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Short Communication

Spectrophotometric determination of nizatidine in pharmaceutical formulations

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1. Introduction

Nizatidine is *N*-[2-[[[2-[(dimethylamino)-methyl]-4 - thiazolyl]methyl]thio]ethyl] - *N'* - methyl-2-nitro-1,1-ethenediamine and belongs to the new generation of anti-ulcer agents. It is a competitive inhibitor of histamine H_2-receptors [1,2]. Monitoring of nizatidine in biological fluids and its metabolic pathway has been carried out mainly by HPLC with UV detection [3–6]. Studies of the stability of nizatidine in oral liquid preparations and total nutrient admixtures were also carried out by HPLC [7,8]. Nizatidine was determined in pharmaceutical formulations by reversed-phase HPLC and derivative UV spectrophotometry [9]. As secondary and tertiary amines [10] tend to form coloured ion-pair complexes with acid dyes, it was suggested that nizatidine, a free base, may form a stable coloured ion-pair complex with bromophenol blue (BPB).

The main aim of this work was to investigate the ion-pair complex formation between nizatidine and BPB, develop a spectrophotometric method for the determination of nizatidine in pharmaceutical formulations.

2. Experimental

2.1. Apparatus

All spectral and absorbance measurements were made on a Specord M40 UV visible spectrophotometer (Carl-Zeiss, Jena) with 10-mm matched cells.

2.2. Chemicals and pharmaceutical formulations

Nizatidine, obtained from Ely Lilly and Company, (Indianapolis, USA) was used as a working standard. Galitidin® capsules (150 and 300 mg of nizatidine) and Galitidin® injection (25 mg ml^{-1}) were supplied by ICN-Galenika (Belgrade, Serbia). Bromophenol blue BPB and citric acid monohydrate were manufactured by Fluka; chloroform (stabilized with ethanol), anhydrous sodium sulphate, hydrochloric acid and sodium hydroxide were all manufactured by Merck. Water was doubly-distilled and stored in a glass container. All chemicals were of analytical grade.

2.3. Solutions

All citrate buffers (pH 2.50, 3.0, 3.25, 3.50, 4.0 and 4.50) were prepared according to Lurje [11]. Citrate buffer (pH 3.25; 0.2 M) was used as the solvent for preparing solutions A, B, C

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and D. BPB solution (A): 0.134 g of BPB was dissolved in 100 ml of citrate buffer. BPB solution (B): 67.0 mg of BPB was dissolved in 100 ml of citrate buffer. Standard solution of nizatidine (C): 16.6 mg of nizatidine was dissolved in 100 ml of citrate buffer. Standard solution of nizatidine (D): 33.15 mg of nizatidine was dissolved in 100 ml of citrate buffer.

Solutions A and B were extracted with 20 ml of chloroform to remove chloroform-soluble impurities. Stored in the dark at 4–8 °C the solution were stable for two weeks.

2.4. Preparaton of sample solutions

Capsules

An appropriate sample of powdered Galitidin® capsules (150 and 300 mg) containing 16.6 mg of nizatidine was transferred to a calibrated flask and 50 ml of citrate buffer (pH 3.25; 0.2 M) was added. The mixture was homogenized by ultrasonication for a few minutes and diluted to 100 ml with citrate buffer.

Injection

An appropriate volume of Galitidin® injection (0.75 ml containing 20.0 mg of nizatidine) was transferred to a 100 ml-calibrated flask and dissolved in citrate buffer.

2.5. General procedure

3 ml of nizatidine standard solution (C) was placed in a 50 ml-Erlenmeyer flask fitted with a glass stopper; 7 ml of BPB solution (A) and 5 ml of citrate buffer (pH 3.25; 0.2 M) were added. 10 ml of chloroform was then added and the reaction mixture was gently shaken for 3 min. The yellow chloroformic phase was separated form the aqueous phase in a separating funnel and filtered through 1.5 g of anhydrous sodium sulphate. The absorbance of the chloroformic extract was measured at 417 nm against the blank. The extraction with chloroform (10 ml) was repeated on the remainder of the reaction mixture (aqueous phase). The blank contained 10 ml of the chloroformic extract of an aqueous mixture of 8 ml of citrate buffer and 7 ml of BPB solution.

2.6. Investigation of the nizatidine–BPB ion-pair complex

The composition of the nizatidine–BPB ion-pair complex was determined by Job's method of equimolar solutions [12] and the Bent–French method of logarithmic analysis [13]. Experiments for Job's method was conducted according to the general procedure on nizatidine (D) and BPB solution (B). Nine reaction mixtures were prepared containing nizatidine and BPB solutions in addition to 5 ml of citrate buffer (pH 3.25; 0.2 M). The volumes of the nizatidine solution used varied from 0.5 to 4.5 ml and that of BPB solution from 4.50 to 0.50 ml; thus the total volume was always 5 ml.

2.7. Calibration curve

The reaction mixture containing 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 ml of nizatidine standard stock solution (C) were treated by the general procedure. The volume of each aqueous phase was adjusted with citrate buffer (pH 3.25; 0.2 M) to 15 ml. Three experiments were performed with each concentration and the absorbance was measured at 417 nm.

3. Results and discussion

In order to optimize formation of the ion-pair complex, the effects of pH, ionic strength and concentration of the BPB reagent have been investigated. Citrate buffer was chosen to study the effect of ionic strength in the pH range 2.50–4.50. Citrate buffers of 0.1, 0.2 and 0.3 M were tested. The best results were obtained with 0.2 M citrate buffer (pH 3.25).

