Validated selective spectrophotometric methods for the kinetic determination of desloratidine in tablets and in the presence of its parent drug

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Abstract: Two novel selective validated methods have been developed for analysis of desloratidine (DSL) in its tablets formulation. Both were kinetic spectrophotometric methods, depend on the interaction of the secondary amino group in DSL with acetaldehyde to give N-vinylpiperidyl product. The formed N-vinylpiperidyl compound was reacted with 2,3,5,6-tetrachloro-1,4-benzoquinone (chloranil) to form colored N-vinylpiperidyl-substituted benzoquinone derivatives. The formed blue-colored derivative was measured at 672 nm. The reaction conditions were carefully studied and all factors were optimized. The molar ratio between the reactants was estimated and a suggested reaction mechanism was presented. The analysis was carried out using initial rate and fixed time (at 6 min) methods. The linear concentration ranges were 3–50 and 10 – 60 µg mL^{-1} with limits of detection of 3.2 and 2.2 µg mL^{-1} for the initial rate and fixed time methods, respectively. ICH guidelines were applied for analytical performance validation of the proposed methods. The presence of common excipients in the pharmaceutical formulation did not produce any significant interference, as well as from loratadine, which is the parent compound of DSL. Different commercially available tablets formulations containing were successfully analyzed, with, the percentage recovery ranging from 97.28–100.90 ± 0.7 2–1.41%. The obtained results were compared statistically with the reported method results. The proposed methods have similar accuracy and precision as the reported as indicated from the F- and t-test data.

Keywords: Desloratidine; kinetic spectrophotometry; initial rate method; fixed time method; pharmaceutical analysis.

INTRODUCTION

Desloratadine (DSL), the descarboethoxy form and the major active metabolite of loratadine, is a non-sedating antihistamine (fig. 1). DSL is a selective peripheral H_1 receptor antagonist, which do not produce any substantial effect on either the central or autonomic nervous systems. It is used in the treatment of allergic condition symptoms such as urticaria and rhinitis (Sweetman, 2009).

Several analytical techniques were applied for the analysis of DSL in bulk, dosage forms or different biological fluids. Among these reported analytical methods are spectrofluorometry (El-Enany *et al*., 2007), liquid chromatography (El-Enany *et al*., 2007; Liu *et al*., 2004; Qi *et al*., 2005; Razib *et al*., 2007; Sutherland *et al*., 2001; Yang *et al*., 2003; Zheng *et al*., 2010), UPLC (Rao *et al*., 2010; Shen *et al*., 2006), HPTLC (Talele *et al*., 2005), densitometry (Sumarlik *et al*., 2005) and capillary isotachophoresis (Kubacák *et al*., 2005).

Spectrophotometric methods have several advantages including; simplicity, low cost of analysis, and the availability of tools in most quality control laboratories. These advantages make these techniques are used widely in pharmaceutical analysis. However, it has been described a few spectral methods to identify.

Survey of the scientific literature shows that there are a few published spectrophotometric methods for analysis of DSL. The reported methods include; charge-transfer reaction of the secondary amino group with TCNQ (Cağlar *et al*., 2007), reaction with NBD-Cl or 2,4-dinitrofluorobenzene (El-Enany *et al*., 2007), and ion-pair formation (Patel *et al*., 2006). The selectivity of these methods was low because they cannot differentiate between DSL and other members of the tricyclic antidepressants (such as loratadin). Furthermore, the procedures were in many of these methods are tedious and time-consuming.

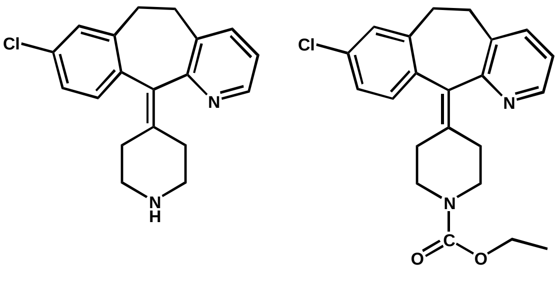

Fig. 1: Chemical structures of desloratidine (DSL) and its parent drug, loratadine.

