

Research Article

Uv Spectrophotometric Method For Determination Of Bifonazole

Mr. Koustubh S. Pachkor¹, Miss. Bhuwaneshwari V. Asekar², Miss. Shilpa S. Borkar³

^{1,2,3}Department of Pharmaceutics, At. Kamla Nehru Collage of Pharmacy, Butibori, Nagpur.

Received: 05.01.26, Revised: 07.02.26, Accepted: 24.03.26

ABSTRACT

A simple, precise, accurate and reproducible UV-spectrophotometric method has been developed and validated for quantification of Bifonazole in bulk. Bifonazole is soluble in 0.1M HCl. The UV spectrum of Bifonazole in 0.1M HCl shows maximum absorbance at 254nm after scanning in 200-400nm range. The concentration range for analysis was 0.02-0.12 µg/ml. The method was validated for linearity, accuracy, precision, robustness, LOD, LOQ and ruggedness. The linearity equation was found to be $y = 11.13x + 0.0294$ with correlation coefficient $R^2 = 0.9978$. LOD and LOQ were found to be 0.123612255µg/ml and 0.374582589µg/ml respectively. The %recovery was found to be in range 91-103.91%. Based on the validation results the method can be successfully used for determination of Bifonazole in bulk.

Keywords: Method DEVELOPMENT, Bifonazole, Uv Spectrometry, Lod AND Loq.

INTRODUCTION

Bifonazole 1-[biphenyl1-4-yl(phenyl)methyl]imidazole is an imidazole derivative having an antifungal activity against skin or mucosal mycoses [1]. Local therapy formulations are preferable in order to maximize medication penetration deep into the epidermis, which is the site of action. However, these medications are primarily maintained in the stratum corneum (SCR) and are noticeably lipophilic [2]. Bifonazole is active in- vitro against dermatophytes, provocations, moulds, dimorphic fungi and some Gram-positive bacteria [3]. When compared with majority of other topical antifungal drugs needed to be applied at least twice daily, bifonazole offers the convenience of once daily administration, which improves patient compliance. Bifonazole workshop by inhibiting product of ergosterol, an

essential element of fungal cell membranes. Bifonazole acts by destabilizing fungal cytochrome- P450 51 enzyme which is vital in fungal cell membrane structure, leading to cell lysis. dislocation in product of ergosterol disrupts the cell membrane and causes holes to appear in cell membrane, essential ingredients of fungal cells blunder out, leading to fungal cell death. Bifonazole is used for the treatment of colourful topical fungal infections, including athlete's bottom (tinea pedis) [4]. Bifonazole was determine by various chromatographic method HPLC (5,6), HPTLC (7), GC (8,9), electrolytically by using ion selective electrodes (10) and spectrophotometrically (11, 12) [13]. Spectrophotometry is one of the most widely used drug testing techniques due to its affordability, ease of use, and high specificity.

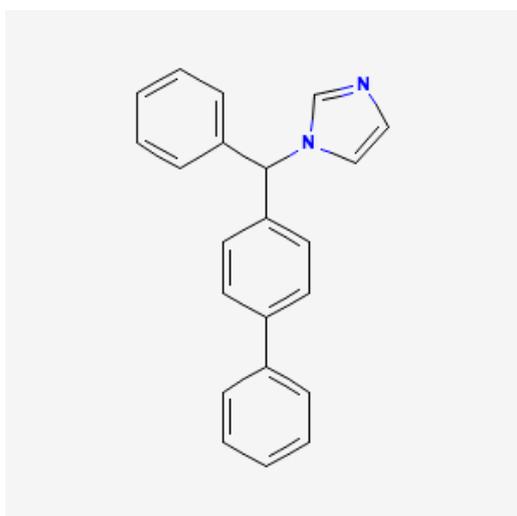


Fig. 1: 2d Structure [14]

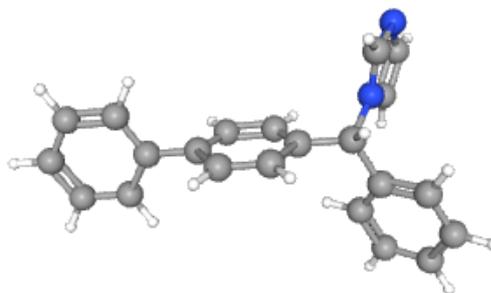


Fig. 2: 3d Structure [15]

MATERIALS AND METHODS

Chemicals and Reagents

Bifonazole pure drug was a gift sample from Srikem Laboratories Pvt. Ltd, Mumbai; Concentrated HCl and distilled water laboratory.

