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Protolytic equilibria in homogeneous and heterogeneous systems of ketoconazole and its direct spectrophotometric determination in tablets

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Abstract: The acid-base equilibria of a diprotic, slightly hydrosoluble base ketoconazole were studied in homogeneous and heterogeneous water systems. The determinations were performed at 25 ºC at a constant ionic strength of 0.1 M (NaCl). The acidity constant K_{a1} was determined by potentiometric (pK_{a1} 3.20) and spectrophotometric (pK_{a1} 3.26) methods. A pK_{a2} constant of 6.10 was obtained based on the equilibrium constants pK_{s0} 4.84 and pK_{s1} –1.26, determined in a heterogeneous ketoconazole system. The obtained values of the constants served to calculate the solubility and the distribution of the equilibrium forms of ketoconazole as a function of pH. On the basis of the distribution of the equilibrium forms of ketoconazole, a spectrophotometric method for the determination of its content in commercial tablets was developed. The determinations were performed at 225 nm in 0.1 M HCl. The method is simple and rapid and enables the direct spectrophotometric determination of the content of ketoconazole without previous isolation.

Keywords: ketoconazole, acidity constants, heterogeneous equilibria, spectrophotometric determination, pharmaceuticals.

INTRODUCTION

Ketoconazole (*cis*-1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazine) is an orally active, antimycotic agent from the class of imidazole derivatives. It is active against *Candida* spp., *Cryptococcus neoformans* and *Pseudallescheria boydii*.[1,2] It is also effective in the treatment of *pityriasis versicolor*. Ketoconazole is the only member of the imidazole derivatives currently used for the treatment of systemic infections. The continually increasing number of patients suffering from mycoses due to decreased immunity (AIDS, organ transplantations, *etc.*) has led to a rather frequent application of pharmacologically active agents from this class.

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Chemically, ketoconazole is a diprotic base and its molecular form is slightly hydrosoluble. Knowledge about the distribution of the equilibria and the solubility of ketoconazole within the physiological pH range could help to understand better the details of the mechanism underlying its action as a drug. Namely, the absorption of drugs in the gastrointestinal tract, as well as their transport through the cell membranes depend on the properties of the chemical species involved. However, there are no data in the available literature on the protolytic equilibria of ketoconazole in homogeneous and heterogeneous aqueous systems and a part of this work was devoted to these studies.

In addition, this study was aimed at demonstrating that examination of protolytic equilibria might be of importance in the analytics of ketoconazole, concerning the choice of methodological approaches and the conditions for its direct spectrophotometric determination. Several spectrophotometric methods for the determination of ketoconazole have been described to date.[3–11] These methods were based on the formation and extraction of ionic pairs or complexes by a suitable reagent. Only Kedor-Hackmann *et al*.[3] proposed a direct spectrophotometric method for the determination of ketoconazole in pharmaceutical preparations.

EXPERIMENTAL

Apparatus

For the spectrophotometric measurements, a GBC Cintra 40 spectrophotometer with 1.0 cm quartz cells was used. A PHM240 pH meter (Radiometer) with a combined GK2401B electrode (Radiometer) served to determine the pH values. Titrations were performed with a TTT-60 titrator and an ABU-12 autoburette (Radiometer).

Reagents

Ketoconazole (KC) of pharmaceutical purity, a product of Medilom & Co. (Antwerpen, Belgium) was kindly supplied by The Institute of Pharmacy of Serbia (Belgrade, Serbia and Montenegro). All other reagents (HCl, NaOH and NaCl), products of Merck (Darmstadt, Germany), were of analytical grade purity. Solutions of HCl and NaOH were standardized potentiometrically.

The examined tablets, Mycoseb® ("Zorka Pharma", Šabac, Serbia and Montenegro), Nizoral® (Janssen Pharmaceutica, Beerse, Belgium) and Oronazol® ("Krka", Novo Mesto, Slovenia), contained 200 mg ketoconazole each.

Determination of the equilibrium constants

The equilibrium constants in homogeneous and heterogeneous systems of ketoconazole were determined at 25.0 ± 0.1 ºC and constant ionic strength of 0.1 M (NaCl).