The reaction of halogenated quinones with secondary amines in the presence of acetaldehyde produces mono quinonic derivatives (Alnabari *et al*., 2000). This reaction was utilized for the quantitative determination of a variety of secondary amines such as sympathomimetic amines

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(Amer *et al*., 1982), some antidepressants (Darwish, 2005) and trimetazidine (Ganesh *et al*., 2011).

In the present work, the free secondary amino group in the piperidine ring of DSL, was reacted with acetaldehyde (ACD) to produce N-vinylpiperidyl compound, followed by reaction with 2,3,5,6-tetrachloro-1,4-benzoquinones (chloranil) to form the N-vinylpiperidyl-substituted benzoquinone derivative. The formation of the blue colored product was observed by measuring the absorbance at 672 nm.

The use of kinetic spectrophotometric methods as analytical technique becomes of a great interest in the analysis of many pharmaceutical compounds because of its inherent advantages. These advantages include; selectivity improvement as a result of measuring the absorption intensity as a function of reaction time. Also, it eliminates interference arise from color or turbidity of samples and from the presence of other drugs co-formulated in pharmaceutical dosage forms.

The only published kinetic spectrophotometric method for the analysis of DSL was based on the formation of colored product between DSL and 1,2-Naphthoquinone-4-sulfonic acid sodium (Ashour *et al*., 2010). In the current work, two simple methods based on kinetic spectrophotometric measurements were developed for DSL. The suggested methods are highly selective for DSL determination either alone or in the presence of its parent drug (loratadine). The proposed kinetic methods are the initial rate and fixed time methods. The methods are developed, optimized, fully validated, and successfully applied for the analysis of DSL in its commercially available tablets and in synthetic mixture containing DSL and loratadine.

MATERIALS AND METHODS

Apparatus

Double beam Spectronic™ genesys™ (Milton Roy Co, Westhaven, USA) ultraviolet-visible spectrophotometer was used for carrying out the spectrophotometric measurements throughout this work.

UV-Visible spectrophotometer (Jennway® 6505, London, U.K.), was used for analytical method validation.

All calculations including; linear and non-linear regression analysis, and statistical treatments of the data, were carried out using "Statistical Methods in Analytical Chemistry (SMAC) software" designed by P.C Meier and R.E. Zund (Meier *et al*., 1990).

Chemicals and dosage forms

Desloratidine (DSL) was obtained as a gift from Delta-pharm., (Cairo, Egypt) and used without further purification. Acetaldehyde and 2,3,5,6-Tetrachloro-1,4-benzoquinone (chloranil) were purchased from Sigma Chemical Co., (St. Louis, USA). Acetaldehyde was prepared as 4% (v/v), in methanol and chloranil was prepared as 2×10^{-2} mol L^{-1}, in dioxane. Other chemicals and solvents used in this work were of analytical grade.

The following commercially available tablets are investigated; Delarex® tablets (Global Napi. pharm., Cairo, Egypt), Desa® tablets (Delta-pharm, 10th of Ramadan City, Cairo, Egypt) and Deslorate® tablets (BIG Pharma, 6th of October City, Cairo, Egypt), all are labeled to contain 5 mg of DSL per tablet. Synthetic mixture was prepared in the laboratory to contain 5 mg of DSL and 10 mg loratadine per 100 mg mixture.

Preparation of standard drug solution

An accurately weighed amount (50 mg) of DSL was transferred into 50-mL calibrated flask, 25 mL of methanol was added and the flask was sonicated for 5 min. The solution was completed to 50 mL with methanol. This gives a stock solution containing 1.0 mg mL^{-1} of DSL. Working standard solutions (50–1500 µg mL^{-1}) were prepared by diluting a portion of the stock solution with methanol.

