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ORIGINAL ARTICLE

Novel spectrophotometric methods for determination of desloratidine in pharmaceutical formulations based on charge transfer reaction

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Abstract A facile, rapid, sensitive and validated spectrophotometric methods for the determination of desloratidine (DES) in pure and in dosage forms is described. The methods are based on the formation of charge transfer complexes between DES and the chromogenic reagents alizarin (I), alizarin red S (II) and quinalizarin (III) producing charge transfer complexes in alcoholic medium which showed an absorption maximum at 528, 505 and 560 nm for I, II and III, respectively. The optimization of the reaction conditions such as the type of solvent, reagent concentration and reaction time was investigated. Beer's law is obeyed in the concentration ranges 1.0–16, 0.5–12 and 2.0–20 μg mL^{-1} for I, II and III, respectively. The molar absorptivity, Sandell sensitivity, detection and quantification limits are also calculated. The correlation coefficient was $\geqslant 0.9993$ ($n = 6$) with a relative standard deviation (R.S.D.) of $\leqslant 1.13$. The methods are successfully applied to the determination of DES in pharmaceutical formulations and the validity assesses by applying the standard addition technique, which compared with those obtained using the reported method.

1. Introduction

Desloratadine (DES), 4-(8-chloro-5,6-dihydro-11*H*-benzo-[5,6]cyclohepta [1,2*b*]pyridin-11-ylidene)-1-piperidine, (DES is the descarboethoxy form of loratadine) (Fig. 1). An orally active major metabolite of the nonsedating antihistamine loratadine, is a selective, potent, peripheral H1 receptor antagonist (Kleemann and Engels, 2000; Graul et al., 2000). Desloratadine exhibits qualitatively similar pharmacodynamic activity with a relative oral potency in animals, two to three-fold greater than its parent analog loratadine, probably due to a higher affinity for histamine H1 human receptors (Hand-

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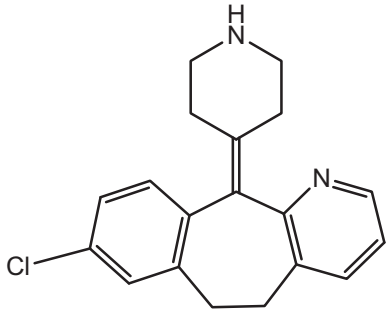

Figure 1 Structural formula of desloratadine (DES).

ley et al., 1997). Nevertheless, the development of drugs with increased potency will continue to challenge the analytical chemist to lower the limit of quantitation (LOQ).

Several analytical methods have been reported for the determination of DES in biological samples and applied in pharmacokinetic studies. These methods include gas chromatography with nitrogen phosphorous detection (Johnson et al., 1994), liquid chromatography with fluorescence detection (Zhang and Blume, 1994; Yin et al., 2004), ultraviolet detection (Liu et al., 2004; Qi et al., 2005; EL-Enany et al., 2007; El-Sherbiny et al., 2007; Zheng and Rustum, 2010), or mass spectrometric detection (Yang et al., 2003; Srinubabu et al., 2007; Shen et al., 2006; Zhang et al., 2003). However, DES was determined in pharmaceutical preparations using capillary isotachophoresis (Kubacak et al., 2005) and stability-indicating UPLC method (Rao et al., 2010).

For determination of DES in tablets using spectrophotometric technique; ultraviolet spectrophotometric methods at 282.5 nm (Patel et al., 2004) and 242 nm (Bondili and Reddy, 2011) were reported. Visible spectrophotometric methods used eosin (Abd El-Hay et al., 2011), 2,4-dichloro-6-nitrophenol (DCNP), 2,4-dinitrophenol (DNP) and picric acid (PA) reagents (Mohamed et al., 2011), 7,7,8,8-tetracyanoquinodimethane (TCNQ, π-acceptor) (Cağlar and Oztunç, 2007), 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) and 2,4-dinitrofluorobenzene (DNFB) (EL-Enany et al., 2007). Also Spectrofluorimetric methods were reported for determination of DES (EL-Enany et al., 2007; Walash et al., 2011). Comparison between the reported spectrophotometric methods for determination of DES and the proposed methods was shown in Table 1. The proposed methods are highly specific for DES in the presence of the parent drug (loratadine) which contains a single basic center pyridine nitrogen atom. The developed three methods are very simple and less time consuming than other methods. Also, it does not require extraction and heating).

