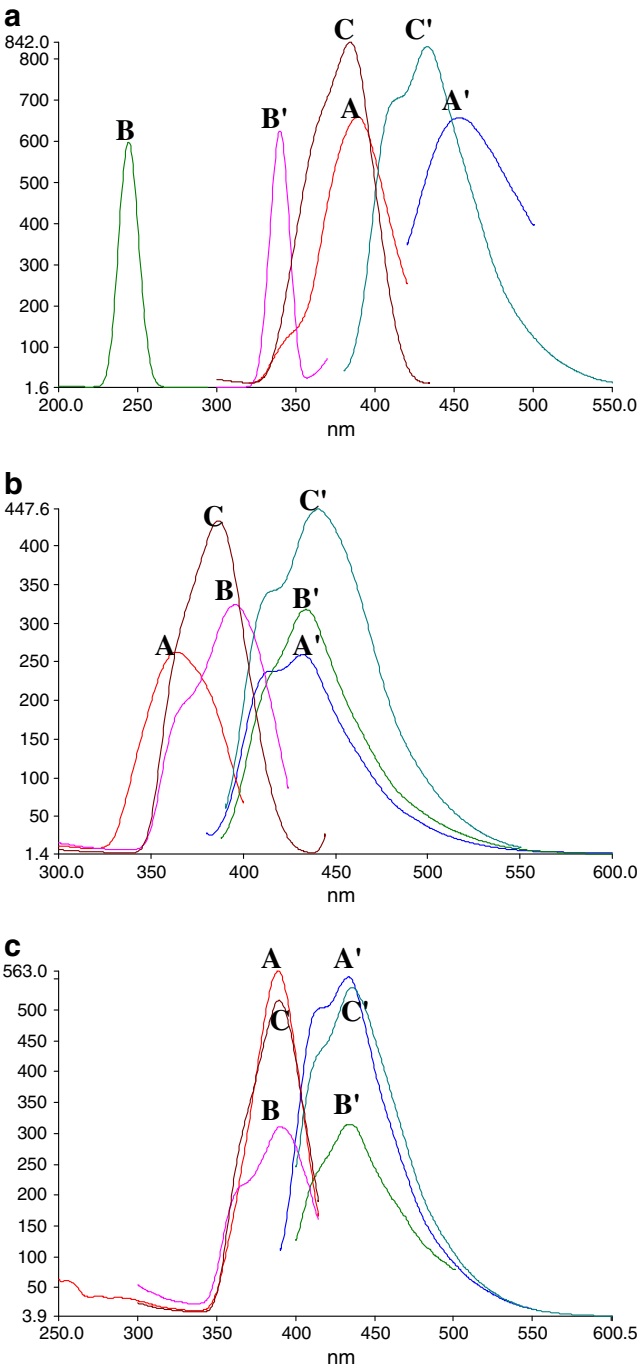


Fig. 4 **a**. Fluorescence spectra of: A and A′ are the excitation and emission spectra respectively of the formed EBS-CLA charge transfer complex. B and B′ are the excitation and emission spectra respectively of the formed EBS-DDQ charge transfer complex. C and C′ are the excitation and emission spectra respectively of the formed EBS-TCNE charge transfer complex. **b**. Fluorescence spectra of: A and A′ are the excitation and emission spectra respectively of the formed CTZ-CLA charge transfer complex. B and B′ are the excitation and emission spectra respectively of the formed CTZ-DDQ charge transfer complex. C and C′ are the excitation and emission spectra respectively of the formed CTZ-TCNE charge transfer complex. **c**. Fluorescence spectra of: A and A′ are the excitation and emission spectra respectively of the formed FXD-CLA charge transfer complex. B and B′ are the excitation and emission spectra respectively of the formed FXD-DDQ charge transfer complex. C and C′ are the excitation and emission spectra respectively of the formed FXD-TCNE charge transfer complex

Table 2 Effect of diluting solvents on the RFI of the formed charge transfer complexes

Solvent	EBS			CTZ			FXD		
	CLA	DDQ	TCNE	CLA	DDQ	TCNE	CLA	DDQ	TCNE
Acetone	645	630	706	240	300	430	520	300	500
Methanol	520	562	800	150	280	381	443	264	483
Dimethylsulfoxide	310	426	658	90	160	210	284	132	346
Dimethylformamide	214	497	592	83	175	260	361	146	354
Acetonitrile	442	534	685	120	243	340	480	248	470

thermostatically controlled water bath. Maximum relative fluorescence values were obtained at room temperature. The results are shown in Fig. 5a–c.

Effect of Time on the Formation and Stability of the Complexes

The formation and stability of the formed complexes was also studied by measuring the RFI readings every 10 min interval, the consistency of the measured values indicated that charge transfer complexes were formed instantaneously and remained stable for at least 90 min. (Fig. 6a–c).

Analytical Performance and Application

Using the above spectrofluorimetric method, linear regression equations were obtained. The relative fluorescence—concentration plots were found to be linear over the ranges mentioned in Table 3.

The applications of the proposed method were extensively studied to cover many aspects including sensitivity, validation, pharmaceutical applications, interferences and reactions mechanisms. Such throughout study adds advantages to the proposed method and make it suitable for quality control laboratories.

These parameters could be fully discussed as follows:

Sensitivity

Detection limit (LOD) is the lowest concentration of the drug that can be detected, but not necessarily quantitated, under the stated experimental conditions. The limit of detection is generally quoted as the concentration yielding a signal-to-noise ratio of 3:1[32] and is confirmed by analyzing a number of samples near this value using the following equation:

$$\text{The signal to noise ratio} = H/h$$

Where

H height of the spectrum corresponding to the drug
h absolute value of the largest noise fluctuation from the baseline of the spectrum of a blank solution.