Preparation of tablets sample solution

An accurately weight amount of the finely powdered tablets (or laboratory made mixture) equivalent to 50 mg of DSL was transferred into 50-mL volumetric flask and 25 ml of methanol was added. The solution was sonicated for 5 min., completed with methanol to 50 mL, shacked well, and filtered. The first part of the filtrate was rejected. Working solutions of samples (50–1500 µg mL^{-1}) were prepared by diluting a portion of the filtrate with methanol.

General analytical procedures

One milliliter of standard or sample solution containing 50-1500 µg mL^{-1} of DSL was transferred into 10-ml calibrated flask. One milliliter of ACD solution (4%, v/v, in methanol) followed by 1.0 ml of chloranil (2×10^{-2} M in dioxane) were added. The flask contents were mixed thoroughly and completed to 10 mL with methanol. The absorbance of the resulting solution was measured at 672 nm as a function of time against reagent blank.

Determination of molar ratio of the reactions

a. Molar ratio between DSL and ACD

Molar ratio was estimated using the limiting logarithmic method (Rose, 1964). The general recommended procedure was carried out using two different reaction conditions. At first, the procedure is performed using different ACD concentrations (0.6×10^{-2} to 1.6×10^{-2} mol L^{-1}) and a constant DSL concentration (12.9×10^{-5} mol L^{-1}). Then, the procedure is performed using different DSL concentrations (3.2×10^{-5} to 19.3×10^{-5} mol L^{-1})

and constant ACD concentration (1.6×10^{-2} mol L^{-1}). Two graphs were established, the first graph represents the plot of the logarithms of absorbances as a function of the logarithms of ACD concentration. The second one is plot between logarithms of absorbances and logarithms of DSL concentration. The molar ratio was nominated from the slopes of the two graphs.

b. Between DSL and chloranil

This molar ratio is obtained similarly as the drug and ACD. The general recommened procedure is performed at first using different chloranil concentrations (0.9×10^{-3} to 1.6×10^{-3} mol L^{-1}) at constant DSL concentration (12.9×10^{-5} mol L^{-1}). Then, the procedure is carried out using different DSL concentrations (3.2×10^{-5} to 19.3×10^{-5} mol L^{-1}) at constant chloranil concentration (2.0×10^{-3} mol L^{-1}). Here, also two logarithmic graphs were obtained; one represents the relationship between the absorbance and chloranil concentration and the second between the absorbance and DSL concentration. The molar ratio was postulated from the slopes of the two graphs.

RESULTS

The involved reaction and absorption spectra

The current study is based on the condensation reaction of the free secondary amino (NH) group in the piperidins ring of DSL with ACD forming N-vinylpiperidyl compound. The resulting N-vinylpiperidyl product was reacted with chloranil. The formed blue-colored vinylimino-substituted benzoquinone derivative was measured at 672 nm. Fig 2 shows the absorption spectrum for the reaction product of DSL, ACD and chloranil.

Optimization of reaction conditions

Factors affecting the interaction between DSL, ACD and chloranil have been studied in order to achieve the optimum reaction conditions. The studied factors included the concentrations of ACD and chloranil reagents, reaction time and temperature, and diluting solvent. In this study, one variable changed in turn while keeping the others constant. Finally, the optimal conditions were applied in the development of recommended analytical procedures.

Effect of ACD and chloranil concentrations

Increasing the concentration of either reagents, increased the color intensity of the resulting reaction product (fig. 3). The maximum absorbance intensity was obtained when the final concentrations of ACD and chloranil were 0.1–0.8 % (v/v) and 1.0–5× 10^{-3} mol L^{-1}, respectively. The selected ACD and chloranil concentrations were 0.4% (v/v) and 2×10^{-3} mol L^{-1} respectively.

Effect of reaction time and temperature

The rate of color formation increases by increasing the temperature of the reaction vessel. The maximum absorption intensity was obtained at room temperature after 10 min and did not change for at least 30 min. Increasing the temperature higher than room temperature (i.e. 40°C), make the maximum absorbance values comes earlier (after 5 min), but the absorbance becomes unstable and decreased rapidly which affect the precision of the obtained results. Consequently, subsequent experiments were performed at room temperature (25°C).

