Research Article

Validation of Developed Method of Fluconazole in Solid Dosage Form by RP-HPLC

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ABSTRACT

Fluconazole is an antifungal medication used to treat cutaneous and superficial infections brought on by Candida species. For the quantitative detection of fluconazole in pharmaceutical solid dosage forms, such as capsules, chromatographic methods were verified using RP-HPLC. The purpose of this study is to validate the formulation of fluconazole in capsules. For the estimation of these medications in pharmaceutical dose form, the suggested method was validated utilising factors like System suitability, Specificity, Method precision, Linearity and range, Robustness, Accuracy. Research was conducted with the Shimadzu LC-2010C HPLC. The fluconazole product sold under the brand name Flucomex capsule by Glumex Pharmaceuticals was utilised. For HPLC, a 25cm by 4.6 mm, octadecylsilane-packed, porous silica (5 mm) column has been employed. The buffer and methanol in a 60:40 molar ratio, and orthophosphoric acid was used to adjust the pH to 3.6 at a flow rate of 1.0 ml/min at an operating temperature of 25 °C. The validation process followed ICH norms.

Keywords: Fluconazole, Method Validation, RP-HPLC, Antifungal, Precision.

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INTRODUCTION

Fluconazole, α-(2.4-diflurofenil)-α-(1H-triazol-1-methyl)-1H-1,2,4-triazol-1-ethanol, is a triazole antifungal agent available for oral or intravenous use in the treatment of a number of localized and disseminated mycoses.[1,2] Its chemical composition is $C_{13}H_{12}F_2N_6O$ and its molecular weight is 306.271 g/mol. Fluconazole is a white or almost white, crystalline powder, slightly soluble in water (1mg/mL), and soluble in ethanol (61mg/mL), ethyl acetate and methanol.[3] Compared to intravenous delivery, fluconazole taken orally has a bioavailability of > 90%.

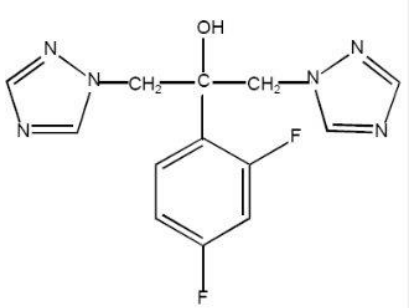

Figure 1: Structure of Fluconazole.[5]

Fluconazole half-life is 30 hours and excreted through renal route.[4] The most common adverse effects of fluconazole include chest tightness, difficulty with swallowing, fast heartbeat, hives, itching, skin rash. Fluconazole can interact with drug such as clopidogrel, pimozide, quinidine, macrolide antibiotics.[5]

The analytical technique of High Performance Liquid Chromatography (HPLC) is used extensively throughout the pharmaceutical industry. The information gathered may be quantitative, providing the precise amounts of compounds in the sample, or qualitative, indicating which compounds are actually present in the sample.[6] HPLC is used at all the different stages in the creation of a new drug, and is used routinely during drug manufacture. The aim of the analysis depends on both the nature of the sample and the stage of development. Since HPLC is a chromatographic technique, it is vital to understand the fundamentals of chromatography in order to comprehend how it functions.[7]

The process of proving an analytical method is appropriate for the purpose for which it was designed. Analytical processes are frequently created and applied internally, and validation is a fundamental necessity to ensure the quality and dependability of the results for all applications of analysis.[8,9,10] Therefore, the degree of knowledge and expertise is initially much larger compared with standard methods and hence it can be defined as "The process of proving an analytical method is appropriate for the purpose for which it was designed."[11,12]

MATERIALS AND METHODS

1. Chemicals and Reagents:

Fluconazole (Glumex Pharmaceuticals Manufacturing Pvt. Ltd Hamrapur, Thane) was used as a model drug, 0.1 M hydrochloric acid was used for disintegration time and potassium dihydrogen orthophosphate were selected as a buffer, methanol was added as solvent. Orthophosphoric acid was used as a retarding agent to control the drug release.

2. Instruments:

HPLC System used in study was UV – Visible Spectrophotometer (Double beam UV- Visible Spectrophotometer) in Model has LC-2010C and make by Shimadzu. HPLC System (HPLC Binary Gradient System), Analytical Balance (wenser), pH Meter (Digital pH meter), Sonicator (Bio-technic Ultra Sonicator), Filter paper (Whatman filter paper No. 1).

3. Selection of Chromatographic Conditions:

Column : 25cmX4.6-mm, packed with octadecylsilane bonded to porous silica (5µm)

Flow rate : 1.0 ml/min

Operating temperature : 25°C

Selected wave length : 261 nm

Injection volume : 50 µL

Buffer Solution: Weigh 1.36 gm of potassium dihydrogen orthophosphate in 1000 ml of water.