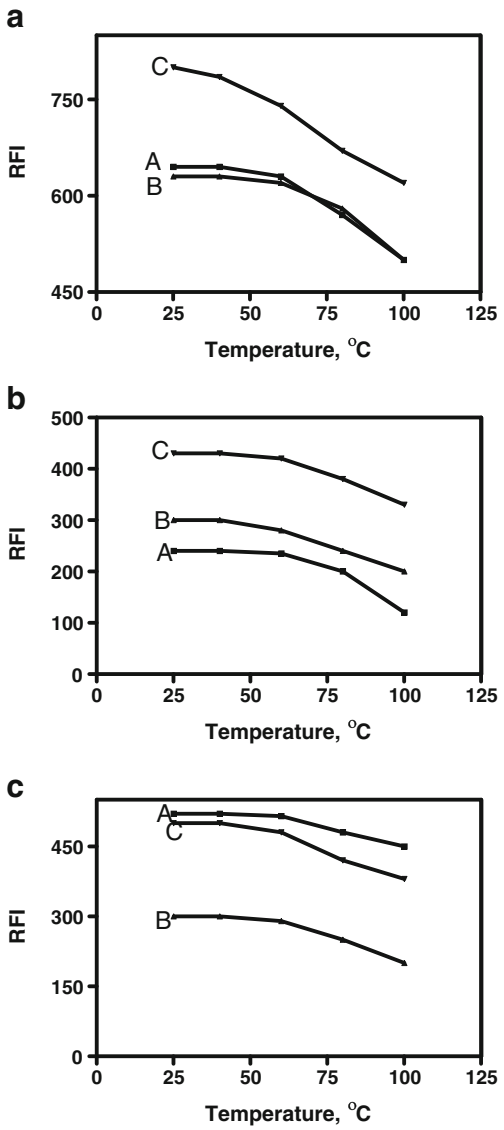


Fig. 5 **a**. Effect of different temperature settings on the stability of the formed charge transfer complex of EBS (2.0 μg/ml) where: A: CLA, B: DDQ, C: TCNE. **b**. Effect of different temperature settings on the stability of the formed charge transfer complex of CTZ (2.0 μg/ml) where: A: CLA, B: DDQ, C: TCNE. **c**. Effect of different temperature settings on the stability of the formed charge transfer complex of FXD (2.0 μg/ml) where: A: CLA, B: DDQ, C: TCNE

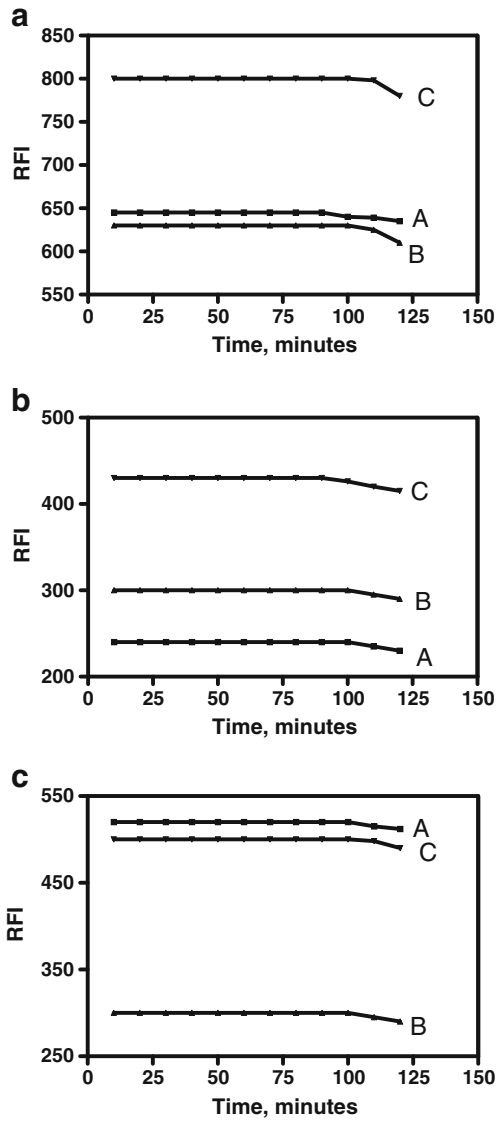


Fig. 6 **a**. Effect of time on the formation and stability of the formed charge transfer complex of EBS (2.0 μg/ml) where: A: CLA, B: DDQ, C: TCNE. **b**. Effect of time on the formation and stability of the formed charge transfer complex of CTZ (2.0 μg/ml) where: A: CLA, B: DDQ, C: TCNE. **c**. Effect of time on the formation and stability of the formed charge transfer complex of FXD (2.0 μg/ml) where: A: CLA, B: DDQ, C: TCNE

While the limit of quantification (LOQ); is the lowest concentration of the analyte that can be determined with acceptable precision and accuracy. It is quoted as the concentration yielding a signal-to-noise ratio of 10: 1 and is confirmed by analyzing a number of samples near this value [32].The obtained values of concentration ranges, LOD, and LOQ listed in Table 3 are comparable with those values of the reported spectrofluorimetric method of the concerned drugs [4], and also with those in the report dealing with the use of Π acceptors as fluorigenic agent for drug determination [29].

Validation of the Method

The method was tested for linearity, selectivity, accuracy and precision. Using the above spectrofluorimetric method, linear regression equations were obtained. The regression plots showed that there was a linear dependence of the fluorescence intensity value on the concentration of the drug over the ranges cited in Table 3. The validity of the proposed method was evaluated by statistical analysis of the regression data regarding the standard deviation of the residual ($S_{y/x}$), the standard deviation of the intercept (S_a), and standard deviation of the slope (S_b) [33]. The results are shown in Table 3. The small values of the figures point to the low scattering of the points around the calibration graph and high precision of the proposed method.

Accuracy

The accuracy of the proposed method was evaluated by analyzing standard solutions of the drugs under investigation. The results obtained by the proposed method were favorably compared with those obtained by the comparison methods [4, 16]. Statistical analysis [33] of the results obtained by the proposed and comparison methods using student's *t*-test and variance ratio F- test, showed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively (Table 4).

Precision

Repeatability

The repeatability was evaluated through analysis of different concentrations of the studied drugs in pure or in dosage forms on 3 successive times. The mean percentage recoveries listed in Table 5 indicate the high precision of the proposed method.

Intermediate Precision

It was performed through repeated analysis of variable concentrations of the drugs either *per se* or in dosage forms on three successive days. The results are abridged in Table 5.

Robustness of the Method

The robustness of the method adopted was demonstrated by the consistency of the relative fluorescence values with the deliberately minor changes in the experimental parameters such as volumes of the reagents used which did not greatly affect the fluorescence intensities of the formed complexes.

