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Spectrophotometric Determination of Nizatidine and Ranitidine Through Charge Transfer Complex Formation

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Two Spectrophotometric procedures are presented for the determination of two commonly used H2-receptor antagonists, nizatidine (I) and ranitidine hydrochloride (II). The methods are based mainly on charge transfer complexation reaction of these drugs with either *p*-chloranilic acid (*p*-CA) or 2, 3 dichloro-5, 6-dicyanoquinone (DDQ). The produced colored products are quantified spectrophotometrically at 515 and 467 nm in chloranilic acid and DDQ methods, respectively. The molar ratios for the reaction products and the optimum assay conditions were studied. The methods determine the cited drugs in concentration ranges of 20-200 and 20-160 μg/mL for nizatidine and ranges of 20-240 and 20-140 μg/mL for ranitidine with chloranilic acid and DDQ methods, respectively. A more detailed investigation of the complexes formed was made with respect to their composition, association constant, molar absorptivity and free energy change. The proposed procedures were successfully utilized in the determination of the drugs in pharmaceutical preparations. The standard addition method was applied by adding nizatidine and ranitidine to the previously analyzed tablets or capsules. The recovery of each drug was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drug. The results of analysis of commercial tablets and the recovery study (standard addition method) of the cited drugs suggested that there is no interference from any excipients, which are present in tablets or capsules. 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. There is no significant difference between the reported and proposed methods with regard to accuracy and precision.

Key words: Ranitidine, Nizatidine, Charge transfer complexation

INTRODUCTION

Nizatidine (I) and ranitidine (II) (Scheme 1) are specific potent H2-receptor antagonists. Unlike cimetidine, which contains an imidazole ring, nizatidine and ranitidine have a thiazole and a furan ring, respectively. These drugs are more potent than cimetidine in inhibition of gastric acid secretion induced by various stimuli and they lack cimetidine's *anti*-androgenic and hepatic microsomal enzyme inhibiting effects. Nizatidine has been determined in pharmaceutical preparations using spectrophotometry (Walash *et al.*, 2002), potentiometric titration (Koricanac *et al.*, 1995), HPTLC (Kelani *et al.*, 2002) and HPLC (Mathew *et al.*, 1993).

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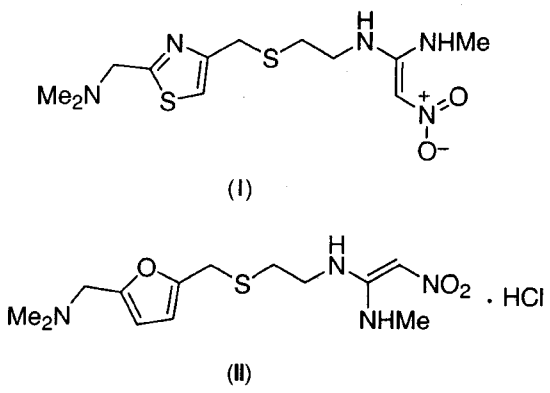
For nizatidine and ranitidine, the authentic crude drug and the pharmaceutical preparations are determined in the USP XXV using an HPLC method (The United States Pharmacopoeia, The National Formulary USP XXVNFXX, United States Pharmacopoeial convention Inc. 2002).

Various methods have been reported for the determination of ranitidine as a pure drug and in pharmaceutical preparations. These methods include colorimetric (Al-Ghannam *et al.*, 2002), spectrophotometric (Bourne and Burgess, 1995), potentiometric (Nikolic *et al.*, 1995) and HPLC methods (Rustum, 1988).

Searching the published methods for the determination of the cited drugs shows that the charge transfer complexation technique has been previously used, but with a different reagent (Al-Ghannam *et al.*, 2002). The reagents used here have been previously used with different drugs (Du *et al.*, 2003); consequently the present work describes new colorimetric methods, which are cheaper, simpler and less time consuming than the official and the published



(I)

(II)

Scheme 1. Compound I and II

chromatographic methods for nizatidine and ranitidine.

