IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF BIFONAZOLE IN BULK

Perampalli N.L*, Ansari A.M, Patki P.R, Khare A.S

Department of Pharmaceutical Quality Assurance, D.S.T.S. Mandal's College of Pharmacy, Solapur-413004, Maharashtra, India.

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 ethanol: water (9:1) mixture. The UV spectrum of Bifonazole in ethanol: water (9:1) mixture shows maximum absorbance at 254nm after scanning in 200-400nm range. The concentration range for analysis was 6-10 µg/ml. The method was validated for linearity, accuracy, precision, robustness, LOD, LOQ and ruggedness. The linearity equation was found to be y=0.1016x - 0.117 with correlation coefficient 0.995. LOD and LOQ were found to be 1.098µg/ml and 3.328µg/ml respectively. The %recovery was found to be in range 98.81-100.32%. Based on the validation results the method can be successfully used for determination of Bifonazole in bulk.

KEYWORDS:

Bifonazole, Method development, UV Spectrophotometer, Validation.

INTRODUCTION:

Bifonazole 1-[biphenyl-4-yl(phenyl)methyl]imidazole is an imidazole derivative having an antifungal activity against skin or mucosal mycoses[1].Bifonazole is active in-vitro against dermatophytes, yeasts, moulds, dimorphic fungi and some Gram positive bacteria[2].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[3].Bifonazole works by inhibiting production of ergosterol, an essential component 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. Disruption in production of ergosterol disrupts the cell membrane and causes holes to appear in cell membrane, essential constituents of fungal cells leak out, leading to fungal cell death. Bifonazole is used for the treatment of various topical fungal infections, including athlete's foot (tinea pedis)[4].Bifonazole was determined by various chromatographic methods (HPLC[5],[6]), (GC[7],[8]), electrolytically by using ion selective electrodes and spectrophotometrically[9].Among the various methods available for determination of drugs, spectrophotometry is very popular because of its simplicity, specificity and low cost.

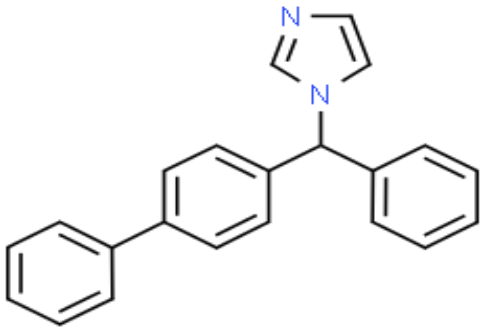

Fig. 1: Chemical structure of Bifonazole

MATERIALS AND METHODS:

Chemicals and reagents:

Bifonazole pure drug was a gift sample from Srikem Laboratories Pvt. Ltd, Mumbai. Methanol and water were used of HPLC grade

Instruments:

Analytical balance (Acet CY224C) and UV-Visible double beam spectrophotometer (Labman LMSP-UV1900) instruments were used.

Method:

Preparation of standard stock solution:

Accurately weighed 10mg of Bifonazole was transferred in 10ml volumetric flask. Sufficient quantity of mixture of ethanol: water (9:1) was added to dissolve the Bifonazole. The volume was made up to mark using same solvent (Conc. Of Bifonazole: 1000µg/ml). 1ml of above solution was transferred into 10ml volumetric flask and volume was made up to mark using mixture of ethanol: water (9:1) (Conc. Of Bifonazole: 100µg/ml)

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 ethanol and water (9:1) mixture to give concentration of 10µg/ml. The resulting solution was scanned in the UV range (200-400nm), bifonazole showed absorbance maximum at 254nm.