The absorption spectrum of the ion-pair complex formed between nizatidine and BPB was measured under optimal experimental conditions. The chloroformic extract showed maximum absorbance at 417 nm (Fig. 1, curve 1). The absorbance of the repeated chloroformic

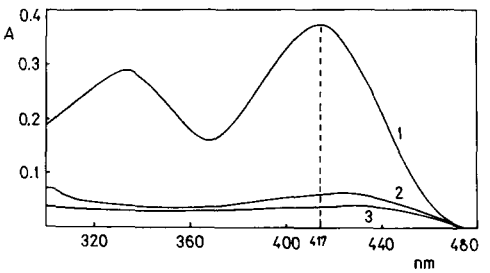

Fig. 1. Absorption spectra of: chloroformic extract of the ion-pair nizatidine–BPB complex (curve 1); repeated chloroformic extract of the remainder of the reaction mixture (curve 2); the blank reagent (curve 3). $C = 1.50 \times 10^{-5}$ M (pH 3.25 ± 01; 0.2 M).

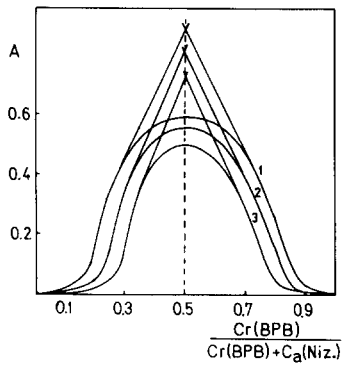

Fig. 2. Job's curves of equimolar solutions for the nizatidine–BPB ion-pair complex in chloroformic extracts; C_a (nizatidine) = C_r(BPB) = 1.00×10^{-3} M (pH 3.25 ± 0.1; 0.2 M). Curves 1, 2 and 3 at 417, 400 and 440 nm respectively.

extract of the remainder of the reaction mixture and that of the blank reagent (Fig. 1, curves 2 and 3, respectively) were insignificant.

The composition of the nizatidine–BPB ion-pair complex was determined by Job's method of equimolar solutions and the Bent–French method of logarithmic absorbances analysis. The molar ratio of nizatidine and BPB in an ion-pair complex determined by Job's method is shown in Fig. 2. The curves obtained exhibit a maximum $x_{max} = 0.50$, which means that the components of the ion-pair complex react in a 1:1 stoichiometric ratio. The shapes of the graphs and values of the exhibited maxima are independent of the wavelengths (417 nm, 440 nm and 400 nm) that are used for absorbance measurements. The number of nizatidine molecules in the ion-pair complex was determined by the Bent–French method. It was found that $-\log A$ (absorbance) varied linearly with $-\log C$ (nizatidine) for the range of investigated concentrations from 2.50×10^{-5} to 3.50×10^{-4} M. The Bent–French equation was $y = -4.31 + 0.9819\,x$; the correlation coefficient (r) was 0.9998. The slope of the straight line was 0.9819, which means that only one molecule of nizatidine takes part in the formation of the ion-pair complex. Thus the molar ratio of the nizatidine–BPB ion-pair complex was confirmed to be 1:1.

The graph of absorbance against concentration was linear in the range of 8.3–116.2 μg ml^{-1}. Beer's law was given by the equation $y = 0.01155 + 0.0074x$; the correlation coefficient (r) was 0.9990; the molar absorptivity was 2.35×10^4 l mol^{-1} cm^{-1}. The intercept a was 0.01155 and was statistically insignificant. The lower detection limit was 2.5×10^{-5} M of nizatidine. The reliability of the proposed method was checked at three different concentrations of nizatidine; the RSD ($n = 6$) varied from 0.68 to 1.48% for concentrations of nizatidine from 1.5×10^{-4} to 3.0×10^{-4} M. The precision of the method was calculated from results of the determination of nizatidine in laboratory-made excipient mixtures (150 mg g^{-1}) spiked with a known quantity of nizatidine. The recovery was 98.85% ($n = 6$); the RSD was 1.96%. Results and statistical parameters for the determination of nizatidine in pharmaceutical formulations are given in Table 1. The recoveries for Galitidin® capsules (150 mg and 300 mg) and Galitidin® injection (25 mg ml^{-1}) were 98.9, 98.0 and 98.3% (RSD 1.78, 2.01 and 1.72%), respectively, in relation to the labelled content.

Table 1
Results of the determination of nizatidine in pharmaceutical formulations

Sample ($n = 6$)	Taken (μg)	Found (Mean ± SD) (μg)	Recovery (%)	RSD (%)
Gal.® Caps. I [a]	498.0	492.5 ± 8.76	98.9	1.78
Gal.® Caps. II [b]	498.0	488.0 ± 9.80	98.0	2.01
Gal.® Inject. [c]	600.0	589.7 ± 10.1	98.3	1.72

[a] Gal.® Caps. I — Galitidin® capsules (150 mg).
[b] Gal.® Caps. II — Galitidin® capsules (300 mg).
[c] Gal.® Inject. — Galitidin® injection (25 mg ml^{-1}).

4. Conclusions

The results suggest that because of its sensitivity and precision, the proposed method may be suitable for the routine quality control of nizatidine in pharmaceutical formulations.

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