SPECTROPHOTOMETRIC DETERMINATION OF CINNARIZINE THROUGH CHARGE-TRANSFER COMPLEX FORMATION WITH POLYNITRO COMPOUNDS

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Spectrophotometric studies were carried out to investigate the charge-transfer reaction between cinnarizine and dipicrylamine (DPA) or 2,6-dinitrophenol (DNP). Simple and rapid spectrophotometric methods were suggested for the determination of cinnarizine (CN) in pure and in its pharmaceutical dosage forms. The colored products are quantified spectrophotometrically at 430 and 440 nm for cinnarizine complexes with DPA in a mixture of 15% dioxane in dichloroethane (I) and 15% ethyl acetate in chloroform (II), respectively. On the other hand, cinnarizine complexes formed with DNP in acetonitrile (III) and in a mixture of 30% dichloroethane in ethyl alcohol (IV) were quantified at 460 and 430 nm, respectively. The proposed procedures were successfully utilized for the determination of the drug in its pharmaceutical formulation using standard addition and calibration curve methods. Beer's law was obeyed in the concentration ranges 1.00-22.00, 1.50-36.80, 1.50-36.80 and 3.60-36.80 µg mL-1 cinnarizine with molar absorptivities of 1.64×10^4, 1.43×10^4, 5.16×10^4 and 3.60×10^4 (L mol-1 cm-1), for CN-DPA in mixture (I), CN-DPA in mixture (II), CN-DNP in acetonitrile (III) and CN-DNP in mixture (IV), respectively. The relative standard deviations are less than 1.02%. The results of analysis of commercial tablets (Cinnarizine-25) showed that there is no interference from any excipient. Statistical comparison of the results was performed with regard to accuracy and precision using student's t-test and F-ratio at 95% confidence level.

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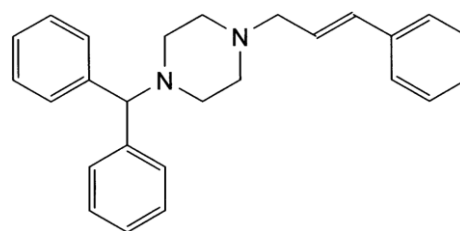[c]   National Organization for Drug Control and Research, Giza, Egypt

Introduction

Cinnarizine (CN), 1-(diphenylmethyl)-4-(3-phenyl-2-propenyl) piperazine (Fig. 1), is a piperazine derivative with antihistaminic, sedative, and calcium channel blocking activity. It is used for the symptomatic treatment of nausea and vertigo caused by Menier's disease and other vestibular disorders. It is also used for the prevention and treatment of motion sickness.[1,2] Several analytical methods have been reported for the determination of CN, either in pure form or in pharmaceutical preparations and biological fluids. These methods include non-aqueous titration,[3] spectrophotometry,[4–8] electrometry,[9,10] high-performance liquid chromatography (HPLC),[11–14] gas chromatography (GC),[15] titrimetry[16] and capillary electrophoresis.[17]

The molecular interactions between electron donors and acceptors are generally associated with the formation of intense colored charge-transfer complexes, which absorb radiation in the visible region. The photometric methods based on these interactions are usually simple and convenient because of the rapid formation of the complexes.

Figure 1. The structural formula of cinnarizine (CN).

The present article deals with the spectrophotometric determination of cinnarizine (CN) in pure form and in pharmaceutical preparations using dipicrylamine and 2,6-dintirophenol as electron acceptors.

Experimental

Materials and reagents

All reagents and chemicals used were of analytical grade, and the solvents were of spectroscopic grade. Dipicrylamine and 2,6-dinitrophenol chemicals were provided by BDH chemical company. Cinnarizine standard powder was kindly provided by Edwic Chemical Company, Egypt and used as received and cinnarizine-25 tablets (1 mg/tablet) produced by Adco Drug Company were obtained from local market.

Stock solution of dipicrylamine, 10^{-2} mol L^{-1}, was freshly prepared by dissolving 0.4392 mg in 100 mL of 15% dioxane in dichloroethane, mixture (I) and 15%

ethyl acetate in chloroform, mixture (II). For 2,6-dinitrophenol reagent, a 10^{-2} mol L^{-1} solution was prepared by dissolving 0.1841 mg in 100 mL acetonitrile (III) and in 30% dichloroethane in ethyl alcohol mixture (IV). These solutions are stable for 2 weeks. 10^{-2} mol L^{-1} cinnarizine stock solutions were prepared by dissolving 0.3685 mg in a 100 mL of mixture (I), mixture (II), acetonitrile (III) and mixture (IV). These solutions are stable for 2 weeks.