The charge transfer complex formation with alizarin derivatives by some pharmaceutical compounds was studied such as; sulfamethoxazole and trimethoprim (Issa and Amin, 1994); piroxicam and tenoxicam (Amin, 2002) and nefopam, mebevrine and phenylpropanolamine hydrochloride (Shama and Amin, 2004).

In the present work, we report a very simple, rapid, accurate, and sensitive visible spectrophotometric method to assay DES in raw material in some commercial pharmaceutical preparations (tablets). The proposed colorimetric method involves the formation of charge transfer complex between DES and alizarines derivatives; alizarin (I), alizarin red S (II) and quinalizarin (III) as chromogenic reagents.

2. Experimental

2.1. Apparatus

All the absorption spectral measurements were made using Varian double beam UV–Vis spectrophotometer (Tokyo, Japan) equipped with 10 mm matched quartz cells.

2.2. Materials and reagents

All solvents (methanol, dimethyl sulfoxide, ethanol, acetone and acetonitrile) used in this work were of HPLC grade.

Desloratadine reference standard and bulk powder was supplied from Delta Pharma, Egypt. Its purity was found to be 99.78% according to the manufacturer's method. The commercial formulations used included Aerius tablets labeled to contain (5.0 mg/tablet, Schering-Plough Cooperation, USA) and Desa tablets labeled to contain (5.0 mg/tablet, Delta Pharma, Egypt).

2.2.1. Standard solutions

A standard stock solution of the studied drugs containing 100 μg mL^{-1} was prepared by dissolving 10 mg of pure drug in 20 mL methanol and was further diluted to 100 mL with the same solvent to obtain the working concentration. The standard solution was kept in refrigerator and was found to be stable for at least 1 week if they had been stored in a cool (< 25 °C) and dark place.

2.2.2. Reagents

Alizarin, 1,2-dihydroxyanthraquinone (I), alizarin red S, 3,4-dihydroxy-9, 10-dioxo-2-anthracene sulfonic acid (II) and quinalizarin 1,2,5,8-tetrahydroxy-anthraquinone (IV) were Aldrich products and used without further purification. A stock solution 3.0×10^{-3} M was prepared by dissolving the appropriate weight of the reagent in approximately 25 mL of methanol. After obtaining a solid-free solution, it was transferred to a 100 mL volumetric flask and the volume was completed to the mark with methanol. This solution was stable for 1 week, at least.

2.3. Construction of calibration curves

Aliquots of methanolic solutions containing (0.05–2.0 mL) of a standard solution of DES (100 μg mL^{-1}) were transferred to separated 10 mL calibrated flasks. To each flask was added 2.0 mL of (3.0×10^{-3} M) chromogenic reagent (I–III) solution. Afterward, the obtained mixture was shaken in order to promote the reaction and the volume was completed to the mark with methanol. The absorbance of this final solution was measured at 528, 505 and 560 nm for I, II and III, respectively against a reagent blank. Perform a linear regression analysis using absorbance data *vs.* concentration of the drug. Use the slope and intercept data obtained from linear regression analysis of the calibration graph to calculate the concentration of an unknown sample.

Absorbance = intercept + (slope X concentration)

Alternatively, the concentration of the unknown may be directly obtained by calibration graphing.

Table 1 Comparison between the reported spectrophotometric methods for determination of DES.

Reagent	λ_{max} nm	Concentration range (μg mL^{-1})	Molar absorptivity (L mol^{-1} cm^{-1})	LOD (μg mL^{-1})	Refs.
UV-spectrophotometry	282.5	16–24	–	–	Patel et al. (2004)
	242	2.0–10	–	0.11	Bondili and Reddy (2011)
Eosin	549	0.31–2.81	–	–	Abd El-Hay et al. (2011)
2,4-Dichloro-6-nitrophenol (DCNP)	402	3.11–93.35	$6.14x10^5$	2.132	Mohamed et al. (2011)
2,4-Dinitrophenol (DNP)	426	3.11–62.17	13.72×10^5	1.884	
Picric acid (PA)	352	3.11–43.44	17.08×10^5	0.559	
7,7,8,8-Tetracyanoquinodimethane (TCNQ)	843	1.5–13	2.2968×10^4	0.35	Cağlar and Oztunç (2007)
4-Chloro-7-nitrobenzo-2-oxa-1, 3-diazole (NBD-Cl)	485	0.5–6	–	0.112	EL-Enany et al. (2007)
2,4-Dinitrofluorobenzene (DNFB)	375	1.0–10	–	0.172	
Alizarin (I)	528	1.0–16	1.6078×10^4	0.21	Proposed methods
Alizarin red S (II)	505	0.5–12	2.1836×10^4	0.08	
Quinalizarin (III)	560	2.0–20	1.5213×10^4	0.40	

