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Development and validation of UV spectrophotometric procedure for estimation of bifonazole in bulk

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ABSTRACT

A simple, precise, accurate, reproducible and low cost UV-spectrophotometric method has been developed and validated for the quantification of Bifonazole in bulk. The linearity is found at the 256 nm for 0.0006-0.001% Bifonazole solutions in 0.1 M HCl according to Beer–Lambert–Bouguer law. The determination coefficient is 0.999, LOD - 1.50 μ g/mL, and LOQ - 4.54 μ g/mL. The exact ready-to-use methodic for quantity determination is proposed, which can be successfully used for determination of Bifonazole in bulk and for future studies in pharmaceutical formulations.

Keywords: Bifonazole, validation, UV-spectrophotometry

INTRODUCTION

(±)-Bifonazole (Mycospor®) is a substituted imidazole analogue, chemically known as 1-(biphenyl-4-yl(phenyl)methyl)-1*H*-imidazole (Fig. 1) is broad spectrum imidazole antimycotic, that has broad spectrum activity against dermatophytes, molds, yeasts, dimorphic fungi, and some Grampositive bacteria [1]. Which like Clotrimazole, interfers with sterol biosynthesis by inhibition of the cytochrome P₄₅₀-dependent hydroxylation, but also additionally inhibits directly HMG-CoAreductase [2]. Bifonazole is also a potent inhibitor of cytochrome P_{450} aromatase (K_i = 68 nM, IC₅₀ = 270 nM), which catalyzes the biosynthesis of estrogens from androgens [3]. It is found to have activity against seborrhoeic dermatitis [4] and Tinea infections [5].

In pharmaceutical formulations, bifonazole was reported to be determined by chromatographic methods (HPLC [6,7], HPTLC [8], GC [9,10]), electroanalytically by using ion-selective electrodes [11,12] and spectrophotometrically [13,14, 16]. For instance, among the latest studies, there was one, based on the charge transfer complexation reaction of bifonazole with 2,5-dichloro-3,6-dihydroxy-1,4benzoquinone (CAA) or with 2,3-dichloro-5,6dicvano-1,4-benzoquinone (DDQ), resulting in the formation of colored complexes, quantitated at the 517 nm and the 457 nm for bifonazole-CAA and bifonazole-DDQ, respectively [13]. A second order derivative spectrophotometric method of its determination in the presence of methyl and propyl *p*-hydroxybenzoate as preservatives has been performed in a 0.1 M HCl solution at the 241.5 nm, a wavelength corresponding to the intersection of the second order derivative spectra of methyl and propyl *p*-hydroxybenzoate with the axis [14]. Also a simple, precise and sensitive RP-HPLC method has been developed for the quantitation of bifonazole simultaneously with benzyl alcohol in methanol - ammonium acetate (pH 2; 65 mM) (65:35, v/v, pH=3.6) as mobile phase with flow rate of 1 mL min⁻¹ and variable UV detection at the 220 and the 252 nm [15].

But our interest was attracted by the simplest and accurate method with the usage of just 0.1 M HCl solution to develop ready-to-be used methodic for

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Bifonazole quantity determination by UV maximum absorbance at the 255 nm [16]. Hence, such procedure was proposed and validated.

MATERIALS AND METHODS

Instrumentation: Substance was weighed using analytical balances Kern ABT 120-5DM, KERN&Sohn GmbH, Germany. UV spectra were recorded on Analytic Jena UV-vis spectrophotometer Specord 200 (190-400 nm), Germany.

Reagents and solutions: All of the chemicals were of the highest purity available from the LAB-SCAN (Ireland) and were used without any further purification. The distilled water was used throughout the experiments. Working substance of (\pm) -Bifonazole was purchased from Changzhou Pharmaceutical Factory (China) (CAS 60628-96-8).

Validation

Calibration curve: Standard solution (0.0008%) was prepared by dissolving 0.0500 g of Bifonazole in the 100.0 mL flask with 0.1 M solution of HCl. Stirred for 5 min. Then 0.80 mL of obtained solution was quantitatively transferred into the 25.00 mL flask, added 0.1 M solution of HCl. Stirred for 5 min. The amount of Bifonazole was determined by employing UV absorption at the wavelength of the 256 nm. The five working standard solutions (0.0006-0.001%) were made quantitatively in the same way, diluting from 0.0462 g to 0.0604 g of Bifonazole. All solutions were stored at 18-20 °C. The calibration curve of was constructed by a UV-vis Bifonazole spectrophotometer absorption data monitored 5 times for each sample at the wavelength of maximum absorbance at the 256 nm in comparison to 0.1 M solution of HCl in 3 mL cuvette with 1 cm layer. The regression equation was obtained by the method of least squares for n=5. Regression equation: Y = slope*x + intercept. Slope, intercept and determination coefficient were determined from the regression analysis' calculations in Microsoft Excel 2007 [17]. Using this linear equation, determination coefficient (r^2) and the detection limits were determined.