Preparation of solid complexes

A solution contains 1 mmol CN was prepared in solvent mixture (I), (II) or (IV) and pure acetoitrile (III), while the DPA solutions contain 1.0 and 0.5 mmol were prepared in solvent mixtures (I) and (II), respectively. For DNP, solutions contain 1.0 and 2.0 mmol were prepared in pure acetonitrile (III) and solvent mixture (IV), respectively. The reagent solutions were added individually with constant stirring and left until solvent evaporated and then washed by petroleum ether.

Apparatus

The spectral measurements were carried out using a single beam spectrophotometer model 22 Spectro Labomed, with quartz cells of 1-cm optical path length. Elemental analysis of the resulting reaction products was carried out by atomic CHN analyzer (Perkin Elmer model 2400) in the Micro analytical Center, Faculty of Science, Cairo University, Giza, Egypt.

General recommended procedures

Using dipicrylamine (DPA)

Aliquots of CN solution containing 1.0-36.8 µg were transferred into 10 mL measuring flasks. To each flask, 0.1 mL of DPA in mixture of 15% dioxane in dichloroethane or in mixture of 15% ethyl acetate in chloroform was added. The reaction mixture was mixed well and the volume was completed to 10 mL with the same solvent. The absorbance was measured against a blank solution, containing all additives except the drug, at 430 and 440 nm, in mixture (I) and mixture (II) respectively.

Using 2,6-dinitrophenol (DNP)

Different aliquots of the working standard solution containing 3.6-36.8 µg were transferred into 10 mL measuring flasks. To each flask, 0.2 mL of DNP in pure acetonitrile or in 30% dichloroethane in ethyl alcohol was added. The reaction mixture was completed to 10 mL with the same solvent. The absorbance was measured against a blank, containing all additives except the drug, at 460 and 430 nm, in acetonitrile (III) and mixture (IV) respectively.

Pharmaceutical analysis

20 tablets were weighed and finely ground, then a weight equivalent to one tablet was dissolved in 15% dioxane in dichloroethane or in pure acetonitrile and

transferred into a 25 mL measuring flask. The solution was shacked and filtered to remove any undissolved excipients substances. The resulting solution was subjected to analysis as described in the general procedures.

Determination of the stoichiometric ratio of the complexes

The stoichiometry of the charge-transfer complexes formed between cinnarizine and both PDA and DNP were studied by the aid of continuous variation method,[18] molar ratio method[19] and elemental analysis.

For the Job's method of continuous variation, master equimolar (2×10^{-4} mol L^{-1}) aqueous solutions of CN and DPA or DNP were prepared. Series of 10 mL portions of the master solutions of CN and DPA or DNP were made up comprising different complementary proportions (0:10, 1:9, . . ., 9:1, 10:0) in 10 mL calibrated flasks. For the molar ratio method, the concentration of CN was kept constant by adding 0.2 mL of 2×10^{-4} mol L^{-1} of CN while the concentration of the acceptors (DPA or DNP) was changed over a wide range. All solutions were further manipulated as described under the general recommended procedures. The solid charge-transfer complexes were subjected to elemental analysis and the results are presented in Table 1.

Table 1. Elemental analysis of the formed CN-DPA and CN-DNP charge-transfer complexes.

Formed complex	Molar ratio	(Found)% Calculated%		
		C	**H**	**N**
CN-DPA[a]	1:1	(55.10)	(3.78)	(14.22)
		56.40	3.83	15.59
CN-DPA[b]	2:1	(65.91)	(5.95)	(11.81)
		65.29	5.18	13.09
CN-DNP[c]	1:1	(69.99)	(5.95)	(10.04)
		69.48	5.60	10.13
CN-DNP[d]	1:2	(56.50)	(3.75)	(11.93)
		61.80	4.62	11.40

[a]15% Dioxane in dichloroethane; [b]15% Ethyl acetate in chloroform; [c]Acetonitrile; [d]30% Dichloroethane in ethyl alcohol.

Results and discussion

The charge-transfer complex formation reactions are based on the π-acceptors that react with the basic nitrogenous compounds as donors to form charge-transfer complexes or radical anions according to the polarity of the solvent used.