2.4. Applications to pharmaceutical formulations (tablets)

The contents of ten tablets were removed and finely powdered using an agate mortar. The combined contents were mixed and weighed accurately. A portion of the powder equivalent to 50 mg of DES was accurately weighed and exactly 25 mL of methanol was added, sonicated for about 20 min, left for a time in a refrigerator to allow any insoluble matter to settle down and then filtered into a 50 mL volumetric flask. The solution was then completed to volume with methanol and the procedure was completed as described for preparing the calibration graph. The nominal contents of the tablets were determined either from the calibration graph or using the corresponding regression equation.

2.5. Stoichiometric relationship

The stoichiometric ratios of the charge transfer complexes formed between DES and I–III reagents were determined by applying the continuous variation method attributable to Job (Job, 1939) and modified by Vosburgh and Coober (1941) at the optimum wavelengths of maximum absorbance. Job's method of continuous variation was employed, a 3.0×10^{-3} M standard solution of DES and 3.0×10^{-3} M solution of reagent (I–III) were used. A series of solution were prepared in which the total volume of drug and reagent was kept at 2.0 mL. The reagents were mixed in various proportions with drug and diluted to volume in a 10 mL calibrated flask with methanol following the above mentioned procedures.

3. Results and discussion

Alizarin derivative reagents were utilized for the determination of DES. The procedure depends on the formation of charge transfer complex upon the reaction of these reagents with DES alcoholic medium. The reaction proceeds through the formation of a charge transferred colored product, which was measured spectrophotometrically.

The study and development of the methods for the determination of DES in bulk powders and pharmaceutical formulations, exploring its charge transfer reaction with alizarin derivatives (I–III), were performed through two steps: (*i*) optimization of the experimental conditions in order to achieve both maximum sensitivity and selectivity. This step comprised the evaluation of the effect of the solvent nature, investigation of the influence of the reagent concentration and evaluation of the time required to complete the reaction and; (*ii*) study and characterization of the reaction, which were carried out by the evaluation of the reaction stoichiometry (Job's continuous variation method) and the verification of the proposed reaction mechanism.

At optimum conditions, the radical anion (absorbing species) was formed in the medium immediately after mixing of the reagents and showed maximum absorption at 528, 505 and 560 nm for I, II and III, respectively in methanol medium. Thus, these wavelengths were chosen for all further measurements in order to obtain highest sensitivity for the proposed methods. It is important to point out that I, II and III alone, in methanol medium, exhibit maximum absorption at 428, 421 and 491 nm, respectively. The high difference between maxima of the reagent and the product absorption bands ~100, 84 and 69 nm for I, II and III, respectively allowed the measurement of the products with only a small contribution of the reagents that were added in excess in the medium (Fig. 2).

3.1. Evaluation of the effect of the solvent nature

The first parameter evaluated in the optimization of the experimental conditions was the nature of the solvent employed. The solvent plays an important role in some charge transfer reactions, since it must be able to facilitate the total charge transfer and then allow the complex dissociation and stabilization of the radical anion formed, which is the absorbing species. According to the literature, solvents with high dielectric constant are more effective to execute this task (Kelani et al., 1997; Huang et al., 2006). Taking this fact into account, water would be an excellent solvent for the procedure. However, the poor solubility of the reagents in water did not allow its use in the present case. So, the reaction was tested in ethanol, methanol, acetone, DMSO and acetonitrile media. Although the highest dielectric constant of DMSO and acetonitrile and best sensitivity were achieved with methanol, probably because of the capacity of this solvent to form stable hydrogen bonds with the radical anion. Then, methanol was chosen for further experiments (Fig. 3).

