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Short communication

Spectrophotometric and titrimetric determination of nizatidine in capsules

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Abstract

Four simple and accurate methods are described for the determination of nizatidine (NIZ) in pharmaceutical preparations. The first method is based on the formation of an ion-pair complex between the drug and either of bromocresol purple or picric acid with subsequent measurement of the developed colors at 411 and 400 nm, respectively. The second method depends on the condensation of mixed anhydrides of citric acid/acetic anhydride, with the tertiary amino group of the drug, where the developed color is measured spectrophotometrically at 545 nm. The oxidation of nizatidine by *N*-bromosuccinimide was utilized as a basis for the titrimetric method for its assay in capsules. The last method depends on the oxidation of nizatidine by ammonium cerium IV sulfate in the presence of perchloric acid with subsequent measurement of the absorbance at 314 nm; this principle is adopted to develop a kinetic method for the determination of NIZ in capsules. All the reaction conditions have been studied. The detection limits were varied from 0.44 to 0.78 μg ml^{-1}. The proposed methods were successfully applied to the assay of nizatidine in capsules.

Keywords: Nizatidine; Ion-pair complex; Spectrophotometry; *N*-Bromosuccinimide; Titrimetry; Capsules

1. Introduction

Nizatidine (NIZ) is a specific H_2-receptor antagonist. It is more potent than cimetidine in inhibition of gastric acid secretion induced by various stimuli and it lacks cimetidine's anti-androgenic and hepatic microsomal-inhibiting effect [1].

Nizatidine has been determined in pharmaceutical preparations using spectrophotometry [2,3], potentiometric titration [4], coulometry [5], HPLC [6], and polarographic [7] methods. In biological fluids, it was determined using HPLC [8] and polarographic methods [9]. A kinetic spectrophotometric method has been reported for the determination of nizatidine in pharmaceutical

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Table 1
Statistical data of the regression equations for color formed between NIZ and reagents in proposed methods

Parameters	Method A		Method B (CAA)	Method D (cerric)
	BCP	PCA		
Concentration range (μg ml^{-1})	4–10	5–30	5–14	5–15
ε	4.56×10^4	1.107×10^4	2.11×10^4	2.25×10^4
a	−0.23	−0.0038	5.2×10^{-3}	−0.0053
b	0.1376	0.0334	0.064	0.068
r	0.9990	0.9997	0.9992	0.9993
S_a	0.0207	0.0066	0.014401	0.01486
S_b	0.00298	0.00035	0.0014	0.00142
S_b^2	8.88×10^{-6}	1.22×10^{-7}	1.96×10^{-6}	2.01×10^{-6}
Detection limit	0.78	0.59	0.44	0.66
Quantitation limit	2.59	1.96	1.47	2.13

ε, apparent absorptivity; a, intercept; b, slope; r, correlation coefficient; S_a, standard deviation of intercept; S_b, standard deviation of slope; and S_b^2, variance around slope.

preparation [10]. An extended bibliography of nizatidine can be found in the comprehensive analytical profile [11]. NIZ and its pharmaceutical preparations are official in USP 24, where an HPLC procedure for its assay has been described [12].

Many drugs have been determined by the formation of an ion-pair complex [13,14]. This technique depends on the reaction of a drug that has basic cationic nitrogen and an anionic dye, where a highly colored ion-pair complex is formed.

Drugs containing tertiary amino group can be determined through base-catalyzed condensation of mixed anhydrides of organic acids, where the tertiary amine acts as the base catalyst with the formation of colored condensation products [15].

Organic sulfides can be oxidized fairly readily to the sulfoxide forms and further to the sulfone forms [16,17]. Therefore, it was thought that this technique could be employed for the determination of nizatidine as it contains a methyl thio group. The oxidation can be carried out either by *N*-bromosuccinimide (NBS) or with ammonium cerium IV sulfate (Ce IV).

In this work, nizatidine was determined by four techniques. The first one depends on the formation of colored ion-pair complex either with bromocresol purple (BCP) or picric acid (PCA). The second method depends on the reaction of the tertiary amino group of NIZ with citric acid/acetic anhydride (CAA) with the formation of a colored condensation product. The third one is a direct titrimetric assay based on the oxidation of the drug by NBS in acidic medium. Finally, a kinetically based method was developed through the reaction of the drug with Ce IV in the presence of perchloric acid at room temperature.