Effect of diluting solvent

Several solvents were used in the dilution of the formed chromophore before the absorbance measurements. The tested solvents were 1,4-dioxane, acetone, acetonitrile, chloroform, ethanol, ethyl acetate, methanol and methylene chloride. Methanol gave the highest absorbance reading and was used for subsequent works.

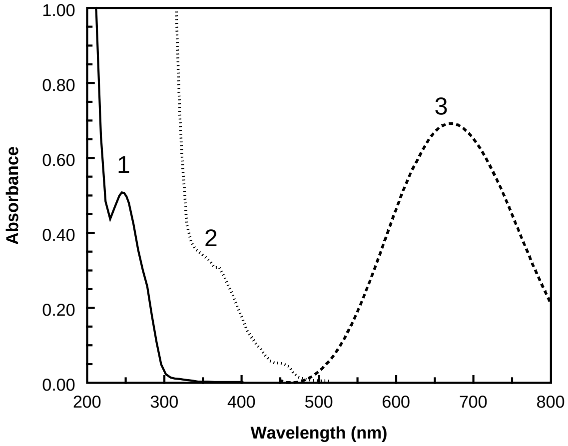

Fig. 2: Absorption spectra of DSL (1), chloranil (2) and the reaction product (2) of DSL (30 µg mL^{-1}) with ACD (0.4%, v/v) and chloranil (2×10^{-3} mol L^{-1}).

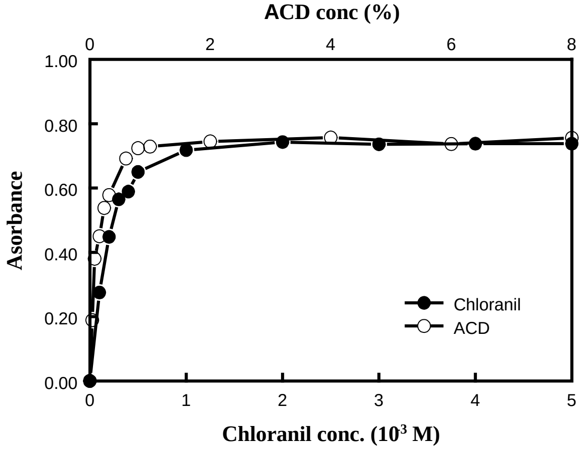

Fig. 3: Effect of ACD (-○-) and vvvvchloranil (-●-) concentrations on the absorption intensity of their reaction with DSL (30 µg mL^{-1}).

Table 1: Effect of solvents on the absorption intensity of the reaction product of DSL (30 μg/ml) with ACD and chloranil reagent.

Solvent	Di-electric constant (Mandip *et al.*, 2006)	λ_{max}	Absorbance
1,4-Dioxan	2.25	653	0.650
Acetone	20.7	668	0.530
Acetonitrile	37.5	674	0.554
Chloroform	4.81	680	0.692
Ethanol	24.3	671	0.731
Ethyl acetate	6.02	662	0.670
Methanol	32.7	671	0.740
Methylene chloride	8.93	680	0.656

After optimization of the reaction condition, the absorbance was measured as a function of time at different DSL concentrations (3.2×10^{-5} to 19.30×10^{-5} mol L^{-1}), keeping the concentration of both chloranil and ACD at 2×10^{-3} mol L^{-1} and 4%, v/v respectively. The obtained results are represented in Fig 4. In the initial rate (*K*) method, the slope of the tangent to the absorbance-time curve was calculated for each DSL concentration. The logarithm of the obtained slopes (which equal to the initial rate), (log K) was plotted as a function of logarithm of DSL molar concentration (log *C*) in order to construct the calibration graph. Equation (1) was applied to perform curve fitting of the data:

$$\log K = \log k' + n \log C \qquad (1)$$

where *k'* is the rate constant and *n* is slope which represent the order of the reaction.