Instruments

100ct Diamond Pocket Scale and UV-visible double beam spectrophotometer (Shimadzu UV-1800) instruments were used.

METHOD

Preparation of Standard Stock Solution:

Accurately weighed 1.0 mg of bifonazole was transferred into 10 ml 0.1 M HCl in 25 ml volumetric flask (Conc. Of Bifonazole: 0.1000 μ g/ml). After homogenization by a warm water bath for (10 min.), the sample was cooled to at room temperature. After filtration, 2 ml of the solution was diluted in to 20 ml with 0.1 M HCl (Conc. Of Bifonazole: 0.01000 μ g/ml). The effects of the acidity and temperature on Bifonazole were examined UV-spectrometry at 254 λ^{\max} .

Selection of wavelength for analysis of Bifonazole

Appropriate volume 1ml of standard stock solution of Bifonazole was transferred in a 10ml of volumetric flask, diluted to a mark with 0.1 M HCL to give concentration of 0.00100 μ g/ml. The resulting solution was scanned in the UV range (200-400nm), bifonazole showed absorbance maximum at 254nm.

Method Validation

The method was validated as per ICH guidelines Q2(R1) in terms of linearity, accuracy, precision, LOD, LOQ, ruggedness, robustness and range [15]

1. Linearity

Different aliquots of bifonazole in the range 1-6ml of stock solution were transferred into series of 10 ml volumetric flasks and volume was made up to the mark with 0.1 M HCL to get concentrations respectively. The solution was analyzed in the UV range on spectrophotometer. The spectrum was recorded at 254nm and calibration plot was plotted as absorbance vs concentration.

2. Range

The range of an analytical procedure is interval between upper and lower concentration of analyte in sample for which it has been shown that analytical procedure has a suitable level of

linearity, accuracy, precision. The obtained range of an analyte is 0.02 to 0.12 μ g/ml.

3. Accuracy

Accuracy was determined by preparing solution of different concentration that is 80, 100 and 120%. The percentage recovery was calculated.

4. Precision

Precision of analytical method was studied by performing repeatability. Repeatability studies were carried out by estimating responses of working standard solution (conc. of Bifonazole: 0.06 μ g/ml) for 5 times. The results were reported in terms of percentage relative standard deviation(%RSD).

5. Robustness

Robustness of the proposed method is determined for 0.06 μ g/ml concentration of bifonazole by analysing aliquots from a homogenous slot of two different wavelengths, at two different temperatures using the same environmental conditions.

6. Limit of Detection (LOD)

The limit of detection (LOD) was calculated by using equation

$$LOD = 3.3 * \sigma / S$$

Whereas, σ = Standard deviation and S= Slope of regression coefficient.

7. Limit of Quantification (LOQ)

The limit of quantification (LOQ) is an individual analytical procedure, it is the lowest amount of analyte in the sample. LOQ was calculated by using equation.

$$LOD = 10 * \sigma / S$$

Whereas, σ = Standard deviation and S= Slope of regression coefficient.

The linearity equation was found to be $y = 11.13x + 0.0294$

The LOD and LOQ of bifonazole was found to be 0.123612255 μ g/ml and 0.374582589 μ g/ml respectively.

8. Ruggedness

Ruggedness is determined by analysing aliquots from a homogenous slot by two analysts for 0.06 μ g/ml concentration of bifonazole using same operational and environmental conditions.

RESULTS AND DISCUSSION:

The absorption spectrum shows λ_{\max} of Bifonazole at 254nm.

Method Validation

1. Linearity

The absorbance is proportional to the concentration and linearity is in the range of

0.02-0.12µg/ml (Table: I). The value of r^2 was 0.9978 which is well within acceptance limit ($r^2 < 1$).