2. Experimental

2.1. Apparatus

The spectrophotometric determinations were performed using a Perkin–Elmer lambda EZ 201 (Version 1.0), connected to a Panasonic 24 Pin Quit KX 3626 printer.

2.2. Materials and reagents

All experiments were performed with analytical reagent grade chemicals.

- NIZ was kindly provided by El: Pharonia Pharm. and used as received.
- BCP (Aldrich Chem. Co., USA), 2×10^{-3} M in chloroform.
- PCA (Aldrich Chem. Co., USA), 2×10^{-3} M in chloroform.
- Saturated solution of citric acid (Merck) in acetic anhydride (CAA) was prepared by add-

ing 100 ml acetic anhydride to excess solid citric acid in stoppered conical flask and the mixture was shaked for 2 h. The flask was left to stand for 15 min to allow sedimentation of the solid, and then the supernatant solution was decanted.
- NBS (Prolabo, Paris, France), 2×10^{-3} M in water.
- Methyl red (BDH), 40 mg% in water.
- Ce IV (Prolabo, Paris, France) was prepared as 1×10^{-2} M in 0.5 M sulfuric acid, and then step-dilution with 1 M perchloric acid to obtain 1×10^{-3} M of cerium IV.
- Perchloric acid, chloroform, ethanol, and sulfuric acid were of analytical grade (BDH Chem. Ltd., Poole, UK).

2.3. Standard solution

Stock solution of NIZ containing 0.1 mg ml^{-1} was prepared in chloroform for Method A and in ethanol for Methods B and D. For Method C, stock solution containing 2×10^{-3} M of NIZ was prepared in water (0.66 mg ml^{-1}).

2.4. Construction of calibration graphs

2.4.1. Method A

Accurate portions from stock standard solution (0.5–3.0 ml) within the concentration range stated in Table 1 were transferred into two separate sets of 10 ml volumetric flasks, and 6 ml of 2×10^{-3} M BCP was added to one set and 5 ml of PCA solution was added to the other set. The flasks were completed to the volume with chloroform, and the absorbance of each solution was measured at 411 and 400 nm for BCP and PCA, respectively, against a reagent blank.

2.4.2. Method B

Into a set of wide-mouthed thick-walled test tubes, aliquots of stock standard solution of drug (0.4–1.5 ml), within the concentration range stated in Table 1, were pipetted and evaporated to dryness in a hot waterbath; then, 2.5 ml of CAA reagent was added and the set was allowed to stand in a boiling waterbath for 15 min. After cooling, the contents of each test tube were transferred quantitatively into 5 ml volumetric flask and the volume was completed with ethanol. The intensity of the developed violet color was measured at 545 nm against a blank solution treated similarly.

2.4.3. Method C

Different portions from stock standard solution (0.2–5.0 ml) in the range 0.13–3.3 mg were transferred into 50 ml conical flask followed by 5 ml of 2 M sulfuric acid, two drops of methyl red indicator, and titrated with NBS solution by dropwise addition with constant stirring until the red color changes to faint yellow. A blank experiment was carried out.

2.4.4. Method D

Aliquot portions of stock standard solution (0.5–1.5 ml), within the range stated in Table 1, were transferred into a set of 10 ml volumetric flasks; then, 3 ml of 1×10^{-3} M Ce IV was added to each flask. The contents of each flask were mixed well, completed to the mark with distilled water, and left to stand for 60 min at room temperature. The absorbance of the blank was measured against each solution at 314 nm.

2.5. Assay of drug in pharmaceutical formulation

An accurately weighed amount of the mixed contents of 10 capsules, equivalent to 25 mg of NIZ, was transferred into 25 ml volumetric flask using ethanol (chloroform in case of method A or distilled water for method C). The content of the flask was sonicated for 10 min, and then the volume was completed with the same solvent. The content of the flask was mixed well and filtered. The procedures were completed on the filtrate as described in Section 2.4.

3. Results and discussion

3.1. Method A

Containing basic cationic nitrogen, NIZ reacts with BCP, an acid dye, to form a colored product.

