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DEVELOPMENT AND VALIDATION OF RP-HPLC AND UV METHODS OF ANALYSIS FOR FLUCONAZOLE IN PHARMACEUTICAL SOLID DOSAGE FORMS

Pothana Sadasivudu, Nalini Shastri*, M.Sadanandam

Sri Venkateshwara College of Pharmacy, 86 Madhapur, Hitech city road ,

Hyderabad 500 081, India.

E-mail: svcphod@yahoo.co.in

ABSTRACT: A RP-HPLC and an UV spectrophotometric assay method were developed and validated for quantitative determination of fluconazole in pharmaceutical solid dosage forms like capsules, uncoated tablets, and dispersible tablets. The chromatography was carried out on a C-18 (150 mm x 4.6 mm, 5 µm) column with water and acetonitrile (65:35 v/v) as mobile phase at 260 nm detector wave length. The UV method was performed at 260 nm using 0.1M HCl as solvent. The linearity was established in the range of 1 to 100 µg/ml and 50 to 400 µg/ml for HPLC and UV methods respectively. The HPLC method was accurate and precise for all the dosage forms studied with a recovery of 98 to 102%. The UV method correlated well with HPLC for the analysis of fluconazole only in capsule dosage form.

Key words: Fluconazole, Reversed Phase High Performance Liquid Chromatography, Ultraviolet Spectrophotometry, Analytical method validation, Pharmaceutical solid dosage forms.

INTRODUCTION

Fluconazole[1] is chemically 2-(2,4-difluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)-2-propanol, a synthetic triazole derivative antifungal agent that has been shown to be effective against a wide range of systemic and superficial fungal infections, following both oral and intravenous administration[2]. GC[3,4] and HPLC[5-7] methods for the determination of fluconazole in biological fluids, HPLC for eye drops[8] and creams[9], UV spectrophotometry for syrups[10], capsules and intravenous solution[11], and microbiological assay for capsules[12] are some of the methods reported for analysis of fluconazole.

There is however no reported HPLC method for the analysis of fluconazole in solid dosage forms (capsules and tablets). This paper describes a validated HPLC method for the quantitative determination of fluconazole in solid dosage forms. This paper also reports a new validated UV spectrophotometric method for the quantitative determination of fluconazole in capsule dosage forms. The proposed HPLC method fulfilled the requirements of analytical parameters necessary to be applied to the content uniformity tests for finished pharmaceutical products in the study and hence can be successfully applied for routine quality control. The proposed UV method however was found satisfactory only for capsule dosage forms.

MATERIALS AND METHODS

MATERIALS

Standard fluconazole was kindly supplied by Sunrise International Labs Ltd., Hyderabad, India. Acetonitrile (HPLC grade) and Hydrochloric acid (AR grade) were purchased from SD Fine Chem., Mumbai, India. Pharmacopoeial grades of excipients were procured from Nehal traders, Hyderabad, India. In-house triple distilled water and distilled water was used for HPLC and UV method respectively. Marketed dosage forms of fluconazole capsules; Flucan (Bombay Tablets Mfg Co.), Fluzide (Concept Pharmaceuticals ltd.), Cancap (Ind Swift ltd.), Antican-O (Baroda India) uncoated tablets (Zefun, SH Pharmaceuticals ltd.), and dispersible tablets (AF-150, Systopic Laboratories) were purchased commercially. Each unit dose claimed to contain 150 mg of the fluconazole USP.

PLACEBO CAPSULE MATRIX AND PLACEBO TABLET MATRIX

Excipients for placebo capsule matrix were lactose anhydrate and talc and for placebo tablet matrix were lactose, dibasic calcium phosphate, PVP, gelatin, sodium starch glycolate, methyl paraben, propyl paraben, talc, and magnesium stearate.

ANALYTICAL CONDITIONS

The HPLC method was performed on a Shimadzu HPLC system equipped with LC-10ATVP pump, SPD-10AVP

UV detector, and Rheodyne injector system fitted with 20 µl loop. The HPLC analysis was performed on reversed-phase high-performance liquid chromatographic system with isocratic elution mode using a mobile phase of water: acetonitrile (65:35 v/v), on Gemini C-18 column (Phenomenix, 150×4.6 mm, 5 µm particle size) with 1 ml/min flow rate at 260 nm using UV detector. Spinchrom software was used for the data interpretation.

