IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF ANTIFUNGAL DRUG IN BULK AND PHARMACEUTICAL DOSAGE FORM

[1]Omkar D. Ghatage, [2]Pramod B. Patil, [3]Sachinkumar V. Patil

[1]Author: Omkar D. Ghatage, Student, Department of quality assurance, Ashokrao Mane College of Pharmacy, Pet-Vadgaon, Maharashtra- 416112, India.

[2]Author: Associate Professor, Department of quality assurance, Ashokrao Mane College of Pharmacy, Pet-Vadgaon, Maharashtra- 416112, India.

[3]Author: Professor and Principal, Department of Pharmaceutics, Ashokrao Mane College of Pharmacy, Pet-Vadgaon, Maharashtra- 416112, India.

Abstract: Fluconazole in pharmaceutical matrices may be quantified using a simple HPLC approach established in current work. The lack of extra peaks in the chromatogram suggests that the common excipients utilised in the tablets are not interfering. The validation test findings, taken together, indicated a technique with a relatively large linear range, adequate precision and accuracy, and practically dependable sensitivity. The approach allows for easy, selective, sensitive, and specific drug analysis and may be utilised for regular pharmaceutical quality control analysis in a short amount of time. A truthful along with reliable RP-HPLC technique for the analysis of fluconazole active pharmaceutical ingredient has been established. All together, we created 5 distinct samples with doses ranging from 80% to 120%. It was revealed that between 120 and 180 µg/ml, a linear liaison existed between drug concentrations with peak area on the calibration graph. The blank shows no peak and the Drug Product shows the RT 2.43. The result suggests that the developed approach is an incredibly, another acceptable strategy for investigation, immaculateness, and soundness, which may aid in the investigation of Fluconazole.

***Keywords*:** Fluconazole, RP-HPLC, Method development, Validation.

I. INTRODUCTION

Investigation of a pharmaceutical invention entails the steps needed to ascertain its true nature, potency, quality, and purity. The chemical discipline known as pharmaceutical analysis Quality assurance as well as quality control for pharmaceutical formulations and bulk drugs also heavily rely on it. Every pharmaceutical formulation requires analysis, from purchasing raw materials to finishing the product. Pharmaceutical analysis is a crucial part of both the manufacturing process and quality control for pharmaceuticals. The need for innovative analytical methods in the pharmaceutical sector has grown in tandem with the global expansion of the pharmaceutical industry and medication manufacturing [1]. Analytical monitoring of the product or its individual ingredients is required to guarantee the safety, efficacy, and clinical relevance of a drug product during its entire shelf life, from manufacturing to storage to distribution. This method is used to determine the chemical composition of a substance and to isolate its active ingredients from a solution or mixture. The primary goals of pharmaceutical analysis are quality control and efficacy evaluation [2]. Also included are numerical values and a succinct description of the medicine in question. There would be no drug evolution or discovery without the contributions of pharmaceutical analysis. Analytical techniques are used to perform quantitative and qualitative evaluations of pharmaceutical formulations. Various techniques are used for the determination of the concentration of a compound; these techniques are titration, spectroscopy, chromatography, and gravimetric analysis [3].

Fluconazole is a synthetic triazole derivative antifungal agent that has been demonstrated to be effective against a broad spectrum of systemic and superficial fungal infections after both oral and intravenous treatment. 2-(2, 4-difluorophenyl)-1, 3-bis (1H-1,2,4-triazol-1-yl)-2-propanol [4] is its chemical name. In this study, we provide a validated HPLC technique for the quantitative measurement of fluconazole in semi-solid dosage forms. In the research, the analytical parameters needed to use the proposed HPLC method to test for content uniformity in finished pharmaceutical products were met. This means that the method can be used effectively for regular quality control.

II. MATERIALS AND METHODS

MATERIALS

Fluconazole was received as gift sample from Cipla pvt. Ltd. Chemicals required for study was procured from Research lab Mumbai. All chemicals are of analytical grade.

