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Journal of Pharmaceutical and Biomedical Analysis
33 (2003) 131–136
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

Determination of bifonazole in creams containing methyl- and propyl *p*-hydroxybenzoate by derivative spectrophotometric method

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Received 15 January 2003; received in revised form 26 March 2003; accepted 26 March 2003

Abstract

A second order derivative spectrophotometric method for the determination of bifonazole in the presence of methyl- and propyl *p*-hydroxybenzoate as preservatives has been developed. The determination was performed in a 0.1 M HCl solution at 241.5 nm, a wavelength corresponding to the intersection of the second order derivative spectra (^{2}D) of methyl- and propyl *p*-hydroxybenzoate with the axis (zero-crossing point). On the basis of the knowledge of acidity constants and solubility, as well as of the investigations of zero-order and ^{2}D spectra of bifonazole and preservatives, these conditions were chosen as optimal ones. A calibration curve constructed for bifonazole concentrations ranging from 1.5 to 15 μg/ml had a correlation coefficient of 0.9998. Reliability and reproducibility of the method was checked by analyzing laboratory mixtures of bifonazole and preservatives (recovery 99.97–102.7%; RDS 0.48–1.46%). The proposed method was applied for the determination of bifonazole in a commercial cream formulation. The mean value of bifonazole obtained per 100 g cream was 1.029 g (102.9% of the labeled claim) with a RSD of 0.60%.
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Keywords: Bifonazole; Spectrophotometric determination; Derivative spectrophotometry; Cream formulation

1. Introduction

Bifonazole (1-[(1,1′-biphenyl)-4-ylphenylmethyl]-1H-imidazole) is a member of the group of antimycotic imidazole derivatives with a broad spectrum of action. It is applied against infections of the skin and nails caused by the fungi *Malassezia furfur* and *Candida* spp. It also shows in vitro antibacterial activity to some gram-positive cocci. During recent years, the application of imidazole derivatives is increasing due to their efficiency in the treatment of mycoses, especially in patients with a decreased immunity (e.g. after organ transplantations, AIDS, etc.). Bifonazole is official in the European Pharmacopoeia [1]. Pharmaceutical formulations containing bifonazole exist in the form of lotion, powder or cream.

In pharmaceutical formulations, bifonazole has been determined till present by chromatographic methods (HPLC [2,3], HPTLC [4], GC [5]),

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doi:10.1016/S0731-7085(03)00228-0

electroanalytically by using ion-selective electrodes [6,7] and spectrophotometrically [8,9] (based on the formation and extraction of an ion pair or by derivative spectrophotometry after supercritical fluid extraction).

In the present study a second order derivative spectrophotometric method for a direct determination of bifonazole in creams in the presence of methyl *p*-hydroxybenzoate (methylparaben) and propyl *p*-hydroxybenzoate (propylparaben) as preservatives is described. Due to their absorption in the UV region, the presence of such preservatives prevents a direct conventional spectrophotometric determination of bifonazole in pharmaceutical formulations. Moreover, turbidity of water suspensions of creams represents an additional drawback. The proposed second order derivative spectrophotometric method overcomes in a relatively simple way the problem of overlapping of the spectral bands and the effect of the background scattering signal caused by the turbidity of sample solutions.

2. Experimental

2.1. Reagents and apparatus

Bifonazole, methyl 4-hydroxybenzoate and propyl 4-hydroxybenzoate of pharmaceutical purity grade were kindly provided by 'Srbolek' Pharmaceutical Works (Belgrade, Yugoslavia). All other reagents were of analytical grade of purity (Merck).

The investigated Bicutrin cream (a product of 'Srbolek' Pharmaceutical Works) was claimed to contain 1.0 g bifonazole in 100 g cream.

Spectrophotometric measurements were performed on a GBC Cintra 20 spectrophotometer with 1.0 cm quartz cuvettes. Instrumental conditions were: wavelength range 200–500 nm; scan rate 1000 nm/min; slit 1.0 nm; data interval 2.56 nm.