The slope of the constructed calibration plot was 0.9780 (≈1). This value indicated that the reaction was first order (Fig 5). Actually, the used concentrations of ACD and chloranil in the recommended analytical procedure were greatly exceeded that of DSL in the reaction solution. Hence, the reaction was considered a pseudo-first order reaction.

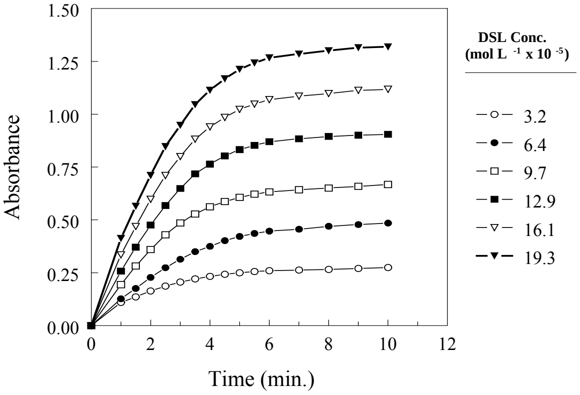

Fig. 4: Absorbance–time curves for the reaction product of different concentrations of DSL with ACD (0.4%, v/v) and chloranil (2×10^{-3} M).

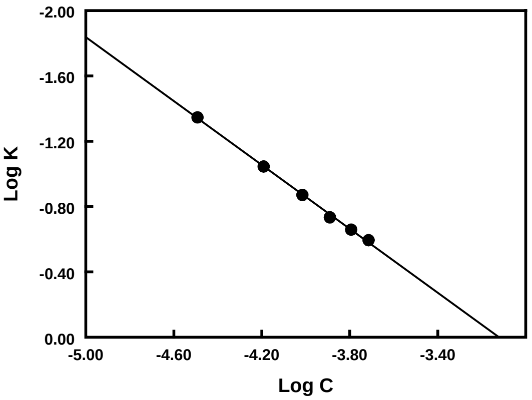

Fig. 5: Linear plot for log *C* vs. log *K* for the kinetic reaction of DSL with ACD (4%, v/v) and chloranil (2×10^{-3} mol L^{-1}). *C* is [DSL]: (3.2×10^{-4} to 19.3×10^{-5} mol L^{-1}); *K* is the reaction rate (per s).

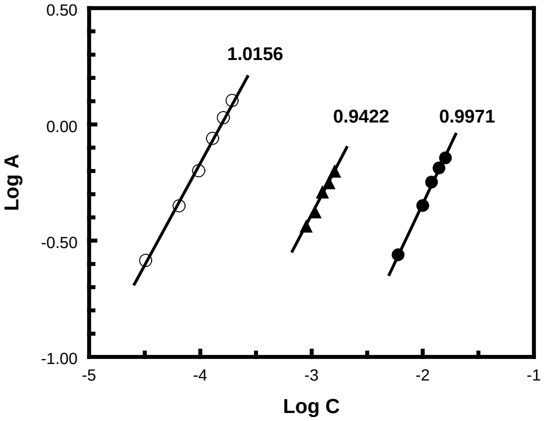

Fig. 6: Limiting logarithmic plots for molar reactivity of DSL with ACD and chloranil. C and A are the concentration and absorbance, respectively. The first line (○): log *A* vs. log[DSL]; The second line (▲): log *A* vs. log [chloranil]; The third line (●): log *A* vs. log[ACD]. The figures on the lines are their slopes.

Reaction stoichiometry

The molar ratio of the reaction of DSL with either ACD or chloranil was studied using limiting logarithmic method (Rose, 1964). Figure 6, shows three linear plots of the logarithm of the absorbance with the logarithm of the molar concentration of either, DSL, chloranil or ACD. These straight lines have comparable slopes (1.0156, 0.9422 and 0.9971 respectively) which revealing 1:1 ratio for the reactions of DSL with either ACD or chloranil. According to the obtained ratio, a suggested reaction mechanism was presented in fig.7.