The UV method was performed on a UV – Visible Spectrophotometer 2201 (Systronics) using 1 cm quartz cells (Systronics). Systronics software was used for absorbance measurements. The UV spectrophotometric method was performed at 260 nm using 0.1M HCl as solvent for the preparation of standard and sample solutions.

PREPARATION OF STANDARD SOLUTIONS

HPLC method

Twenty five mg of accurately weighed standard fluconazole was dissolved and made up to mark with water, in a 50 ml volumetric flask, to get primary stock solution of 500 µg/ml. Serial dilutions were made to obtain 1, 2, 4, 8, 20, 40 and 100 µg/ml using mobile phase. All solutions were filtered through 0.45 µ membrane filter prior to use.

UV method

About 100 mg of accurately weighed standard fluconazole was dissolved and made up to mark with 0.1M HCl solution, in a 100 ml volumetric flask, to give primary stock solution of 1000 µg/ml. From this stock solution, dilutions were made to obtain 50, 100, 200, 300 and 400 µg/ml using 0.1M HCl solutions.

PREPARATION OF THE SAMPLE SOLUTIONS

HPLC method

The contents of 20 fluconazole capsules / uncoated tablets / dispersible tablets were taken and powdered. The powder equivalent to 25 mg of fluconazole was accurately weighed and transferred into a 50 ml volumetric flask. To this, 30 ml of water was added and sonicated for 10 min with occasional shaking to disperse and dissolve the contents. The volume was made up to 50 ml with water to give 500 µg/ml of fluconazole solution. This solution was filtered through 0.45 µ membrane filter and diluted suitably using mobile phase to obtain 40 µg/ml solution.

UV method

The contents of 20 fluconazole capsule were taken and powdered. The powder equivalent to 100 mg of fluconazole was accurately weighed and transferred into a 100 ml volumetric flask. To this, 50 ml of 0.1M HCl solution was added and sonicated for 10 min with occasional shaking to disperse and dissolve the contents. The volume was made up to 100 ml with 0.1M HCl solution to give 1000 µg/ml of fluconazole solution. This solution was filtered through 0.45 µ membrane filter and further diluted with 0.1M HCl solution to give 200 µg/ml.

METHOD VALIDATION

The methods were validated according to International Conference on Harmonisation (ICH) guidelines for validation of analytical procedures[13, 14].

Specificity

The specificity of the methods were evaluated by comparing the chromatograms (HPLC) and scans (UV) obtained from the standard solution, sample solutions (of capsule/uncoated tablet/ dispersible tablet), placebo capsule matrix solution and placebo tablet matrix solution (these matrices solutions were prepared in a manner similar to that of sample solution using placebo capsule / tablet matrix instead of fluconazole capsule or tablets).

Linearity

Seven concentrations of the standard solutions in 1-100 µg/ml range were analyzed by HPLC. Calibration curves were constructed by plotting average peak areas *versus* concentrations. Five concentrations of the standard solutions in the range of 50 - 400 µg/ml were analyzed for UV method. Calibration curves were constructed by plotting average absorbance *versus* concentrations. Linearity was determined by regression equations for both methods. This experiment was repeated six times for both methods.

Precision

Repeatability was evaluated by analysing six independent fluconazole standard solutions (40 µg/ml for HPLC method and 200 µg/ml for UV method). The intermediate precision was evaluated on three independent fluconazole standard solutions per day for three different days.

Accuracy (by standard addition method)

For the HPLC method, an accurately weighed amount of powder (capsule/ uncoated tablets/ dispersible tablets) equivalent to 25 mg of fluconazole was transferred to 50 ml volumetric flask and dissolved water. Aliquot of 2 ml of this solution were transferred into 25 ml volumetric flasks containing 2, 4 and 6 ml of fluconazole standard solution (50 µg/ml) and mobile phase was added to make up the volume to give a final concentrations of 44, 48, and 52 µg/ml. For the UV method, an accurately weighed amount of capsule powder equivalent to 100 mg of fluconazole was transferred to 100 ml volumetric flask and dissolved in 0.1M HCl. Aliquots of 2 ml of this solution were transferred into 10 ml volumetric flasks containing 2, 4 and 6 ml of fluconazole standard solution (200 µg/ml) and was added to make up the volume with 0.1M HCl to give final concentrations of 240, 280, and 320 µg/ml. All solutions were prepared in triplicate and assayed. The percentage recovery of added fluconazole standard was calculated.

Limit of detection (LOD) and limit of quantification (LOQ)

The parameters LOD and LOQ were determined using signal to noise ratio.