2.2. Derivative spectrophotometric procedure

2.2.1. Standard solution

Stock solution of bifonazole (150 μg/ml) was prepared in 0.1 M HCl. Aliquots of 0.25, 0.50, 1.00, 1.50 and 2.50 ml of this solution were transferred into 25-ml volumetric flasks and diluted with 0.1 M HCl to 25 ml. The spectra were recorded using 0.1 M HCl as a blank. The amplitude of the second order derivative spectrum (smooth 21 points) was measured at 241.5 nm ($^2D_{241.5}$).

2.2.2. Analysis of bifonazole cream

The amount of the cream containing approximately 4.0 mg bifonazole was transferred into a 25-ml volumetric flask and 20 ml 0.1 M HCl was added. After homogenization by a warm water bath (10 min), the sample was cooled to ambient temperature and the volume adjusted to 25 ml with 0.1 M HCl. After filtration (blue strip filter paper), 1.0 ml of the solution was diluted to 25 ml with 0.1 M HCl. The resulting solution was slightly turbid. The ^2D spectra were recorded as described above for the series of standards.

The effects of the acidity and temperature on bifonazole and preservatives stability were examined spectrophotometrically. Applying the same procedure as that used for the analysis of the cream, no changes in the absorption spectra of the control samples containing these compounds individually were detected.

For the investigation of turbidity influence on the accuracy, solution of the cream with a standard bifonazole addition was prepared. The amounts of the cream and bifonazole stock solution each containing approximately 2 mg bifonazole were transferred into a 25-ml volumetric flask and 20 ml 0.1 M HCl were added. Upon homogenization in a warm water bath the volume was adjusted to 25 ml with 0.1 M HCl and filtered. One milliliter of the filtrate was diluted with 0.1 HCl to the volume of 25.0 ml. The spectrum of the resulting solution was recorded using 0.1 M HCl as a blank.

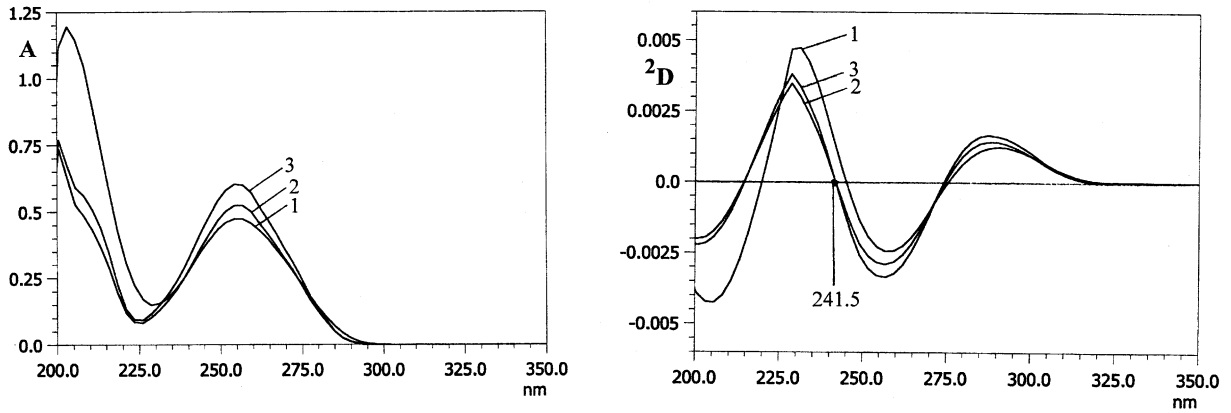

Fig. 1. Absorption (left) and second order derivative (right) spectra of: (1) bifonazole (6 μg/ml), (2) propylparaben (6 μg/ml), (3) methylparaben (6 μg/ml), in 0.1 M HCl.