METHODS

Chromatographic Conditions: [5-10]

The following criteria were selected for method development on a trial and error basis. The oven temperature was kept at 30°C, the flow rate was 1 ml/min, the runtime was 10 minutes, the injection volume was 10 l, the wavelength was 220 nm, and the Mobile Phase-0.1% perchloric acid in methanol (40:60% v/v)Methanol: 0.1% Perchloric Acid (50:50 v/v) Diluentand Agilent Zorbax Bonus-RP (250 x 4.6 mm, 5) column

Standard Preparation:

Standard Stock Solution-I (SSS-I):

Initially Prepare a Standard Stock Solution (SSS-I) of Fluconazole by adding 15mg in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Fluconazole = 1500µg/ml).Further pipette out 1.0 ml of SSS-I in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Fluconazole = 150µg/ml).

Preparation of Drug Product Sample

6gmof cream (Equivalent to 1.5 mg of Fluconazole) was accurately weighed into 10 ml volumetric flask & add 5 ml diluent, Sonicated for 10 minutes and make the volume to 10 ml with diluent. (Conc. of Fluconazole = 150µg/ml).

Selection of Wavelength:

The sample was scanned from 200-400 nm with PDA detector. The Wavelength selected for analysis chosen was 220 nm on basis of appropriate intensity of both the peaks.

Method Validation:

Specificity& Assay:

Individual working standards of Fluconazole of150µg/ml, and peaks was for identified in Mixture working standard from Retention Time. Blank was injected to ensure there is no blank peak interfering with the main analyte peaks. 6gmof cream (Equivalent to 1.5 mg of Fluconazole) was accurately weighed into 10 ml volumetric flask & add 5 ml diluent, Sonicated for 10 minutes and make the volume to 10 ml with diluent. (Conc. of Fluconazole = 150µg/ml).

Instrument Precision& System Suitability:

A single sample was prepared as described and 6 injections were made from same sample and checked for system suitability. System suitability parameters are as below:

1. Retention Time,
2. Theoretical plates,
3. Asymmetry (Tailing factor),
4. Resolution.

b. Linearity& Range:

5 samples of varying concentrations ranging from 80-120% were made.

The concentrations are given below:

Table no. 1 Linearity concentration

% Level	Fluconazole Conc. (µg/ml)
80	120
90	135
100	150
110	165
120	180

The sample preparations are given as below; X ml of SSS-I was added to 10 ml diluent to make up the concentrations given above:

Table no. 2 Sample preparation

X ml of SSS-I	Diluted to
0.8	10 ml
0.9	10 ml
1.0	10 ml
1.1	10 ml
1.2	10 l

Accuracy:

Samples were prepared of 80%, 100% and 120% concentration by spiking the same amount of concentration given above in table for both Fluconazole. Samples were injected in Triplicate to calculate % RSD. % recovery was also calculated.

LOD/ LOQ:

LOD/LOD was being calculated for both drugs by using ANOVA technique.

Formula:

$$LOD = \frac{3.3 \times Std.Error\ of\ Intercept}{Coefficients\ of\ X\ Variable\ 1}$$

$$LOQ = \frac{10 \times Std.Error\ of\ Intercept}{Coefficients\ of\ X\ Variable\ 1}$$

III. RESULT AND DISCUSSION

RP-HPLC Estimation of Fluconazole in Bulk and from its Formulation

Materials and methods

All chemicals are used of an analytical grade. High performance thin layer chromatography is used to develop precious, cost effective method development of fluconazole.