Stability of standard and sample Solution

The standard solution of fluconazole (40 µg/ml for HPLC method and 200 µg/ml for UV method) and sample solution of fluconazole capsules (40 µg/ml for HPLC method and 200 µg/ml for UV method) were prepared in triplicate and analyzed after 48 hrs by storing the solutions at room temperature.

Analysis of marked fluconazole formulations by RP-HPLC and UV Methods

Fluconazole in three different solid dosage forms, capsules (brands Cap-A, Cap-B, Cap-C, and Cap-D), uncoated tablets, and dispersible tablets were analyzed by optimized RP-HPLC method. Each product was analyzed by six independent determinations. Four brands of fluconazole capsules (brands Cap-A, Cap-B, Cap-C, and Cap-D) were analyzed by optimized UV method. Each brand was analysed by 6 independent determinations.

RESULTS AND DISCUSSION

OPTIMIZATION OF HPLC METHOD

Optimization of mobile phase was performed based on peak symmetry, peak width, and run time. The mobile phase of water and acetonitrile (65:35 v/v) was found to be satisfactory. The Fig.1 shows typical chromatograms obtained from the analysis of a standard and sample (fluconazole capsules, uncoated tablets and dispersible tablets) solutions of fluconazole using the proposed method. The retention time observed (2.47 min) permits a rapid determination of the drug, which is important for routine analysis. System suitability parameters for this method are reported in Table1. The parameters were within the acceptance limits.

VALIDATION OF HPLC METHOD

The described reversed-phase HPLC method was found to be specific for fluconazole, as none of the excipients interfered with the estimation of fluconazole (Fig. 2). The method was found linear over the range 1 to 100 µg/ml (Table 2). The LOD and LOQ were found to be 0.10 µg/ml and 0.25 µg/ml, respectively, indicating a high sensitivity of the method. The results for accuracy and precision are summarized in Table 1 and Table 2. The results of recovery studies indicate a high agreement between the true value and the estimated value. The standard and sample solutions were stable for 48 hrs (Table 3).

VALIDATION OF UV METHOD

The proposed UV spectrophotometric method was found to be specific for analysis of fluconazole in capsules, as no interference was observed at 260 nm in placebo capsule matrix when compared with standard fluconazole solution (Fig.3). But this method is not suitable for other solid dosage forms like uncoated and dispersible tablets, as some of the normally used tablet excipients like dibasic calcium phosphate, methyl paraben, propyl paraben and gelatin, showed significant absorbance at 260 nm and thus interfered with the estimation of the fluconazole.

The UV method hence permits a rapid and economical quantitation of fluconazole in capsule dosage form only. The absorption spectra of fluconazole in 0.1M HCl solution is shown in Fig.3. The λmax was found to be 260 nm. The calibration curves were constructed in the range of 50 to 400 µg/ml. Beer's law was obeyed over this concentration range (Table 1). The LOD and LOQ were found to be 5.0 µg/ml and 15 µg/ml, respectively. The repeatability was 0.75% and 0.78% respectively, demonstrating high precision of the method. The accuracy of the proposed method by standard addition method was determined for capsules and the mean recovery was found to be 100.06% (Table 2). The standard and sample solutions were stable for 48 hrs (Table 3).

ASSAY OF MARKETED FLUCONAZOLE FORMULATIONS

Results of assay on different solid dosage forms of fluconazole by proposed HPLC and UV method is reported in Table 4. The assay results of proposed RP-HPLC and UV methods when compared using Student's t-test does not reveal significant difference between the experimental values obtained in the standard and sample analysis by the two methods (P>0.05).

CONCLUSIONS

The HPLC method for the determination of fluconazole in capsules, uncoated tablets and dispersible tablets and the UV method for fluconazole in capsules were found to be simple, rapid, precise, accurate and sensitive. A good agreement was observed between HPLC and UV method. The validated HPLC and UV methods can be used for the drug analysis in routine quality control for bulk and dosage forms.