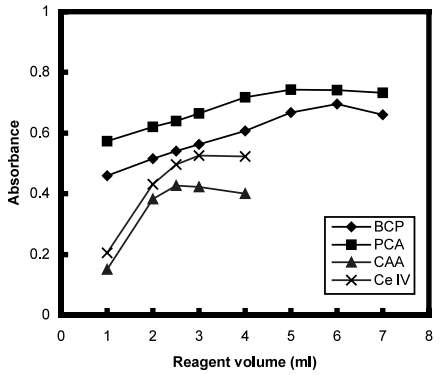

Fig. 1. Effect of the reagent volume on the reaction products formed between 2×10^{-3} M BCP, 2×10^{-3} M PCA, CAA reagent, and 1×10^{-3} M Ce IV, and 7, 22, 7, and 8 μg ml^{-1} of NIZ, respectively.

Therefore, when a solution of BCP (in the sulfophthalein form) in chloroform is added to NIZ solution in the same solvent, an intense yellow color is produced. This is due to conversion of the dye into an open quinonoidal anionic derivative, which forms ion-pair with NIZ.

In the proposed method, the reaction is carried out in organic solvent (chloroform) and hence is pH-independent; so, the ionization of the dye depends on the concentration of the basic drug, NIZ.

On the other hand, NIZ forms an ion-pair product with PCA in organic solvent (chloroform). The product was found to have an intense color, which can be measured at 400 nm. The high absorptivity of the formed product was due to the resulted negatively charged picrate ion, which is intensely colored.

To establish the most favorable conditions with respect to maximum sensitivity and obedience to Beer's law, the effect of concentration of BCP and PCA was studied (Fig. 1). It was found that the yellow-colored products were formed instantly at room temperature and stable for at least 60 min.

The continuous variation method [18] was applied to determine the stoichiometry of the reaction between NIZ and BCP or PCA. The method showed a ratio of 1:1 under the described conditions.

3.2. Method B

Under suitable conditions, citric acid and acetic anhydride condensed with NIZ (containing tertiary amino group) to give a violet-colored condensation product with maximum absorbance at about 545 nm.

The reaction was studied as a function of the reagent volume, reaction temperature, and time with respect to maximum sensitivity and low blank reading.

Maximum color intensity was achieved by using 2.5 ml of the reagent (Fig. 1). A boiling waterbath temperature gave the highest color intensity. The optimal heating time was 15 min, and shorter or longer periods of time resulted in lower color intensities. The produced color was found to be stable for at least 30 min.

3.3. Method C

N-Bromosuccinimide was found to react quantitatively with NIZ in acidic medium. A study of the stoichiometry of the reaction in different acids with different concentrations revealed that 2 M sulfuric acid gave the best results. Four moles of NBS was required for complete oxidation of each mole of NIZ. The concentration of the drug can be obtained from the following equation:

$$\text{nizatidine (mg)} = MVG,$$

where M is the concentration in mol l^{-1}, V the volume of NBS used in the titration in ml, and G the number of moles of drug reacting with 1 mol of NBS.

3.4. Method D

Ce IV has been used for the quantitative determination of some organic compounds [19,20]. Due to its high oxidation potential and excellent solution stability, this reagent has been utilized to develop a quantitative method for the analysis of NIZ in dosage forms. The method depends on measuring the absorbance of the consumed Ce IV at 314 nm. This was achieved by measuring the absorbance of a blank containing solution of certain concentration of Ce IV

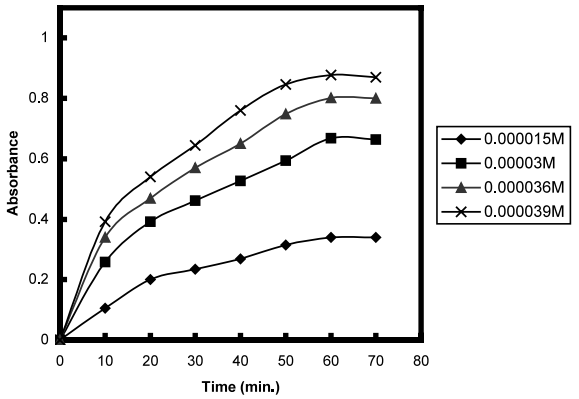

Fig. 2. Absorbance versus time graphs for the reaction between 1.5×10^{-5}, 3×10^{-5}, 3.6×10^{-5}, and 3.9×10^{-5} M NIZ and Ce (IV) at room temperature.