In addition, the spectrophotometric technique continues to be the most preferred method for the assay of different classes of drugs in pure, pharmaceutical formulations and in biological samples (Nagaralli *et al.*, 2002), because of its simplicity, reasonable sensitivity and significant economical advantages. The spectrophotometric methods reported for the assay of the cited drugs are not satisfactory for routine quality assurance for one or another reason. Some of these methods involve heating (Hassan and Belal, 2002) or tedious treatments or requirement of more time for analysis (Guvener, 1986).

Hence, a modest attempt has been made to develop a simple, fast and accurate spectrophotometric method for the determination of the cited drugs in pure form and in pharmaceutical formulations.

MATERIALS AND METHODS

Instrument

A Shimadzu (Model 1601PC) UV-Visible spectrophotometer (Shimadzu, Koyoto, Japan) was used to measure the absorbance at 515 nm and 467 nm.

Samples

Nizatidine and ranitidine hydrochloride standards were kindly provided by South Egypt Drug Industry Co. (SEDICO), Egypt and used as received.

Nizatine capsules (150 mg, HI PHARM, Industrial zone, Elobour City, Cairo, Egypt) and zantac tablets (150 mg, Glaxo Wellcome Egypt S.A. E. Elsalam City, Cairo, Egypt) were obtained from the market.

Reagents and chemicals

All reagents and chemicals used were of analytical grade and the solvents were of spectroscopic grade.

1. Chloranilic acid (Aldrich), 0.35% w/v solution in acetonitrile.
2. DDQ (Aldrich), 0.4% w/v solution in acetone.

Standard solutions

Stock standard solutions for chloranilic acid and DDQ methods

Nizatidine stock solution:

It was prepared by dissolving 200 mg of nizatidine in acetonitrile (ρ-CA method) or in acetone (DDQ method) in 100 mL volumetric flask and diluting to volume with the same solvent. These stock solutions were used as it is for the corresponding methods.

Ranitidine stock solution:

It was prepared by dissolving an accurately weighed amount of (II) equivalent to 400 mg of ranitidine base in 20 ml distilled water. This solution was quantitatively transferred into a 125-mL separating funnel, rendered alkaline with ammonia solution and extracted with 4×20 ml chloroform. The extract was washed with 20 mL water, filtered through anhydrous sodium sulphate into a 100-mL volumetric flask and made up to volume using chloroform (4 mg/mL).

Working standard solutions

M1 (ρ-CA method):

Evaporate 50 mL of the stock solution of ranitidine, and dissolve the residue in 10 mL acetonitrile. Transfer quantitatively into a 100 mL volumetric flask and make up to volume with the same solvent (2 mg/mL of ranitidine).

M2 (DDQ method):

Evaporate 25 mL of stock solution of ranitidine, dissolve the residue in 25 mL acetone, transfer quantitatively into a 50-mL volumetric flask and make up to volume with the same solvent (2 mg/mL).

Procedures

Construction of calibration curves

Different aliquots of working standard solutions were transferred into separate 10-mL volumetric flasks; to each flask, 1.2 mL of ρ-CA or 2 mL of DDQ were added. The reaction mixture was mixed well and the volume was completed to 10 mL with acetonitrile (M1) or acetone (M2). The absorbance was measured against a blank prepared in the same manner except addition of the drug at 515 and 467 nm for M1 and M2, respectively, as shown in Fig. 1 and Fig. 2.

Analysis of dosage forms

Nizatine capsules:

10 nizatidine capsules were accurately weighed; the contents of the capsules were removed as completely as possible and mixed. The capsule shells were cleaned and accurately weighed, and the net weight of the capsule

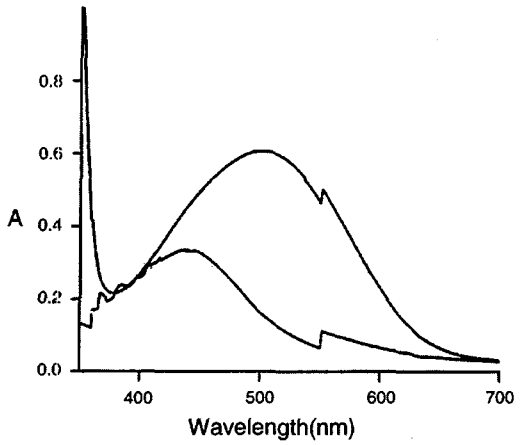


Fig. 1. Absorption spectrum of nizatidine and ranitidine-chloranilic acid charge transfer complexes (__), reagent blank (----).