2.3. Chromatography

Sample of the cream containing 500 μg bifonazole was dissolved in a mixture chlorophorm–ethanol (1:1; v/v) in a 10-ml volumetric flask. Sample solution (1.0 μl) and standard solution (50 μg/ml; 1.0 μl) were applied to 20 × 10 cm HPTLC silica gel $60F_{254}$ plates (Merck, Germany) using Nanomat II (Camag, Muttenz, Switzerland). The chromatograms were developed in twin-through chamber using: *n*-hexane–ethyl acetate–acetone–diethylamine (4.5:4.5:1:0.4; v/v/v/v), as a mobile phase. The plates were air-dried and the bifonazole spots scanned (TLC Scanner II, Camag, Muttenz, Switzerland) in the reflectance/absorbance mode at 260 nm. Quantitative evaluation was done by using peak area.

3. Results and discussion

Bifonazole is a monofunctional base with the pK_a value of 5.72 [10]. Parabens, as phenol derivatives with an alkoxy carbonyl group in the p-position, have a pK_a of about 8.0 [11]. Consequently, it may be concluded that at pH <4 and pH >10, all of the investigated compounds exist as a single form (at pH <4 bifonazole in protonated and parabens in molecular form; at pH >10 bifonazole in molecular and parabens in deprotonated form). Bifonazole is sparingly soluble in water in its molecular form (0.3 μg/ml) and the protonation of the imidazole nitrogen results in an increase of its solubility. This was the reason why an acidic medium was chosen for the determination of bifonazole performed throughout the present study. The absorption spectra of bifonazole and parabens in 0.1 M HCl are presented in Fig. 1. There is a strong overlapping of these spectra (Fig. 1, left). However, the influence of parabens was completely eliminated in the second order derivative spectra (^{2}D spectra). The ^{2}D spectra of both parabens intersect with the *x*-axis in the same point (zero crossing point) at 241.5 nm (Fig. 1, right). Therefore, 0.1 M HCl and a wavelength of 241.5 nm were selected as the optimal conditions for the determination of bifonazole in the presence of parabens.

A calibration curve was constructed using bifonazole concentration range from 1.5 to 15.0 μg/ml:

$$^2D_{241.5} = (1.717\times10^{-5}\pm2.157\times10^{-5}) + (2.247\times10^{-4}\pm2.565\times10^{-6})c, \quad (r = 0.9998)$$

where c is the concentration (μg/ml).

The accuracy and precision of the method were checked by determining bifonazole in the absence and in the presence of either methyl- or propylpar-

Table 1
Determination of bifonazole (Bf) in experimental mixtures with methylparaben (Mp) and propylparaben (Pp)

Bf taken (μg/ml)	Paraben taken (μg/ml)	Bf found (μg/ml)	RSD ($n = 5$) (%)	Recovery (%)
6.000	–	6.005	0.47	100.1
	Mp			
6.000	1.8	6.036	0.60	100.60
6.000	3.0	5.999	1.18	99.98
6.000	6.0	6.162	0.48	102.70
	Pp			
6.000	1.8	5.998	0.56	99.97
6.000	3.0	6.024	0.79	100.40
6.000	6.0	6.140	0.9	102.30
	Mp+Pp			
6.000	0.9+0.9	6.046	0.83	100.80
6.000	1.5+1.5	6.099	1.46	101.60
6.000	3.0+3.0	6.118	0.51	101.90