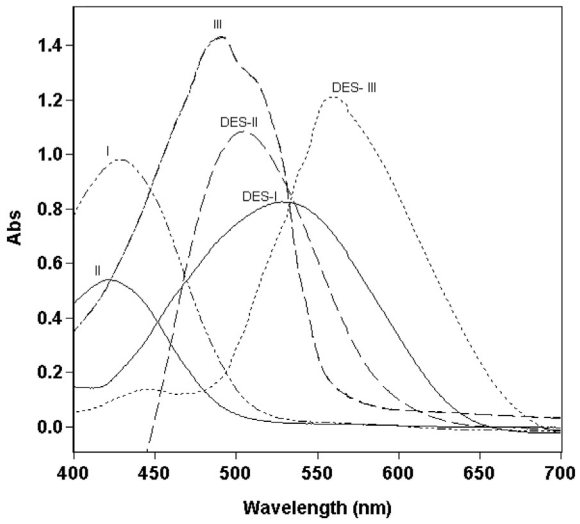

Figure 2 Spectra of charge transfer complexes of 10 μg mL^{-1} DES with (3.0×10^{-3} M) alizarine derivatives (I–III) in methanol solvent obtained against the reagent solutions also prepared in the same solvent.

3.2. Effect of the reagents concentration

In spectrophotometric analytical methods where maximum sensitivity is desired, the reagent concentration in solution is an important parameter to be studied, since the maximum conversion of the analyte into absorbing species depends on the amount of the reagent available in the solution for reaction and the equilibrium involved. In order to achieve this objective, an experiment was performed when various concentrations of reagents (I–III) solution (3.0×10^{-3} M) in the range of 0.25–4.0 mL were added to a fixed drug concentration (10 μg mL^{-1}) (Fig. 4). The results showed that 2.0 mL of (3.0×10^{-3} M) reagents solution (I–III) was enough to develop the color to its full intensity. As it can be seen, remarkable increase of the absorbance was verified up to respectively, after this point, it only suffered a slight increase or constant absorbance.

3.3. Effect of the reaction time

The optimum reaction time was determined by continuous monitoring of the absorbance at optimum wavelengths of a solution containing 10 μg mL^{-1} DES plus 2.0 mL of (3.0×10^{-3} M) (I–III) reagent, respectively at laboratory ambient temperature (25 ± 2 °C). On raising the temperature, the absorbance of the charge transfer complex was decreased with a hypochromic shift, until decayed at 60 °C. Stable absorbance values were observed from the beginning of the experiment up to 12 h. After this time, absorbance suffered a slight decrease. In view of these results, all spectral measurements were carried out after 3.0 min of mixing of the reagents and 25 ± 2 °C in order to make the method faster.

3.4. Sequence of additions

Drug–reagent–solvent was the favorable sequence of addition for complete color development and highest absorbance at the recommended wavelength.

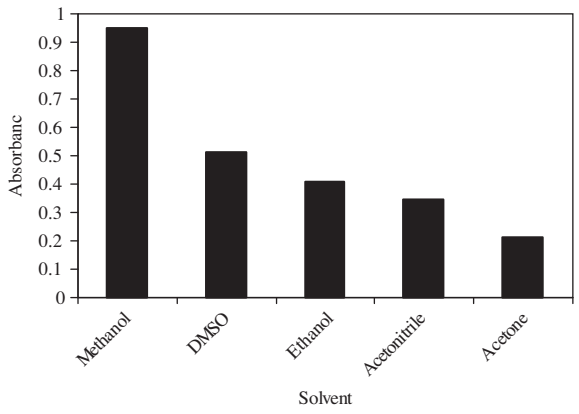

Figure 3 Effect of different solvents on the charge transfer complex of DES-III complex obtained against III reagent solution also prepared in each solvent. DES concentration = 10 μg L^{-1} and reagent III concentration = (3.0×10^{-3} M).

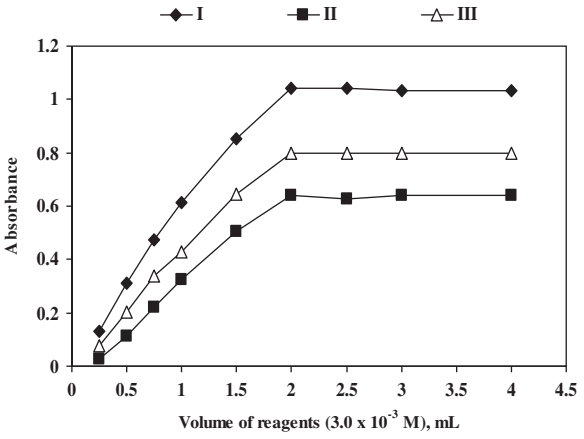

Figure 4 Effect of (3.0×10^{-3} M) reagent concentration on the absorbance of charge transfer complexes formed between DES (10 μg mL^{-1}) and the reagents at the optimum wavelengths.