Table I: Linearity of Bifonazole

concentration	absorbance
0.02	0.232
0.04	0.492
0.06	0.702
0.08	0.915
0.1	1.168
0.12	1.342

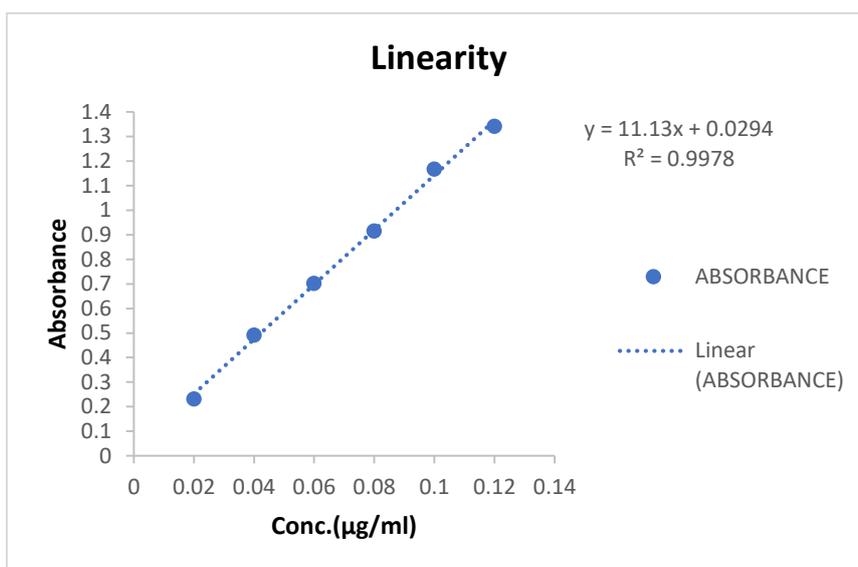


Fig. 3: Linearity Curve of Bifonazole

2. Range

The range of Bifonazole is found to be 0.02 - 0.12 µg/ml.

The percentage recoveries of the results indicate that the recoveries are well within acceptance range ($RSD < 2$), therefore method is accurate.

3. Accuracy

Table II: Accuracy Results of Bifonazole

Name of Drug	concentration (µg/ml)	absorbance	found concentration (µg/ml)	recovery (%)	STDEV
Bifonazole	0.02	0.232	0.018203055	91.01527403	4.515645633
	0.04	0.492	0.041563342	103.9083558	
	0.06	0.702	0.060431267	100.7187781	
	0.08	0.915	0.079568733	99.46091644	
	0.1	1.168	0.10230009	102.3000898	
	0.12	1.342	0.117933513	98.27792752	

4. Precision

The % RSD < 2 values obtained shows that method developed is precise. Absorbance of

standard solution were recorded (Conc. of Bifonazole=0.06µg/ml)

Table III: Precision Results of Bifonazole

Responses	Absorbance
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1	0.702
2	0.703
3	0.702
4	0.701
5	0.702
Average	0.702
STDEV.	0.000707107
%RSD	0.100727462

5. Robustness

Deliberate changes were made in the wavelength keeping other operational

parameters same and effect on the results were observed. (Table IV)

Table IV: Result of Robustness of Bifonazole

Maximum wavelength (nm)	254 nm	256 nm
Absorbance	0.702	0.704
	0.703	0.705
	0.702	0.704
Average	0.702333333	0.704333333
STDEV	0.00057735	0.00057735
%RSD	0.082204595	0.081971169

6. LOD

LOD of Bifonazole was found to be 0.123612255µg/ml.

7. LOQ

LOQ of Bifonazole was found to be 0.374582589µg/ml.

8. Ruggedness

The change in analyst and laboratories with the same concentration of 0.06 µg/ml gave reproducible results. Hence the parameter was found to be validated.

Table V: Result of Ruggedness of Bifonazole

Concentration	Absorbance (Analyst1)	Absorbance (Analyst2)
0.06 µg/ml	0.702	0.703
	0.703	0.702
	0.702	0.703
	0.701	0.702
	0.702	0.702
Average	0.702	0.7024
STDEV	0.000707107	0.000547723
%RSD	0.100727462	0.077978724

CONCLUSION

An explanatory UV spectrophotometric strategy was developed and validated for linearity, accuracy, precision, range, LOD, LOQ, ruggedness and robustness. The approval affirms that this is a suitable strategy that can be utilized for assurance of bifonazole in bulk in expository research facilities and pharmaceutical industrial facilities.

Acknowledgement

The researchers are grateful thanks to Srikem Laboratories, Pvt. Ltd. for giving gratis sample of Bifonazole API.

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