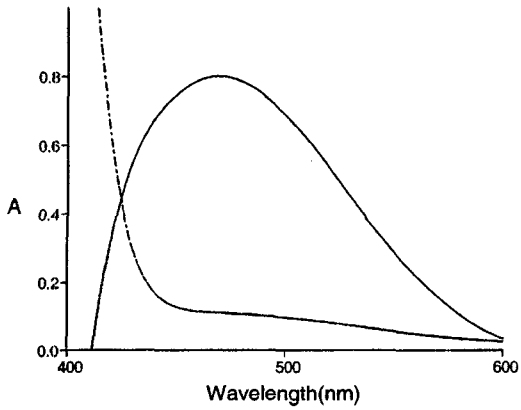


Fig. 2. Absorption spectrum of nizatidine and ranitidine-DDQ charge transfer complexes (__), reagent blank (----).

contents was calculated. An accurately weighed portion of the mixed capsule contents equivalent to 200 mg nizatidine was transferred into 100 mL volumetric flask. 50 mL portion of acetonitrile (*p*-CA method) or acetone (DDQ method) was added. The mixture was sonicated for few minutes, diluted to volume with the same solvent, mixed and filtered. A portion of the filtrate was transferred into 10 mL volumetric flask and used for the determination of the drug content following the procedures described above.

Zantac tablets:

An accurately weighed amount of the finely powdered tablets equivalent to 400 mg of the drug base (II) was transferred into a 125-mL separating funnel containing 20 mL distilled water and rendered alkaline with ammonia. Extract the drug base as under (Standard solutions). Prepare working test solution of the same concentration as that mentioned under working standard solutions. A portion of this solution was transferred into 10 mL volumetric flask and used for the determination of the drug content following the procedures described above.

Stoichiometric relationship

Job's method of continuous variation was used (A. Martin, 1993) Fig. 3, 4.

Association constant and free energy change for DDQ method

Serial volumes of 1-5 mL of 6.03, 6.36×10^{-3} M solutions of nizatidine and ranitidine in acetone were transferred into 10-mL standard flasks. To each flask, 2 mL of 1×10^{-3} M DDQ in acetone was added and continued as directed under construction of calibration curves.

RESULTS AND DISCUSSION

The charge transfer complex forming reactions are based on that π acceptors react with the basic nitrogenous compounds as *n*-donors to form charge transfer complexes or radical anions according to the polarity of the solvent used. Hence *p*-CA and DDQ used in the proposed methods are selective reagents for the determination of the cited drugs.

Some hydrochloride salts of amines do not react with π acceptors. To determine amine-HCl, it is necessary to first neutralize the hydrochloride and then extract the amine into a non-aqueous solvent.

Ibrahim et al described an extracting procedure for the neutralized amine into chloroform and then evaporating the chloroform.

Suggested mechanism for the reaction of DDQ and p-CA with the cited drugs

For *p*-CA method (M1)

Some of the literature reveals that the reaction of *p-CA* (HA) with certain basic nitrogenous compounds (B) is probably due to charge transfer complexation reaction (Agarwal and ElSayed, 1981). Also other literature explains the reaction to be first a proton transfer from *p-CA* to the basic center of the drug. Dissociation of the obtained ion pair salt was enhanced in the highly polar solvent acetonitrile to give the purple anion form of *p-CA* (HA). This was confirmed by IR spectrum, electron spin resonance and NMR (Abdel-Hamid *et al.*,1985).