Mobile phase: A mixture of 60 volume of buffer and 40 volumes of methanol, adjust pH 3.6 with orthophosphoric acid.

Standard solution (0.05%w/v): Weigh 25.0 mg of Fluconazole WS in 100ml of volumetric flask, and make up the volume 100ml with mobile phase, sonicate the solution up to dissolve, if necessary.

Sample Solution: Weigh a quantity of 25 mg equivalent to fluconazole the powder in 100 ml of volumetric flask, add 70ml of mobile phase, sonicate for 15 min, and dilute to 100ml with the mobile phase and filter. **Procedure:** Separately inject 20 µl of diluent once, five replicate injections of standard Preparation and sample preparation in duplicate into the chromatograph, record the chromatograms, and measure the responses for Fluconazole.

RESULT AND DISCUSSION

Validation:

To the validation of analytical procedures RP-HPLC method developed was according to International Conference on Harmonization (ICH) and USP guidelines.[13] Various parameters or criteria are used for the method of validification such as specificity, precision, linearity and range, accuracy, robustness, filter evaluation and solution stability.[14]

1. System Suitability Test: The relative standard deviation for replicate injections is not more than 2.0%.

Procedure: System suitability data are evaluated on the basis of the chromatogram of the solution.

Observation:

Table 1: System suitability parameter

Sr. No.	Injection	RT	Area
		Fluconazole	Fluconazole
1	1	6.289	1501511
2	2	6.280	1499532
3	3	6.280	1499700
4	4	6.276	1497857
5	5	6.279	1497370
6	6	6.279	1497341
Average		**6.28**	**1498885**
%RSD		**0.07**	**0.11**

Interpretation: Based on the verification parameters, it is established that method is able to identify the subjected substance within acceptable parameters for retention time and the RSD

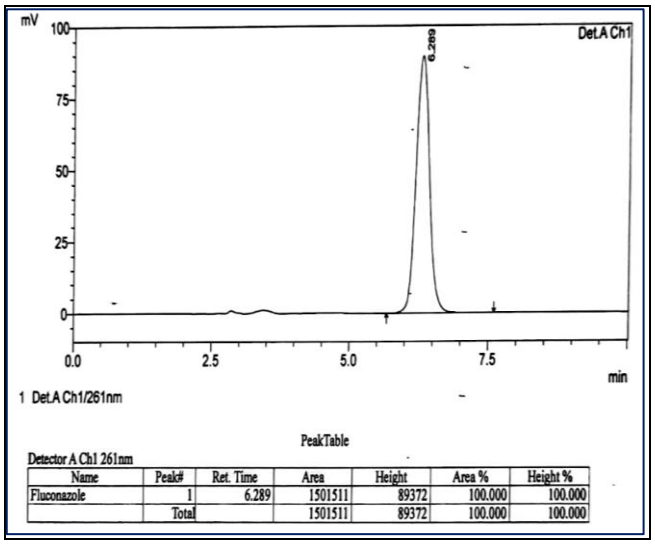

Figure 2: System Suitability Standard

2. Specificity: Specificity is defined as ability to assess unequivocally the analyte the presence of components that may be expected be present, such as impurities, degradation products and matrix components.[15]

Procedure: Inject these solutions once into the chromatograph in the following sequence.

1. Blank (Mobile Phase)

2. Placebo solution

3. Standard solution

4. Test solution

Table 2: Specificity observation

Active Ingredient	RT	Placebo Interference
Fluconazole Standard	6.275	Nil
Fluconazole sample	6.277	

Table 3: Specificity observation

Parameter	Acceptance criteria	Observation
Interference	There should be no interference at retention time of Fluconazole due to Diluent and Placebo solution	No interference at retention time of Fluconazole due to Diluent and Placebo solution.
Applied solvent (Diluent)	Must not give any detectable chromatographic sign at the retention times of the main component	Not giving any detectable chromatographic sign at the retention times of the main component.
Selectivity	The method must make selective separation other potential peak in the elution pattern	The method makes selective separation other potential peak in the elution pattern

Placebo Preparation:

Excipients for placebo preparation were 24.785g of Dicalcium Phosphate, 5.000 g of Talcum Powder, 2.500 g of Magnesium Stearate, 1.000 g of Aerosil 200 and total placebo content was 33.285g.

Acceptance criteria:

1. The system suitability criteria should pass as per analytical method

2. The blank and placebo solution should not show any peak at retention time of peak due to fluconazole. The peak fluconazole should be well resolved from any other peaks.

