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### Development and Validation of UV Spectroscopic Method for Estimation of Fluconazole in Tablet Dosage Form

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#### ABSTRACT

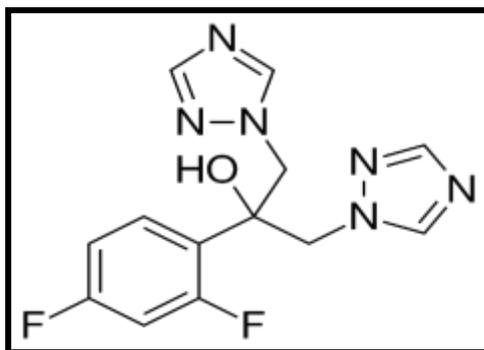
To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Fluconazole in tablet dosage form. The drug is freely soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide. The drug was identified in terms of solubility studies and on the basis of melting point done on Melting Point Apparatus of Equiptronics. It showed absorption maxima were determined in Ethanol. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity. The UV spectroscopic method was developed for estimation of Fluconazole in tablet dosage form and also validated as per ICH guidelines. The drug is freely soluble in organic solvents such as Ethanol, DMSO, and Dimethyl Formamide. So, the Analytical Grade Ethanol is used as a diluent in method. The melting point of Fluconazole was found to be 139-140°C (uncorrected). It showed absorption maxima 252 nm in Ethanol. On the basis of absorption spectrum the working concentration was set on 60µg/ml (PPM). The linearity was observed between 20-100 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 98.75, 101.00 and 100.83% for three levels respectively. The % RSD for precision was found to be 0.78%. A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Fluconazole in tablet dosage form. The method could be considered for the determination of Fluconazole in quality control laboratories.

**Keywords:** Fluconazole, Development, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

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## INTRODUCTION

Chemically Fluconazole is 2-(2,4-difluorophenyl)- 1,3-bis(1H-1,2,4-triazol-1-yl)propan-2-ol <sup>[1]</sup>. This drug is a broad spectrum anti-fungal agent and recommended for the treatment and prophylaxis of disseminated and deep organ candidiasis <sup>[2]</sup>. Fluconazole is a triazole antifungal drug used in the treatment and prevention of superficial and systemic fungal infections. In a bulk powder form, it appears as a white crystalline powder, and it is very slightly soluble in water and soluble in alcohol <sup>[3,4]</sup>. It has a desirable pharmacological properties including relatively long half-life, the ability to be administered either orally or parentally. Like other imidazole's and triazole's - class antifungal, fluconazole inhibits the fungal cytochrome P450 enzyme 14 $\alpha$ -demethylase <sup>[5]</sup>. This inhibition prevents the conversion of lanosterol to ergosterol, an essential component of the fungal cytoplasmic membrane.



**Figure 1: Chemical Structure of Fluconazole**

Major advantage of Fluconazole over other antifungal agents is that it can cross the blood-brain barrier. Fluconazole is primarily fungi static; however, it may be fungicidal against certain organisms in a dose-dependent manner, specifically *Cryptococcus* <sup>[6,7]</sup>.

From literature review it's found that few GC <sup>[4,5]</sup>, Microbiological method <sup>[6]</sup>, HPLC Detection methods <sup>[7-9]</sup> was performed on fluconazole with different formulation. Also there is method for estimation of fluconazole in single or in combination from biological fluids like serum <sup>[10-12]</sup>. Simultaneous UV spectrophotometric methods <sup>[13]</sup> was also reported for determination of Fluconazole in eye drops alone or in combination. But no method was found on estimation of Fluconazole in tablet dosage form for UV spectroscopic method. This indicates that so far no UV method exists for the estimation and determination of Fluconazole in tablet dosage forms in single without combination.

## MATERIALS AND METHOD

**Instruments:**

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair. Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance. Melting point apparatus of Equiptronics were used.