For DDQ method

The mechanism of the reaction produced by the proposed method (DDQ method) depends on the formation of an original donor-acceptor (DA) complex through the interaction between tertiary amine moiety of the selected drugs as n-electron donor and DDQ as π acceptor. The dissociation of DA complex was promoted by the high ionizing power of the solvent acetone where complete

electron transfer from the donor to the acceptor moiety takes place. This is followed by formation of the DDQ radical anions as a predominant chromogen (Abdel Hamid et al., 1985) (Scheme 2).

The stoichiometry of the reactions was studied by Job's method. It was found that the ratio was 1:1 (donor/acceptor) for (I) and (II) with both *p-CA* and DDQ reagents (Fig. 3, 4).

The spectrophotometric properties of the color species formed with *p-CA* and DDQ as well as the different parameters affecting the color development were extensively studied to determine the optimum conditions for the assay procedures. The reactions were studied as a function of the volume of the reagents, nature of the solvents, and effect of temperature on the formation of the complex (Table I). Stability of colors and the molar ratio were also studied.

The absorbances of (I) and (II) were used to calculate the association constant using the Benesi-Hildebrandt equation which depends on the experimental condition that one of the two component species should be present in large excess, so that its concentration is virtually unaltered on formation of the complex. Where [A°] and [D°] are the total concentrations of the interacting species, A^{AD} and ξ^{AD} are the absorbance and molar absorptivity of the complex at the specified ëmax and Kc^{AD} is the association constant of the complex. A line was obtained (Fig. 5) when plotting the values of $[A^{\circ}]/A^{AD}$ *vs.*$1/[D^{\circ}]$ according to the following equations:

$$[A^{\circ}]/ A^{AD} = 5.47 \times 10^{4} + 1/[D^{\circ}]\ (8.15\times10^{-9}) \quad (1)$$

$$[A^{\circ}]/ A^{AD} = 4.84 \times 10^{4} + 1/ [D^{\circ}]\ (8.63 \times 10^{-9}) \quad (2)$$

From Eqs. (1) and (2), the association constants K, the molar absorptivities ξ^{AD} and free energy change ΔG° for the cited drugs were calculated (Table III).

However, it should be noted that the value of ξ^{AD}, which is the molar absorptivity of the complex itself, should not

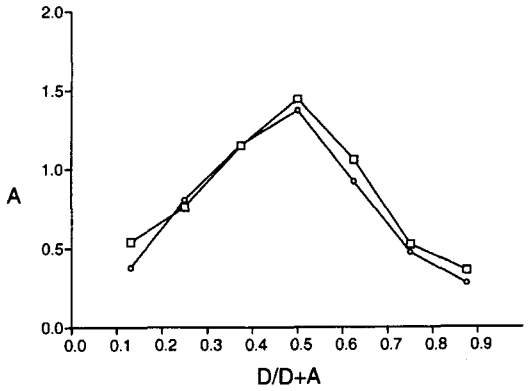
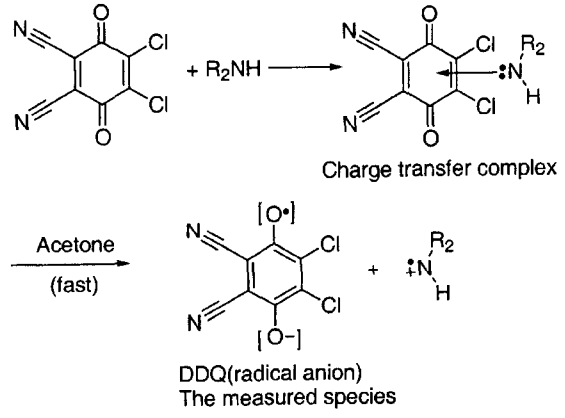


Fig. 3. Continuous variation plot for : Nizatidine(D)-Chloranilic acid(A) complex (-○-), 6.03×10^{-3} M and ranitidine(D)-Chloranilic acid(A) complex (-□-), 6.36×10^{-3} M



Scheme 2.