Interpretation:

In blank and placebo solution no any peak observed at the retention time of peak due to fluconazole. The peaks Fluconazole should be well resolved from any other peaks.

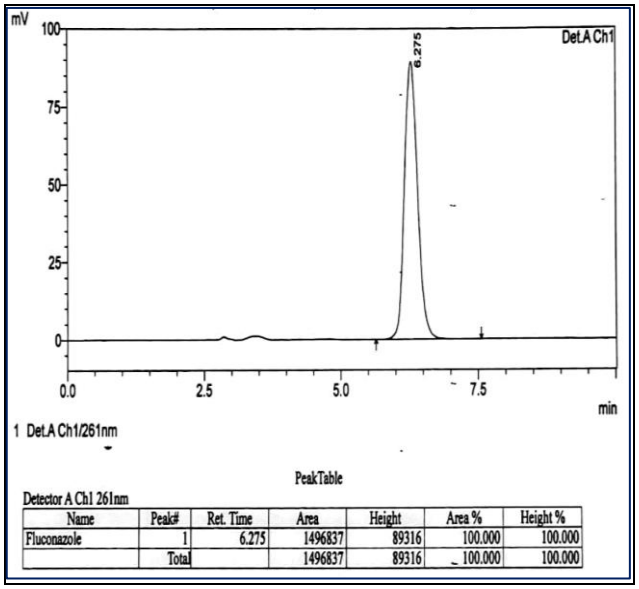

Figure 3: Specificity Standard

3. Precision:

System precision: From the Six injections of the standard, the RSD of peak area, tailing factor and theoretical plates will be calculated for system suitability.

Observation:

Table 4: System precision

Sr no.	Injection	Fluconazole (Area)
1	1	1481221
2	2	1480070
3	3	1481836
4	4	1480447
5	5	1480773
6	6	1480139
	Average	**1480747.7**
	% RSD	**0.05**

Acceptance criteria: Relative standard deviation (% RSD) from the six injections of the standard peak area should not be more than 2.0%.

ii. Method precision: Evaluate the Method Precision by quantitative analysis of Fluconazole in Fluconazole Capsules. Analyse six different preparations of a homogeneous batch of Fluconazole Capsules. Prepare blank, standard solution and sample solutions as described in the test procedure. Calculate % Assay of above six different sample preparations. Calculate the average, standard deviation and % RSD of % Assay obtained from six different sample preparations and records the results in observation table.

Acceptance Criteria:

1. The System Suitability criteria should pass as per analytical method.

2. The % RSD of the % Assay of six different sample preparations should not be more than 2.0.

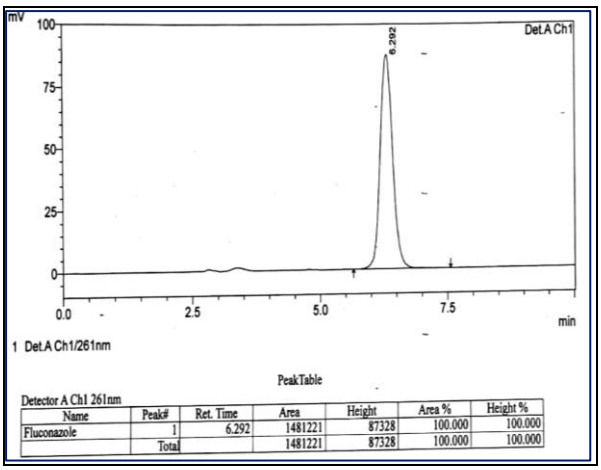

Figure 4: method Precision Standard

iii) Intermediate Precision: The intermediate precision of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as different analysts, different days, etc. Intermediate precision is a measure of reproducibility of test results under the variations in conditions normally expected from laboratory and from analyst to analyst. Two analysts A and B should perform Intermediate precision study. Prepare and inject six individual samples by each analyst and calculate the overall %RSD of assay of all samples. The % RSD should not be more than 2.0.