Accuracy: mean \pm SD; Linearity (lowest – highest concentration while curve is linear); SE of intercept: $\sqrt{}$ of $\sum(y-y^2/n)$, where y - standard concentration, y' - found concentration; SD of intercept: SE of intercept* \sqrt{n} .

The limit of detection (LOD): 3.3*(SD of intercept / slope); and the *limit of quantitation* (LOQ): 10*(SD of intercept / slope). The LOD was defined by the

concentration with a signal-to-noise ratio of 3. The analyte peak in the LOQ sample should be identifiable, discrete, and reproducible with a precision of $\pm 20\%$ and accuracy within 80%–120%. The deviation of standards other than LOQ should not be more than $\pm 15\%$ of the nominal concentration.

Precision (repeatability of the method) was evaluated by repeated absorbance detection and the results were expressed as the mean standard deviation (SD) and the percent relative standard deviation RSD (%) = SD/Mean. For intra-day analysis the samples were analyzed six times a day at 09:00 am, 11:00 am, 01:00 pm, 03:00 pm, 05:00 pm, and 07:00 pm, while for inter-days stability was analyzed for 6 consecutive days at 09:00 am.

RESULTS AND DISCUSSION

Among all the reported procedures of Bifonazole determination, UV detection in 0.1 M HCl was chosen as the cheapest and simplest one with the linearity range of 0.0001-0.0012 g/100mL (1-12 μ g/mL) [18]. After the series of dilutions it was found, that 0.0008% solution of Bifonazole in 0.1 M HCl solution had two absorbance maximums at the 205 and 256 nm wavelengths of appropriate value of the last one (0.6623) according to the Beer–Lambert–Bouguer law (**Fig. 2**). Thus, it was chosen for validation studies for quantity determination

The five samples of 0.0400-0.600 g were taken to measure the linearity and the next calibration curve was obtained (**Fig. 3**).

The linearity was evaluated by linear regression analysis by the least-square regression analysis. The calibration curve of Bifonazole had good determination coefficient 0.999 (Fig. 3). Thus, it was established, that 0.0006-0.001% solution of Bifonazole in 0.1 M HCl, but not mentioned by Wahab 0.0001-0.0012%, was appropriate for quantity determination as well as maximum absorbance the 256 nm, but not the 255 nm [16]. Validation of the method was prepared in accordance to the analytical methods validation parameters [18]. The samples of Bifonazole, taken to make working solutions, method's calculated accuracy and recovery data are presented in the Table 1. The accuracy of the method was proven by recovery of five different concentrations of the $(100.00\pm0.33\%)$ calibration range with 100.01±0.003% percent of recoveries.

Slope, intercept and determination coefficient were found from the regression analysis' calculations (**Table 2**).

The limit of detection and limit of quantification were also higher if represented in μ g/mL to be compared with reported earlier: LOD – 1.4981 against 0.3 μ g/mL and LOQ 4.5397 – 1.1 μ g/mL (**Table 3**) [16].

Investigations of the ruggedness revealed method's good reproducibility: during the day RSD was 0.01% and during the week -0.02% (**Table 3**).

The robustness of the method was studied through temperature influence, stirring and usage of different 0.1 M HCl solutions. It was detected, that usage of the cuvette without glass cap, or even with cap, due to water evaporation or condensation at the cap, slightly resulted the absorption results, if cuvette was left for at least one hour in spectrometer because of lamp heating. The mechanical stirring for 10 min of each flask and temperature of 20-25°C were the best conditions to obtain accurate data.

Hence, to detect the amount of Bifonazole in pure substance the next procedure is proposed. Quantitatively place 0.0500-0.0600 g of Bifonazole in the 100.0 mL flask, dissolve it with 0.1 M HCl. Stir for 10 min. Quantitatively transfer 0.80 mL of obtained solution into the 50.00 mL flask, dissolve it with 0.1 M HCl. Stir for 10 min. Determine the amount of Bifonazole by employing UV absorption at the wavelength of 256 nm in comparison to 0.1 M HCl in 3 mL cuvette with 1 cm layer.