Absorption spectra

The absorption spectra of CN-DPA and CN-DNP complexes were scanned in different solvents (acetonitrile, 1,4-dioxane, ethyl acetate 1,2-dichloroethane, and their mixtures with ethyl alcohol and chloroform). The results showed that a mixture of 15% (v/v) 1,4-dioxane in 1,2-dichloroethane and 15% ethyl acetate in chloroform are

the most suitable solvents for CN-DPA while pure acetonitrile and 30% dichloroethane in ethyl alcohol are the most suitable solvents for CN-DNP complexes. High molar absorptivity values (1.64×10^4, 5.16×10^3 L mol^{-1} cm^{-1}) for CN-DPA in mixture (I) and CN-DNP in acetonitrile have been obtained. The reaction between CN and DPA or DNP was performed and the absorption spectra of the products were recorded against reagent blank (Figs. 2 and 3). It was found that intense yellow-colored complexes, exhibiting λmax at 430 and 460 nm have been obtained for CN-DPA and CN-DNP in mixture (I) and acetonitrile (III), respectively.
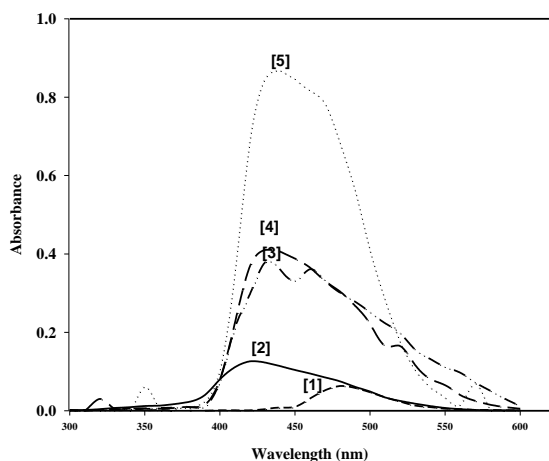

Figure 2. Absorption spectra for CN-DPA complexes in different solvents, [CN] =5x10^{-5} mol L^{-1}; at room temperature. (1) 15% (v/v) Dioxane in ethyl alcohol, (2) Dioxane, (3) Ethyl acetate, (4) 15% (v/v) Ethyl acetate in chloroform, and (5) 15% (v/v) Dioxane in dichloroethane.

Spectral characteristics of the CT complex

The oscillator strength (*f*), which is a dimensionless quantity used to express the transition probability of the CT band,[20] and the transition dipole moment (μ$_{EN}$) of the CT complexes[21] were calculated from the following expressions:

$$f = 4,32 \times 10^{-9} \left[\varepsilon_{max} \Delta \upsilon_{1/2} \right] \qquad (1)$$

$$\mu_{EN} = 0,0958 \left[\frac{\varepsilon_{max} \Delta \upsilon_{1/2}}{\upsilon_{max}} \right] \qquad (2)$$

where

Δν$_{1/2}$ is the band width at half absorbance and

ε$_{max}$ and ν$_{max}$ are the molar absorptivity and wave number at the absorption maximum of the complex, respectively.

In these equations, ν$_{max}$ has been expressed in cm^{-1}. The relative low values of *f* indicate a weak interaction between the donors–acceptor pairs.[22] The R_N is the resonance energy of the complex in the ground state,

which is obviously a contributing factor to the stability constant of the complex (a ground state property), can be determined by the following equation[23]:

$$\varepsilon_{max} = \frac{7.7 \times 10^4}{h\upsilon_{CT} / |R_N| - 3.5} \qquad (3)$$

where

ν$_{CT}$ represents the frequency at maximum absorbance of the CT band.

Resonance energies for the CT complexes of CN with different electron acceptors, Table. 2, indicate that the probability and dipole moment of transition in case of CN-DPA in mixtures (I) and (II) are higher than those of CN-DNP in acetonitrile (III) and mixture (IV).
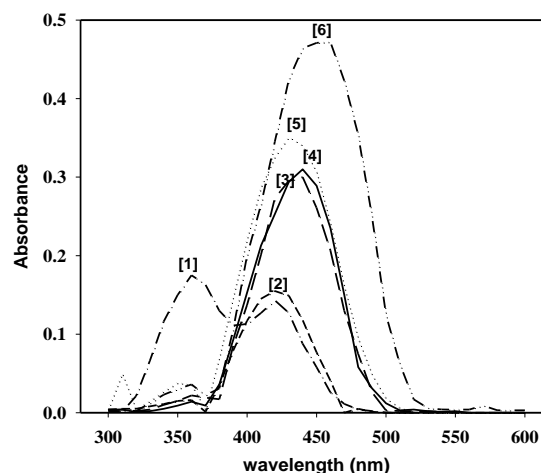