Quantitation methods

Initial rate method: As it was mentioned later, DSL reaction was considered a pseudo-first order. Equation (1) could be written in the following formula:

$$K = \Delta A/\Delta t = k' C^n \qquad (2)$$

Table 2: Analytical parameters for the proposed fixed time method for determination of DSL

Reaction time (min)	Linear range (μg/ml)	Intercept ± SD*	Slope ± SD	Correl. Coeff. (r)	LOD (μg/ml)	LOQ (μg/ml)
1.5	15-150	0.0191 ± 0.0194	0.0063 ± 0.00050	0.9757	10.2	30.8
2.0	10-120	0.0220 ± 0.0173	0.0089 ± 0.00044	0.9901	6.4	19.4
3.0	8-70	0.0283 ± 0.0164	0.0135 ± 0.00042	0.9961	4.0	12.2
4.0	5-60	0.0331 ± 0.0150	0.0169 ± 0.00039	0.9979	2.9	8.9
5.0	4-50	0.0389 ± 0.0137	0.0188 ± 0.00035	0.9986	2.4	7.3
6.0	4-50	0.0428 ± 0.0130	0.0200 ± 0.00034	0.9989	2.2	6.5
7.0	4-50	0.0455 ± 0.0132	0.0207 ± 0.00034	0.9989	2.1	6.4
10	3-40	0.0514 ± 0.0099	0.0215 ± 0.00026	0.9994	1.5	4.6

* SD is Standard deviation

where A is the absorbance and t is the measuring time. Figure 5, shows the linear plot for log C of DSL vs. log K for the reaction. Least square method was performed for regression analysis of the data. The correlation coefficient was 0.9992 in the concentration range of 10-60 μg mL^{-1} of DSL. The calculated limit of detection (LOD) was 3.2 μg mL^{-1}. This low value confirmed the good sensitivity of the initial rate method.

Fixed time method: The calibration plot was established between the absorbance of the reaction product and the concentration of DSL. The result of regression analysis of this plot is summarized in table 2. Poor correlations were obtained using fixed times of 1.5 and 2 min. The correlation became stronger at fixed time of 3 and 4 min, but LOD is still relatively high. Finally, at time more than 4 min., low LOD and high correlation were observed. Consequently, a fixed time of 6 min was chosen for the analysis (International Conference on Harmonization, 1995) using the fixed time method.

Validation of the proposed methods

Precision: Three concentration levels of DSL (low, medium, and high of 10, 20, and 40 μg mL^{-1}, respectively) were analyzed using the initial rate and fixed time methods. Each concentration was investigated in three replicates. The results were summarize in table 3. The proposed methods showed high reproducibility and good precision as the relative standard deviations did not exceed 2%.

Table 3: Precision for the initial rate and fixed-time methods for determination of DSL

Concentration (μg mL^{-1})	% Recovery (± RSD)[a]	
	Initial rate method	Fixed time method
10	99.4 ± 1.36	99.8 ± 1.18
20	98.7 ± 0.76	100.4 ± 1.23
40	100.5 ± 0.88	101.5 ± 1.06

[a]Values are mean of three determinations.

Accuracy: The performance of proposed methods was checked by analyzing a known amount of the pure DSL in the presence of certain amount of the commonly used tablets excipients. The percentage recoveries and relative standard deviations for each excipient were calculated. The presence of these excipients did not produce any significant interference and the % recoveries were in the range 98.52 ± 1.05–100.65 ± 0.58 % (table 4).

Table 4: Analysis of DSL in the presence of commonly used pharmaceutical tablets excipients.

Excipients	Amount added (mg)	% Recovery (± RSD)[a]	
		Initial rate method	Fixed time method
Gelatin	5	99.32±0.76	99.98±0.68
Gum acacia	5	100.14±1.04	100.65±0.58
Lactose	20	99.64±0.56	100.1±0.21
Starch	30	100.02±0.56	99.20±0.38
Sucrose	20	99.47±0.25	100.25±0.64
Talc	5	98.96±0.82	98.52±1.05

[a] Values are mean of three determinations.