Table 3 Performance data of the proposed method

Parameter	EBS			CTZ			FXD		
	CLA	DDQ	TCNE	CLA	DDQ	TCNE	CLA	DDQ	TCNE
Concentration range (μg/ml)	0.2–3.0	0.2–2.5	0.15–2.0	0.5–7.0	0.5–6.0	0.2–4.0	0.2–3.5	0.5–6.0	0.2–3.5
LOD (μg/ml)	0.11	0.16	0.11	0.26	0.33	0.09	0.14	0.29	0.11
LOQ (μg/ml)	0.15	0.23	0.14	0.45	0.48	0.17	0.21	0.45	0.23
Correlation coefficient (r)	0.9999	0.9998	0.9996	0.9997	0.9998	0.9999	0.9999	0.9999	0.9998
Slope	324	315	401	120	150	215	258	150	250
Intercept	1.308	2.726	3.454	1.627	2.663	2.284	3.916	3.369	2.887
$S_{y/x}$,S.D of the residuals	2.033	3.719	5.420	5.166	4.076	3.451	3.419	4.718	4.326
S_a,S.D. of the intercept of the regression line	0.158	0.264	−0.199	0.186	0.132	0.369	0.228	0.156	0.107
S_b,S.D. of the slope of the regression line	0.767	1.721	3.171	0.831	0.779	0.925	1.09	0.937	1.381

Pharmaceutical Applications

The proposed method was successfully applied to the determination of the concerned drugs in their dosage forms. The results are summarized in Table 6. No interference from the sample matrix was observed, the results were found to be in good agreement with the labeled amount.

Interferences

Drugs which are co-formulated with CTZ; (pseudoephedrine hydrochloride and paracetamol), and others that are frequently co- administered with the investigated drugs such as ketoconazole and erythromycin were carefully tested using the proposed method. All of the studied compounds did not form any fluorescent products upon reaction with the concerned Π acceptors, and hence, showed no interference.

Mechanism of the Reaction

A charge-transfer complex or electron-donor-acceptor complex is an association of two or more molecules, or of different parts of one very large molecule, in which a fraction of electronic charge is transferred between the molecular entities [34]. The resulting electrostatic attraction provides a stabilizing force for the molecular complex. The nature of the attraction in a charge-transfer complex is not a stable chemical bond, and is much weaker than covalent forces. The attraction is created by an electronic transition into an excited electronic state, and is best characterized as a weak electron resonance. The excitation energy of this resonance occurs very frequently in the visible region of the electro-magnetic spectrum, which produces the usually intense color characteristic for these complexes [34]. These optical absorption bands are often referred to as charge-transfer bands. Optical spectroscopy is a powerful technique to characterize charge-transfer bands.

The charge-transfer association occurs in a chemical equilibrium with the independent donor (D) and acceptor (A) molecules:

$$\mathbf{D + A} \rightleftharpoons \mathbf{DA}$$

The intensity of charge-transfer bands in the absorbance spectrum is strongly dependent upon the degree (equilibrium constant) of this association reaction. Methods have been developed to determine the equilibrium constant for these complexes in solution by measuring the intensity of absorption bands as a function of the concentration of donor and acceptor components in solution.

Based on these facts, and by analogy to a previous report [35], the charge transfer complexes of concern are postulated o be formed through the electron rich groups of the studied drugs as electron donor and the electron-acceptor reagents (CLA, DDQ, and TCNE). The structure of the complexes formed between the drugs under study and the different reagents is shown in Schemes 1, 2 and 3.

The stoichiometry of the reaction between the studied drugs and the electron acceptors was studied using Job's continuous variation method [36] where all the plots (Fig. 7a–c) reached a maximum value at a mole fraction of 0.5, which indicated the formation of a 1:1 complex.

The formation constant of the reaction product was calculated according to the following equation [37]:

$$K_f = \frac{F/F_m}{\left[(1 - F/F_m)^{n+1}\right] c^n n^n}$$

where F and F_m are the observed maximum relative fluorescence and the relative fluorescence obtained from

Table 4 Determination of the studied drugs in pure form using the proposed method

Studied Drug	Amount taken, μg/ml			Amount found, μg/ml			% Recovery			Comparison method [16]
EBS	CLA	DDQ	TCNE	CLA	DDQ	TCNE	CLA	DDQ	TCNE	
	0.2	0.2	0.15	0.199	0.198	0.149	99.72	99.12	99.12	100.62
	0.5	0.5	0.2	0.498	0.496	0.199	99.64	99.24	99.46	99.15
	0.7	1.0	0.5	0.702	1.001	0.501	100.26	100.13	100.24	99.34
	1.0	1.2	0.7	1.003	1.203	0.701	100.33	100.22	100.19	
	1.5	1.5	1.0	1.514	1.496	1.003	100.92	99.72	100.34	
	2	2.0	1.2	1.994	2.002	1.197	99.72	100.11	99.77	
	2.5	2.2	1.5	2.491	2.204	1.497	99.62	100.19	99.83	
	3.0	2.5	2.0	2.983	2.506	1.984	99.43	100.24	99.21	
$\overline{X} \pm SD$							99.96±0.51	99.87±0.46	99.77±0.47	99.71±0.79
t test							0.56[a]	0.59	0.29	
F test							2.39[a]	1.79	2.83	Comparison method [4]
CTZ	CLA	DDQ	TCNE	CLA	DDQ	TCNE	CLA	DDQ	TCNE	
	0.5	0.5	0.2	0.496	0.496	0.198	99.11	99.17	99.16	100.32
	1.0	1.0	0.5	0.997	0.992	0.497	99.72	99.22	99.33	99.51
	2.0	1.5	1.0	2.007	1.511	0.997	100.34	100.67	99.72	99.31
	3.0	2.0	2.0	3.005	2.009	2.005	100.17	100.44	100.24	
	4.0	3.0	2.5	4.002	3.006	2.516	100.55	100.19	100.62	
	5.0	4.0	3.0	4.977	4.009	3.023	99.54	100.24	100.76	
	6.0	5.0	3.5	5.989	4.968	3.498	99.82	99.36	99.93	
	7.0	6.0	4.0	6.997	5.984	4.014	99.90	99.74	100.34	
$\overline{X} \pm SD$							99.89±0.46	99.88±0.58	100.01±0.58	99.71±0.53
t test							0.31	0.58	0.14	
F test							1.33	1.19	1.19	Comparison method [4]
FXD	CLA	DDQ	TCNE	CLA	DDQ	TCNE	CLA	DDQ	TCNE	
	0.2	0.5	0.2	0.199	0.497	0.198	99.28	99.39	99.11	100.25
	0.5	1.0	0.5	0.499	1.002	0.499	99.87	100.29	99.76	100.12
	1.0	2.0	1.0	1.008	2.012	1.007	100.78	100.58	100.74	100.99
	1.5	3.0	1.5	1.515	3.018	1.514	100.97	100.61	100.93	
	2.0	3.5	2.0	2.013	3.522	2.011	100.65	100.62	100.54	
	2.5	4.0	2.5	2.489	3.994	2.496	99.55	99.84	99.83	
	3.0	5.0	3.0	3.019	4.987	2.974	100.65	99.73	99.14	
	3.5	6.0	3.5	3.512	6.053	3.524	100.34	100.89	100.69	
$\overline{X} \pm SD$							100.26±0.62	100.24±0.53	100.09±0.73	100.40±0.47
t test							0.11	0.19	0.61	
F test							1.74	1.27	2.41	

