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U.V. SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF FLUCONAZOLE

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ABSTRACT

A rapid, simple, selective and precise U.V. Spectrophotometric method for estimation of Fluconazole content has been developed. The spectrophotometric detection was carried out at maximum absorption wavelength 260.8nm in water. Linearity was observed at concentration range of 25 to 500µg/ml with correlation coefficient of 0.999. LOD and LOQ were found to be 1.59 and 4.82µg/ml. The developed method was validated according to ICH guidelines for linearity, precision, accuracy, robustness, LOD and LOQ.

KEYWORDS: U.V. Spectroscopy, Fluconazole, ICH guideliness, Validation.

1. INTRODUCTION

Fluconazole is a synthetic antifungal agent belonging to the group of triazoles. This drug is structurally related to the antifungal agents that are imidazole derivative. Drug was approved by FDA in the United States on January 9, 1990.[2] Fluconazole is a hygroscopic, crystalline powder freely soluble in methanol and slightly soluble in water. Fluconazole occurred in polymorphic form with different melting points, Form I : 135-136^{o}C, Form II : 138 – 140^{o}C, Form III : 137 – 138^{o}C.[2] Fluconazole is chemically 2-(2,4-Difluorophenyl)-1,3-bis(1*H*-1,2,4-triazol-1-yl)propan-2-ol and is available in tablet, suspension, parentral and gels for treatment of local, systemic fungal infection and deep organ candidiasis.[2,7] It acts as fungistatic agent by inhibiting fungal cytochrome P450 enzyme14α- demythylase and prevents the conversion of lanosterol to ergosterol, an essential component of the fungal cytoplasmic membrane, and subsequent accumulation of 14α-methyl sterols.[25] Mammalian demethylase activity is much less sensitive to Fluconazole than fungal demethylase.[6,25]

Fungal resistance to drugs (azole class) tends to occur gradually over the course of prolonged drug therapy, resulting in clinical failure in immune-compromised patients (e.g., patients with advanced HIV receiving treatment for thrush or esophageal Candida infection).[5] In C. albicans, resistance occurs by way of mutations in the ERG11 gene, which codes for 14α-demethylase. These mutations prevent the azole drug from binding, while still allowing binding of the enzyme's natural substrate, lanosterol. Development of resistance to one azole confers resistance to all drugs in the class. Another resistance mechanism employed by both C. albicans and C. glabrata is increasing the rate of efflux of the azole drug from the cell, by both ATP-binding cassette and major facilitator superfamily transporters. Other gene mutations are also known to contribute to development of resistance.[5]

Adverse drug reactions associated with Fluconazole therapy include: rash, headache, dizziness, nausea, vomiting, abdominal pain, diarrhea, and/or elevated liver enzymes.[30] Fluconazole is secreted in human milk at concentrations similar to plasma. Therefore, the use of Fluconazole in lactating mothers is not recommended.[26] Some people are allergic to azoles. Some azole drugs may disrupt estrogen production in pregnancy, affecting pregnancy outcome.[22]

Fluconazole is an inhibitor of the human cytochrome P450 system, particularly the isozyme CYP2C9 (CYP3A4 to lesser ex-tent), therefore, fluconazole decreases the metabolism and increases the concentration of any drug metabolized by these enzymes. (e.g. Phenytoin, Ca^{++} channel blocker, Warfarin, and other anticoagulant, Sulphonylurea, HMG CoA reductase inhibitor etc). In addition, it prolong QT interval increases the risk of cardiac arrhythmia.[7]

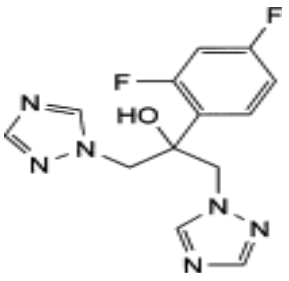

Fig. 1- Chemical Structure of Fluconazole

Numerous method has been reported in literature for analysis of Fluconazole in Pharmaceutical preparation and biological sample such as UV spectrophotometric[10,20,26], High Performance Liquid Chromatography in pharmaceutical dosage form[1,3,24] and High Performance Liquid Chromatography biological fluid[8,9], IR-spectroscopic[28], TLC-

densiometry[14], Spectrofluorimetry[11], Gas Liquid Chromatography[15] and Micro-biological assay.[15,21]

2. MATERIALS AND METHODS

2.1. Materials

Fluconazole was obtained as gift sample from Symed Labs Limited, Hyderabad. Methanol, Potassium Dihydrogen ortho-Phosphate, Sodium Hydroxide was purchased from SD Fine Chemicals, Mumbai. Distilled water was used for the experiment. All the materials used were of analytical grade.