**Materials:**

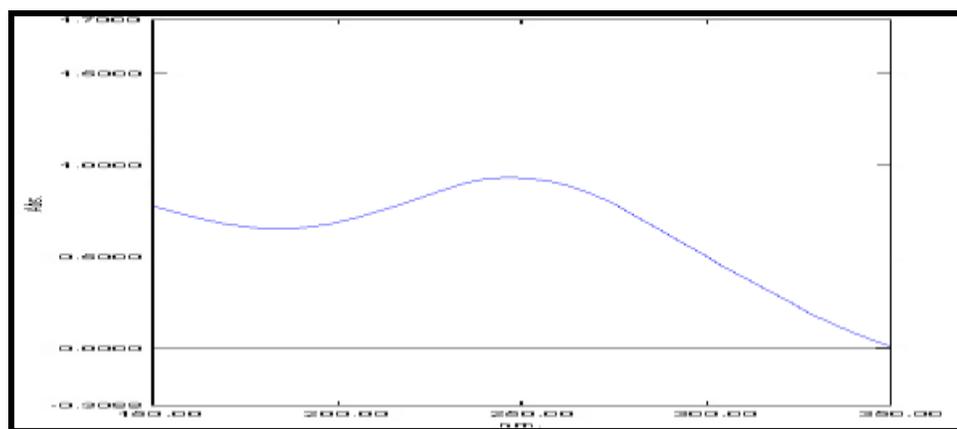
Fluconazole was obtained as a gift sample. Fluconazole tablets were procured from local pharmacy. Analytical grade Ethanol were used throughout the experiment. Freshly prepared solutions were employed.

**Diluent:**

Analytical Grade Ethanol is used as a diluent.

**Method development** [15, 16]:**Determination of  $\lambda$  max (100 PPM)**

100 mg weighed amount of Fluconazole was dissolved into 100 ml of volumetric flask with diluent. Pipette out 10 ml and added in 100 ml of volumetric flask, dissolved and diluted up to the mark with diluent. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined.



**Figure 2: Calibration Curve**

**Preparation of Working concentration****Preparation of Standard stock solution:**

Standard stock was prepared by dissolving 100 mg of Fluconazole in 100 ml of diluent to get concentration of 1000  $\mu\text{g/ml}$  (PPM).

**Preparation of Standard solution:**

Pipette out 6 ml from standard stock solution and diluted up to 100 ml with diluent to get concentration of 60  $\mu\text{g/ml}$  (PPM).

**Procedure for UV reading****Blank Solution:** (For Auto zero)

Fill the cuvette with Diluent. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

**Standard Solution:**

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

**Sample Solution:**

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

**Procedure for sample preparations** <sup>[14]</sup>

For analysis of commercial formulations; twenty tablets are taken weighed it and powdered. The powder equivalent to 100 mg of Fluconazole was accurately weighed and transferred into the 100 ml of volumetric flask, added 60 ml of diluent; the solution was sonicated for 20 min. After sonication cool the flask and diluted upto 100 ml with diluent. Filtered the solution through whatmann filter paper. Pipette out 6 ml of the above solution and diluted up to 100 ml with diluent. The absorbance was measured at 252 nm. The absorbance was recorded:

**Table 1: Absorbance of Dosage Form**

Pfizer Pharma Limited (DIFLUCAN® 100 mg)		
Sr. no.	Sample	Absorbance
1	Blank Solution (Auto zero)	0.0000
2	Standard Solution	0.5754
3	Sample Solution	0.5671

**Table 2: Dosage Form Specifications with % Assay**

Sr No	Company	M.D.	E.D.	Batch No.	Average weight(g)	Assay (%)
1	Pfizer Pharma Pvt. Ltd (100mg)	06/2018	11/2021	PAA 08764	0.1945	98.56

**Method of validation** <sup>[17, 18, 19]</sup>

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

**Linearity:**

The linearity of the proposed assay was studied in the concentration range 20 - 100 PPM at 252nm. The calibration data showed a linear relationship between concentrations.

**Table 3: Linearity Studies**

Sr. no.	Sample Concentration	Absorbance
1	20 PPM	0.1882
2	40 PPM	0.3775
3	60 PPM	0.5747
4	80 PPM	0.7608
5	100 PPM	0.9412
<b>Correlation coefficient</b>		<b>0.9998</b>

**Accuracy:**

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 100ml of volumetric flask with analytical grade water and measuring the absorbance at 252nm.

**Table 4: Accuracy Studies**

<b>Spectrophotometric Method</b>			
Accuracy (%)	Qty weighed (mg)	Qty found (mg)	Recovery (98-102%)
80	0.8	0.79	98.75
100	1	1.01	101.00
120	1.2	1.21	100.83

**Precision:**

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the % RSD was calculated.

