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The voltammetric behavior of nizatidine and its determination in biological fluids

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Abstract

The voltammetric behavior of nizatidine (a newly introduced antiulcer drug) was studied using direct current (DC_t), alternating current and differential pulse polarography (DPP). Well-defined cathodic waves were obtained over the whole pH range in Britton–Robinson buffers, in addition to 0.1 and 1 M HCl media. The main reduction wave was characterized as being irreversible and diffusion-controlled, although adsorption phenomena played a limited role in the electrode process. The current–concentration relationship was found to be rectilinear over the range 1×10^{-5}–6×10^{-4} and 2×10^{-6}–2×10^{-4} M using DC_t and DPP modes respectively, with a minimum detectability ($S/N = 2$) of 2×10^{-7} M using the latter technique. The number of electrons involved in the reduction process was established, and the mechanism of electrode reaction was verified. The proposed method was successfully applied to determination of nizatidine in spiked human plasma and urine and the percentage recoveries were 96.12 ± 0.40 and 97.12 ± 0.17, respectively.

Keywords: Nizatidine; Urine; Plasma; Polarography; voltammetry

1. Introduction

Nizatidine (Axid®) is a specific potent H_2-receptor antagonist [1]. Unlike cimetidine, which contains an imidazole ring structure, nizatidine has a thiazole ring. This drug 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 enzyme inhibiting effects [2]. The drug is widely used in the treatment of doudenal and peptic ulceration. It is administered as a 150- or 300-mg capsule.

Because nizatidine is newly introduced into the market, little has been published concerning its determination. It has been determined in pharmaceutical preparations using spectrophotometric methods [3,4] potentiometric titration [5], coulometry [6] and HPLC [7,8]. As for biological fluids, it has been determined adopting HPLC methods [9–13]. Kapetanovic et al. [14] described the DP polarographic determination of nizatidine in pharmaceutical formulations.

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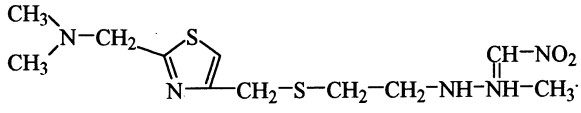
All the reported methods for the determination of nizatidine in biological fluids rely on the use of HPLC [9–13] after a lengthy extraction procedure with immiscible organic solvents. This led us to develop an alternative method, which is equally sensitive, but more simple and time saving than the reported methods. In the present work, no prior extraction step is required. The results obtained are promising; the percentage recoveries are sufficiently high; the accuracy and precision are satisfactory.



Nizatidine

2. Experimental

2.1. Reagents and materials

Nizatidine was kindly provided by Eli Lilly (Indianapolis, USA) and used as received. Plasma was obtained from King Khalid University Hospital, Riyadh, Saudi Arabia and urine was obtained from healthy volunteers.

1. Britton–Robinson buffers (BRb) [22] covering the pH range 2.1–12.0.
2. Methanol (AR grade, Aldrich).
3. Hydrochloric acid, 1 and 0.1 M solutions.

A stock solution of nizatidine (4×10^{-2} M) was prepared in water. This solution was further diluted with water to give the appropriate concentrations for the working solutions. The solutions were purged with pure nitrogen gas, then polarographed at ambient temperature.

2.2. Apparatus

The polarographic study and the differential pulse polarography (DPP) measurements were carried out using the Polarecord E506 Metrohm (Herisau, Switzerland). The drop-time of 1 s. was electronically controlled using a 505 Stand from the same company. The polarograms were recorded using a potential scan of 10 mV s^{-1}. A three-electrode system, a DME as the working electrode, an Ag^0/AgCl reference electrode and a platinum wire as the auxiliary electrode, were used. Phase selective alternating current (AC_t) polarograms of 1×10^{-4} M solutions were recorded using the same instrument, the superimposed alternating voltage being 15 mV at a frequency of 75 Hz and a phase angle of 90°.