Potentiometric determination of K_{a1}. 20 mL of solution containing 1×10^{-3} M of ketoconazole and 5×10^{-3} M of HCl was titrated with standard NaOH solution (0.07072 M) in 0.050 mL aliquots. The function of formation ($\bar{n}$) was calculated using the following equation:

$$\bar{n} = \frac{c_{\mathrm{HCl}} - c_{\mathrm{NaOH}} - [\mathrm{H_3O^+}]}{c_{\mathrm{KC}}} \tag{1}$$

where c_{KC}, c_{HCl} and c_{NaOH} represent the concentration of ketoconazole, HCl and NaOH in the titrated solution, respectively; $[H_3O^+]$ is the equilibrium concentration of hydronium ions obtained from the measured pH values. The obtained pH values were converted into pc_H according to equation:[12] $pc_H = -\log[H_3O^+] = pH - A$. For an ionic strength of 0.1 M (NaCl) at 25 ºC, the correction factor A was 0.12.

Spectrophotometric determination of K_{a1}. A series of ketoconazole solutions of the same stoichiometric concentration ($c_{KC} = 4\times10^{-5}$ M) within the pH range from 1.0 to 4.2 was prepared. The acidity was adjusted with HCl. The spectra of the solutions were recorded against the corresponding blanks. The absorbances measured at 242 nm were further used for the calculations of the acidity constants.

Determination of the equilibrium constants in heterogeneous system. Saturated ketoconazole solutions of pH 5.5–6.5 were prepared by treating an excess of the solid base with an HCl solution and adding NaCl up to an ionic strength 0.1 M, while those having pH of 8.5–10 were obtained by treating an excess of the solid base with 0.1 M NaCl and adding NaOH solution. The samples were thermostated at 25 ºC with occassional stirring until complete equilibration was achieved (4 h). After pH measurement, the solutions were separated from the insoluble part by filtration. Aliquots of the filtrate were diluted with 0.1 M HCl. The concentration of the diprotonated form of ketoconazole (BH_2^{2+}) was determined spectrophotometrically at 225 nm, conformity with Beer's law having been previously verified.

Determination of ketoconazole in tablets

Standard solutions. A stock ketoconazole solution (500 μg/mL) was prepared by dissolving the solid substance in 0.1 M HCl. Aliquots of 0.15, 0.25, 0.50, 0.75, 1.00 and 1.50 mL of this solution were transferred into 25 mL volumetric flasks and 0.1 M HCl was added to the volume. The absorbances of the solutions were measured at 225 nm against 0.1 M HCl as the blank.

Sample solutions. Ten tablets were weighed and powdered; the mass containing 50 mg ketoconazole was taken and quantitatively transferred into a 100 mL volumetric flask containing approximately 80 mL 0.1 M HCl. After 5-min-treatment in an ultrasonic bath, 0.1 M HCl was added to the volume. The resulting suspension was filtered (blue stripe filter paper) and the first 10 mL of the filtrate were discarded. Aliquots of 0.5 mL were taken from the remaining filtrate, transferred into 25 mL volumetric flasks and diluted with 0.1 M HCl to the volume. Absorbances of the solutions were measured at 225 nm against 0.1 M HCl as the blank.

RESULTS AND DISCUSSION

Protolytic equilibria in homogeneous and heterogeneous system

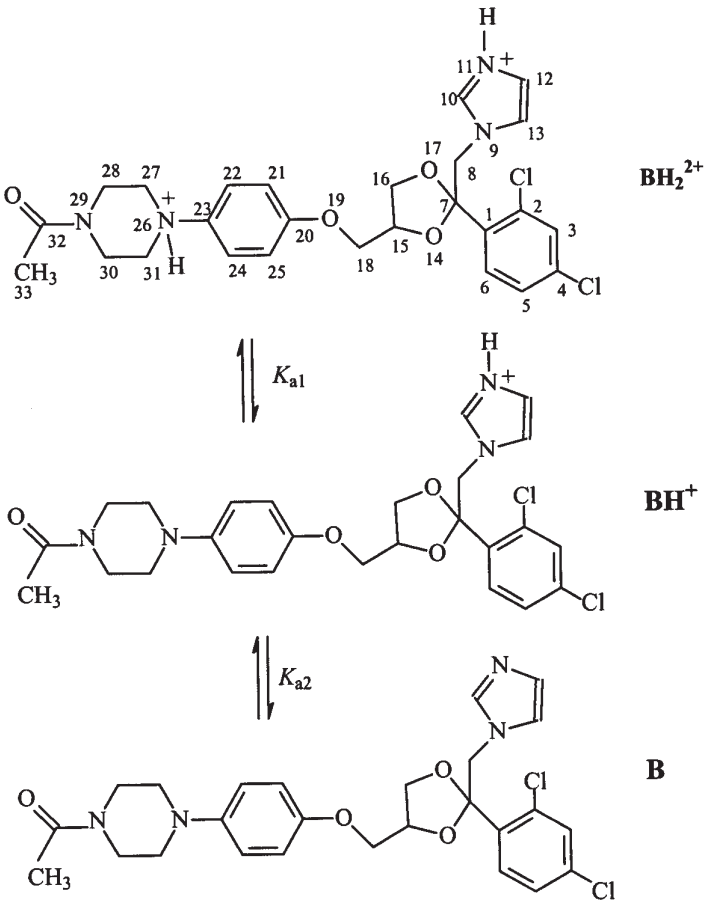
The piperazine nitrogen atom N-26 and the imidazole nitrogen atom N-11 represent the two basic centers of a ketoconazole molecule which participate in protolytic reactions wihtin the pH range from 0 to 14. Based on the comparison with aniline (pK_a 4.6) and imidazole (pK_a 6.9) as model compounds, it can be concluded that the imidazole nitrogen atom has a higher affinity for a proton (see Scheme 1).