Selectivity: The interference from the closely related drug (loratadine) which is the parent drug of DSL was studied through determination of DSL in the presence of different concentration of loratadine. Furthermore, the possible interference of loratadine is also examined through analysis of synthetic mixture, prepared by mixing DSL and loratadine in a 1:2 ratio as in their individual tablets. Each 100 mg of the prepared powdered mixture contain 5 mg DSL and 10 mg loratadine in addition to the tablet excipients mentioned in table 4. The analysis of the prepared mixture for DSL content gave a good percentage recovery that is considered as an indication about the absence of any possible interference from the presence of loratadine with the proposed methods (table 5).

Table 5: Analysis of DSL in the presence of different added amounts of loratadine

DSL (mg)	Loratadine added (mg)	% Recovery (±RSD)	
		Initial rate method	Fixed time method
5	5	97.83±1.52	98.21±0.50
5	10	99.25±1.24	100.09±0.1.36
5	15	97.58±0.21	98.67+1.58
5	20	101.86±1.15	100.56±0.97
Synthetic mixture [a]	…	99.69±1.32	101.13±1.15

[a] Synthetic mixture contain 5 mg DSL and 10 mg loratadine in addition to the tablet excipients

Application of the proposed methods

Commercially available tablets were analyzed for their contents of DSL using both initial rate and fixed time methods. The concentration was calculated from the regression equations of DSL. The analysis is carried out in triplicate and the mean % recovery and standard deviation were calculated. The values were in the range of 97.28 ± 1.36 - 100.90 ± 1.16% (table 6).

DISCUSSION

The condensation reaction of DSL with ACD and chloranil has not yet been reported for analysis of DSL, therefore, the present work was directed toward the study of this interaction and utilize it in the development of a