2.3. Polarographic procedures

2.3.1. Recommended procedure

Transfer aliquots of nizatidine stock solution into a set of 25-ml measuring flasks so that the final concentration is in the range 1×10^{-5}–6×10^{-5} M. Add BRb of pH 5.0 to bring the volume to 25 ml. Transfer the whole contents of the flask to the polarographic cell. Pass pure nitrogen for 5 min. Record the polarogram over the range -0.8–1.6 V vs. Ag^0/AgCl.

2.3.2. Construction of calibration curve for urine

Transfer 1.0 ml of urine sample into a series of centrifugation test tubes. Add aliquots of nizatidine stock solution to each tube, so that the final drug concentration is in the range 2×10^{-6}–2×10^{-4} M (0.7–66 μg ml^{-1}). Mix well using a vortex mixer. Transfer the contents of the test tubes quantitatively into 25-ml measuring flasks. Wash the test tubes with BRb of pH 5.0 and transfer the washings to the measuring flasks. Complete to the mark with the same buffer. Transfer the contents of the measuring flask into the polarographic cell and pass nitrogen for 5 min. Record the polarogram and measure the current. Plot the values of the current (i_p) versus the corresponding concentration to get the calibration graph. A blank experiment is carried out simultaneously.

2.3.2.1. Construction of the calibration curve for plasma. Transfer 1.0 ml of plasma samples into a series of centrifugation tubes. Add aliquots of nizatidine stock solution to each tube, so that the final concentration of the drug is in the range 2×10^{-6}–2×10^{-4} M (0.7–66 μg ml^{-1}). Mix well using a vortex mixer. Add 5 ml of methanol

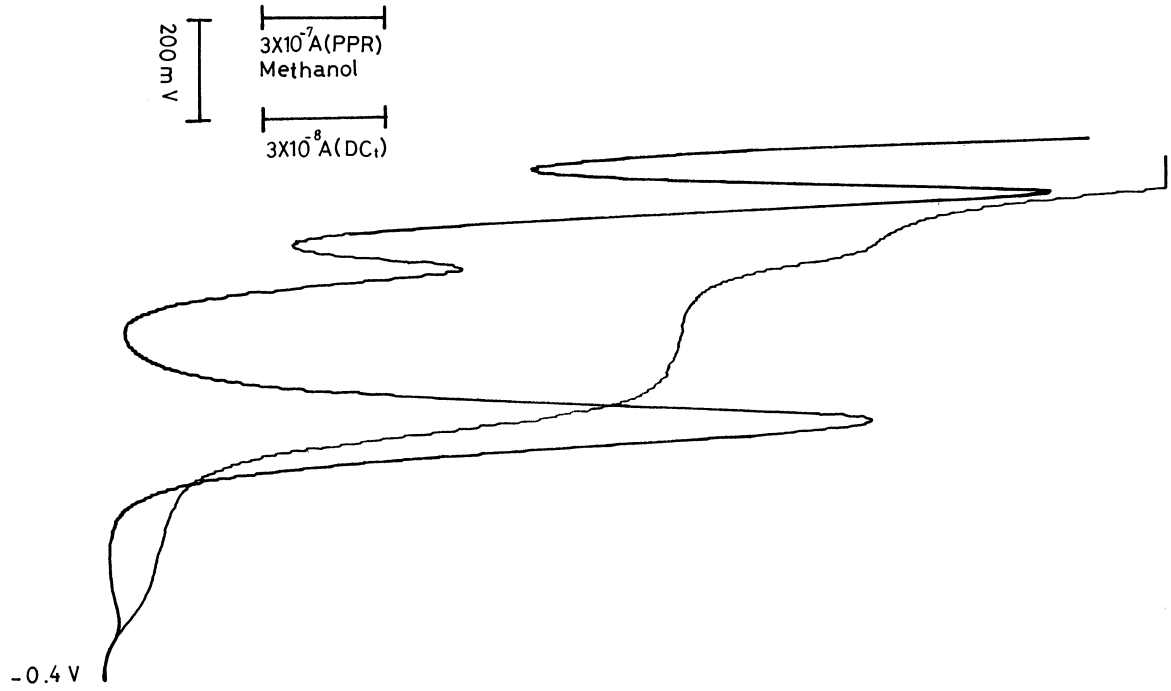


Fig. 1. Typical DC_t and DPP polarogram of nizatidine (1×10^{-4} M) in BRb of pH 5.0. Scan rate: 10 mV s^{-1}, drop time: 1 s.