3.5. Stoichiometry of the reaction

Job's method of the continuous variation (Job, 1939) was employed to determine the stoichiometry of the charge transfer reaction in methanol medium. Keeping the sum of the molar concentrations of the investigated drug (DES) and (I–III) reagents fixed, the ratio of the concentrations of the two substances in the mixture was varied and the absorbances of the mixtures were recorded at optimum wavelengths against a convenient blank solution prepared for each point of the experiment. As shown in Fig. 5, the molar ratio which gave maximum absorbance was 0.33, indicating that they react with (I–III) in a proportion of (1:2) (Drug:reagent) and confirming the assumption raised before when the effect of reagent concentration was studied. In view of this result a reaction mechanism was proposed considering the transfer of free electron of the two nitrogen atoms present in one molecule of drug to the charge-deficient center of the reagent molecule.

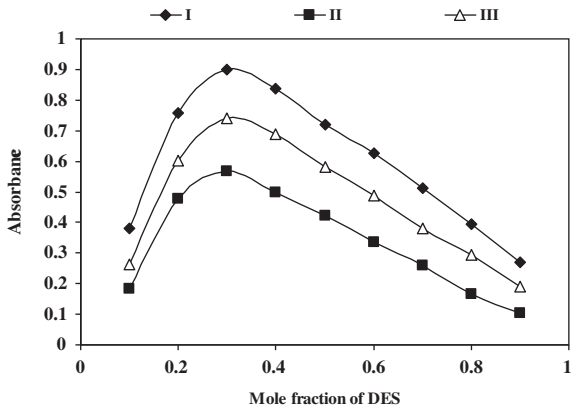

Figure 5 Job's method of continuous variation between DES and Alizarines reagents (I–III).

3.6. Mechanism of the reaction

Solutions of reagents in methanol exhibit an absorption band with a well defined maximum at 428, 421 and 491 nm, respectively, while the drug solution in methanol showed no absorption in the 400–700 nm range. The addition of drug to the reagent solution in methanol caused an immediate change in the absorption spectrum with the appearance of a new characteristic band with maximum absorption at optimum wavelengths recorded in Table 2.

According to (Ayad et al., 1984) molecular charge-transfer complexes are formed in non-polar solvents while radical anion species are predominant in polar solvents. Also, it is believed that the addition of basic compounds that contain a lone pair of electrons, such as DES, results in the formation of charge-transfer complexes of n–π type. This kind of complexes can be considered as an intermediate molecular-association compound that forms a corresponding radical anion in polar solvents. In this case, radical anions result from the total transfer of charge (Fig. 6).

3.7. Validation of the proposed methods

The validity of the methods was tested regarding linearity, specificity, accuracy, repeatability and precision according to International Conference on Harmonization (ICH, 1996) and United States Pharmacopeia (USP, 1999) guidelines.

Linearity – By using the above procedures, linear regression equations were obtained. The regression plots showed that there was a linear dependence of the analytical response in the three methods to the concentration of the drug over the ranges cited in Table 2. Linear regression analysis of the data gave the following equations. For Method I, $A = 0.0007 + 0.0521C$, $r = 0.9998$, for method II, $A = 0.0072 + 0.0617C$, $r = 0.9993$ and for method III, $A = -0.0084 + 0.0509C$, $r = 0.9998$. Where A is the absorbance, C is the concentration of the drug ($\mu g\ mL^{-1}$), and r is the correlation coefficient.

The limit of quantification (LOQ) was determined by establishing the lowest concentration that can be measured according to ICH, 1996. The results are shown in Table 2. The limits of detection (LOD) were determined by establishing the minimum level at which the analyte can be reliably detected, and the results are also summarized in Table 1. LOQ and LOD were calculated according to the following equation (USP, 1999):

$$LOQ = 10\ s/b$$

$$LOD = 3.3\ s/b$$

where s is the standard deviation of the intercept of the regression line. b: is the slope of the calibration curve.

Table 2 Spectral characteristics of the colored products of DES with the reagents (I–III).