The corresponding acidity constants are defined as:

$$K_{a1} = \frac{[H_3O^+][BH^+]}{[BH_2^{2+}]} \tag{2}$$

$$K_{a2} = \frac{[H_3O^+][B]}{[BH^+]} \tag{3}$$

Since ketoconazole is slightly hydrosoluble in its molecular form, in the heterogeneous system between the solid base (B_s) and the saturated aqueous solution, the following equilibria are established:



Scheme 1.

$$B_s \rightleftharpoons B \qquad K_{s0} = [B] \tag{4}$$

$$B_s + H_3O^+ \rightleftarrows BH^+ + H_2O \qquad K_{s1} = \frac{[BH^+]}{[H_3O^+]} \tag{5}$$

$$B_s + 2\,H_3O^+ \rightleftarrows BH_2^{2+} + 2H_2O \qquad K_{s2} = \frac{[BH_2^{2+}]}{[H_3O^+]^2} \tag{6}$$

The following relations between the acidity constants and the constants in a heterogeneous system exist:

$$K_{a1} = \frac{K_{s1}}{K_{s2}} \tag{7}$$

$$K_{a2} = \frac{K_{s0}}{K_{s1}} \tag{8}$$

Taking into account the expected difference in the basicity of N-26 and N-11, it was possible to determine the acidity constants of ketoconazole independently within different pH ranges with a single acid-base pair predominating.

Since ketoconazole is stable in acidic medium and its protonated forms BH_2^{2+} and BH^+ are soluble, it was possible to determine the acidity constant K_{a1} by a potentiometric and a spectrophotometric method.

For potentiometric determination of K_{a1}, the method of bound protons[13] was applied. The determinations were performed within the pH range from 3.0–4.0 where acid-base pair BH_2^{2+} – BH^+ is dominant. The formation function ($\bar{n}$), *i.e.*, the average number of protons bound to the base, can be expressed by the following equation:

$$\bar{n} = \frac{2[BH_2^{2+}] + [BH^+]}{[BH_2^{2+}] + [BH^+]} \tag{9}$$

By combining Eqs. (2) and (9), the following equation can be obtained:

$$\frac{2 - \bar{n}}{\bar{n} - 1} = K_{a1} \frac{1}{[H_3O^+]} \tag{10}$$

On the basis of the experimentally determined value of the formation function at different pH values and Eq. (10), the value of K_{a1} was calculated by regression analysis from the slope of the plot of $(2–\bar{n})/(\bar{n}–1)$ against $1/[H_3O^+]$ (Fig. 1). The obtained pK_{a1} was 3.20.

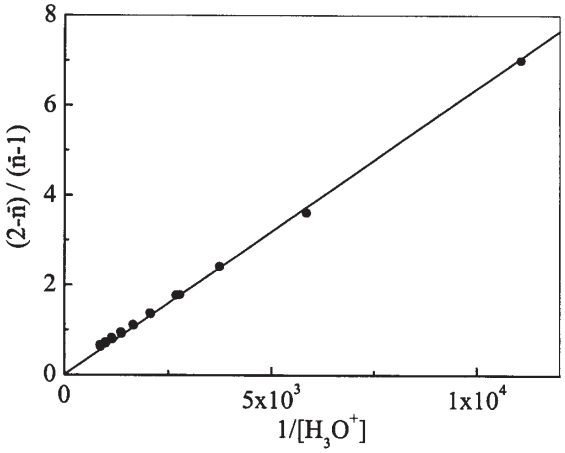


Fig. 1. Potentiometric determination of K_{a1} constant of ketoconazole applying Eq. (10).