for deproteination then centrifuge for 5 min. Transfer the clear supernatants to 25-ml measuring flasks and complete to the mark using BRb of pH 5.0. Transfer the contents of the measuring flask to the polarographic cell then proceed as described above.

3. Results and discussion

3.1. Influence of pH on the reduction peaks

Fig. 1 shows a typical polarogram of nizatidine in BRb of pH 5.0. A well defined cathodic wave followed by a more negative ill-defined one were produced. The ratio of the height of the first wave to the second one is 2:1. The two waves showed negative shift upon increasing the pH of the medium as shown in Fig. 2. The relation between the half-wave potential ($E_{1/2}$) of the main reduction wave and pH is expressed by the following regression equations:

Over the pH range 2.1–7.0:

$$E_{\frac{1}{2}} = -0.59 - 0.11 \text{ pH} \quad (R = 0.9946).$$

Over the pH range 8.0–12.0:

$$E_{\frac{1}{2}} = -1.08 - 0.04 \text{ pH} \quad (R = 0.99812)$$

At lower pH values (0.0 and 1.0 obtained in 1 M and 0.1 M HCl, respectively), an early-developed wave was observed, as the pH value increased. This wave began to decrease in height and a more negative one began to develop up to pH 5, whereby the first wave completely disappeared and the typical behavior of the nitro group was the predominant one (i.e. two waves). The first wave is of double height relative to the second. The early-developed wave may be attributed to the reduction of the double bond in conjugation with the nitro group in the side chain. Thus, the plot of $E_{1/2}$ of the main reduction wave vs. pH gave three segments (Fig. 3), their points of intersection are at pH values of 2.0 ± 0.05 and 6.8 ± 0.05. These values are in agreement with the