Figure 3. Absorption spectra for CN-DNP complexes in different solvents, [CN]=1x10^{-4} mol L^{-1}; at room temperature, (1) 30% (v/v) Acetonitrile in dichloroethane, (2) 30% (v/v) dichloroethane in chloroform, (3) 30% (v/v) Acetonitrile in dichloromethane, (4) Dichloroethane, (5) 30% (v/v) Dichloroethane in ethyl alcohol and (6) Acetonitrile.

The probability of transition is the lowest for CN-DNP in mixture (IV), also values of resonance energy show that the stability of CN-DPA complexes in both mixtures (I) and (II) are higher than the stability of CN-DNP complexes in acetonitrile (III) and mixture (IV), and the CN-DNP complexes prepared in mixture (IV) exhibit the lowest stability.

Optimization of reaction variables

The spectrophotometric properties of the colored species as well as the different parameters affecting the color development were extensively studied to determine the optimum conditions for the analytical determination of the drug. The reactions were studied as a function of the volume of the reagents, nature of the solvents, effect of temperature and effect of time on the formation of the complex. Stability of colors and the molar ratio were also studied.

Table 2. Spectral properties obtained for CN-DPA and CN-DNP charge-transfer complexes.

Parameter	Method			
	DPA		DNP	
	I	II	III	IV
λ_{CT}, nm	430	440	460	430
$h\nu_{CT}$, eV	4.62	4.51	4.30	4.62
ε_{max}, L mol^{-1}cm^{-1}	1.43×10^4	1.64×10^4	5.16×10^3	3.60×10^3
Stoichiometry (drug:reagent)	1:1	2:1	1:1	1:2
R_N (eV)	51.77×10^{-2}	55.08×10^{-2}	23.34×10^{-2}	18.56×10^{-2}
F	6.18×10^{-10}	7.08×10^{-10}	2.01×10^{-10}	1.09×10^{-10}
$\mu_{EN\ Depye}$	2.38×10^{-4}	2.57×10^{-4}	1.40×10^{-4}	9.97×10^{-4}

λ_{CT} - absorption maxima, ε_{max} -molar absorptivity, R_N - the resonance energy of the complex in the ground stat stoichiometry, f - the oscillator strength, μ_{EN} - the transition dipole moment. I-15% dioxane :dichloroethane; II-15% ethyl acetate :chloroform; III-Acetonitrile; IV-30% dichloroethane: ethyl alc.

Effect of diluting solvent

Upon diluting the reaction solution with water, turbid solution with low color intensity was obtained indicating the incomplete solubility of CN-DPA or CN-DNP complexes in water. Therefore, water could not be used for dilution. In order to select the most appropriate solvent for diluting the reaction solution, different solvents were tested e.g. 1,4-dioxane, ethyl acetate, chloroform, acetone, acetonitrile, and mixture of these solvents with different proportions. The highest absorbance values were obtained when mixture of 15% dioxane in dichloroethane and pure acetonitrile were used as diluting solvents for CN-DPA and CN-DNP, respectively, Figs. 2 and 3.

Effect of reagent concentration

The effect of the reagents concentration on the intensity of the color production was studied by adding various amounts of the reagents to a fixed concentration of CN (1.0×10^{-4} mol L^{-1}). The results showed that, in case of using DPA in both mixtures (I) and (II), maximum and constant absorbance values were obtained using reagent concentration at least three times that of CN. While, in using DNP in acetonitrile (III) and mixture (IV), the maximum reproducible absorbances were obtained by using reagent concentration at least six times that of CN, Fig. 4.

Effect of temperature

The effect of temperature on the reaction was studied by carrying out the reaction at temperatures between 25-70 °C. The results revealed that complete color development is obtained at room temperature up to 30 °C and absorbance values decrease with increasing the temperature of the reaction medium. The decrease in the absorbance values may be attributed to the instability of the reaction product at high temperature.
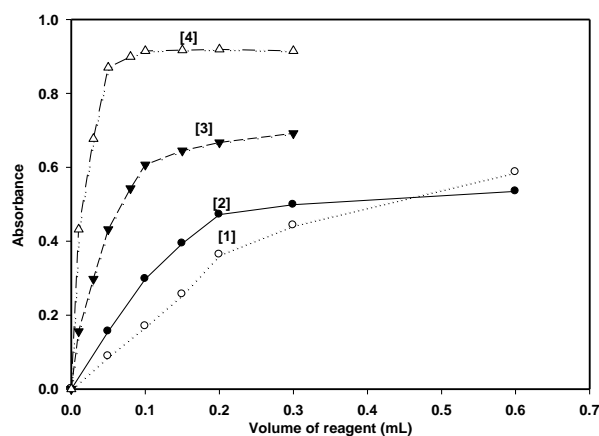