[a] 1.89 and 19.35 are the tabulated t and F values at $p=0.05$ [33]

Table 5 Validation of the studied drugs in pure and dosage forms using the proposed method

Preparation	Repeatability,% found			Intermediate precision,% found		
	CLA (0.2 μg/ml EBS)	DDQ (0.2 μg/ml EBS)	TCNE (0.15 μg/ml EBS)	CLA (2.0 μg/ml EBS)	DDQ (0.5 μg/ml EBS)	TCNE (1.5 μg/ml EBS)
EBS pure form	100.25	99.94	100.17	99.14	100.48	99.64
	100.68	99.57	99.84	99.81	100.99	99.41
	100.07	100.48	99.78	100.72	99.42	100.18
$\overline{X} \pm SD$	100.33±0.31	99.99±0.46	99.93±0.21	99.89±0.79	100.29±0.81	99.74±0.39
Bastab® tablets	CLA (1.0 μg/ml EBS)	DDQ (1.0 μg/ml EBS)	TCNE (1.0 μg/ml EBS)	CLA (2.5 μg/ml EBS)	DDQ (1.5 μg/ml EBS)	TCNE (0.5 μg/ml EBS)
	100.22	100.45	100.11	99.51	100.16	99.51
	100.68	100.37	100.58	100.81	99.23	99.72
	99.85	100.68	100.34	100.26	99.72	100.16
$\overline{X} \pm SD$	100.25±0.42	100.50±0.16	100.34±0.23	100.19±0.65	99.70±0.47	99.79±0.33
Evastine® syrup	CLA (1.5 μg/ml EBS)	DDQ (2.0 μg/ml EBS)	TCNE (0.2 μg/ml EBS)	CLA (3.0 μg/ml EBS)	DDQ (2.5 μg/ml EBS)	TCNE (2.0 μg/ml EBS)
	100.15	100.11	100.84	100.55	100.82	100.61
	100.54	100.38	100.76	100.06	100.03	100.05
	100.34	100.46	100.66	99.51	99.42	99.24
$\overline{X} \pm SD$	100.34±0.19	100.32±0.18	100.75±0.09	100.04±0.52	100.09±0.71	99.97±0.69
CTZ pure form	CLA (0.5 μg/ml CTZ)	DDQ (3.5 μg/ml CTZ)	TCNE (0.5 μg/ml CTZ)	CLA (1.0 μg/ml CTZ)	DDQ (5.0 μg/ml CTZ)	TCNE (1.5 μg/ml CTZ)
	100.25	99.61	100.78	99.75	99.61	100.34
	100.55	99.51	100.16	100.93	99.94	100.78
	100.64	99.13	100.61	100.46	100.76	99.34
$\overline{X} \pm SD$	100.48±0.21	99.42±0.25	100.52±0.32	100.38±0.59	100.10±0.59	100.15±0.74
Zyrtec®oral solution	CLA (1.5 μg/ml CTZ)	DDQ (3.0 μg/ml CTZ)	TCNE (1.0 μg/ml CTZ)	CLA (7.0 μg/ml CTZ)	DDQ (1.0 μg/ml CTZ)	TCNE (2.5 μg/ml CTZ)
	100.25	100.17	99.25	99.12	99.64	100.48
	100.68	100.67	99.61	99.34	99.15	100.67
	99.86	100.81	100.27	100.48	100.47	100.49
$\overline{X} \pm SD$	100.26±0.41	100.55±0.34	99.71±0.52	99.65±0.73	99.75±0.67	100.55±0.11
Zyrtec®oral drops	CLA (6.5 μg/ml CTZ)	DDQ (0.5 μg/ml CTZ)	TCNE (4.0 μg/ml CTZ)	CLA (2.0 μg/ml CTZ)	DDQ (4.0 μg/ml CTZ)	TCNE (3.5 μg/ml CTZ)
	100.16	99.56	99.32	99.12	99.32	100.68
	100.54	99.35	99.48	99.64	99.82	100.12
	100.89	100.15	99.64	100.84	100.64	99.55
$\overline{X} \pm SD$	100.53±0.37	99.69±0.41	99.48±0.16	99.87±0.88	99.93±0.67	100.12±0.57
Cetrak® syrup	CLA (3.0 μg/ml CTZ)	DDQ (4.5 μg/ml CTZ)	TCNE (1.5 μg/ml CTZ)	CLA (4.0 μg/ml CTZ)	DDQ (2.5 μg/ml CTZ)	TCNE (1.0 μg/ml CTZ)
	100.12	100.16	100.45	99.65	100.95	100.75
	100.66	100.67	100.68	99.12	100.12	99.32
	99.82	99.86	100.28	100.57	99.32	100.46
$\overline{X} \pm SD$	100.21±0.43	100.23±0.41	100.47±0.21	99.78±0.73	100.13±0.82	100.18±0.76
Cetrak® tablets	CLA (5.5 μg/ml CTZ)	DDQ (5.5 μg/ml CTZ)	TCNE (0.4 μg/ml CTZ)	CLA (2.5 μg/ml CTZ)	DDQ (1.5 μg/ml CTZ)	TCNE (3.0 μg/ml CTZ)

Table 5 (continued)