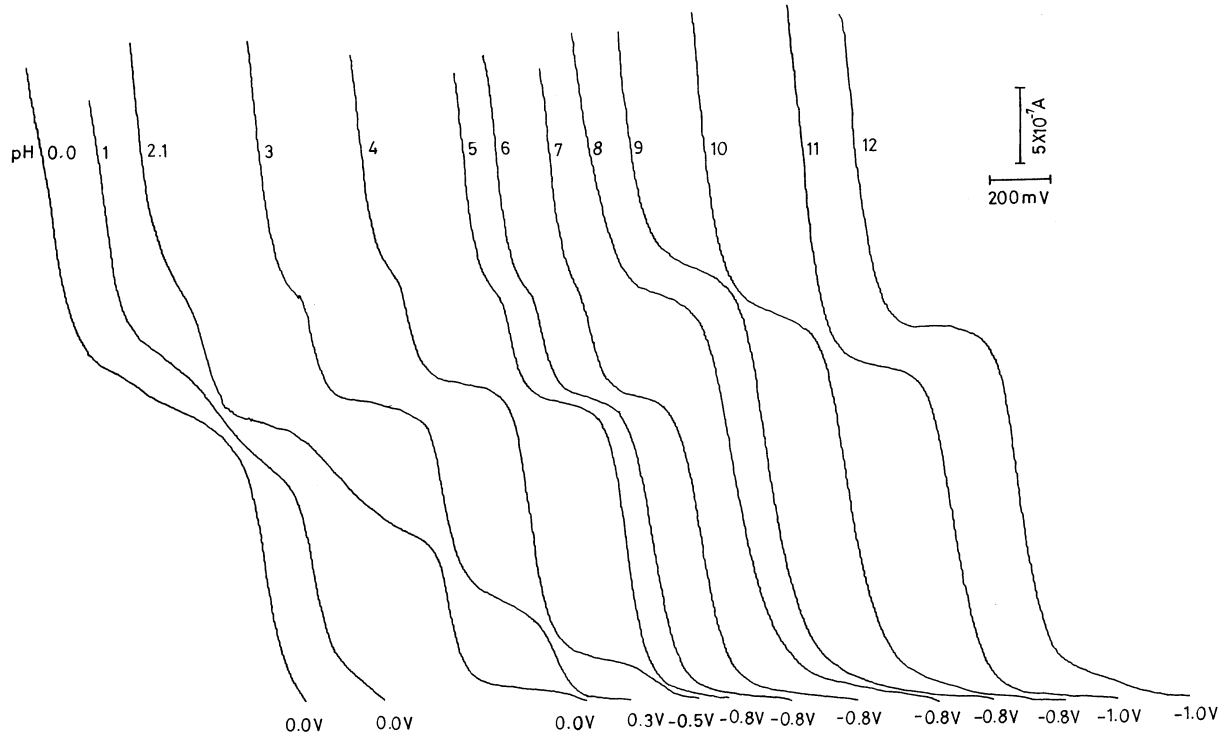


Fig. 2. Effect of pH on the development of the DC_t polarographic waves of nizatidine (1×10^{-4} M). Scan rate: 10 mV s^{-1}, drop time: 1 s.

reported values of pK_{a_1} and pK_{a_2} (2.1 and 6.8) for nizatidine in aqueous media [15]. Similarly, these values were found to be in close agreement with the recent data reported by Dumanovic et al. [16] adopting a spectrophotometric method, the reported values are 1.95 and 6.67. Logarithmic analysis of the main reduction wave (i.e. the first wave) obtained in BRb of different pH values using direct current (DC_t) mode, resulted in straight lines. Assuming that the rate-determining step involves the transfer of two electrons (a free radical, one electron transfer is not likely to occur) the values of slopes suggest that the reduction process is completely irreversible in character. The αn_a values were calculated using the treatment of Meites and Israel [17] and are listed in Table 1. It is noticed that, with the exception of pH 0.0 and 1.0, the degree of reversibility increases as the pH is increased up to pH 5.0, after which the degree of reversibility decreases and remains almost constant (Table 2).

3.2. Study of the wave characteristics

Increasing mercury height (h) resulted in a corresponding increase in wave height (w); a plot of $\sqrt{h}$ versus w gave a straight line, also a plot of log h versus log w gave a straight line with a slope of 0.5. Changing the buffer concentration over the range 0.01–0.08 M resulted in a negligible increase in wave height. These two characteristics point out to a diffusion-controlled process.

The relation between the wave height (w) and $\sqrt{h}$ is given by the following equation:

$$\sqrt{h} = 5.77 + 0.63w \quad (R = 0.9995)$$

while the relation between log w and log h is given by the following equation:

$$\log h = 1.6 + 0.48 \log w \quad (R = 0.9995)$$

The alternating current behaviour (AC_t) behavior of nizatidine (1×10^{-4} M solution) was studied using a phase-selective angle of 90° in BRb of