Analytical wavelength

The λ max observed at the wavelength for Fluconazole is 220nm.The absorption spectra of Fluconazole are as follows:

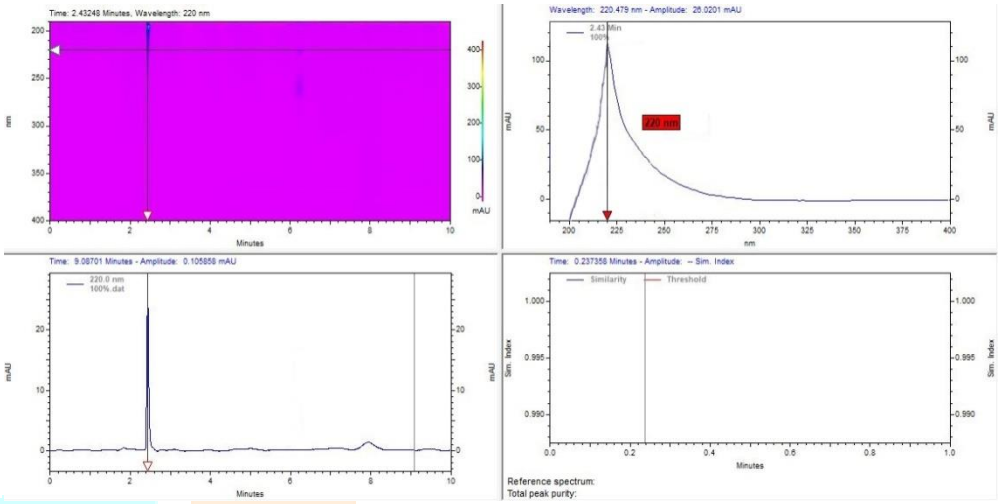

Fig. no. 1. Absorption spectra of Fluconazole

Method development

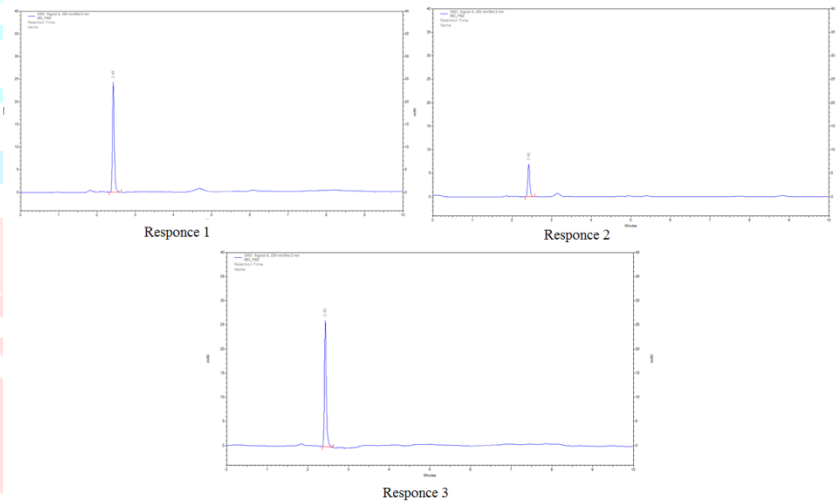

Fig. no. 2. Method development responses

Linearity and Range:

We created 5 samples with concentrations ranging from 80% to 120% of the total volume. A 40:60 mixture of methanol and perchloric acid was used and dilutions were made from Range 120-180 µg/ml for Fluconazole. The linearity of the calibration graph, which was constructed by plotting the concentration of the drug against the peak area, was discovered to exist in the concentration range of 120–180 g/ml. Beer's law is satisfied over the whole concentration range. We determined that the RT at 100% was 2.43. The regression equation and the correlation coefficient were found as in fig no.3

Table no. 3. Different concentration of fluconazole

Fluconazole		
% Level	**Conc (µg/ml)**	**Area**
80	120	1713258
90	135	1913258
100	150	2143641
110	165	2352648
120	180	2584259