Preparation	Repeatability,% found			Intermediate precision,% found		
	CLA (0.2μg/ml EBS)	DDQ (0.2μg/ml EBS)	TCNE (0.15μg/ml EBS)	CLA (2.0μg/ml EBS)	DDQ (0.5μg/ml EBS)	TCNE (1.5μg/ml EBS)
	100.11	100.55	100.45	99.54	100.45	99.45
	99.32	99.61	100.56	99.45	100.32	100.97
	99.65	99.73	99.34	100.33	99.42	100.26
$\overline{X} \pm SD$	99.69±0.39	99.96±0.51	100.12±0.67	99.77±0.48	100.06±0.56	100.23±0.76
Clearest® capsules	CLA (3.5 μg/ml CTZ)	DDQ (6.0 μg/ml CTZ)	TCNE (2.5 μg/ml CTZ)	CLA (4.5 μg/ml CTZ)	DDQ (5.0 μg/ml CTZ)	TCNE (4.0 μg/ml CTZ)
	100.22	99.95	100.44	99.52	100.75	100.14
	100.45	100.46	100.36	99.47	100.12	100.99
	100.85	100.77	100.46	99.93	99.32	100.43
$\overline{X} \pm SD$	100.51±0.32	100.39±0.41	100.42±0.11	99.64±0.25	100.06±0.72	100.52±0.43
Allercet® capsules	CLA (5.0 μg/ml CTZ)	DDQ (2.0 μg/ml CTZ)	TCNE (0.2 μg/ml CTZ)	CLA (6.0 μg/ml CTZ)	DDQ (1.0 μg/ml CTZ)	TCNE (2.0 μg/ml CTZ)
	100.47	100.48	100.78	99.25	99.64	99.66
	100.86	100.88	100.86	100.67	100.98	100.37
	100.28	100.25	100.57	100.17	100.27	100.88
$\overline{X} \pm SD$	100.54±0.29	100.54±0.32	100.74±0.15	100.03±0.72	100.29±0.67	100.31±0.61
FXD pure form	CLA (0.2 μg/ml FXD)	DDQ (4.5 μg/ml FXD)	TCNE (1.5 μg/ml FXD)	CLA (2.0 μg/ml FXD)	DDQ (1.0 μg/ml FXD)	TCNE (2.5 μg/ml FXD)
	100.15	100.68	100.68	99.45	100.11	100.46
	100.65	100.44	100.48	99.37	100.97	99.15
	99.58	99.57	99.46	100.82	99.34	100.33
$\overline{X} \pm SD$	100.13±0.54	100.23±0.58	100.21±0.65	99.88±0.82	100.14±0.82	99.98±0.72
Fastofen® tablets (60 mg FXD/tablet)	CLA (2.5 μg/ml FXD)	DDQ (0.5 μg/ml FXD)	TCNE (3.0 μg/ml FXD)	CLA (3.0 μg/ml FXD)	DDQ (6.0 μg/ml FXD)	TCNE (2.0 μg/ml FXD)
	100.12	100.45	99.54	99.65	99.12	100.95
	100.86	100.66	99.12	100.45	100.26	100.23
	100.45	100.48	100.24	100.67	99.53	99.14
$\overline{X} \pm SD$	100.48±0.37	100.53±0.11	99.63±0.57	100.26±0.53	99.64±0.58	100.11±0.91
Fastofen® tablets (120 mg FXD/tablet)	CLA (0.5 μg/ml FXD)	DDQ (4.0 μg/ml FXD)	TCNE (1.0 μg/ml FXD)	CLA (1.5 μg/ml FXD)	DDQ (3.0 μg/ml FXD)	TCNE (0.5 μg/ml FXD)
	100.36	99.61	99.45	99.85	99.51	100.54
	99.65	100.14	99.61	99.16	99.83	100.29
	99.45	100.89	100.24	100.56	100.79	99.78
$\overline{X} \pm SD$	99.82±0.48	100.21±0.64	99.77±0.42	99.86±0.71	100.04±0.67	100.21±0.39
Fexodine® capsules	CLA (3.5 μg/ml FXD)	DDQ (2.0 μg/ml FXD)	TCNE (0.2 μg/ml FXD)	CLA (1.0 μg/ml FXD)	DDQ (5.0 μg/ml FXD)	TCNE (3.5 μg/ml FXD)
	99.65	100.25	100.12	100.45	100.45	99.14
	99.45	100.34	100.23	99.13	100.95	99.89
	100.32	99.77	99.62	99.72	99.13	100.88
$\overline{X} \pm SD$	99.81±0.46	100.12±0.31	99.99±0.33	99.77±0.67	100.18±0.94	99.97±0.87

Table 6 Determination of the studied drugs in their dosage forms using the proposed method

Dosage form	Amount taken, μg/ml			Amount found, μg/ml			% Recovery			Comparison method [16], % recovery
Bastab® tablets	CLA	DDQ	TCNE	CLA	DDQ	TCNE	CLA	DDQ	TCNE	
	0.2	0.2	0.15	0.198	0.198	0.151	99.23	99.21	100.24	99.23
	0.5	1.0	0.5	0.502	0.992	0.504	100.46	99.24	100.77	99.61
	1.0	1.5	1.0	1.006	1.509	1.006	100.62	100.66	100.61	100.61
	2.0	2.0	1.5	2.017	2.011	1.503	100.84	100.57	100.23	
	3.0	2.5	2.0	3.028	2.521	2.002	100.92	100.82	100.11	
$\overline{X} \pm SD$							100.42±0.69	100.11±0.81	100.39±0.29	99.82±0.71
t test							0.14[a]	0.79	0.02	
F test							1.06[a]	1.3	5.9	
Evastine® syrup	0.2	0.2	0.15	0.198	0.198	0.149	99.24	99.13	99.24	100.12
	0.5	1.0	0.5	0.499	1.006	0.496	99.78	100.62	99.11	99.61
	1.0	1.5	1.0	1.006	1.512	1.002	100.62	100.78	100.24	99.51
	2.0	2.0	1.5	1.985	2.002	1.506	99.27	100.12	100.39	
	3.0	2.5	2.0	2.998	2.491	2.009	99.94	99.64	100.44	
$\overline{X} \pm SD$							99.77±0.57	100.06±0.68	99.88±0.65	99.75±0.33
t test							0.46	0.64	0.36	
F test							2.9	4.2	3.9	
Zyrtec®oral solution	CLA	DDQ	TCNE	CLA	DDQ	TCNE	CLA	DDQ	TCNE	Comparison method [4], % recovery
	1.0	1.0	0.5	0.991	1.004	0.499	99.12	100.42	99.72	100.12
	2.0	2.0	1.0	2.009	1.997	1.005	100.44	99.87	100.46	99.51
	3.0	3.0	2.0	3.021	3.029	2.018	100.67	100.99	100.88	99.34
	5.0	5.0	3.0	5.036	5.028	3.028	100.72	100.55	100.92	
	7.0	6.0	4.0	7.009	6.037	4.006	100.14	100.62	100.14	
$\overline{X} \pm SD$							100.22±0.66	100.49±0.41	100.42±0.51	99.66±0.41
t test							0.15	0.07	0.09	
F test							2.6	1.1	1.5	
Zyrtec® oral drops	1.0	1.0	0.5	1.001	1.003	0.496	100.12	100.29	99.14	100.25
	2.0	2.0	1.0	2.004	2.003	1.003	100.19	100.14	100.33	99.24
	3.0	3.0	2.0	3.022	3.028	2.008	100.73	100.92	100.39	99.62
	5.0	5.0	3.0	4.982	5.049	3.011	99.63	100.98	100.34	
	7.0	6.0	4.0	7.027	6.013	4.014	100.39	100.22	100.36	
$\overline{X} \pm SD$							100.21±0.41	100.51±0.41	100.11±0.54	99.70±0.51
t test							0.42	0.09	0.11	
F test							1.5	1.5	1.1	