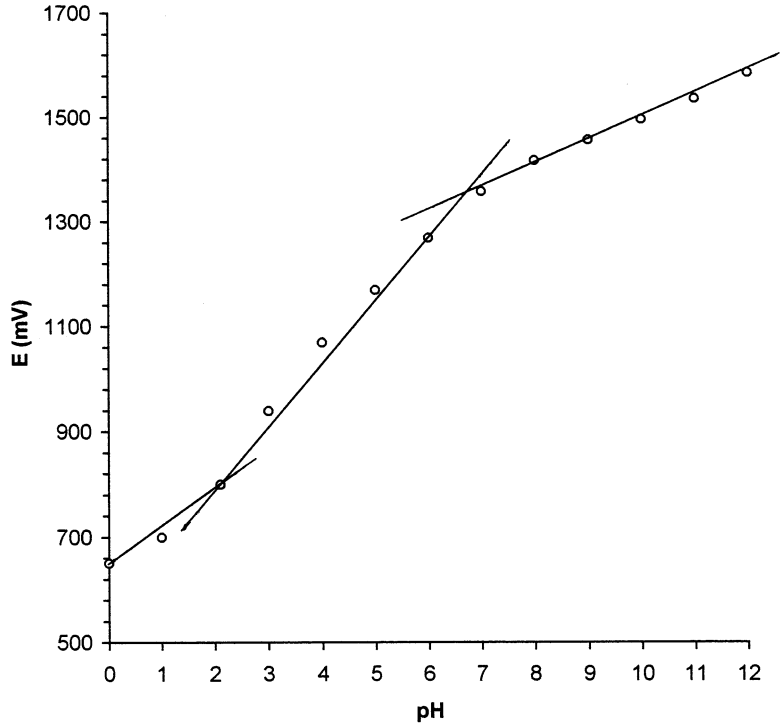


Fig. 3. Plot of the half-wave potentials versus pH obtained for nizatidine (1×10^{-4} M) using DC_t mode.

pH values of 5.0, 7.0 and 10.0, the summit potentials were 100, 130 and 140 mV more positive than the corresponding $E_{1/2}$ values, respectively.

Table 1
Effect of pH on the development of the DC polarographic waves of nizatidine (1×10^{-5} M)

pH	$-E_{1/2}$ (mV)	$\Delta E_{1/2}/\Delta$pH (mV)	$W_{1/2}$[a] (mm)	αn_a
0.0	650	–	100	0.75
1.0	700	50	115	0.79
2.1	800	91	115	0.53
3.0	940	156	115	0.69
4.0	1070	130	115	0.70
5.0	1170	100	110	0.84
6.0	1270	100	125	0.63
7.0	1300	90	130	0.60
8.0	1420	60	140	0.59
9.0	1460	40	145	0.60
10.0	1500	60	125	0.58
11.0	1540	50	120	0.59
12.0	1590	40	120	0.79

[a] Half-peak width in the DPP mode.

Fig. 4 demonstrates that at pH 5.0 neither the depolarizer nor its reduction product are adsorbed, while at pH 7, both are strongly adsorbed; as for pH 10: both are adsorbed to some extent. The adsorption of nizatidine on the surface of the mercury electrode may be attributed to the presence of two sulfur atoms in its molecule.

The diffusion coefficient of nizatidine in BRb of pH 5 was calculated according to Ilkovic equation [18] and was found to be 7.5×10^{-6} cm^2 s^{-1}. This small value may be attributed to the bulky nature of its molecule.

3.3. Number of electrons involved in the electrode reaction

The number of electrons transferred during the reduction process was accomplished through comparing the wave height of nizatidine with that obtained from an equimolar solution of a previously studied compound having the same functional group (nitro group) and of nearly the same

Table 2
Correlation between the concentration of nizatidine and the limiting current in the DC_t mode IN BRb pH 5

No.	Concentration (C) (mM)	Current (id) (μA)	id/C (μA mM^{-1})	$I_d/C\ m^{2/3}t^{1/6}$
1	0.01	0.08	8.40	6.93
2	0.02	0.17	8.50	7.02
3	0.04	0.33	8.25	6.81
4	0.08	0.65	8.13	6.71
5	0.10	0.81	8.13	6.71
6	0.12	0.97	8.13	6.71
7	0.16	1.30	8.13	6.71
8	0.20	1.63	8.15	6.73
9	0.30	2.43	8.10	6.69
10	0.40	3.25	8.13	6.71
11	0.48	3.85	8.02	6.62
12	0.60	4.84	8.07	6.66
$\bar{x}$	–	–	8.18	6.75
± S.D.	–	–	0.14	0.12