System suitability table:

Table 5: System suitability

Sr.no	Std Area	Spl Area	% Assay
1	1474679	1536387	98.12
2	1473473	1557163	100.11
3	1473745	1576496	100.02
4	1472618	1574553	99.61
5	1471636	1590257	99.58
6	1473031	1585678	99.62
Average	**1473197.0**	**1570089.0**	**99.5**
%RSD	**0.07**	**1.28**	**0.72**

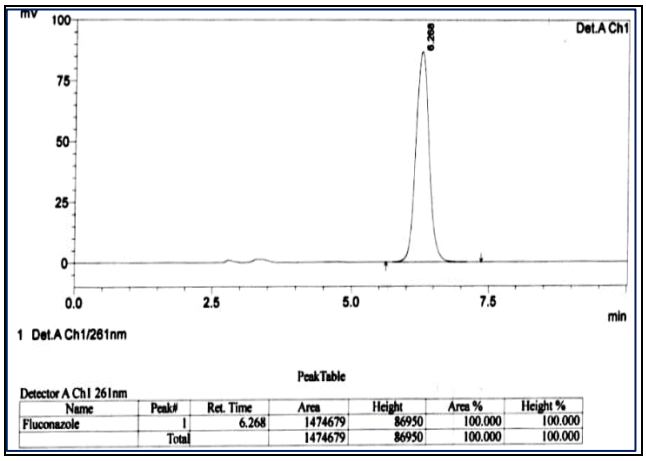

Figure 5: Intermediate Precision Standard

Observation Table:

Table 6: Precision

Sample		Fluconazole		
		Std Area	Spl Area	% Assay
Method Precision Sample	**1**	1481221	1532051	99.25
	2	1480070	1559372	98.68
	3	1481836	1579046	99.27
	4	1480447	1580183	100.33
	5	1480773	1597071	99.74
	6	1480139	1586558	100.40
Intermediate Precision Sample	**7**	1481221	1532051	99.25
	8	1480070	1559372	98.68
	9	1481836	1579046	99.27
	10	1480447	1580183	100.33
	11	1480773	1597071	99.74
	12	1480139	1586558	100.40
Average		**1480747.7**	**1572380.2**	**99.60**
% RSD		**0.05**	**1.48**	**0.68**

Acceptance Criteria:

1. The System Suitability criteria should pass as per analytical method.

2. The RSD of the area should not be more than 2.0%.

3. Overall % RSD for assays of Fluconazole in Repeatability and Intermediate precision should not be more than 2.0.

Interpretation: Based on the data interpretation analytical procedure expresses the closeness of agreement (degree of scatter) between 6 injections of independent weighing's at different days and different instruments.

4. Linearity and Range: The linearity of an analytical method is its ability to elicit test results that are directly, or by a well- defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.

For Fluconazole:

Table 7: Linearity & Range

Sr. No.	Weight of Fluconazole	Diluted up to (ml)	Conc. In %	Area
1	12.5	100	50	769011
2	20.0	100	80	1199826
3	25.0	100	100	1483620
4	30.0	100	120	1768299
5	37.5	100	150	2191653
Correlation coefficient = 1.0 (NLT: 0.990)				

Procedure:

Various concentrations of Fluconazole in standard solution from 50% to 150% i.e., 5 should inject and the correlation coefficients obtained for the analyte from the curve (Concentration v/s. AUC) are determined. The value of Correlation coefficient should be >0.990. The range of an analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined with a suitable level of precision, accuracy and linearity using the prescribed method. Upper and Lower concentration of six replicate standard injections of a solution containing Fluconazole in Fluconazole Capsules, having concentrations of should be injected in the chromatographic system and the % RSD of the area under the curve obtained for the main peaks i.e., analyte peaks should determine. The %RSD should not be more than 2.0%.

Observation Table:

Acceptance Criteria:

1. The system suitability criteria should pass as per analytical method.

2. Correlation coefficient (r): [Limit: NLT 0.990]

Interpretation:

1. Based on data interpretation analytical procedure expresses the closeness of agreement (degree of Scatter) Between 3 Independent Injection of different strength.