Calculate the sample concentration in accordance to the absorbance of standard solution:

$$=\frac{Ai \cdot 0.0008}{}$$

C, % 0.6623

where Ai – absorbance of the final experimental sample solution;

0.0008 - concentration of the standard solution of Bifonazole with absorbance of 0.6623 in 0.1 M solution of HCl at the 256 nm, %.

Or in accordance with sample weight calculate the initial pure substance:

C, % in bulk =
$$\frac{Ai \cdot Co \cdot 100.0 \cdot 50.00}{A_0 \cdot \eth \cdot 0.80 \cdot l},$$

where Ai – absorbance of the final experimental sample solution;

 C_0 – concentration of the standard solution of Bifonazole is 0.0008, %;

100.0, 50.00 – flasks dilutions volume, mL;

 A_0 – maximum absorbance of 0.0008% standard solution at the 256 nm is 0.6623,

p - taken sample weight (0.0500-0.0600) to obtain maximum absorbance (0.6-0.8) at the 256 nm, g,

0.80 – sample volume taken by pipette, mL;

l – cuvette layer, 1 cm;

CONCLUSION

It was found, that in Bifonazole 0.1 M HCl solution could be simply, low-cost, fast and accurately quantitatively determined by UV spectroscopy in bulk by the maximum absorbance at the 256 nm. Validation of the proposed method showed, that calibration curve had good linearity ($R^2 = 0.999$) in the concentration range 0.0006-0.001%. The LOD was found to be 1.50 µg/mL, and LOQ - 4.54 µg/mL. Such criteria like accuracy, precision, robustness and ruggedness also showed high validity and reproducibility. The proposed simple technique could be successfully used for determination Bifonazole in bulk in analytical laboratories and pharmaceutical factories.

Fable 1. Sample mass of Bifonazole	, final solutions absorbance,	recovery and accuracy data.
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# Sample a	ΔAmax	%RSD,	^a Sample found a	^b Recovery	
# Sample, g		of 5 meas.	of Amax	Sample Toulid, g	Recovery
1	0.0462	0.6091	0.0065	0.0462	100.0212
2	0.0500	0.6623	0.0062	0.0500	100.0071
3	0.0558	0.7458	0.0048	0.0560	100.2833
4	0.0576	0.7645	0.0055	0.0573	99.4646
5	0.0604	0.8101	0.0023	0.0605	100.2374
				Mean	100.0027
				SD	0.3255
				%RSD	0.0029
				Accuracy, %	100.0027±0.3255
				Recovery, %	100.0027±0.0029

^aFound sample mass = (absorbance – intercept)/slope;

^bRecovery = found sample mass / labeled concentration*100.

Parameters	Data
Slope	14.023
Intercept	0.0389
Linearity (g/100mL/50mL)	0.4500-0.6000
Regression equation	y = 14.023x - 0.0389
r^2	0.999
SE of intercept	0.0142
SD of intercept	0.0318
LOD (g to be diluted in 100mL and 50mL)	0.0075
LOD (µg/mL)	1.4981
LOQ (g to be diluted in 100mL and 50mL)	0.0227
LOQ (µg/mL)	4.5397

Lyudmyla *et al.*, J Pharm Biol Sci 2016; 4(4): 111-115 Table 2. Linearity data, accuracy and precision of investigated Bifonazole solutions in 0.1 M HCl.

Table 3. Intra-day and inter-day precision data of 0.0008% Bifonazole solution in 0.1 M HCl.

#	Amax at the 256 nm			
	Intra-day precision	Inter-day precision		
1	0.6604	0.6604		
2	0.6603	0.6475		
3	0.6595	0.6348		
4	0.6579	0.6221		
5	0.6529	0.6612		
6	0.6505	0.6552		
Mean	0.6569	0.6469		
SD	0.0042	0.0156		
%RSD	0.0058	0.0220		



Fig. 1. Bifonazole structure.



Fig. 2. UV spectrum of 0.0008% solution of Bifonazole in 0.1 M HCl with the 205 and 256 nm absorbance maximums.





Fig. 3. Calibration curve of 0.0006-0.001% solutions of Bifonazole in 0.1 M HCl by UV absorbance at the 256 nm.

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