Figure 4. The effect of reagent concentration on the formation of the complexes. [CN] = 0.1×10^{-3} mol L^{-1}, (1) DNP in 30% (v/v) dichloroethane in ethyl alcohol, 430 nm, (2) DNP in acetonitrile, 460 nm, (3) DPA in 15% (v/v) ethyl acetate inchloroform, 430 nm, and (4) DPA in 15% (v/v) dioxane in dichloroethane, 440 nm.

Effect of time

Under the above mentioned optimum conditions, the reaction between CN and DPA or DNP was completed immediately at 25±5 °C, and the absorbance no longer changes for standing up to 24 hours. The effect of time on the stability of the product was studied by following the absorbance intensity of the reaction solution (after dilution) at different time intervals. It was found that the absorbance of the product remains stable for at least 24 hours. This allows the processing of a large number of samples, and their comfortable measurements with convenience.

Stoichiometry of the reaction

Under the optimum conditions, the stoichiometry of the formed complexes was investigated using Job's continuous variation and molar ratio methods.

The results indicated that CN-DPA complex in mixture (I) and CN-DNP complex in acetonitrile (III) were formed in the ratio of 1:1. On the other hand, the ratio of the complexes CN-DNP formed in mixture (IV) and CN-DPA formed in mixture (II) are 1:2 and 2:1 drug : reagent ratio, respectively. The results obtained were confirmed by elemental analysis for the solid charge-transfer complexes, Table 1. The results are shown in Figs. 5 and 6.
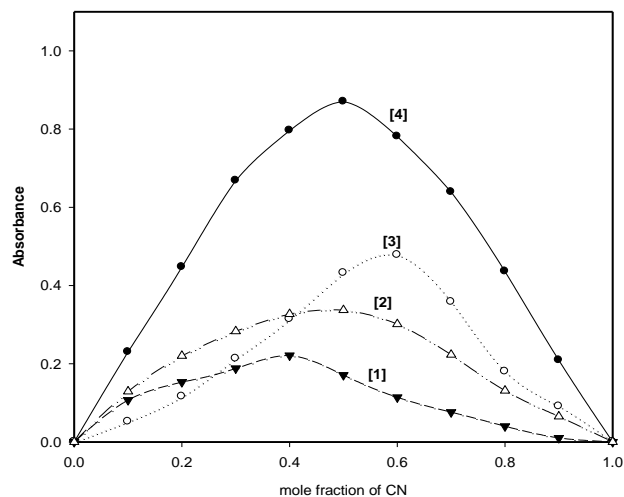

Figure 5. Continuous variation plots of CN-DPA and CN-DNP complexes; total molar concentration = 2×10^{-4} mol L-1. (1) DNP in 30% (v/v) dichloroethane in ethyl alcohol, 430 nm, (2) DNP in acetonitrile, 460 nm, (3) DPA in 15 % (v/v) ethyl acetate in chloroform, 430 nm, and (4) DPA in 15% (v/v) dioxane in dichloroethane, 440 nm.

Figure 6. Molar ratio plots of CN-DPA and CN-DNP complexes. (1) DNP in 30% (v/v) dichloroethane in ethyl alcohol, 430 nm, (2) DNP in acetonitrile, 460 nm, (3) DPA in 15% (v/v) ethyl acetate in chloroform, 430 nm, and (4) DPA in 15% (v/v) dioxane in dichloroethane, 440 nm.
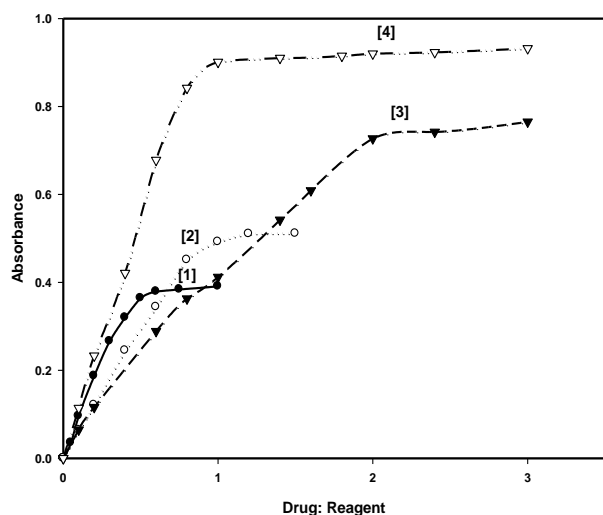
Association constants and standard free energy change