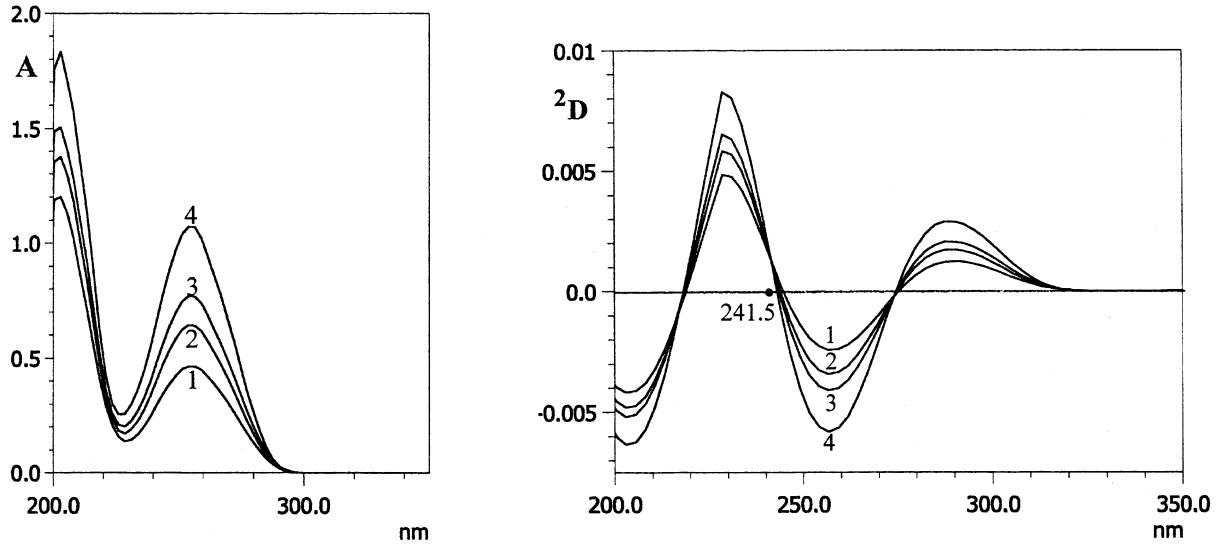

Fig. 2. Absorption (left) and second order derivative (right) spectra of bifonazole and its mixtures with methylparaben: (1) bifonazole (6 μg/ml), (2) bifonazole (6 μg/ml) and methylparaben (1.8 μg/ml), (3) bifonazole (6 μg/ml) and methylparaben (3 μg/ml), (4) bifonazole (6 μg/ml) and methylparaben (6 μg/ml) in 0.1 M HCl.

aben, as well as in the presence of their mixture. The results of these investigations are summarized in Table 1. Since 1% bifonazole and maximum 0.25% parabens [12] are contained in pharmaceutical preparations, laboratory mixtures were prepared with mass ratios of bifonazole to paraben of 1:0.3, 1:0.5 and 1:1. For comparison, the absorption and ^{2}D spectra of bifonazole and methylparaben mixtures are presented in Fig. 2. It can be seen (Fig. 2, right) that the ^{2}D spectra of either bifonazole alone or its mixtures with methylparaben intersect at 241.5 nm, i.e. that the amplitudes