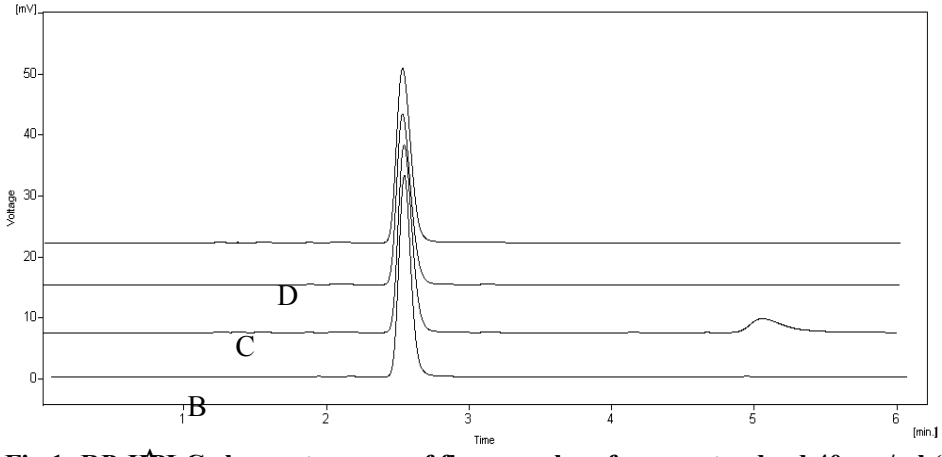

Fig 1: RP-HPLC chromatograms of fluconazole reference standard 40 mg/ml (A), fluconazole Dispersible tablet (B), Uncoated tablet (C), and Capsule (Cap-A) (D).

Table 1: Regression analysis and system suitability parameters for the quantification of fluconazole by HPLC and UV

Parameter	HPLC method	UV method
System precision	0.16% RSD	--------
Peak asymmetry	1.22 – 1.36	--------
Peak Width (min)	0.09	--------
Theoretical plates	4290	--------
Linearity range (µg/ml)	1 to 100	50 to 400
Regression equation	y = 5043.9 x + 1364.6	y = 0.002 x + 0.0053
Correlation coefficient (r^2)	0.9991	0.9997
Standard error of line	0.017	0.0058
Standard error of slope	0.079	0.0082
95% confidence interval for slope	5059.91 to 5027.60	0.00201 to 0.00197
Standard error of intercept	0.035	0.0020
95% confidence interval for intercept	2083.03 to 654.60	0.0096 to 0.0013
Repeatability [a] (%RSD)	0.59	0.75
Intermediate Precision [b] (%RSD)	0.58	0.78
LOD (µg/ml)	0.1	5.0
LOQ (µg/ml)	0.25	15.0

[a] RSD of 6 independent determinations in a day.
[b] RSD of 9 independent determinations (3 independent samples per day for 3 days)

Table 2: Accuracy test results for fluconazole dosage forms by HPLC and UV

Method	Product	Sample concentration (µg/ml)	Concentration of added standard (µg/ml)	Recovery ± RSD (%) of added FCZ*	Mean Recovery ± RSD (%) of added FCZ
HPLC Method	Capsule	40	4	100.21 ± 0.33	100.21 ± 0.25
		40	8	100.13 ± 0.26	
		40	12	100.30 ± 0.23	
	Uncoated Tablet	40	4	99.99 ± 0.30	99.98 ± 0.15
		40	8	99.75 ± 0.10	
		40	12	100.27 ± 0.32	
	Dispersible Tablet	40	4	100.05 ± 0.37	100.24 ± 0.32
		40	8	100.20 ± 0.27	
		40	12	100.29 ± 0.21	
UV Method	Capsule	200	20	100.02 ± 0.13	100.06 ± 0.19
		200	40	100.17 ± 0.12	
		200	60	99.98 ± 0.29	

* Average of 3 determinations

Table 3: Stability of the standard and sample solutions of fluconazole

Time interval	RP-HPLC Method				UV Method			
	Standard Solution		Sample solution		Standard Solution		Sample solution	
	Recovery (%)*	Difference (%)	Recovery (%)*	Difference (%)	Recovery (%)*	Difference (%)	Recovery (%)*	Difference (%)
0 hr	100.00	-------	100.00	-------	100.00	-------	100.00	-------
24 hr	100.11	- 0.11	100.21	- 0.21	99.52	0.48	99.00	1.00
48 hr	99.94	0.06	99.82	0.18	98.55	1.45	98.25	1.75

* Average of 3 determinations

Table 4: Assay results of marketed fluconazole dosage forms by HPLC & UV

Dosage forms	HPLC Method* Assay ± SD (%)	UV Method* Assay ± SD (%)
Uncoated Tablet	95.44 ± 0.87	NP
Dispersible tablet	102.28 ± 0.66	NP
Cap-A	100.06 ± 0.37	100.15 ± 0.54
Cap-B	99.98 ± 0.37	99.98 ± 0.45
Cap-C	100.13 ± 0.29	99.56 ± 0.69
Cap-D	101.02 ± 0.56	99.75 ± 0.40