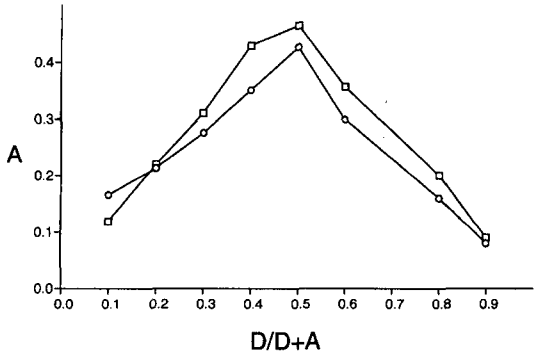


Fig. 4. Continuous variation plot for : Nizatidine(D)-DDQ(A) complex (-○-), 6.03×10^{-3} M and ranitidine(D)-DDQ (A) complex (-□-), 6.36×10^{-3} M

Table I. Optimum conditions used in the proposed methods

Parameter	Nizatidine		Ranitidine	
	Chloranilic acid	DDQ	Chloranilic acid	DDQ
Amount of standard taken (mg)	0.2-2	0.2-1.6	0.2-2.2	0.2-1.4
Amount of reagent (mL)	1.2	2	1.2	2
Solvent	Acetonitrile	Acetone	Acetonitrile	Acetone
Heating temperature	Ambient temperature	Ambient temperature	Ambient temperature	Ambient temperature
λ_{max} (nm)	515	467	515	467
Stability of the colored product (min)	60	More than 120	60	More than 120

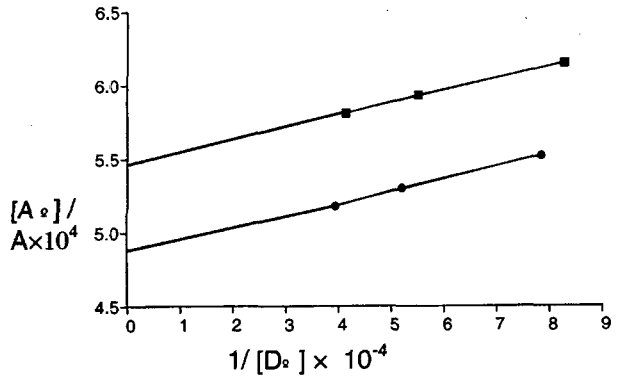


Fig. 5. Benesi-Hildebrandt plot for : Nizatidine(D)-DDQ(A) complex (-●-); λ= 467 nm and ranitidine(D)-DDQ (A) complex (-■-); λ= 467 nm

be confused with any Stoichiometric values calculated with reference to the amount of any analyte being determined. The latter is best described, as Beer's value while the former is Benesi-Hildebrandt's value.

The standard free energy change of complexation (ΔG°) is related to the association constant by the following equation (Martin *et al.*, 1983):

$$\Delta G^\circ = -2.303\, RT \log K$$

Where ΔG°, the free energy change of complex; *R*, the gas constant (1.987 cal $mol^{-1}°C$); T, the temperature in Kelvin degrees (273°C); K, the association constant of drug-DDQ complex (1 mol^{-1}).

The proposed methods were applied to the commercial capsules of nizatidine (Nizatine) and tablets of ranitidine (Zantac). These results were compared with those obtained by the comparison methods (Hassan and F. Belal, 2002) (Table IV). There was no evidence of interference from excipients in the commercial dosage forms analyzed (Table IV). The proposed methods offer the advantage of accuracy and time saving as well as simplicity of the reagents and apparatus.

Table III. Association constant Kc^{AD}, molar absorptivity values ξ^{AD} from Benesi-Hildebrandt plots for the complex and the calculated free energy ΔG°

Parameter	Nizatidine	Ranitidine
Kc^{AD}	6.71×10^4	5.6×10^4
ξ^{AD}	1.828×10^3	2.066×10^3
ΔG °	-6.029	-5.93

Method validation

The proposed methods were found to give linear calibration curves over the concentration ranges of 20-200 μg/mL, 20-240 μg/mL (M1), 20-160 μg/mL, 20-140 μg/mL (M2) with a regression coefficient (r) of 0.9996, 0.9999 (M1), 0.9998, 0.9998(M2) for I and II, respectively, indicating good linearity. The linear regression equations for each drug were listed in Table II. The detection limit (Miller and Miller, 1984).

of the methods were also listed.