The association constants were calculated for 1:1 complexes using Benesi-Hildebrand[24] equation (4):

$$\frac{\left[A_0\right]}{\left[A_\lambda^{AD}\right]} = \frac{1}{\varepsilon_\lambda^{AD}} + \frac{1}{K_C^{AD}\,\varepsilon_\lambda^{AD}} \times \frac{1}{\left[D_0\right]} \qquad (4)$$

where

A_λ^{AD} is the absorbance of the charge-transfer band,

ε_λ^{AD} is the molar absorptivity of the complex,

$[A_0]$ and $[D_0]$ represent the initial acceptor and donor concentrations, respectively, and

K_C^{AD} is the association constant for the equilibrium.

Benesi-Hildebrand equations for 1:1 complexes of CN with DPA and DNP can be represented by equations 5 and 6, respectively.

$$\frac{[A_O]}{A_\lambda^{AD}} = (4.9 \times 10^{-5}) + (0.56 \times 10^{-8}) \times \frac{1}{[D_O]} \qquad (5)$$

$$\frac{[A_O]}{A_\lambda^{AD}} = (1.4 \times 10^{-4}) + (0.27 \times 10^{-7}) \frac{1}{[D_O]} \qquad (6)$$

The formation constants (K_f) of CN-DNP 1:2 ion-associate complexes formed in mixture (IV) and 2:1 CN-DPA complexes formed in mixture (II), were calculated, (Table 3) from the continuous variation data using the following equation[25]:

$$K_f = \frac{\left[\dfrac{A}{A_m}\right]}{\left[\dfrac{1-A}{A_m}\right]^{n+1}} C^n n^n \qquad (7)$$

where

A_m is the maximum absorbance obtained from Job's continuous variation curve,

A is the absorbance corresponding to the intersection of the two tangents of the continuous variation curve,

C is the concentration corresponding to maximum absorbance,

n is the ratio of the drug in the reaction product.

The standard free energy change, $\Delta G°$ of the complex formation is related to the formation constant by the following equation [26]:

$$\Delta G^0 = -2.303 RT \lg K \qquad (8)$$

where

R is the universal gas constant (8.314 J mol^{-1} K^{-1}),

T is the absolute temperature and

K is the formation constant of the CT complex.

The obtained values of, ε_λ^{AD}, K_f and $\Delta G°$ for CN charge-transfer complexes are presented in Table 3. It is clear from the obtained results that, these charge-transfer reactions are spontaneous as indicated by the negative values of $\Delta G°$ and the high values of K_f.

Validation of the methods

Calibration and sensitivity

Calibration curves for the determination of CN by its reaction with both DPA and DNP were constructed by plotting the absorbances as a function of the corresponding concentrations. The regression equations for the results were $A=0.008+0.0425C$ ($r=0.9970$) and $A=0.0031+0.0371C$ ($r=0.9996$) with molar absorptivities (ε) 1.64×10^4 and 1.43×10^4 L mol^{-1} cm^{-1} for DPA-CN complexes prepared in mixture (I) and mixture (II), respectively. The $A = -0.0071 + 0.0101C$ ($r = 0.9954$) and $A = 0.0033 + 0.0143C$ ($r=0.9996$) with molar absorptivities (ε) 5.16×10^3 and 3.60×10^3 L mol^{-1} cm^{-1} for DNP-CN complexes prepared in acetonitrile (III) and mixture (IV) were found, respectively, where A is the absorbance at λ_{max} for each method, C is the concentration of CN in μg mL^{-1}, and r is the correlation coefficient. Optimization of the linear range (Ringbom) was evaluated by plotting the percentage transmittance versus the logarithm of concentration (μg mL^{-1}). The limit of detection (*LOD*) and limit of quantification (*LOQ*) were determined according to the International Conference of Harmonization (ICH) guidelines for validation of analytical procedures.[27] The following formula was used: *LOD* or *LOQ* =K SD_a, where $K=3$ for *LOD* and 10 for *LOQ*, and SD_a is the standard deviation of the intercept. The values of *LOD* were 0.72 and 0.40, *LOQ* were 2.23 and 1.48 for CN-DPA in case of using 15% dioxane in dichloroethane and 15% ethyl acetate in chloroform, respectively. For CN-DNP *LOD* values were 0.54 and 2.08, and *LOQ* values were 2.35 and 5.27 in case of using acetonitrile and 30% dichloroethane in ethyl alcohol, respectively, Table 3.