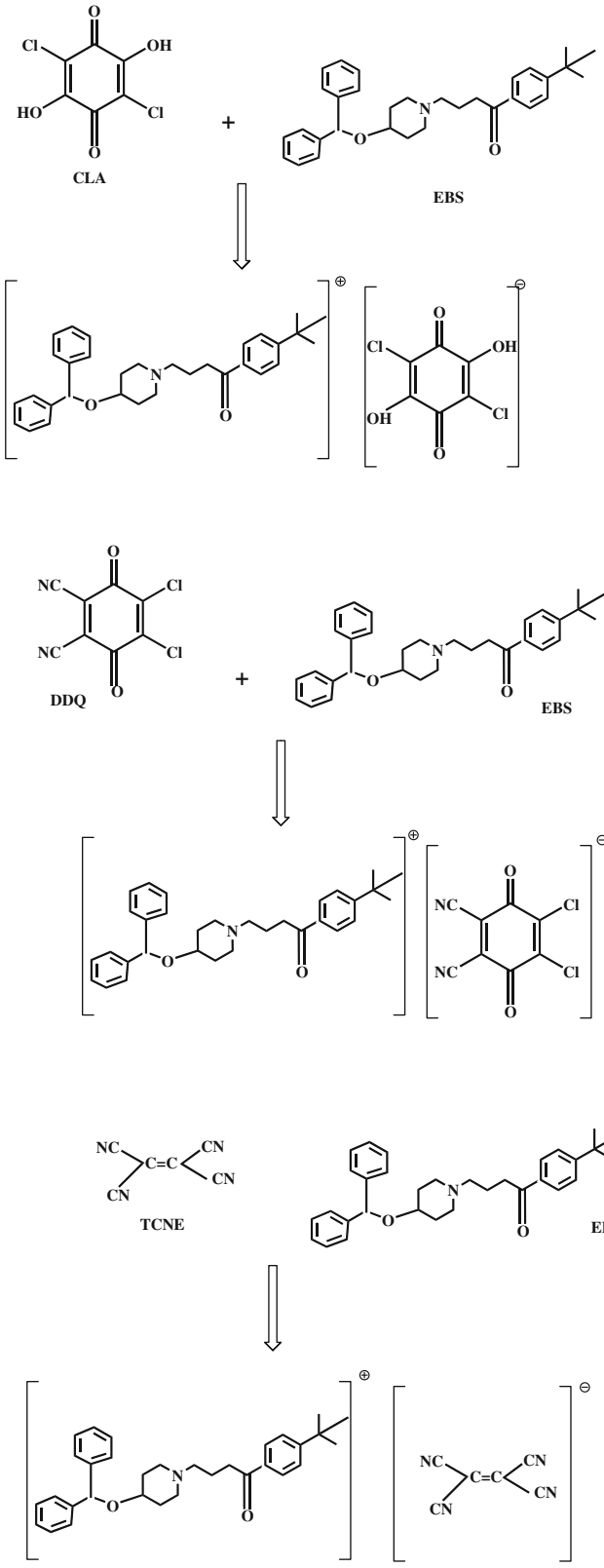
Table 6 (continued)

Dosage form	Amount taken, μg/ml			Amount found, μg/ml			% Recovery			Comparison method [4], % recovery
	CLA	DDQ	TCNE	CLA	DDQ	TCNE	CLA	DDQ	TCNE	
Cetrak® syrup	1.0	1.0	0.5	0.992	0.993	0.505	99.21	99.27	100.92	99.65
	2.0	2.0	1.0	1.996	1.993	1.006	99.78	99.63	100.63	100.21
	3.0	3.0	2.0	3.005	3.013	2.014	100.17	100.42	100.71	100.75
	5.0	5.0	3.0	5.041	5.039	3.013	100.82	100.79	100.42	
	7.0	6.0	4.0	7.065	6.026	4.009	100.93	100.43	100.23	
$\overline{X} \pm SD$							100.18±0.72	100.11±0.63	100.58±0.27	100.20±0.55
t test							0.24	0.23	0.01	
F test							1.7	1.3	4.1	
Clearest® capsules	1.0	1.0	0.5	0.995	0.991	0.497	99.48	99.12	99.32	100.32
	2.0	2.0	1.0	1.982	1.987	0.991	99.12	99.33	99.14	100.95
	3.0	3.0	2.0	3.008	3.014	2.002	100.25	100.47	100.11	99.51
	5.0	5.0	3.0	5.045	5.042	3.011	100.89	100.84	100.35	
	7.0	6.0	4.0	7.016	6.009	4.027	100.23	100.15	100.67	
$\overline{X} \pm SD$							99.99±0.69	99.98±0.74	99.92±0.66	100.26±0.72
t test							0.44	0.43	0.42	
F test							1.09	1.06	1.19	
Allercet® capsules	1.0	1.0	0.5	0.993	0.993	0.502	99.32	99.32	100.45	100.92
	2.0	2.0	1.0	1.991	2.009	1.002	99.54	100.45	100.23	99.65
	3.0	3.0	2.0	3.004	3.019	2.018	100.12	100.62	100.89	99.21
	5.0	5.0	3.0	5.011	5.039	2.987	100.22	100.78	99.57	
	7.0	6.0	4.0	7.025	6.039	3.996	100.35	100.65	99.91	
$\overline{X} \pm SD$							99.91±0.45	100.36±0.59	100.21±0.51	99.93±0.89
t test							0.48	0.09	0.85	
F test							3.9	2.3	3.1	
Cetrak® tablets	1.0	1.0	0.5	1.003	0.994	5.007	100.26	99.44	100.13	100.25
	2.0	2.0	1.0	2.008	1.995	0.997	100.39	99.74	99.73	99.65
	3.0	3.0	2.0	2.978	3.005	1.985	99.24	100.18	99.24	99.15
	5.0	5.0	3.0	4.959	5.041	3.019	99.19	100.82	100.62	
	7.0	6.0	4.0	7.017	6.038	4.037	100.24	100.63	100.92	
$\overline{X} \pm SD$							99.86±0.59	100.16±0.58	100.13±0.67	99.68±0.55
t test							0.55	0.23	0.37	
F test							1.2	1.1	1.5	
Fastofen® tablets (60 mg FXD/tablet)	0.5	1.0	0.5	0.497	0.991	0.501	99.48	99.14	100.24	100.25
	1.0	2.0	1.0	0.991	1.993	1.008	99.13	99.63	100.78	99.34