against the test containing the same concentration of Ce IV. It was found that acid medium is needed to prevent precipitation of hydrated cerric oxide. The reaction of NIZ and Ce IV was found to proceed quantitatively only in the presence of 1 M perchloric acid. In case of other acids such as sulfuric or nitric acid, non-stoichiometric reaction was obtained. The adoption of 3 ml of 1×10^{-3} M solution of Ce IV in the final solution proved to be adequate for maximum concentration of NIZ used in the calibration curve (Fig. 1). The oxidation of NIZ was completed after 60 min at room temperature. The maximum absorbance obtained remained stable for at least 30 min. The stoichiometry of the reaction of NIZ with Ce IV was studied under the described conditions; a molar ratio of 1:4 was obtained.

As the intensity of color increases with time, it was useful to develop a kinetically based method for the assay of NIZ. Therefore, the reaction was investigated under various factors to establish the most favorable conditions.

The rate of the reaction was also found to be NIZ-dependent. The rates were followed at room temperature with various concentrations of NIZ in the range 5–15 μg ml^{-1}, keeping Ce IV and $HClO_4$ acid concentrations constant.

The graphs shown in Fig. 2 clearly indicate that the reaction rate obeys the following equation:

$$\text{rate} = K'[\text{NIZ}]^n, \quad (1)$$

Table 2
Logarithms of rates for different concentrations at room temperature at 314 nm

log $\Delta A/\Delta t$	log [NIZ]
−4.02	−4.82
−3.73	−4.52
−3.65	−4.44
−3.62	−4.40

Table 3

Time (min)	Regression equations	Correlation coefficient, r
20	$13145C + 4.73 \times 10^{-5}$	0.999
30	$16715C + 0.0243$	0.996
40	$19739C + 0.0414$	0.992
50	$21668C + 0.0240$	0.994
60	$22344C + 8.6 \times 10^{-4}$	0.999
70	$22114C + 4.4 \times 10^{-3}$	0.999

where K' is the pseudo-order rate constant and n is the order of the reaction.

The rate could be estimated as $\Delta A/\Delta t$ [21], where A is the absorbance and t the time in seconds. Taking logarithms of rates and concentrations (Table 2), Eq. (1) is transformed into

$$\log(\text{rate}) = \log\left(\frac{\Delta A}{\Delta t}\right) = \log K' + n \log[\text{NIZ}]. \quad (2)$$

Regression of log(rate) versus log[NIZ] gave the regression equation

$$\log(\text{rate}) = 0.613 + 0.961 \log[\text{NIZ}], \qquad r = 0.998.$$

Hence $K' = 4.108\ s^{-1}$ and the reaction is pseudo-first-order ($n = 0.961 \simeq 1$) with respect to NIZ.

3.4.1. Evaluation of kinetic method

The determination of NIZ under the optimized experimental conditions, mentioned above, was achieved by the determination of the reaction rates for different concentrations of NIZ; at a preselected fixed time which was accurately determined, the absorbance was measured. Calibration graphs of absorbance versus initial concentration of NIZ were established at fixed times with the regression

Table 4
Evaluation of the reproducibility of the proposed analytical assays for the determination of NIZ

Method	Mean recovery ± S.D.
Method A	
BCP	99.8 ± 1.57
PCA	99.7 ± 1.71
Method B	
CAA	100.8 ± 1.31
Method C	
Titration with NBS	101.4 ± 1.47
Method D	
Cerric IV	100.3 ± 1.34

equations assembled in Table 3. It is clear that the slope increases with time and the most acceptable values of r and the intercept were obtained for a fixed time of 60 min, which was therefore chosen as the most suitable time interval for measurement. The calibration graphs were linear over the concentration range 5–15 μg ml^{-1}.