value of diffusion coefficient, i.e. oxamniquine [19]. In BRb of pH 5, both compounds gave two waves. The first waves of the two compounds were of the same height corresponding to a four-electron transfer process for the first wave, and consequently two electrons for the second one. It is evident from the experimental results that a slow electron-transfer reaction is involved in the reduction of nizatidine. Logarithmic analysis of the waves established that two electrons are involved in the rate-determining step of the first wave, and the shift in the $E_{1/2}$ potentials with increasing pH indicates that two hydrogen atoms are consumed in this step. Based on these facts, and by analogy to the previously reported mechanism [19], the following pathway may be postulated for the first-wave:

$$\text{R-NO}_2 + 2\text{H}^+ + 2\text{e} \overset{\text{slow}}{\rightarrow} \text{RNO} + \text{H}_2\text{O}$$

$$\text{R-NO} + 2\text{H}^+ + 2\text{e} \overset{\text{fast}}{\rightarrow} \text{RNH-OH}$$

The second wave involves two electrons and is due to a further reduction of the hydroxy-amino group to the primary amine:

$$\text{RNH-OH} + 2\text{H}^+ + 2\text{e} \rightarrow \text{R-NH}_2 + \text{H}_2\text{O}$$

3.4. Analytical applications

Polarograms of nizatidine in BRb of pH 5.0 exhibit a very well-defined cathodic wave. No polarographic maxima were developed; therefore, no maxima suppressor was needed. The current is diffusion-controlled, and proportional to the concentration over a convenient range. At that pH value; the wave in the DC_t mode was the steepest one and the peak in the DPP mode had the least width (Table 1).

To estimate the reproducibility of the electrode response six replicate concentrations were tested at nizatidine concentrations of 3.2×10^{-5}, 6.4×10^{-5}, 8.0×10^{-5} and 1.2×10^{-5} M adopting the DPP mode, mean current values of 0.382 ± 0.003, 0.695 ± 0.004, 0.905 ± 0.005 and 1.22 ± 0.008 μA, respectively were obtained. The precision of these measurements is expressed by the relative standard deviations of 0.78, 0.57, 0.55 and 0.56, respectively.

Solutions of nizatidine in water were found to be stable for 3 days if kept in the refrigerator. Solutions of nizatidine in BRb of pH 5.0 were found to be stable for more than 5 h. The relation between the limiting current, id or the peak current, i_p in DPP (μA) and the concentration (mM) was found to be rectilinear over the concentration range: 1×10^{-5}–6×10^{-4} M and 2×10^{-6}–2×10^{-4} M in the DC_t and DPP modes, respectively, with minimum detectability ($S/N = 2$) of 2×10^{-7} M using the latter technique. Linear regression analysis of the above data gave the following equations:

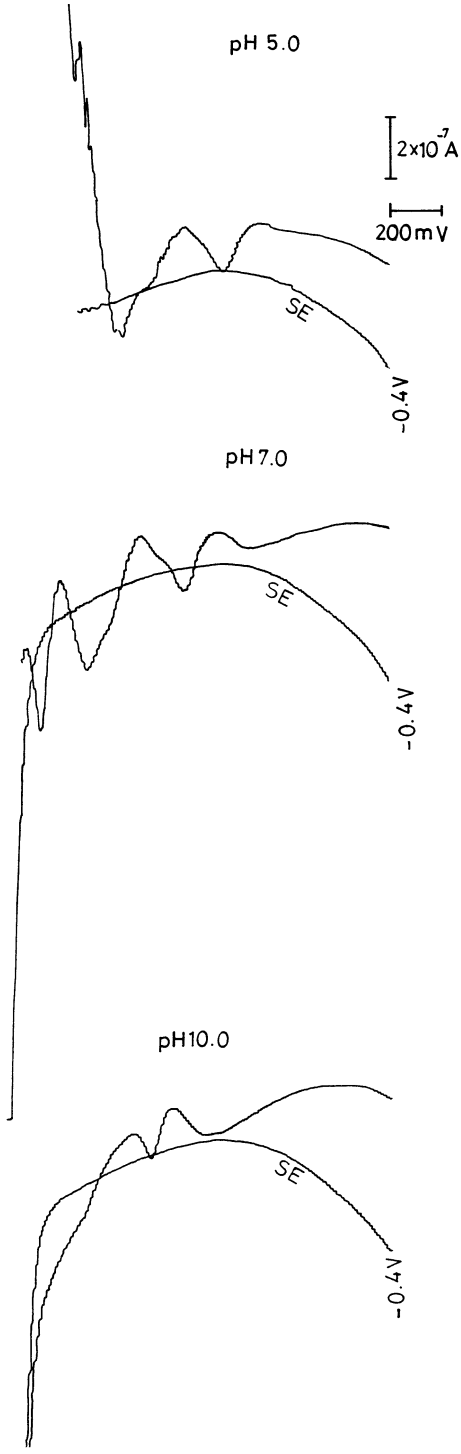


Fig. 4. Alternating current behavior of nizatidine (1×10^{-4} M) in BRb of different pH values. Superimposed alternating voltage: 15 mV; frequency: 75 Hz, phase angle 90° (SE: supporting electrolyte).

$$C = -1.2 \times 10^{-3} + 12.4 \times 10^{-2}\mathrm{id} (r = 0.9991)$$

using the DC_t mode, and

$$C = -6 \times 10^{-3} + 9.2 \times 10^{-2}\mathrm{id} (r = 0.9982)$$

using the DPP mode. where C is the concentration of nizatidine in mM and id is the current in μA.

The diffusion–current constant [$I_d = \mathrm{id}/Cm^{2/3}t^{1/6}$] was calculated at 25°C and was found to be 6.75 ± 0.12. (Table 2).

Bioavailability of nizatidine given orally exceeds 90%. The elimination half-life is 1–2 h. It is excreted primarily (90%) in the urine, and mostly (60%) as unchanged drug [20]. The proposed method was successfully applied to the determination of nizatidine in spiked urine and plasma. The three major metabolites of nizatidine are: N_2-monodesmethylnizatidine; nizatidine–N_2 oxide and nizatidne sulphoxide [23]. Probably, these compounds will interfere with the proposed method. Nizatidine is administered in the form of capsules containing 150 or 300 mg twice daily, leading an overall blood concentration of 30 or 60 μg ml^{-1} (i.e. 9×10^{-5} or 1.8×10^{-4} M), respectively. These values are high above the working concentration range in either DC_t or DPP modes. However, the DPP mode was preferred for the determination as it offers better accuracy and higher sensitivity. The results for the analysis of spiked urine and plasma are abridged in Table 3. No interference was noticed from urine after diluting it with BRb. As for the plasma, it had to be deproteinated by addition of methanol and centrifugation.

The major advantage of the proposed method over the reported HPLC method is that it does not require a prior extraction step; thus it is more simple and time saving. No sophisticated instrumentation is necessary. Moreover, the lower detection limit for the proposed method (2×10^{-7} M, i.e. 0.066 μg/ml) is less than that of the HPLC methods (0.1 μg/ml). The presence of nitro group in the side-chain of nizatidine structural formula (being polar and basic nitrogen) is essential for its maximum reactivity [21], thus the proposed method measures the biological activity of the drug.

Table 3
Application of the proposed method (DPP mode) to the determination of nizatidine in biological fluids

Sample	μg added	μg found	% Recovery[a]
Plasma	6.40	6.18	96.56
	12.80	17.20	95.30
	19.20	18.50	96.35
	32.00	30.80	96.25
$\bar{x}$	–	–	0.40
± S.D.	–	–	0.4
Urine	6.40	6.21	97.03
	17.80	17.40	96.88
	19.20	18.70	97.40
	32.00	31.10	97.18
$\bar{x}$	–	–	97.12
± S.D.	–	–	0.17

[a] Each result is the average of three separate determinations.

4. Conclusion

A simple, rapid and highly sensitive method was developed for the determination of nizatidine in biological fluids. The method is based on the reduction of the nitro group at the DME. The proposed method has some advantages over the reported methods, regarding time-consumption, simplicity and detection limit.

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