**Table 5: Precision studies**

Sr. No.	Sample Solution	Absorbance
1	Sample Solution 1	0.5742
2	Sample Solution 2	0.5785
3	Sample Solution 3	0.5692
4	Sample Solution 4	0.5810
5	Sample Solution 5	0.5755
Mean		0.5757
SD		0.0045
% RSD		0.7791

**Ruggedness:**

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

**Table 6: Results for Ruggedness Studies**

Sr. No.	Analyst	Results	Mean	% Assay	% RSD
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1	Analyst 1	0.5681	0.5686	98.82	0.3426
		0.5691			
2	Analyst 2	0.5715	0.5714	99.31	
		0.5713			

## RESULTS AND DISCUSSION

### Solubility of Fluconazole

Solubility test was passed as per criteria.

**Table 7: Results for solubility studies**

Sr. no.	Title	Result
1	Ethanol, DMSO, Dimethyl formamide	Freely Soluble
2	Water	Sparingly soluble

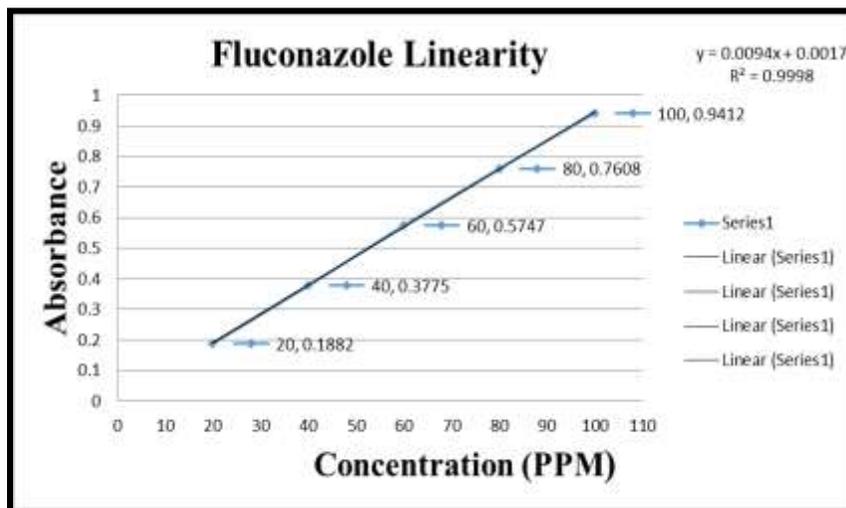
### Melting point of Fluconazole

The Melting Point of Fluconazole was found to be 139-140°C (uncorrected).

### Results for linearity for assay method of Fluconazole

The linearity of method was determined at concentration level ranging from 10 to 30 µg/ml (PPM).

The correlation coefficient value was found to be ( $R^2$ ) **0.9998**.



**Figure 3: Fluconazole Standard Curve**

### Results for accuracy for assay method of Fluconazole

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

### Results for precision for assay method of Fluconazole

The % RSD for different sample of precision was found to be 0.3518 and it is within acceptance criteria represented in Table - 5.

### **Results for ruggedness for assay method of Fluconazole**

The %RSD for different sample of ruggedness was found to be 0.0717 and it is within acceptance criteria represented in Table - 6.

## **CONCLUSION**

A method for the estimation of Fluconazole in tablet form has been developed. From the spectrum of Fluconazole, it was found that the maximum absorbance was 252 nm in diluent. A good linear relationship was observed in the concentration range of 20-100 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Fluconazole in tablet dosage forms. Hence, the method could be considered for the determination of Fluconazole in quality control laboratories in tablet dosage forms.

## **ABBREVIATIONS**

1. PPM - Parts per Million
2. nm - Nanometer
3. HPLC - High Performance Liquid Chromatography
4. UV - Ultra violet
5. DMSO - Dimethyl Sulfoxide
6. GC - Gas Chromatography
7. ICH - International Council for Harmonization
8. RSD - Relative Standard Deviation
9. SD - Standard Deviation
10. Qty - Quantity
11. °C - Degree Celsius
12. Fig. - Figure
13. Qty - Quantity
14. % - Percentage
15. M.D. - Manufacturing Date
16. E.D. - Expiry Date

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