Parameters	Reagents		
	I	II	III
Wavelength (λ_{max}, nm)	528	505	560
Concentration range ($\mu g\ mL^{-1}$)	1.0–16	0.5–12	2.0–20
Ringbom conc. range ($\mu g\ mL^{-1}$)	2.0–14	1.0–11	3.5–18
Molar absorptivity $\times 10^4$ ($L\ mol^{-1}\ cm^{-1}$)	1.6078	2.1836	1.5213
Sandell sensitivity ($ng\ cm^{-2}$)	19.33	14.23	20.43
Regression equation [a]			
Slope	0.0521	0.0617	0.0509
Intercept	0.0007	0.0072	−0.0084
Correlation coefficient	0.9998	0.9993	0.9998
Mean recovery % ± SD	99.53 ± 0.52	99.91 ± 1.13	100.09 ± 0.69
Variance	0.270	1.277	0.476
Relative standard deviation[b] (%)	0.52	1.13	0.69
Detection limits ($\mu g\ mL^{-1}$)	0.21	0.08	0.40
Quantification limits ($\mu g\ mL^{-1}$)	0.67	0.26	1.34
Calculated t-value (2.20)[c]	0.456	0.353	1.14
Calculated F-value (4.39)[c]	3.92	1.20	2.23

[a] $A = a + bC$, where C is the concentration in ($\mu g\ mL^{-1-1}$), A is the absorbance, a is the intercept and b is the slope.
[b] Average of six determinations.
[c] The figures between parentheses are the tabulated t and F values, respectively for five degree of freedom and 95% confidence level at $p = 0.05$ Miller and Miller (2005).