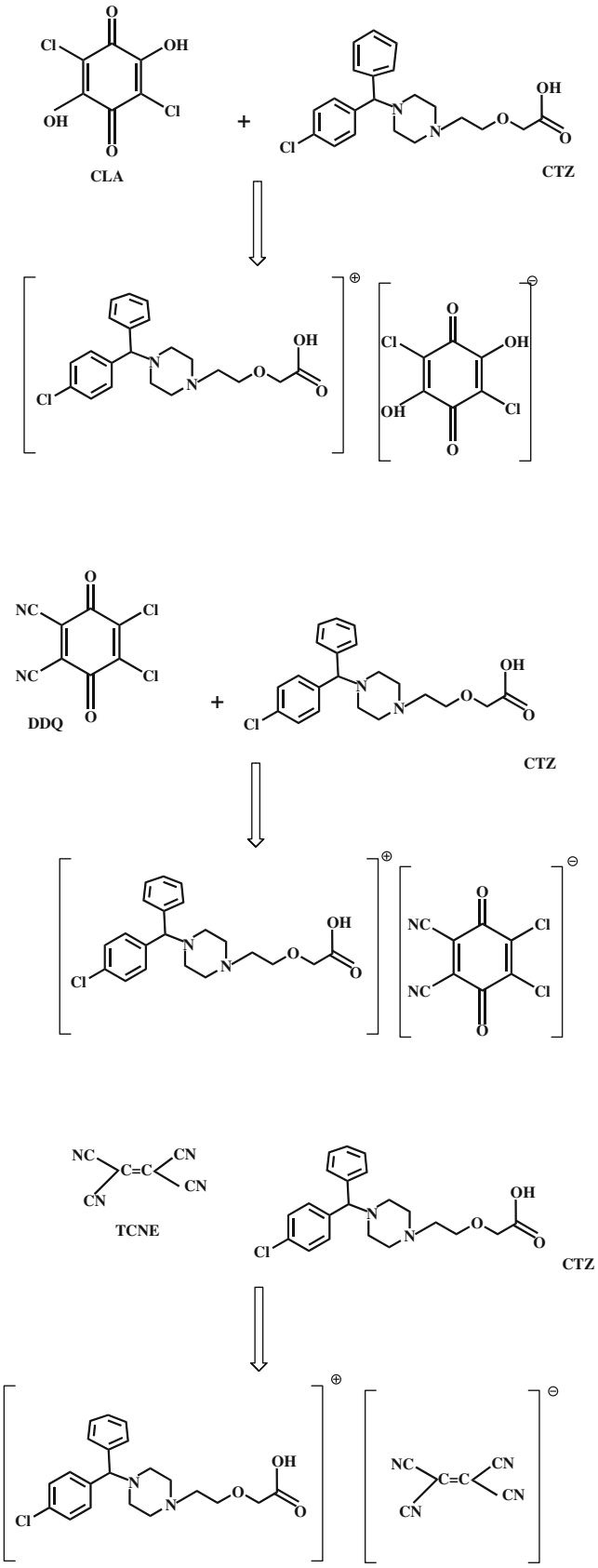
Table 6 (continued)

Dosage form	Amount taken, μg/ml			Amount found, μg/ml			% Recovery			Comparison method [4], % recovery
	CLA	DDQ	TCNE	CLA	DDQ	TCNE	CLA	DDQ	TCNE	
	2.0	3.0	2.0	2.006	3.011	2.015	100.28	100.34	100.77	100.12
	3.0	5.0	3.0	3.017	5.026	2.974	100.55	100.52	99.14	
	3.5	6.0	3.5	3.473	6.017	3.474	99.23	100.29	99.26	
$\overline{X} \pm SD$							99.73±0.64	99.98±0.58	100.04±0.79	99.90±0.49
t test							0.69	0.33	0.55	
F test							1.7	1.4	2.6	
Fastofen® tablets (120 mg FXD/tablet)	0.5	1.0	0.5	0.498	0.991	0.497	99.55	99.06	99.44	100.32
	1.0	2.0	1.0	1.002	2.005	1.006	100.16	100.23	100.62	100.97
	2.0	3.0	2.0	2.006	3.004	2.019	100.29	100.14	100.94	100.05
	3.0	5.0	3.0	3.011	4.989	3.026	100.36	99.77	100.86	
	3.5	6.0	3.5	3.485	6.039	3.519	99.57	100.66	100.53	
$\overline{X} \pm SD$							99.99±0.39	99.97±0.61	100.48±0.61	100.45±0.47
t test							0.93	0.57	0.06	
F test							1.5	1.7	1.7	
Fexodine® capsules	0.5	1.0	0.5	0.499	1.002	0.496	99.92	100.16	99.11	99.97
	1.0	2.0	1.0	1.003	2.015	0.992	100.26	100.74	99.16	99.21
	2.0	3.0	2.0	2.015	3.025	2.003	100.73	100.82	100.13	100.15
	3.0	5.0	3.0	3.025	5.046	3.008	100.84	100.92	100.26	
	3.5	6.0	3.5	3.522	6.008	3.512	100.63	100.13	100.34	
$\overline{X} \pm SD$							100.48±0.38	100.55±0.38	99.80±0.61	99.78±0.49
t test							0.07	0.07	0.58	
F test							1.7	1.7	1.5	