3.5. Validation of the methods

Using the above-mentioned procedures, linear regression equations were obtained over the concentration ranges stated in Table 1. The statistical parameters, regression equations, and standard deviation of the calibration graphs are clearly evident from the values of the variances around

Table 5
Evaluation of the accuracy and precision of the proposed method for the determination of NIZ

Method	Added[a]	Recovery ± S.D.[b]	RSD%	E_r%
Method A				
BCP	4	98.27 ± 1.7	1.73	−1.7
	7	101.86 ± 0.58	0.57	1.86
	10	99.20 ± 1.4	1.41	−0.8
Mean		101.70 ± 0.89	0.88	1.45
PCA	7	99.1 ± 0.56	0.57	−0.9
	20	100.5 ± 0.58	0.58	0.5
	30	99.0 ± 0.69	0.70	−0.46
Mean		99.5 ± 0.69	0.69	−0.46
Method B				
CAA	5	98.04 ± 1.27	1.30	−1.96
	10	98.0 ± 0.98	1.00	−2.0
	14	100.1 ± 0.73	0.73	0.1
Mean		98.7 ± 0.99	1.00	−1.3
Method C				
NBS (mg of NIZ)	0.13	100.1 ± 0.4	0.40	0.1
	0.66	99.8 ± 2.0	2.00	−0.2
	1.32	99.0 ± 1.0	1.01	−1.0
Mean		99.6 ± 1.13	1.13	−0.36
Method D				
Cerric	5	101.1 ± 0.63	0.62	1.1
	10	101.0 ± 0.8	0.79	1.0
	15	99.8 ± 0.73	0.73	−0.2
Mean		100.6 ± 0.72	0.72	0.63

[a] Final concentration in μg ml^{-1}.
[b] Mean ± S.D. of five determinations.

Table 6
Statistical comparison between the determination of NIZ using the proposed and reference HPLC methods in pharmaceutical formulation

Nizatidine capsules (150 mg per capsule)	Reference HPLC method[a]	Method A		Method B (CAA)	Method C (NBS)	Method D (cerric)
		BCP	PCA			
Mean ± S.D.[b]	98.4 ± 0.7	99.8 ± 1.32	98.58 ± 1.2	97.5 ± 0.618	98.9 ± 1.08	98.6 ± 0.43
F		3.4	2.86	1.3	2.30	2.65
t		2.1	0.288	2.14	1.89	0.76

Theoretical value $F = 6.39$ at the 95% confidence level and theoretical value $t = 2.31$ at the 95% confidence level.
[a] HPLC method [12].
[b] Mean% found ± S.D. of five determinations.

the slopes (S_b^2), and the detection limits varied from 0.44 to 0.78 μg ml^{-1}.

The reproducibility of the results was tested by applying the above procedures of the proposed methods for the analysis of solutions of NIZ of different concentrations. The results obtained were summarized in Table 4 indicating good reproducibility.

In order to evaluate the precision of the proposed method, solutions containing three different concentrations of the stated drugs were prepared and analyzed in five replicates. The analytical results obtained from this investigation are summarized in Table 5. The low values of the relative standard deviation (RSD%) and percentage relative error (E_r%) also indicate the high precision and the good accuracy of the proposed methods Table 5.

The influence of commonly used capsule excipients (lactose, starch, magnesium stearate, talc, and microcrystalline cellulose) was investigated before the determination of the drug in dosage forms. No interference could be observed with the proposed methods.

3.6. Analysis of pharmaceutical formulations

The applicability of the proposed methods was tested by the determination of NIZ in commercial dosage form (capsules).

The determination was carried out on the same batch of samples together with HPLC reference method [12]. Statistical analysis of the results obtained by the proposed methods and the reference method was done using the Student's t-test and the variance ratio F-test (Table 6). The calculated values did not exceed the theoretical ones, indicating a good agreement between the proposed methods. The proposed procedures are simple, sensitive, rapid, and accurate, and can be used for routine determination of NIZ in its dosage form.

4. Conclusion

The proposed spectrophotometric methods are simple, of equal sensitivity, and suitable for the analysis of nizatidine in commercial dosage forms. The reaction of NIZ with citric acid acetic anhydride was shown to be sensitive, simple, and inexpensive and selective (for tertiary amino group). The proposed methods offer the advantages of accuracy and time saving as well as simplicity of reagents and apparatus. Also, the reaction with cerric IV shows the suitability of the proposed method for the kinetic determination of NIZ. Compared with kinetic spectrophotometric method [10], the proposed method is more simple that it does not require heating and there is no interference of reagent, since at 314 nm only cerium exhibits maximum absorbance while none of NIZ or cerous moiety showed any absorbance in this region.

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