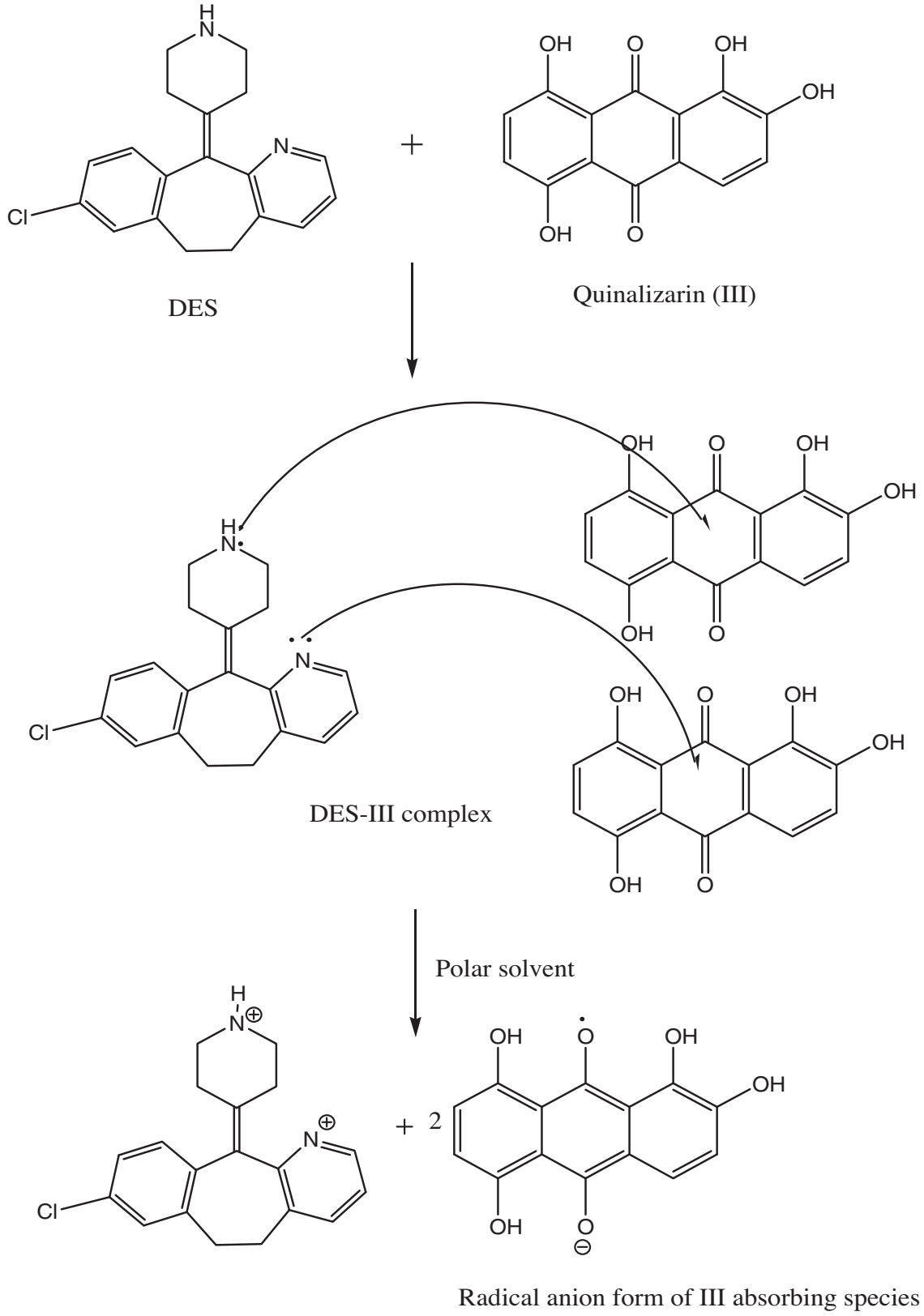

Figure 6 Possible mechanism of radical anion formation from III with DES reaction.

The proposed methods were evaluated for the accuracy as percent relative error (% Er) and the precision as percent relative standard deviation (% RSD) (Tables 3).

3.7.1. Precision

The precision of the proposed methods was investigated by intraday and interday determinations of DES at four different

Table 3 Inter-day and intra-day precision and accuracy for the determination of DES using the proposed methods.

Methods	Added ($\mu g\ mL^{-1}$)	Inter-day				Inter-day			
		Recovery (%)	Precision RSD (%) [a]	Accuracy RE (%)	Confidence limit [b]	Recovery (%)	Precision RSD (%) [a]	Accuracy RE (%)	Confidence limit [b]
I	3.0	99.20	0.50	−0.80	2.976 ± 0.016	100.05	0.68	0.05	3.002 ± 0.021
	6.0	99.40	0.68	−0.60	5.964 ± 0.043	99.30	0.94	−0.70	5.958 ± 0.059
	9.0	99.35	0.71	−0.65	8.942 ± 0.067	100.20	0.73	0.20	9.018 ± 0.069
	12	99.80	0.83	−0.20	11.976 ± 0.104	99.45	1.05	−0.55	11.934 ± 0.132
II	2.0	99.65	0.92	−0.35	1.996 ± 0.019	99.20	0.35	−0.80	1.984 ± 0.007
	4.0	99.70	1.27	−0.30	3.988 ± 0.053	99.80	0.64	−0.20	3.992 ± 0.027
	8.0	100.15	0.45	0.15	8.012 ± 0.038	99.35	1.17	−0.65	7.948 ± 0.098
	10	100.10	0.53	0.10	10.01 ± 0.056	99.85	0.49	−0.15	9.985 ± 0.051
III	4.0	99.25	0.80	−0.75	3.970 ± 0.033	99.15	0.58	−0.85	3.966 ± 0.024
	8.0	99.55	0.39	−0.45	7.964 ± 0.033	99.20	0.52	−0.80	7.936 ± 0.043
	12	99.15	1.42	−0.80	11.898 ± 0.177	99.50	0.79	−0.50	11.94 ± 0.099
	16	99.60	0.60	−0.40	15.936 ± 0.10	99.75	1.15	−0.25	15.96 ± 0.193

[a] Mean of five determination, RSD%, percentage relative standard deviation; RE%, percentage relative error.
[b] Mean ± standard error.

concentrations of DES solution for each method. The intraday studies were performed in one day (for each level $n = 5$) and interday studies in five days. The inter-day and intraday precisions expressed as relative standard deviation (RSD) values were found to be within 0.39–1.42% and 0.35–1.17%, respectively (Table 3). The data proved good precision for the developed methods.

3.7.2. Accuracy

To check the accuracy of the proposed methods, the standard addition method was applied by adding known amounts of DES to a previously analyzed tablet solution. The recovery of the added drug was calculated by comparing the concentration of the spiked mixtures with that of the previously found value. As can be seen from Table 4, satisfactory results better than the reported spectrophotometric methods were obtained.