Reproducibility

The reproducibility of the proposed methods was evaluated by replicate analysis of five separate solutions of the working standard at different concentration levels. The method gave satisfactory results; RSD did not exceed 2% indicating the good reproducibility of the proposed method. This precision level is adequate for routine analysis of the investigated drug in quality control laboratories.

Accuracy and interference liabilities

The accuracy of the proposed method was evaluated by the recovery studies for added concentrations. The recovery values were 98.77±0.9 – 100.00±1.1%, Table 4, indicating the accuracy of the proposed method. Interference liabilities of common excipients: starch, glucose, lactose, talc, and magnesium stearate were not necessary to be tested before proceeding with the analysis of CN in its dosage forms; the drug is extracted firstly by dissolving it in the appropriate solvent then filtering. The excipients are insoluble in such solvents.

Table 3. Analytical parameters for CT complexes of cinnarizine with DPA and DNP.

Parameter	Method			
	DPA		DNP	
	I	II	III	IV
λ_{max}, nm	430	440	460	430
Beer's law limits, μg mL^{-1}	1.00-22.00	1.50-36.80	1.50-36.80	3.60-36.80
Molar absorbitivity, L mol^{-1} cm^{-1}	1.64×10^4	1.43×10^4	5.16×10^3	3.60×10^3
Sandell sensitivity, μg cm^{-2}	0.0020	0.0269	0.0684	1.1111
Ringbom range, μg mL^{-1}	3.00-22.00	3.00-22.00	7.00-26.00	7.00-26.00
Slope	0.0496	0.0371	0.01446	0.0009
Intercept	0.0330	0.0007	0.0002	0.0001
LOD, μg mL^{-1}	0.72	0.40	0.54	2.08
LOQ, μg mL^{-1}	2.23	1.48	2.35	5.27
Correlation coefficient, r	0.9970	0.9996	0.9954	0.9996
K_c^{AD} (L mol^{-1})	4.20×10^3	--	5.36×10^3	--
K_f^{AD}(L mol^{-1})	3.15×10^4	1.05×10^9	1.46×10^4	1.75×10^4
$\Delta G°$, k cal mol^{-1}	-2.59×10^4	-4.63×10^4	-2.40×10^4	-2.44×10^3

LOD: Limit of detection; *LOQ*: Limit of quantification; *n*=5; K_c^{AD}: the association constants calculated from Benesi-Hildebraned equation; K_f: the formation constants calculated from the continuous variation equation; I. 15% dioxane in dichloroethane; II. 15% ethyl acetate in chloroform; III. Acetonitrile; IV. 30% dichloroethane in ethyl alcohol.

Table 4. Recovery studies for determination of CN in standard solutions and pharmaceutical formulation applying calibration curve and standard addition methods.

Reagent	Proposed methods			Recovery±RSD%[a]	*t*-test[b]	*F*-test[b]	Reference method[28]
	Taken, µg	Added, µg	Found, µg				Recovery±RSD%
DPA							
Pure CN in 15% dioxane in dichloroethane							
	5.52	5.52	10.97	99.36±1.20	0.35	6.15	99.09±1.63
	3.68	--	3.67	99.72±0.92	2.25	1.82	99.80±2.01
	5.52	--	5.51	99.81±1.61	1.85	3.56	99.74±1.00
Cinnarizine-25 in 15% dioxane in dichloroethane							
	5.50	5.52	10.98	99.45±0.67	0.69	1.45	99.23±1.05
	18.00	--	17.78	98.77±0.90	2.25	0.96	99.06±0.69
Pure CN in 15% ethyl acetate in chloroform							
	5.52	5.52	10.97	99.36±1.44	1.32	2.09	
	3.68	--	3.66	99.45±1.63	1.51	3.11	
	5.52	--	5.49	99.40±1.81	1.02	2.15	
DNP							
Pure CN in acetonitrile							
	5.52	5.52	11.03	99.90±1.20	1.02	6.34	
	3.68	--	3.65	99.18±0.90	0.63	3.00	
	5.52	--	5.50	99.63±1.81	0.95	4.12	
Cinnarizine-25 in acetonitrile							
	5.50	5.52	10.96	99.27±0.51	1.12	1.24	
	18.00	--	17.84	99.11±1.20	0.83	1.00	
Pure CN in 30% dichloroethane in ethyl alcohol							
	5.52	5.52	11.00	99.63±0.12	0.98	2.51	
	3.68	--	3.68	100.00±1.11	1.01	4.03	
	5.52	--	5.50	99.63±1.80	1.00	3.52	