Assays were performed in triplicate on two samples at different levels. This was repeated with a second instrument, standard and sample preparation on different days. The complete set of validation assays was performed for each drug, determined by the proposed methods.

The measurement precision was determined by performing five replicate measurements of the methods concentrations. The RSD were found to be 1.92, 1.36 (I) and 0.69, 1.64 (II) for M1 and M2, respectively (Table II).

These results of accuracy and precision show that the proposed method has good repeatability and reproducibility. Also the assay results are unaffected by the presence of excipients, this establish specifity of the method. To ensure the validity of the analytical procedure whenever used, the stability of the analytical solutions of I and II during the analytical procedures was studied and the two analytes were stable for at least 24 h when kept in a refrigerator. Also different parameters affecting the procedures were

Table II. Spectral data for the reaction of nizatidine and ranitidine with chloranilic acid and DDQ

Parameters	Nizatidine		Ranitidine	
	M1	M2	M1	M2
λ (nm)	515	467	515	467
Beer's law limits (μg/mL)	20-200	20-160	20-240	20-140
Detection limit (μg/mL)	3.59	1.81	1.18	1.90
Molar absorptivity	1.459×10^3	2.055×10^3	1.052×10^3	2.431×10^3
Slope	4.4×10^{-3}	6.2×10^{-3}	3×10^{-3}	6.93×10^{-3}
S.D. of the slope	4.2×10^{-5}	3.7×10^{-5}	9×10^{-6}	4.9×10^{-5}
Intercept	2.7×10^{-3}	-7.8×10^{-4}	2.9×10^{-3}	6.14×10^{-3}
S.D. of the intercept	5.27×10^{-3}	3.75×10^{-3}	1.18×10^{-3}	4.4×10^{-3}
Correlation coefficient	0.9996	0.9998	0.9999	0.9998
Mean ± R.S.D (%)	100.93±1.92	100.58±0.69	99.78±1.36	99.82±1.64

Table IV. Statistical comparisons between results of analysis of dosage forms of nizatidine and ranitidine applying the proposed and comparison methods.

Parameter	Nizatine capsules			Zantac tablets		
	p-CA method	DDQ method	Comparison method	*p*-CA method	DDQ method	Comparison method
Mean ± R.S.D	100.93±1.9	100.58±0.68	100.83±1.47	99.78±1.36	99.82±1.64	99.8±2
Variance	3.68	0.462	2.22	1.84	2.68	4
F-value	1.66 (6)	4.66 (6.09)		2.16 (3.22)	1.48 (4.28)	
t-value	0.101 (2.16)	0.39 (2.228)		0.025 (2.12)	0.02 (2.179)	

studied, to evaluate robustness and show reliability of the analytical procedure. The proposed method complied with USP validation guidelines.

Analysis of dosage forms

The proposed methods were applied for the determination of the cited drugs in commercial preparations. Five replicate determinations were made. Satisfactory results were obtained for all of them (Table IV).

Moreover, to check the validity of the proposed methods, the standard addition method was applied by adding nizatidine and ranitidine to the previously analyzed capsules or tablets. The recovery of each drug was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drug. The results of analysis of commercial dosage forms and the recovery study (standard addition method) of the cited drugs (Table IV) suggested that there is no interference from any excipients, which are present in tablets or capsules. The results of determination of the two drugs in dosage forms were compared with the reported method (Hassan and Belal, 2002).

Statistical comparison of the results was performed with regard to accuracy and precision using students *t*-test and *F*-ratio at 95% confidence level (Table IV). There is no significant difference between the reported and proposed methods with regard to accuracy and precision.

CONCLUSION

The suggested methods have the advantage of being simple, accurate, sensitive and suitable for routine analysis in control laboratories. These methods utilize a single step reaction and single solvents. No substantial differences among the proposed methods arose from analysis of the experimental results. The DDQ method was more sensitive than the *p-CA* method due to the higher molar absorptivity.

These methods can be used as general methods for the spectrophotometric determination of nizatidine and ranitidine hydrochloride in bulk powder and in pharmaceutical formulations.

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