The optimal conditions for the spectrophotometric determination of K_{a1} were chosen by analyzing the ketoconazole spectra obtained in solutions of different acidity (Fig. 2). From the ketoconazole spectra at pH 1.0 and 4.0, it can be concluded that the biggest difference in the spectra which follows protonation of the

N-26 atom was observed at approximately 240 nm. To calculate K_{a1}, a transformed classical spectrophotometric equation[14] was employed:

$$A = A_{BH^+} - \frac{1}{K_{a1}} [H_3O^+](A - A_{BH_2^{2+}}) \quad (11)$$

where A_{BH^+} and $A_{BH_2^{2+}}$ represent the absorbances of pure ketoconazole forms BH^+ and BH_2^{2+}, and A are the absorbances of their mixtures at a certain $[H_3O^+]$. For the determination of the constant by this equation, it was necessary to know the spectrum of the single "pure" form of BH_2^{2+}. Absorption spectra used to determine K_{a1} are shown in Fig. 3. The absorbances of the ketoconazole solution at 242 nm and Eq. (11) served to calculate the value of K_{a1} by regression analysis from the slope of the linear plot shown in Fig. 4. In this way, a pK_{a1} value of 3.26 was obtained.

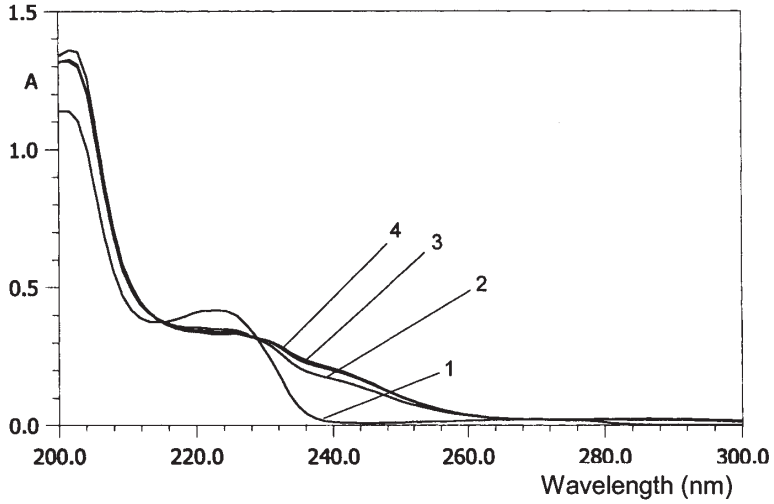


Fig. 2. Absorption spectra of ketoconazole (1.5×10^{-5} M) in solutions of different acidity. 1) 0.1–1 M HCl (BH_2^{2+} form); 2) pH 4.0; 3) pH 6.5; 4) pH 10.0 (B form).

Acidity constant K_{a2} of ketoconazole was impossible to determine spectrophotometrically because of the small differences between the absorption spectra (Fig. 2) which follow protonation of the imidazole nitrogen atom (the spectra of ketoconazole solutions pH 6.5 and 10.0). It was also impossible to determine this constant potentiometrically because the molecular form of ketoconazole is only sparingly soluble in aqueous media. Thus, pK_{a2} was indirectly determined on the basis of the equilibria in a heterogeneous system.

The equilibrium constants in a heterogeneous system were estimated by applying the solubility method. The determination was performed within two different pH ranges from 5.5–6.5 (the BH^+ and B forms of ketoconazole are present in saturated solution) and at pH > 8.5 (the molecular form of ketoconazole, B, is dominant in saturated solution). The relationship between the solubility (S) and the