a. Mean ± relative standard deviation of five replicate analyses; b. Theoretical values for t- and F-tests are 2.77 and 6.39, respectively, for four degree of freedom and 95% confidence limit.

Robustness

Robustness was examined by evaluating the influence of small variation in the method variables on its analytical performance. In these experiments, the absorbances of the formed complex were determined at 5 minutes and at 15 minutes of the reaction time whereas the other conditions were kept unchanged. Similarly the temperature of the reaction was changed and the absorbance of the formed complex determined at 25 °C and 35 °C. The recovery percentage was calculated each time. It was found that small variation in the method variables did not significantly affect the procedures; recovery values were 97.90±1.95 – 100.50±1.02%, Table 5. This indicates the reliability of the proposed method during its routine application for the determination of cinnarizine.

Applications

The proposed methods were applied for the determination of Cinnarizine-25 tablets. Five replicate determinations were performed. Satisfactory results were obtained, Table 4. Moreover, to check the validity of the proposed methods, the standard addition method was applied by adding standard cinnarizine solution to a previously analyzed tablet solution, and the recovery was calculated. The results of analysis of commercial dosage forms and the recovery study of the standard addition method of the cited drug reveals that the suggested methods give high recovery values.

For the sake of determining the accuracy and precision of the suggested methods, they were compared with a previously reported method.[28] Statistical comparison of the results was performed with regard to accuracy and precision using student's t-test and F-test at 95% confidence level, Table 6. The results showed that there is no significant difference between the reported[28] and proposed methods with regard to accuracy and precision.

Conclusion

The suggested methods for the determination of cinnarizine by using dipicrylamine and 2,6-dinitrophenol have the advantages of being simple, accurate, sensitive, rapid and suitable for routine analysis in control laboratories. These methods utilize a single step reaction, and no substantial differences among the proposed methods arise from analysis of the experimental results.

Table 5. Influence of small variations in the assay conditions on the analytical performance of the proposed spectrophotometric method for determination of CN using DPA and DNP.

Method[b]	Recovery% ±SD[a]			
	Temperature		Time	
	25 °C	35 °C	5 minutes	15 minutes
DPA method				
I	99.15±1.02	100.10±0.84	100.02±0.99	100.50±1.02
II	98.20±0.98	98.15±1.01	100.50±1.02	98.21±0.96
DNP method				
III	97.90±1.95	97.90±1.95	97.90±1.95	97.90±1.95
IV	98.80±1.35	99.01±0.89	98.23±1.01	99.56±0.99

a Values are the mean of 3 determinations; b At the optimum conditions; I: 15% dioxane in dichloroethane; II: 15% ethyl acetate in chloroform; III: Acetonitrile; IV: 30% dichloroethane in ethyl alcohol.

Table 6. Statistical tests for accuracy and precision of the proposed methods on samples of pure CN with DPA and DNP, at 95% confidence level and $\upsilon=4$.

Parameters	DPA in 15% dioxane in dichloroethane	DNP in pure acetonitril
SD%	0.64%	0.66%
Coefficient of variation	0.71%	2.53%
RSD%[a]	0.32%	1.02%
F-test[b]	6.15	6.34
t-test[b]	0.35	1.02

a.Relative standard deviation of five replicate analyses; b. Theoretical values for t- and F-tests are 2.776 and 6.39, respectively, for four degree of freedom and 95% confidence level.

The complex formation of CN with DPA in mixture of 15% 1,4-dioxane in 1,2-dichloroethane is more sensitive than the other methods due to the higher molar absorptivity. These methods can be used precisely and accurately with high recovery ranged between 99.18±0.90 and 100.00±1.11, for the spectrophotometric determination of cinnarizine in the range of 1.00 - 36.80 µg both in pure forms and in its pharmaceutical formulations.

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