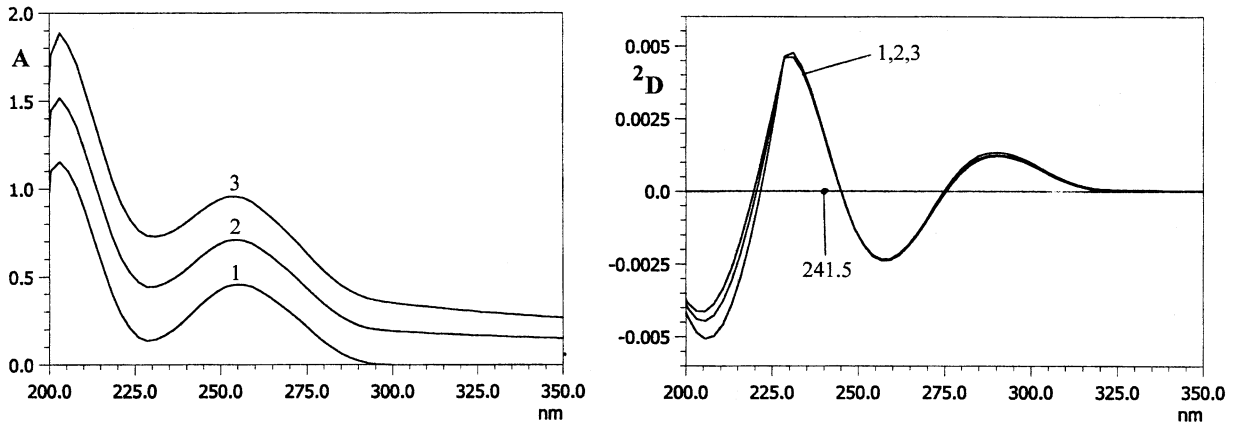

Fig. 3. Influence of turbidity on absorption (left) and second order derivative spectra (right) of: (1) bifonazole (6 μg/ml), (2) cream (3 μg/ml bifonazole)+bifonazole (3 μg/ml), (3) cream (6 μg/ml bifonazole).

Table 2
Bifonazole determination in the commercial cream Bicutrin (label claim: 1 g bifonazole in 100 g cream) applying derivative spectrophotometry, without and with standard addition of bifonazole

Taken[a] (μg/ml)		Found (μg/ml)	Found (g/100 g cream)	RSD (n = 5) (%)	Percentage of label claim
From cream	From stock solution				
6.102	–	6.279	1.029	0.60	102.9
3.036	3.000	6.116	1.013	2.65	101.3

[a] Bifonazole concentration in the working solution.

of the derivative spectra at 241.5 nm of bifonazole and its mixtures with the preservative have the same value, regardless the added amount of methylparaben.

On the basis of the results listed in Table 1, it may be concluded that the application of the proposed method enables the determination of bifonazole in the presence of the maximum permitted amounts of parabens in pharmaceutical formulations, as well as up to 1:1 (w/w) paraben mixtures, with a high accuracy.

The proposed method was applied for the determination of bifonazole in the commercial cream formulation (trade name Bicutrin) which contains methyl- and propylparaben. Since the final solutions subjected to analyses as described in Section 2 were slightly turbid, the influence of the turbidity on the accuracy of bifonazole determination was examined, as well. The absorption and ^{2}D spectra of bifonazole solution, the cream solution and solution of the cream with a standard bifonazole addition are depicted in Fig. 3. It can be seen (Fig. 3) that the influence of the turbidity on the basic absorption bifonazole spectrum (Fig. 3, left) was eliminated in ^{2}D spectra (Fig. 3, right). The data obtained by determining bifonazole in the cream are summarized in Table 2. The results on bifonazole content in the cream in the absence and in the presence of a standard bifonazole amount were in a good accordance. Such a satisfactory accordance clearly demonstrates that the turbidity of the solution did not influence the accuracy of bifonazole determination by the second order derivative spectrophotometric method developed throughout the present study.

HPTLC method [4] was employed as a reference to check the proposed derivative spectrophotometric method (Table 3). The results obtained by

Table 3
Determination of bifonazole in Bicutrin cream by the proposed method and a reference HPTLC method [4]

Percentage of label claim ± SD[a]		t-test[b]	F-test[c]
Derivative method	HPTLC		
102.9 ± 0.62	102.0 ± 1.44	1.395	5.48

[a] Mean and standard deviation for five determinations.
[b] Tabulated value of $t = 2.306$ ($P = 0.05$).
[c] Tabulated value of $F = 6.39$ ($P = 0.05$).

these two methods agreed well as judged by statistical analyses (t- and F-tests). Besides, this accordance of the results demonstrates that the other excipients of the cream do not interfere with bifonazole determination by the proposed derivative spectrophotometric method.

The developed derivative spectrophotometric method for the determination of bifonazole is simple and specific. It can be applied for a direct determination of bifonazole in creams containing methyl- and propylparaben, without tedious and time-consuming bifonazole isolation and application of rather expensive experimental approaches. In addition, the proposed method could be applied for bifonazole determination in other pharmaceutical formulations without preservatives, or containing methyl- and propylparaben (alone or as a mixture).

Acknowledgements

This work was supported by the Ministry for Science, Technology and Development of Serbia, Grant #1458.

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