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RESEARCH ARTICLE

AN RP-HPLC METHOD DEVELOPED FOR DETERMINATION OF BIFONAZOLE IN PHARMACEUTICAL FORMULATION

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ABSTRACT:

A Validated RP-HPLC method has been developed for estimation of Bifonazole in bulk and pharmaceutical cream. The method was developed using C_{18} column (250×4.6 mm; 5µm) with a mobile phase consisting of methanol and 0.1 M sodium acetate in ratio of 70:30 at P^H 3, flow rate of 1 ml/min with retention time found 6.54 min. Detection was carried out at 252 nm with UV detector. The developed method was evaluated for various system suitability parameters and validated for linearity, accuracy, precision, LOD, LOQ as per ICH guidelines. The proposed method can be used for the estimation Bifonazole in their pharmaceutical dosage forms and no interference of excipients found in developed method.

Keyword: Bifonazole, RP-HPLC, Validation, accuracy, precision, LOD, LOQ.

INTRODUCTION

Bifonazole(BIF) is chemically (1-[(1, 1`-biphenyl)-4-ylphenylmethyl]-1H imidazole) particularly useful in Skin and nail fungal infections with broad spectrum activity. Bifonazole is white crystalline powder[1]. Pharmaceutical formulation of Bifonazole is available in cream marketed by Bayer India Ltd. Marketed as Mycospore® [2].

Literature survey reveals some methods for the determination of BIF in pharmaceutical preparations or in biological fluids either alone or in combination with various drugs, such as HPLC [3,4,5,6], spectrophotometry [7, 8, 9].

An attempt was made to develop a new, rapid, sensitive, simple and fully validated RP-HPLC procedure using an mobile phase in combination with Methanol:Sodium Acetate (70:30) at P^H 3 for the determination of BIF. The utility of the developed method to determine the content of drugs in commercial value was also demonstrated. Validation of develop method carryout by following ICH guideline.

EXPERIMENTAL

Chemicals and reagents: Bifonazole was received as gift sample from Encube Ethicals (Mumbai). HPLC grade methanol was purchased from Merck. All other chemicals (analytical grade) were obtained from Merck. Sodium acetate 0.1 M prepared with double distilled water, and filtered through WTP 0.5 mm filters Whatman Grade No. 01

Equipment: Chromatographic separation was performed on a High Performance Liquid Chromatography system Systronic LC-6600 equipped with a LC 6600 solvent delivery system (pump), universal injector with injection volume 20 µL, and UV detector. A Eurosphere C18 (KNAVER, Berlin, Germany) column(25cm × 4.6mm, 5 µm particle size) was used for the separation.

Chromatographic conditions: The proposed method was perform using a reversed- phase technique, UV monitoring at 252 nm A mixture of Methanol: Sodium Acetate (0.1M) (70:30, v/v) at pH 3 was used as a mobile phase. The mobile phase was prepared freshly, filtered, and sonicated before use, delivered at a flow rate of 1 ml per min.

Standard stock solution and calibration curves: Stock solutions of 1 mg/ml of BIF were prepared by dissolving in methanol. Standard solutions for HPLC were prepared with mobile phase by varying the concentration of BIF in the

range of 1.0 to 20 mg/ml Calibration curve for HPLC analysis was obtained by plotting the peak area ratio of the drug against the drug concentration.

Analysis of Marketed sample: An accurately weighed quantity of cream equivalent to about 1 mg of BIF was weighed in to 10.0 ml volumetric flask and dissolved in methanol with shaking for 25 min. The volume was made up to the mark with methanol. The solution was centrifuged and supernatant was used as sample solution.

RESULTS AND DISCUSSION

In analysis of the BIF carried under isocratic conditions, the mixtures of methanol and Sodium Acetate with different combinations were assayed as the mobile phase using C18 packing as stationary phase. Binary mixture of methanol/ Sodium Acetate in proportion of 70:30 (v/v) at pH 3, proved to be better than the other mixture of methanol/ Sodium. Among several flow rates tested 0.5, 2 ml/ml and the rate of 1.0 ml/min was the best with respect to location and resolution of analytical peaks. Using a UV detector at 252 nm, the above described chromatographic conditions allow a better resolution BIF at 6.54 min. System suitability test was applied to a representative chromatogram of freshly prepared standard stock solutions of BIF, to check various parameters such as resolution, selectivity and peak tailing. Resolution and selectivity factors for this system were found 2.23 and 1.11, respectively. Tailing factors were obtained as 1.13 for BIF. The peak area ratios of BIF exhibited linear relationship with their concentrations. The characteristics of regression equations and the working concentrations are given in Table 1. The limit of detection (LOD) and limit of quantitation (LOQ) of the procedure are also shown in Table 1, which were calculated according to the 3s /m and 10s /m criterions, respectively, where s , is the standard deviation of the peak area ratios of the sample and m is the slope of the corresponding calibration curve. The intra- and inter-day variations of the method were determined using three replicate injections of four different concentrations, which were prepared and analyzed on the same day and on three different days (Table 3). These data indicate a considerable degree of precision and reproducibility for the method. Sample solutions analyzed after 72hr did not show any appreciable change in assay values. The results obtained for the recovery of showed that the precision was satisfactory.