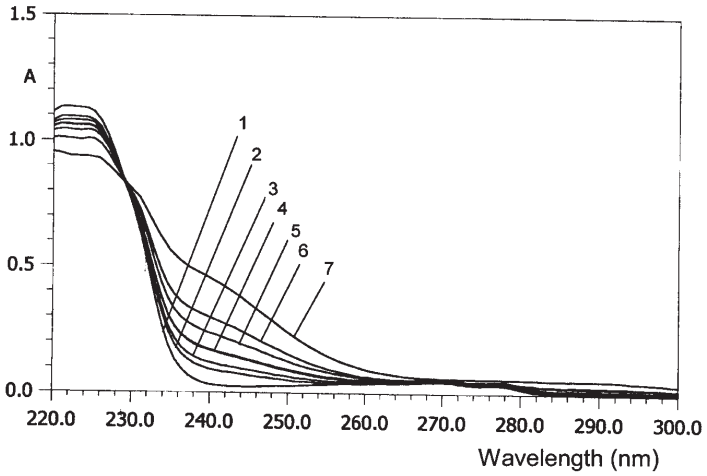


Fig. 3. Absorption spectra of the ketoconazole solution (4×10^{-5} M) used to determine the value of K_{a1}. pH: 1) 1.0 (BH_2^{2+} form); 2) 2.51; 3) 2.71; 4) 2.96; 5) 3.14; 6) 3.36; 7) 4.20.

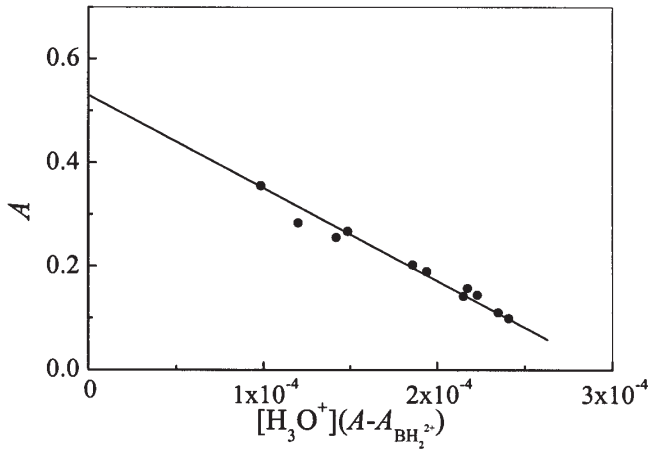


Fig 4. Spectrophotometric determination of K_{a1} constant of ketoconazole applying Eq. (11).

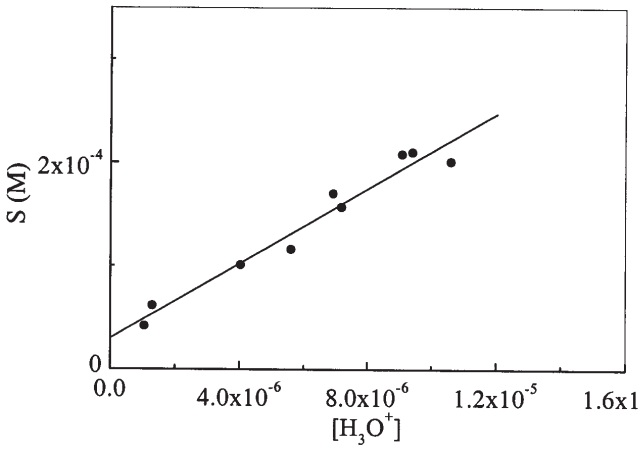


Fig. 5. Determination of the constants K_{s0} and K_{s1} of ketoconazole applying Eq. (13).

corresponding equilibrium constants (Eqs. (4) and (5)) can be expressed by the following equations:

$$S = [B] + [BH^+] = K_{s0} + K_{s1}[H_3O^+] \qquad (pH\ 5.5–6.5) \tag{13}$$

$$S = [B] = K_{s0} \qquad (pH > 8.5) \tag{14}$$

On the basis of experimentally determined solubility within the pH range from 5.5 to 6.5 and by application of Eq. (13), the values of K_{s0} and K_{s1} were determined from the intercept and the slope of the linear plot shown in Fig. 5. Equation (14) shows that the solubility of ketoconazole in alkaline media should be constant (equal to the value of K_{s0}).

The equilibrium constant K_{a2} was calculated according to the determined constants K_{s0} and K_{s1} and Eq. (8).