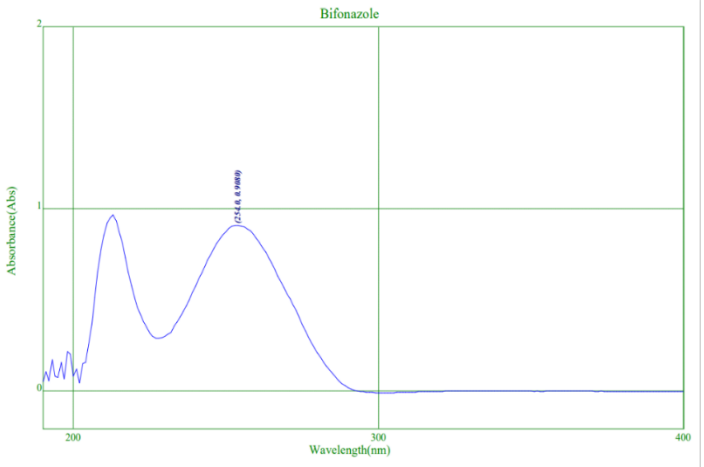

Fig. 2: UV-visible spectra of Bifonazole

Method Validation:

The method was validated as per ICH guidelines Q2(R1) in terms of linearity, accuracy, precision, LOD, LOQ, ruggedness, robustness and range[10].

1. **Linearity:**
Different aliquots of bifonazole in the range 0.6-1ml were transferred into series of 10 ml volumetric flasks and volume was made up to the mark with ethanol: water (9:1) mixture to get concentration 6,7,8,9,10µg/ml 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 6 to 10µg/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: 8µg/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 8µg/ml concentration of bifonazole by analyzing 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=0.1016x - 0.117**

The LOD and LOQ of bifonazole was found to be 1.098µg/ml and 3.328µg/ml respectively

Ruggedness:

Ruggedness is determined by analyzing aliquots from a homogenous slot by two analysts for 8µg/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 6-10µg/ml (Table:I). The value of r^2 was 0.995 which is well within acceptance limit ($r^2 < 1$).

Table I: Linearity of Bifonazole

Concentration (µg/ml)	Absorbance
6	0.5057
7	0.5869
8	0.6812
9	0.7986
10	0.908

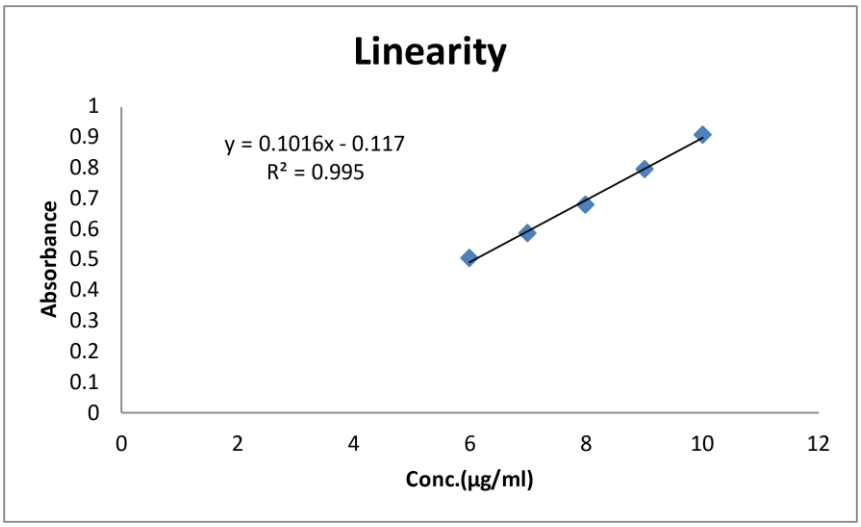

Fig. 3: Linearity curve of Bifonazole

2. Range:

The range of Bifonazole is found to be 6-10µg/ml.

3. Accuracy:

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

Standard absorbance: 0.681

Table II: Accuracy Results of Bifonazole

Name of drug	% recovery	conc (µg/ml)	Abs	Amt recovered	% Recovery	Average % Recovery	STDEV
Bifonazole	80	6.4	0.544	6.39	99.840	99.92	0.11
			0.545	6.40	100		
	100	8	0.682	8.01	100.100	100.05	0.070711
			0.681	8	100		
	120	9.6	0.819	9.62	100.200	100.25	0.070711
			0.820	9.63	100.300		

4. Precision:

The % RSD < 2 values obtained shows that method developed is precise. Absorbance of standard solution were recorded (Conc. of Bifonazole=8µg/ml)

Table III: Precision Results of Bifonazole

Reps	Absorbance
1	0.681
2	0.682
3	0.681
4	0.68
5	0.681
Average	0.681
STDEV	0.000707107
RSD	0.103833595

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	256
Absorbance	0.681	0.683
	0.682	0.684
	0.681	0.683
Average	0.681333333	0.683
STDEV	0.00057735	0.001
% RSD	0.084738298	0.146413

6.LOD:

LOD of Bifonazole was found to be 1.098364187µg/ml.