*Average of 6 independent determinations NP – Not Performed

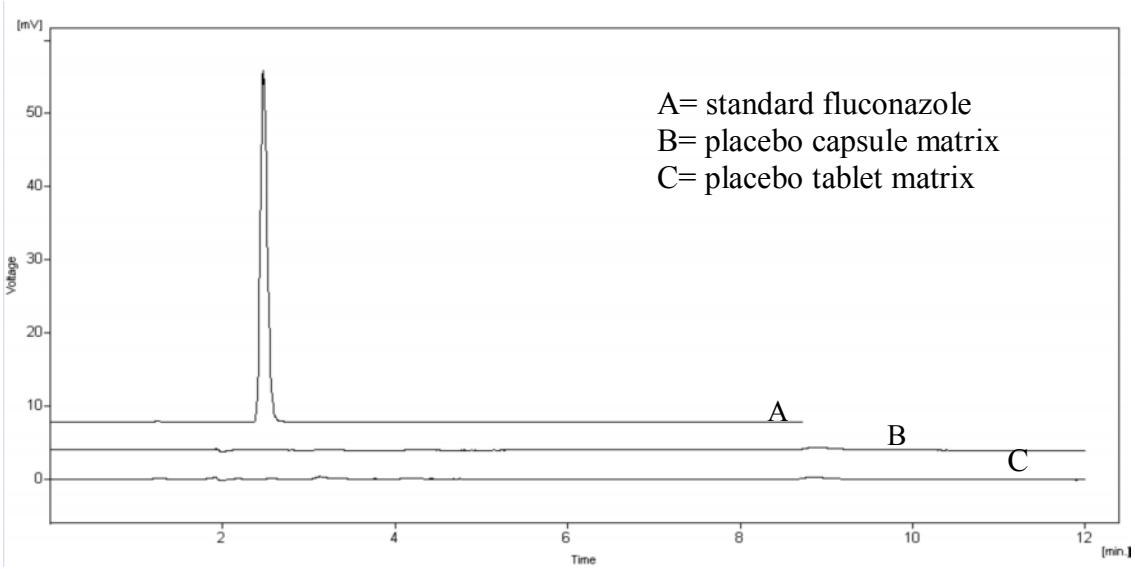

Fig 2: Chromatograms of standard fluconazole with placebo tablet & capsule matrix

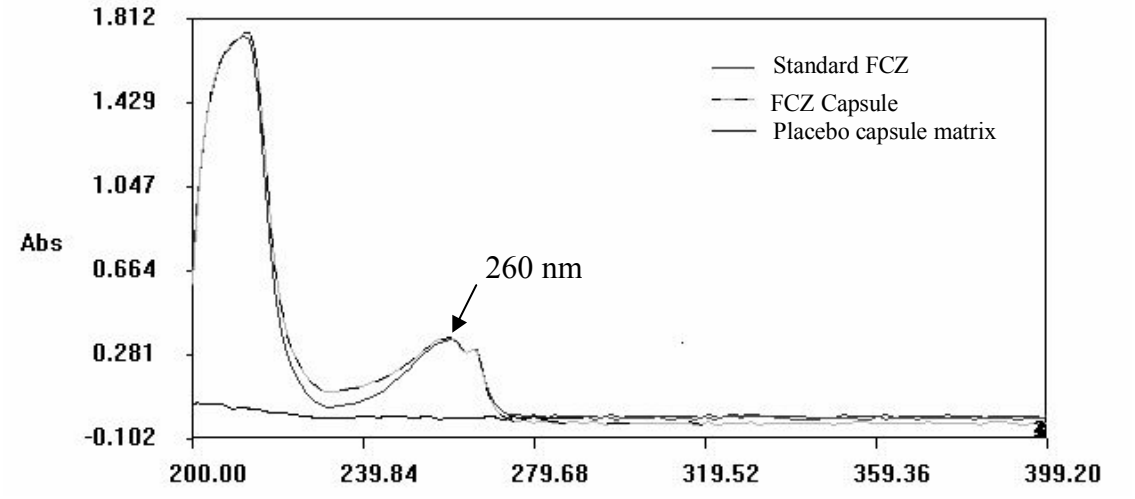

Fig - 3: UV scans of standard fluconazole (FCZ), FCZ Capsule and placebo capsule matrix in 0.1M HCL Solution

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