Table 1: Characteristics of the linear regression analysis of BIF

Parameter	BIF
Linearity range	1.0 to 20 mg/ml
Slope	0.022
Intercept	0.018
Correlation coefficient	0984
RSD of slope	1.07
RSD of intercept	0.26
LOD (mg/ml)	0.33
LOQ (mg/ml)	1.11

Table No. 2: Precision studies.

Component	Amount Present (mg)	Amount Found (%)	Standard Deviation
BIF	10	99.96	0.480

Table No. 3: Intraday and Interday data of Tablet formulation. Method

Comp.	Theoretical concentration (mg/ml)	Intraday measured a concentration (mg/ml)		Interday measured b concentration(mg/ml)	
		Mean	RSD %	Mean	RSD %
BIF	10	9.89	0.32	9.81	0.81
	15	14.57	0.31	14.67	0.51
	20	19.51	0.26	19.35	0.59

Figure No. 1 Structure of Bifonazole[9]

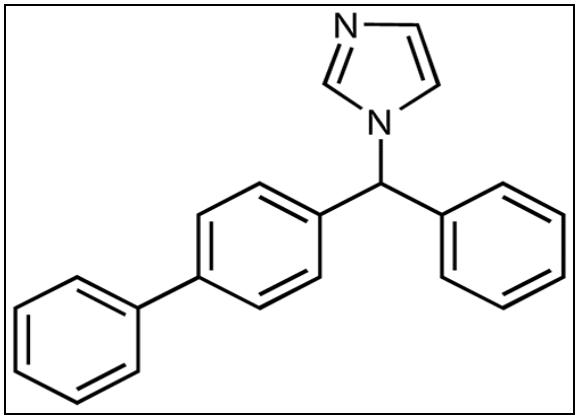
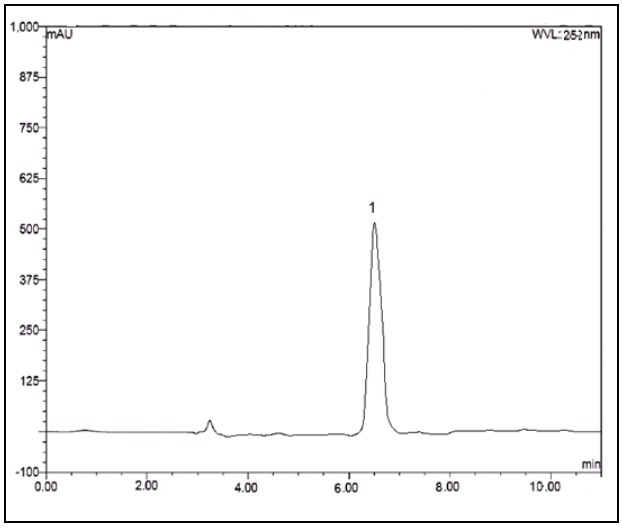


Figure No. 2 Chromatogram of Bifonazole



Assay in pharmaceutical dosage forms: On the basis of above results, the proposed method was applied to the determination of BIF in cream dosage forms which consist of 1% BIF. Fig. 2 shows a typical chromatogram obtained for the analysis of BIF in cream. The differences between the amount claimed and those measured were very low and the RSD values were within the acceptable windows mentioned by pharmacopoeias. The mean percentage recoveries obtained after five repeated experiments were 99.36% with a RSD of 0.47% for BIF, indicating that the results are accurate and precise and there is no interference from the common excipients used in the cream.

CONCLUSION

It can be concluded that the proposed method is sufficiently sensitive and reproducible in the analysis of BIF in pharmaceutical dosage forms within a short analysis time 6.54 min. The proposed HPLC method was validated by evaluation of the validation parameters. The LOD, LOQ values, relative standard deviation of slope and intercept, correlation coefficient, within- and between-day reproducibility, resolution, selectivity, tailing and capacity factors for this technique were obtained. The proposed method is also moreprecise than literature method since the RSD values incream were reported as 1.06 BIF. The applicability of proposed method toserum samples is under investigated.

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