3.7.3. Ruggedness and robustness

The robustness of the proposed methods was examined by evaluating the influence of small variations in the procedure variables, such as time of the reaction (3.0 ± 0.5 min), added reagent volume (2.0 ± 0.1 mL), and using a different instrument, by two different analysts under the same optimized conditions. The obtained reproducible results (Table 5) showed that none of these variables and changes significantly affected the assay of DES.

3.8. Analysis of the pharmaceutical preparation

The proposed methods were applied to the determination of DES in commercial tablets (Aerius and Desa tablets). The methods were tested for linearity, specificity, accuracy, repeatability, and precision according to ICH recommendations. The results of the proposed methods were statistically compared with those obtained using the reference method (EL-Enany et al., 2007). Recovery ± SD values were obtained. Statistical analysis of the results, using Student's *t*-test and the variance ratio *F*-test revealed no significant difference between the performance of the proposed and reference methods regarding the accuracy and precision, respectively (Table 6) (Miller and Miller, 2005).

3.8.1. Specificity and effect of excipients

The specificity of the methods was investigated by observing any interference encountered from the common tablet excipients, such as starch, lactose, glucose, talc, microcrystalline cellulose, titanium dioxide, lactose monohydrate, fructose, sucrose, and magnesium stearate. These excipients did not interfere with the proposed methods.

4. Conclusion

The developed three methods are very simple and rapid. It does not require extraction, heating, a buffer, or any other solutions. The chromophore formed is quite stable. Being simple, rapid, sensitive, accurate, robust, and economic, these characteristics make the proposed methods very suitable for routine analysis of DES in quality control laboratories. Meanwhile, the proposed methods are highly specific for the determination of DES in the presence of the parent drug (loratadine).

Table 4 Application of the standard addition technique for the determination of DES in pharmaceutical preparations using the proposed methods.

Sample	Taken (μg mL^{-1})	Added (μg mL^{-1})	Aerius tablets Recovery[a] (%)	Desa tablets Recovery[a] (%)
I	2.0	–	99.30	100.0
		4.0	100.15	99.10
		6.0	100.70	99.60
		8.0	99.80	99.70
		10	100.30	100.50
		12	99.40	100.80
Mean ± SD			99.94 ± 0.542	99.95 ± 0.622
V			0.294	0.387
RSD			0.542	0.622
S.E			0.222	0.254
II	1.0	–	100.20	99.80
		2.0	100.10	99.00
		4.0	99.55	99.60
		6.0	98.90	100.20
		8.0	99.20	100.50
		10	99.80	99.30
Mean ± SD			99.63 ± 0.510	99.73 ± 0.557
V			0.260	0.311
RSD			0.512	0.559
S.E			0.208	0.228
III	2.0	–	99.60	100.50
		2.0	99.45	100.10
		4.0	99.95	99.10
		8.0	99.20	99.70
		12	100.40	100.30
		16	100.20	99.60
Mean ± SD			99.80 ± 0.462	99.88 ± 0.515
V			0.213	0.266
RSD			0.463	0.516
S.E			0.188	0.210

[a] The average of at least three determinations.

Table 5 Results from robustness experiments.

Changed parameter		The proposed methods					
		I		II		III	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Added reagent volume	2.0 + 0.1	99.96	0.854	99.94	0.746	99.85	0.668
	2.0–0.1	99.90	0.92	100.30	0.882	100.60	0.845
Time of reaction	3.0 + 0.5	100.05	0.680	100.20	0.560	100.10	0.690
	3.0–0.5	100.0	0.759	99.90	0.904	100.40	0.775

Table 6 Application of the proposed methods to the determination of DES in pharmaceutical preparations.

Samples	References method EL-Enany et al. (2007)	Proposed methods[a]		
		I	II	III
Aerius tablets				
X ± SD	99.56 ± 0.95	99.85 ± 0.86	99.65 ± 1.02	99.70 ± 0.78
t-Value (2.26)[b]		0.482	0.136	0.243
F-value (5.19)[b]		1.22	1.15	1.48
Desa tablets				
X ± SD	99.90 ± 0.88	100.10 ± 0.74	99.48 ± 0.93	100.06 ± 0.69
t-Value (2.26)[b]		0.37	0.691	0.305
F-value (5.19)[b]		1.41	1.12	1.63

[a] Average of six determinations.

[b] The figures between parentheses are the tabulated t and F values, respectively for five degree of freedom and 95% confidence level at $p = 0.05$ Miller and Miller (2005).

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