7.LOQ:

LOQ of Bifonazole was found to be 3.328376325µg/ml.

Ruggedness:

The change in analyst and laboratories with the same concentration of 8µg/ml gave reproducible results. Hence the parameter was found to be validated.

Table V: Result of Ruggedness of Bifonazole

Concentration	**Absorbance (Analyst1)**	**Absorbance (Analyst2)**
8µg/ml	0.681	0.682
	0.682	0.681
	0.681	0.682
	0.68	0.681
	0.681	0.681
Average	0.681	0.6814
STDEV	0.000707107	0.000547723
RSD	0.103833595	0.080381943

Summary of Validation Parameters:

Table VI: Summary of Validation Parameters

Parameters	**Values**
Maximum wavelength (nm)	254
Range (µg/ml)	6-10
Regression equation	y=0.1016x - 0.117
Slope	0.1016
Intercept	0.117
Regression coefficient (r^2)	0.995
% RSD	0.103833595
LOD (µg/ml)	1.098364187
LOQ (µg/ml)	3.328376325

CONCLUSION:

An analytical UV spectrophotometric method was developed and validated for linearity, accuracy, precision, range, LOD, LOQ, ruggedness and robustness. The validation confirms that this is an appropriate method that can be used for determination of bifonazole in bulk in analytical laboratories and pharmaceutical factories.

ACKNOWLEDGEMENT:

The authors are thankful to Srikem Laboratories, Pvt.Ltd. for providing gift sample of Bifonazole API.

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

References:

[1] Suñer-Carbó J et al. Biopharmaceutical Development of a Bifonazole Multiple Emulsion for Enhanced Epidermal Delivery. Pharmaceutics. 2019 Feb 2;11(2):66. https://doi: 10.3390/pharmaceutics11020066.

[2] Abdel Naser Zaid, Ayman Qaddomi , Saed Khammash. Formulation of bifonazole powder and development of a new stability indicating HPLC method for its analysis. Journal of Pharmacy Research 2012,5(4),1857-1861.

[3] Lacker TE, Clissold SP. Bifonazole. A review of its antimicrobial activity and therapeutic use in superficial mycoses. Drugs 1989;38(2):24-25.

[4] Bifonazole [Internet]. Drug Bant [cited 2020 Nov 4]. Available from;https://go.drugbank.com/drugs/DB04794.

[5] Pietra AMD et al. HPLC analysis of imidazole antimycotic drugs in pharmaceutical formulations. J Pharm Biomed Anal. 1992;10:873-9.

[6] Guo XJ et al. Determination of bifonazole in creams by High performance liquid chromatography. Sepu 2001;19:279-80.

[7] Kublin E, Kaniewska T. Determination of antimycotic substances, derivatives of imidazole by gas chromatographic method. Chem Analityczna 1996;41:19-25.

[8] Triolo A et al. Gas chromatographic-mass spectrometric determination of two new antimycotic agents, 1-[(5-chloro-2-benzofuranyl)(2-chlorophenyl)methyl]-1H-imidazole and 1-[(5-bromo-2-benzofuranyl)phenylmethyl]-1H-imidazole, in rabbit plasma following topical administration: A preliminary comparison with bifonazole. J Chromat B: Biomed Sci App 1991:567(1):105-12. https://doi:/10.1016/0378-4347(91)80314-3.

[9] Lyudmyla et al. Development and validation of UV spectrophotometric procedure for estimation of bifonazole in bulk. J Pharm Biol Sci 2016; 4(4): 111-115

[10] ICH Guidelines Q2 (R1). Validation of analytical procedures: text and methodology. In: ICH Harmonized Tripartite Guidelines; 2005