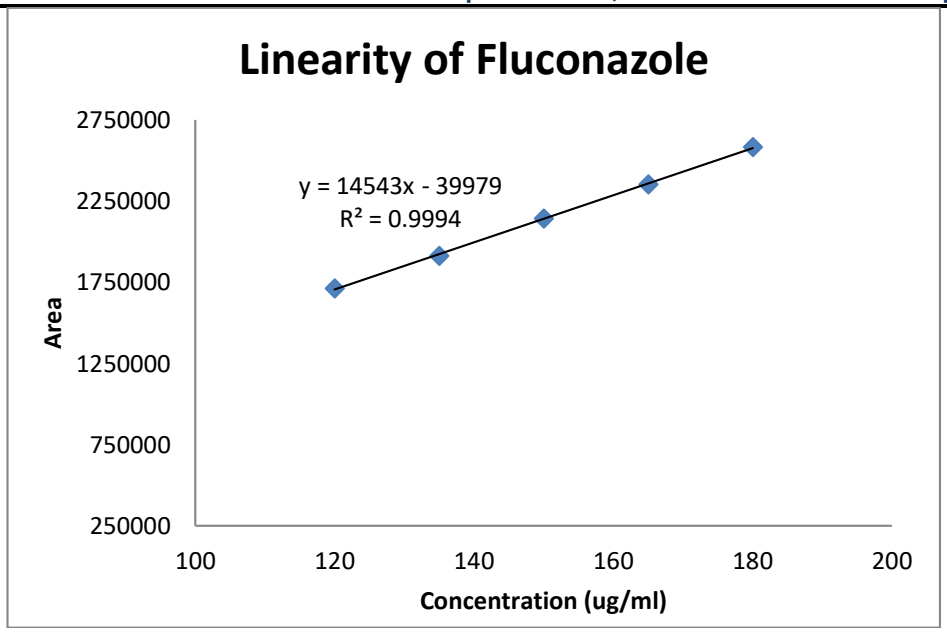

Fig. no.3 linearity of Fluconazole

Specificity& Assay

The specificity of the method was found out by analyzing standard drug and Drug product (Keppra). The blank shows no peak and the Drug Product shows the RT 2.43

Table no. 4. Specificity of fluconazole

Sample ID	Fluconazole		
	RT	Area	% Assay
FCZ WS	2.43	2143641	-
Cream Sample	2.43	2135874	99.64

Accuracy:

Table no. 5. Accuracy data of Fluconazole

Fluconazole								
Std Area				2143641				
% Level	Reps	Spiked Conc (µg/ml)	Area	Amount Recovered (ug/ml)	% Recovery	AVG	STDEV	RSD
80%	Rep 1	120	1722839	120.55	100.46	100.21	0.283869	0.28
	Rep 2	120	1713258	119.88	99.90			
	Rep 3	120	1719548	120.32	100.27			
100%	Rep 1	150	2143641	150.00	100.00	100.19	0.239527	0.24
	Rep 2	150	2153549	150.69	100.46			
	Rep 3	150	2146257	150.18	100.12			
120%	Rep 1	180	2581325	180.63	100.35	100.42	0.060516	0.06
	Rep 2	180	2584259	180.83	100.46			
	Rep 3	180	2583694	180.79	100.44			

Repeatability

Sample of 100% concentration was injected 6 times and calculated % RSD for Area of 6 replicates.

Table no.6 Repeatability data

Fluconazole	
Sample ID	Area
100% Rep 1	2143641
100% Rep 2	2153549
100% Rep 3	2146257
100% Rep 4	2143687
100% Rep 5	2135684

100% Rep 6	2154687
Average	2146251
STDEV	7063.634
RSD	0.33

LOQ/LOD

The Limit of Detection and Limit of Quantitation for Fluconazole was found to be 11.97984 µg/ml and 36.30253 µg/ml respectively.