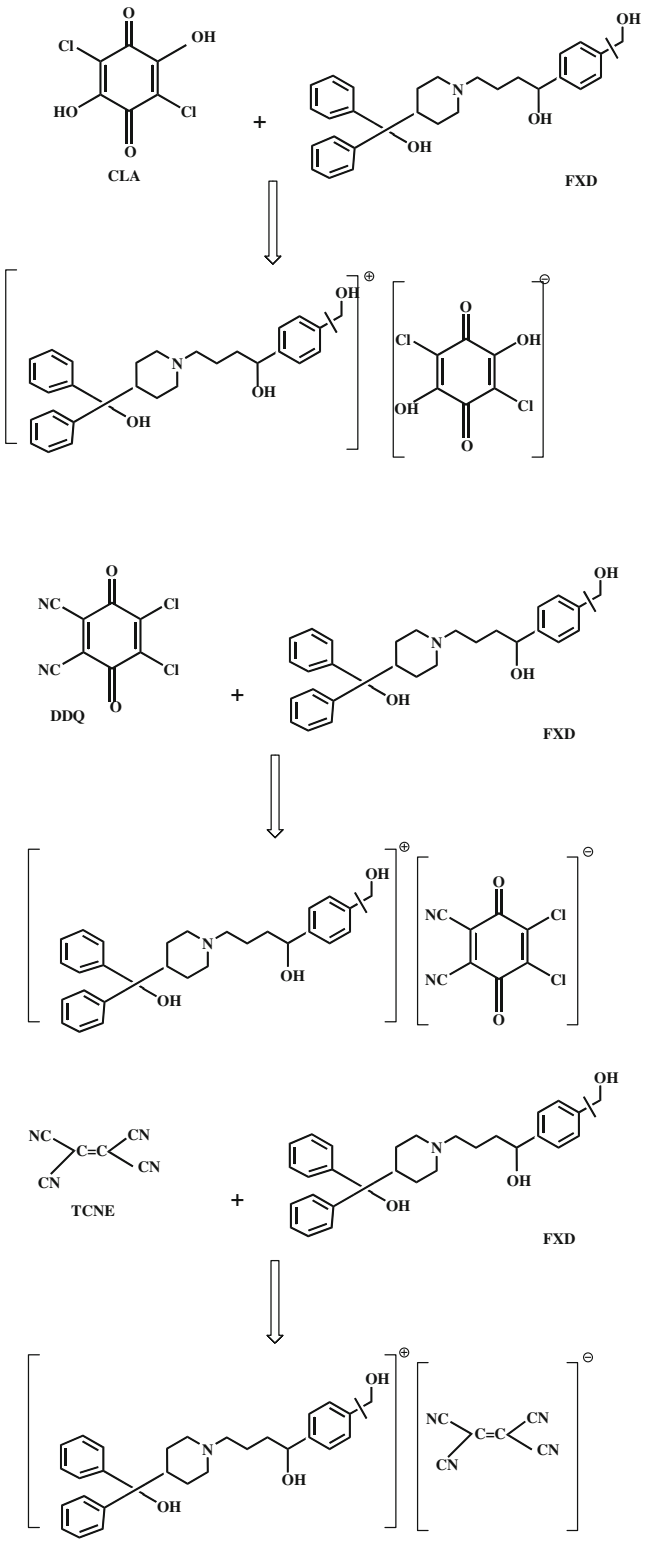
[a] 2.13 and 19.25 are the tabulated t and F values at $p=0.05$ [33]



Scheme 1 Reaction pathway between EBS and Π acceptors



Scheme 2 Reaction pathway between CTZ and Π acceptors



Scheme 3 Reaction pathway between FXD and Π acceptors

the extrapolation of the two lines obtained from Job's continuous variation method, respectively; n is the number of moles of the reagent; n=1; C is the molar concentration of the drug used in Job's continuous variation method.

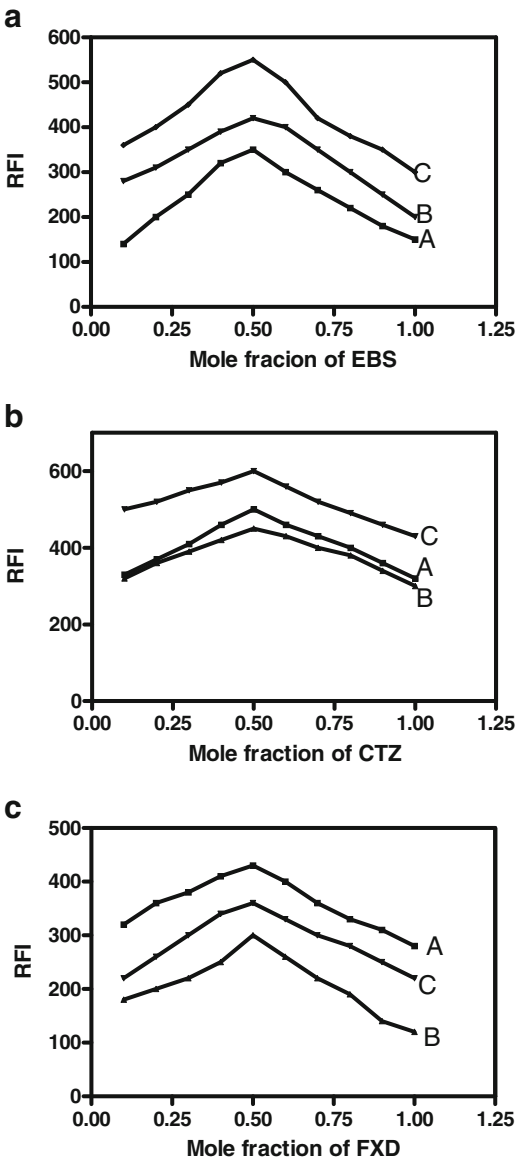


Fig. 7 **a**. Continuous variation graph for EBS with: A: CLA, B: DDQ, C: TCNE (each 3×10^{-3} M). **b**. Continuous variation graph for CTZ with: A: CLA, B: DDQ, C: TCNE (each 3×10^{-3} M). **c**. Continuous variation graph for FXD with: A: CLA, B: DDQ, C: TCNE (each 3×10^{-3} M)

Using the above equation K_f was found to be 1.1×10^5, 7×10^4, and 4.6×10^5 for EBS with CLA, DDQ, and TCNE respectively. Concerning CTZ; K_f was 2.2×10^5, 6.8×10^4, and 3×10^5 respectively. The corresponding values for FXD are 6.5×10^4, 2.7×10^5, and 4.9×10^4.

Also, the Gibbs free energy changes (ΔG) of the reaction were calculated according to the following equation [37]:

$$\Delta G = -2.303\ RT \log K_f$$

Where R is gas constant = 8.3 joule.degree^{-1}.mole^{-1}; T is absolute temperature = °C+273.

Using the above equation ΔG was found to be -2.6×10^4, -2.5×10^4, and -3×10^4 Joule/Mole for EBS with CLA, DDQ, and TCNE respectively. The values of ΔG for CTZ were found to be -2.8×10^4, -2.5×10^4, and -2.9×10^4. The corresponding values for FXD are -2.5×10^4, -2.8×10^4, and -2.4×10^4. The high negative value of ΔG indicates that the reactions are spontaneous.

Conclusion

A validated simple, rapid, and selective spectrofluorimetric method was developed for the determination of some antihistaminic H_1 receptor antagonist drugs based on the reaction of the cited drugs with some Π acceptors . The factors affecting the formation of the reaction products were carefully studied and optimized. The method was successfully applied for the determination of the studied drugs in their dosage forms. The results obtained were in good agreement with those obtained by the comparison methods. Reactions Stoichiometries and stability constants of the formed complexes were also investigated.

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