TABLE I. The concentration equilibrium constants in homogeneous and heterogeneous systems of ketoconazole. t = 25 ºC; I = 0.1 M (NaCl)

Constant	Value*	Equation applied
pK_{a1}	3.20 ± 0.01	(10)
	3.26 ± 0.03	(11)
pK_{a2}	6.10 ± 0.07	(8)
pK_{s0}	4.84 ± 0.06	(14)
pK_{s0}	4.5 ± 0.2	(13)
pK_{s1}	–1.26 ± 0.03	(13)
pK_{s2}	– 4.46 ± 0.03	(7)

*pK ± sd

The equilibrium constants determined in homogeneous and heterogeneous ketoconazole systems are summarized in Table I. As can be seen, the values of K_{a1} obtained by the two independent methods corresponded well. The value of K_{s0} was estimated by two approaches and more precise results were obtained when Eq. (14) was applied. Hence, this value was used to calculate the acidity constant K_{a2}. The equilibrium constants determined in a heterogeneous system can be used to calculate the solubility of ketoconazole in dependence of the acidity:

$$S = [B] + [BH^+] + [BH_2^{2+}] = K_{s0} + K_{s1}[H_3O^+] + K_{s2}[H_3O^+]^2 \tag{15}$$

as well as the distribution of the equilibrium particles:

$$\chi_{BH_2^{2+}} = \frac{[H_3O^+]^2}{[H_3O^+]^2 + K_{a1}[H_3O^+] + K_{a1}K_{a2}} \tag{16}$$

$$\chi_{BH^+} = \frac{K_{a1}[H_3O^+]}{[H_3O^+]^2 + K_{a1}[H_3O^+] + K_{a1}K_{a2}} \tag{17}$$

$$\chi_{BH} = \frac{K_{a1}K_{a2}}{[H_3O^+]^2 + K_{a1}[H_3O^+] + K_{a1}K_{a2}} \quad (18)$$

The distribution of the equilibrium forms of ketoconazole as a function of pH is depicted in Fig. 6. The relative concentrations of the equilibrium forms of ketoconazole at three biologically important pH values are summarized in Table II. At pH 1.0, 99.3 % of the ketoconazole was present as the diprotonated form, while the other forms were only minor components. At pH 7.4 and 9.0, the molecular form of ketoconazole was dominant.

TABLE II. The equilibrium percentage distribution of ketoconazole species at physiologically important pH values

pH	1	7.4	9
$\chi_{BH_2^{2+}}$	99.3	6.0×10^{-3}	1.5×10^{-4}
χ_{BH^+}	6.6×10^{-1}	5.3	1.3×10^{-1}
χ_B	1.1×10^{-5}	94.6	99.8

Spectrophotometric determination of ketoconazole in tablets

The choice of the optimal conditions for the determination of ketoconazole was made based on the distribution of its equilibrium forms (Fig. 6), as well as spectral characteristics (Fig. 2). From Fig. 6, it can be seen that ketoconazole in the form of a single species occurs at pH under 1.2 (BH_2^{2+} form) and over 8.1 (B form). Direct spectrophotometric determination of ketoconazole after Kedor-Hackmann *et al.,*[3] was performed in a solution of pH 2 (0.01 M HCl) at 222 nm. At this pH value, ketoconazole mainly occurs in the diprotonated form. However, the concentration of the monoprotonated form under these conditions can not be neglected. Hence, in the present study, 0.1 M HCl was chosen as the optimal medium. Determinations were performed at 225 nm since this wavelength corresponds to

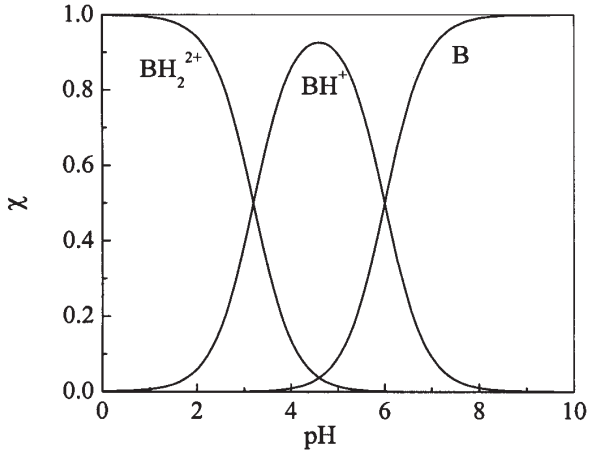


Fig. 6. Distribution of the forms of ketoconazole as a function of pH.

the absorption maximum of the BH_2^{2+} form of ketoconazole. A calibration curve was obtained within the range of ketoconazole concentrations from 3 to 30 μg/mL:

$$A = (-0.003 \pm 0.006) + (0.0525 \pm 0.0004)\, c \qquad (r = 0.9999) \qquad (19)$$

where c is the concentration expressed in μg/mL.