2. A plot of peak area of fluconazole v/s Concentration is linear with correlation coefficient of 1.0.

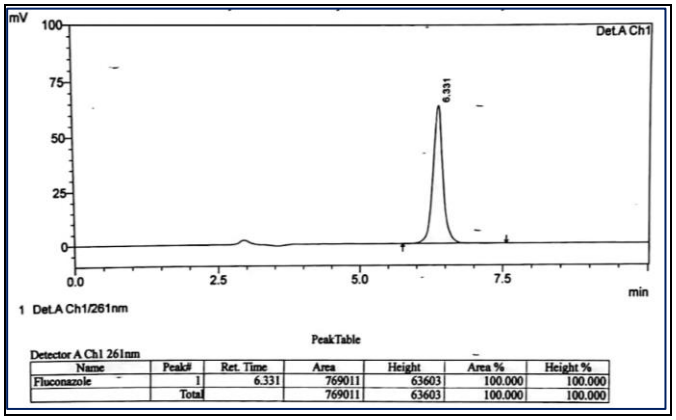

Figure 6: Linearity & Range 50% Concentration

5. Accuracy: The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method should be established across its range.

Table 8: Accuracy

Fluconazole						
Recovery level	**Amount added in ppm**	**Amount found in ppm**	**% Recovery**	**Average (%)**	**Std dev. (%)**	**% RDS**
50%	125	124.83	99.86	99.81	0.04	0.04
	125	124.71	99.77			
	125	123.76	99.81			
100%	250	248.83	99.53	99.55	0.02	0.02
	250	248.85	99.54			
	250	248.95	99.58			
150%	375	368.96	98.39	98.42	0.03	0.03
	375	369.04	98.41			
	375	369.19	98.45			
Mean Recovery: 99.35%						

1) Accuracy: 50% sample 1

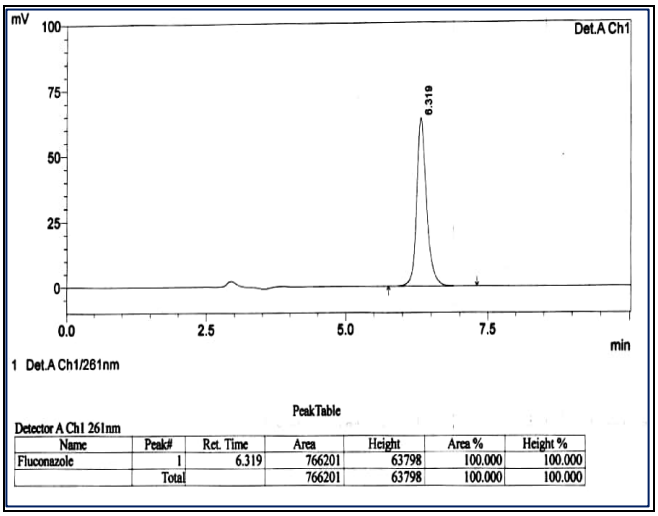

Figure 7: Accuracy 50% sample 1

Acceptance criteria:

1. The system suitability criteria should pass as per analytical methods.

2. The percent recovery for fluconazole should be obtained by the prescribed method is in the range of 98 to 102%.

Interpretation:

1. Percentage relative standard deviation of overall recovery is within the acceptable limit. The percentage recovery results are precise and found well within the acceptable limit of 98% to 102% of the theoretical value showing that the method is accurate.

2. Based on the date interpretation analytical procedures expresses the closeness of agreement (degree of scatter) between 3 independent injections of different strength.

6. Robustness: The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides indication of its reliability during normal usage.

Change in mobile phase PH 3.6 to 4.0

Table 9: Robustness

Sr no.	Fluconazole	
	pH	% Assay
1	3.6	99.60
2	4.0	99.32
Average		99.46
%RSD		0.20

Table 10: Robustness

Mobile phase pH Changed 3.6 to 4.0	
	Absolute Difference
Fluconazole	**0.28%**

1) Robustness: Standard

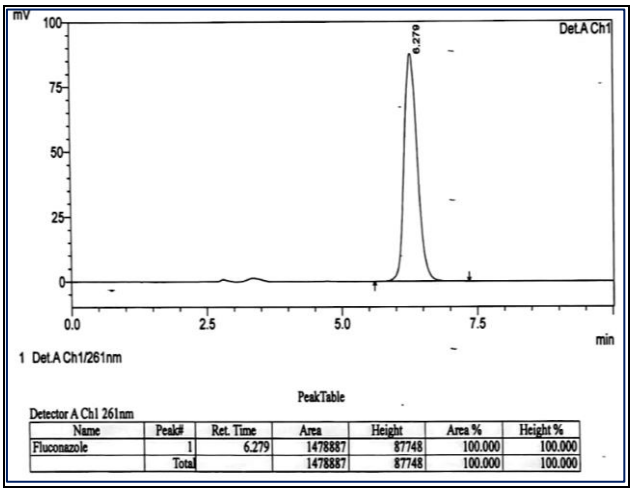

Figure 8: Robustness Standard

Acceptance criteria:

i. The system suitability criteria should pass as per analytical method.

ii. Analytical method should be robust if the % RDS of the % Assay of sample preparation should NMT 2.0.

Interpretation: Based on validation parameters, is established that method is robust for the determination of the subjected substance & there is no changes in assay values on deliberated changes in method parameters.

CONCLUSION

The developed method was validated for determination of Fluconazole in Fluconazole capsules, to rapid, simple, accurate, precise, sensitive, robust and specific that can be successfully applied for routine analysis of determination of Fluconazole on subjected dosage form.

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