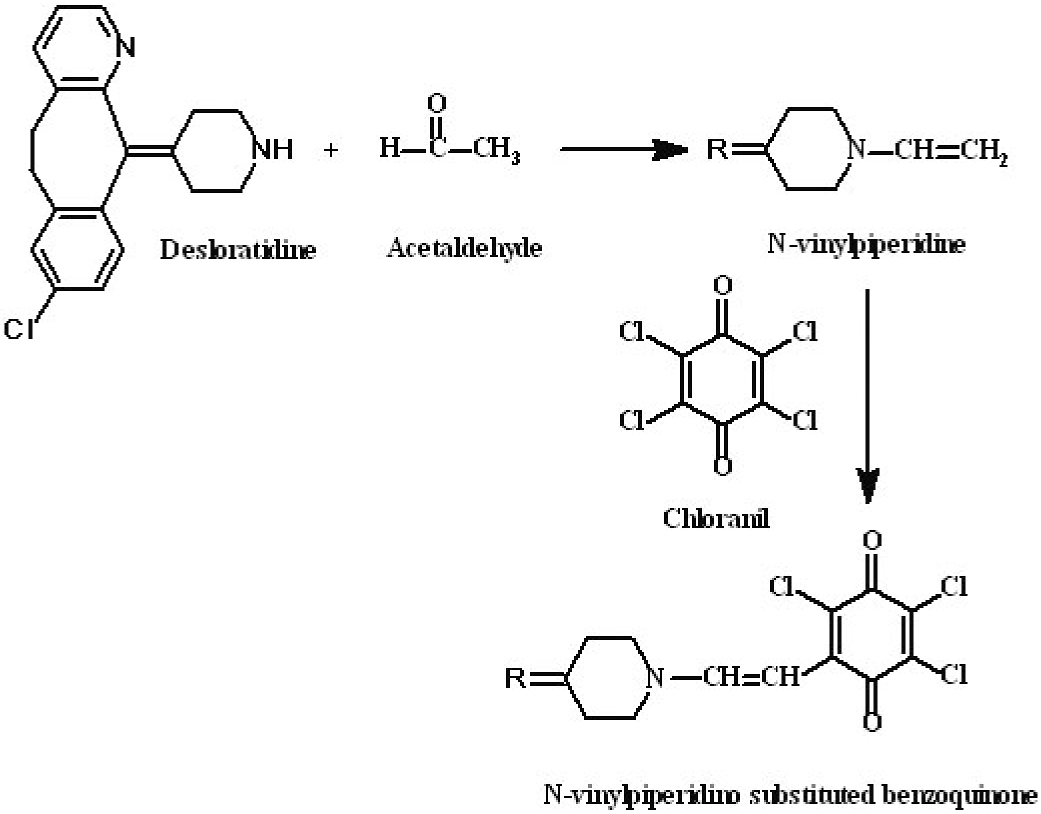

Fig. 7: Scheme for the suggested reaction mechanism of DSL with ACD and chloranil.

Table 6: Determination of DSL in its pharmaceutical dosage form by the reported and the proposed initial rate and fixed time methods.

Dosage forms	Reported	Initial rate method			Fixed time method		
	(% ± RSD)[a]	(% ± RSD)	*t*-value	*F*-value	(% ± RSD)	*t*-value	*F*-value
Delarex® tablet	100.20±1.19	100.46±0.74	0.41	2.59	100.90±1.16	0.94	1.05
Desa® tablets	100.07±0.95	99.50±1.41	0.75	2.2	100.44±1.08	0.58	1.29
Deslorate® tablets	100.36±0.72	100.48±0.99	0.22	1.89	100.32±1.27	0.06	3.11

[a]Reference (Cağlar *et al.*, 2007) values are mean ± RSD of five determinations.
[b]The tabulated values of *t* and *F* at 95% confidence limit are 2.78 and 6.39, respectively.

new spectrophotometric for the kinetic analysis of DSL. Generally, any haloquinone reagent (such as 2,3,5,6-tetrabromo-1,4-benzoquinone, 2,3-dichloronaphthoquinone and chloranil) could be utilized for color formation. However, preliminary experiments showed that chloranil gives highly sensitive results than the other reagents.

At the optimum reaction conditions, the kinetic of the reaction was studied using initial rate method. It was found that the reaction was first order. As the used concentrations of ACD and chloranil greatly exceeded that of DSL, the reaction was considered a pseudo-first order reaction.

The sensitivity of both methods was investigated. The calculated limit of detection (LOD) of the initial rate and fixed time methods were 3.2 and 2.2 and µg mL^{-1}, respectively. These low values confirmed the high sensitivity of the proposed methods.

As mentioned before, the method was based on the reaction of the secondary amino group of DSL, consequently loratadine, the parent drug of DSL, does not contain this NH group (fig. 1), and was expected to not interfere with the analysis. Practically, interference studies show that the methods could selectively determine DSL without interference due to the presence of loratadine or common table excipients.

The obtained percentage recovery for the analysis of DSL was within the acceptable range and the % SD of the results was below 2%. This is an indication for the good accuracy and precision of the developed procedures. The accuracy and precision of the proposed methods were further evaluated by statistical comparison of their results (% recovery and standard deviation) with those of the reported method (Cağlar *et al.*, 2007). As shown in table 6, there is no significant difference between the analytical performance of the two methods with the reported one in respect to the accuracy (student's t-test) and the precision (variance ratio, F-test).

CONCLUSION

The present study describes two new fully validation kinetic methods for spectrophotometric analysis of DSL in bulk powder and commercial tablets. The proposed initial rate and fixed time methods have improved selectivity and do not require sophisticated instruments or expensive chemical reagents. The proposed initial rate could be easily applied within a very short time. Also, the methods are very simple because they do not need tedious extraction procedures. In addition, both methods have reasonable sensitivity which enables the analysis of low amounts of DSL. These advantages make the proposed methods are of great importance which encourage their application for the analysis of DSL in quality control laboratories.

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