TABLE III. Spectrophotometric determination of ketoconazole

Taken μg/mL	Found μg/mL	RSD* %	Recovery %
5.00	4.96	0.98	99.2
10.00	10.05	0.65	100.5
20.00	20.18	0.76	100.9

*$n = 6$

The accuracy and precision of the method were checked by analyzing three ketoconazole solutions of different concentration. The results (6 independent determinations of each ketoconazole solution) are summarized in Table III. The recovery and low values of the relative standard deviation indicate a satisfactory reproducibility of this method.

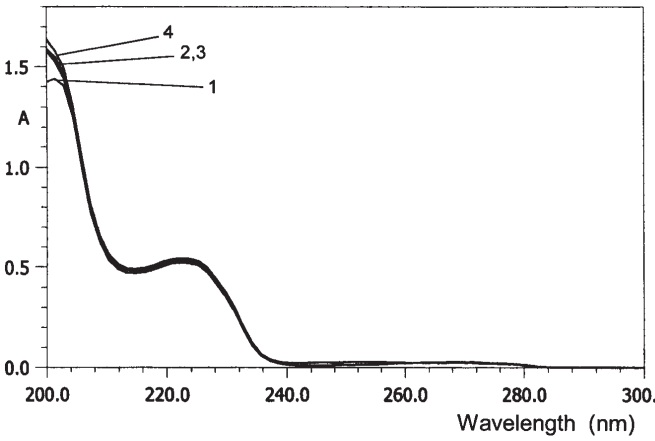


Fig. 7. Absorption spectra of ketoconazole (10 μg/mL) in 0.1 M HCl solutions: 1) Ketoconazole standard, 2) Mycoseb® tablets, 3) Nizoral® tablets, 4) Oronazol® tablets.

TABLE IV. The level of ketoconazole in commercial tablets. Label claim 200 mg ketoconazole per tablet

Preparation	Found mg per tablet	RSD* %	Label claim %
Mycoseb®	199.1	1.39	99.5
Nizoral®	203.2	1.13	101.6
Oronazol®	203.0	1.25	101.5

*$n = 6$

The applicability of the method was tested by determining ketoconazole in the following commercial ketoconazole-containing tablets: Mycoseb®, Nizoral® and

Oronazol®. The absorption spectra of a ketoconazole standard and solutions of the examined tablets are presented in Fig. 7. As can be seen, at wavelengths above 205 nm, the absorption spectra overlap. This demonstrates the absence of interference of other substances present in the examined tablets. The results of ketoconazole determination in tablets are listed in Table IV.

The proposed method for the determination of ketoconazole in pharmaceutical formulations is precise, rapid and simple. It enables the direct spectrophotometric determination of ketoconazole in tablets without previous isolation. In addition, this method does not require the use of expensive equipment and toxic reagents thus fulfilling the concept of Green Chemistry.

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ИЗВОД

ПРОТОЛИТИЧКЕ РАВНОТЕЖЕ У ХОМОГЕНОМ И ХЕТЕРОГЕНОМ СИСТЕМУ КЕТОКОНАЗОЛА И ЊЕГОВО ДИРЕКТНО СПЕКТРОФОТОМЕТРИЈСКО ОДРЕЂИВАЊЕ У ТАБЛЕТАМА

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Проучене су киселинско-базне равнотеже у хомогеном и хетерогеном воденом систему кетоконазола, дипротичне тешко растворне базе. Одређивања су вршена на температури 25 °C и при константној јонској сили 0,1 M (NaCl). Киселинска константа K_{a1} одређена је потенциометријски (pK_{a1} 3,20) и спектрофотометријски (pK_{a1} 3,26). Константа pK_{a2} 6,10 израчуната је из равнотежних константи одређених у хетерогеном систему: pK_{s0} 4,84 и pK_{s1} –1,26. На основу добијених вредности за константе израчуната је растворљивост и расподела равнотежних облика кетоконазола у функцији pH. На бази расподеле равнотежних облика кетоконазола предложена је спектрофотометријска метода за његово одређивање у комерцијалним таблетама. Одређивања су вршена на таласној дужини 225 nm у 0,1 M HCl. Метода је једноставна и брза, и омогућава директно спектрофотометријско одређивање кетоконазола, без претходног изоловања.

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