Table no. 7. Regression Statistics

Regression Statistics	
Multiple R	0.999139756
R Square	0.998280252
Adjusted R Square	0.997707002
Standard Error	2747.801594
Observations	5

ANOVA					
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	13148601210	13148601210	1741.441185	3.02838E-05
Residual	3	22651240.8	7550413.6		
Total	4	13171252451			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	10077.2	8775.774537	1.148297504	0.334105088
X Variable 1	2417.4	57.92874397	41.73057854	3.02838E-05

LOD	11.97984	µg/ml
LOQ	36.30253	µg/ml

IV. CONCLUSION

Fluconazole in pharmaceutical matrices may be quantified using a simple HPLC approach established in current work. The lack of extra peaks in the chromatogram suggests that the common excipients utilised in the cream are not interfering. The validation test findings, taken together, indicated a technique with a relatively large linear range, adequate precision and accuracy, and practically dependable sensitivity. The approach allows for easy, selective, sensitive, and specific drug analysis and may be utilised for regular pharmaceutical quality control analysis in a short amount of time. For the analysis of Fluconazole API, a sensitive and selective RP-HPLC technique has been developed and validated. The λ max observed at the wavelength for Fluconazole is 220nm.5 samples of varying concentrations ranging from 80-120% were made. A 40:60 mixture of methanol and perchloric acid was used and dilutions were made from Range 120-180 µg/ml for Fluconazole. The calibration graph was plotted with concentration of the drug against the peak area was found to be linear in the range of 120-180 µg/ml. It obeys Beer's law over the concentration range. The RT at 100% was found 2.43. The specificity of the method was found out by analyzing standard drug and Drug product (Keppra). The blank shows no peak and the Drug Product shows the RT 2.43.Sample of 100% concentration was injected 6 times and calculated % RSD for Area of 6 replicates; RSD was found 0.33. The Limit of Detection and Limit of Quantitation for Fluconazole was found to be 11.97984 µg/ml and 36.30253 µg/ml respectively. The result suggests that the developed approach is an incredibly, another acceptable strategy for investigation, immaculateness, and soundness, which may aid in the investigation of Fluconazole.

V. ACKNOWLEDGMENT

Authors are thankful to Management of Ashokrao Mane College of Pharmacy, Peth-Vadgaon for providing necessary requirement for research work.

REFERENCES

[1] Doltade M, Saudagar R. The Analytical Method Development and Validation: A Review. Journal of Drug Delivery and Therapeutics. 2019;9(3):563-70.
[2] Rasmussen HT. Method Development. In Ahuja S, Scypinski Sa , editor. A handbook of Modern Pharmaceutical Analysis 1st edn. San Diego: Academics press; 2001.p. 2, 346, 383.
[3] Rashmin. An introduction to analytical method development for pharmaceutical formulations. Pharm Rev 2008; 6: 1-10.
[4] Brittain HG, editor. Profiles of drug substances, excipients, and related methodology. Academic press; 2020 Mar 10.
[5] Bliesner DM. Validating chromatographic methods: a practical guide. John Wiley & Sons; 2006 Sep 11.
[6] Sharma BK. Instrumental methods of chemical analysis. Krishna Prakashan Media; 1981.

[7] Malasiya A, Goyal A. Method development and validation of RP HPLC method for assay and related substances of luliconazole in topical dosage form. International Journal of Pharmaceutical Chemistry and Analysis. 2017;4(2):46-50.
[8] Ivković B, Crevar M, Cvetanović A, Ubavkić K, Marković B. Development and validation of RP-HPLC method for quantification of trace levels of topical corticosteroids in ambiphilic cream. Acta Chromatographica. 2022 Feb 3.
[9] Pradhan M, Alexander A. Development and Validation of a Robust RP-HPLC Method for Analysis of Calcipotriol in Pharmaceutical Dosage Form. Research Journal of Pharmacy and Technology. 2019;12(2):579-83.
[10] Pradhan M, Alexander A. Development and Validation of a Robust RP-HPLC Method for Analysis of Calcipotriol in Pharmaceutical Dosage Form. Research Journal of Pharmacy and Technology. 2019;12(2):579-83.