2.2. Method Development

2.2.1. Instrumentation

Spectroscopy performed using double beam Shimadzu UV-Visible Spectrophotometer, (Model UV1800) installed with software "UV Probe 2.33".

2.2.2. Solvent Selection

Fluconazole is freely soluble in methanol and was used as solvent to aid solubilisation of Fluconazole in distilled water to form homogeneous solution.

2.2.3. Preparation of Standard Stock Solution

Fluconazole (50 mg) was dissolved in 12.5ml methanol and volume was made with distilled water to 100 ml to obtain stock solution of 500µg/ml (stock I). Subsequent concentration of 25, 50, 100, 200, 300, 400, 500µg/ml was prepared by diluting 0.25, 0.5, 1, 2, 3, 4, 5 ml from stock I to 10 ml with water.

2.2.4. Wavelength Selection

Stock solution was scanned in UV range of 200nm to 400nm against water to determine λmax.

2.3. Method Validation

Validation of method was done according to ICH guidelines Q2 (R_1) with respect to linearity, precision, accuracy, robustness, LOD and LOQ.

2.3.1. Linearity

Standard calibration curve of absorbance with working standard of varying concentration (25-500µg/ml) was plotted and linearity was determined with regression coefficient (R^2).

2.3.2. Accuracy

Accuracy was determined with four different level of drug concentrations prepared from three independent stock solution (n=9). Accuracy was validated by % Recovery.

2.3.3. Precision and Repeatability

Intraday precision (repeatability) was determined by analyzing sample in thrice a day at interval of 2hr.

Interday precision (Intermediate precision) was determined by analyzing sample on four different days with four different level of concentration prepared from independent stocks. Data obtain was analyzed by standard deviation, % RSD.

2.3.4. Robustness

Robustness of method was determined with four different level of concentration by varying the wavelength by 1nm.

2.3.5. LOD and LOQ

LOD and LOQ were determined by using standard calibration curve equation and can be expressed as

LOD =3.3r/S and LOQ = 10r/S

Where S is the slope of the calibration curve and r is the standard deviation of y-intercept of regression equation.

3. RESULT AND DISCUSSION

The proposed method was found to be simple, rapid, selective and precise and non expensive for routine analysis of drug content in the formulation.

3.1. Wavelength selection

Scan performed from 200nm to 400nm showed two characteristic adjacent peaks at 265.4nm and 260.8nm (λmax) in water.

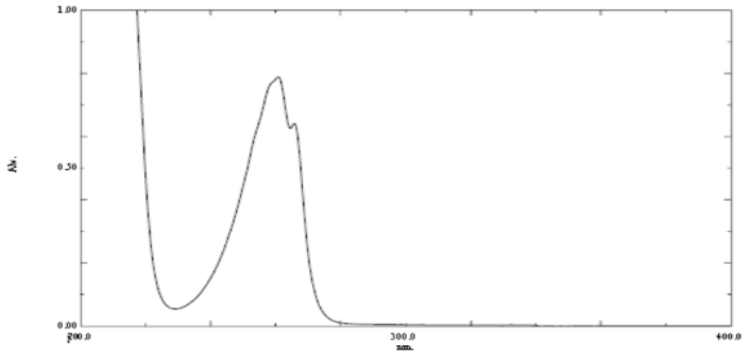

Fig.3.1.1. – Scan of Fluconazole in water

3.2. Linearity

Linearity is range within which the solution concentration is directly proportional to concentration of analyte present in solution.

Linearity was found between 25 to 500µg/ml in water with correlation coefficient of 0.9999.

Table 3.2.1. – Absorbance of Fluconazole in water at 260.8 nm

Conc. (µg/ml)	Mean Abs	S.D	%RSD
0	0	0	0
25	0.056	0.00058	1.04
50	0.105	0.00116	1.10
100	0.207	0.00153	0.74
150	0.310	0.00200	0.65
200	0.403	0.00231	0.57
250	0.500	0.00200	0.40
300	0.600	0.00153	0.26
350	0.695	0.00173	0.25
400	0.804	0.00436	0.54
450	0.896	0.00404	0.45
500	0.992	0.00666	0.67

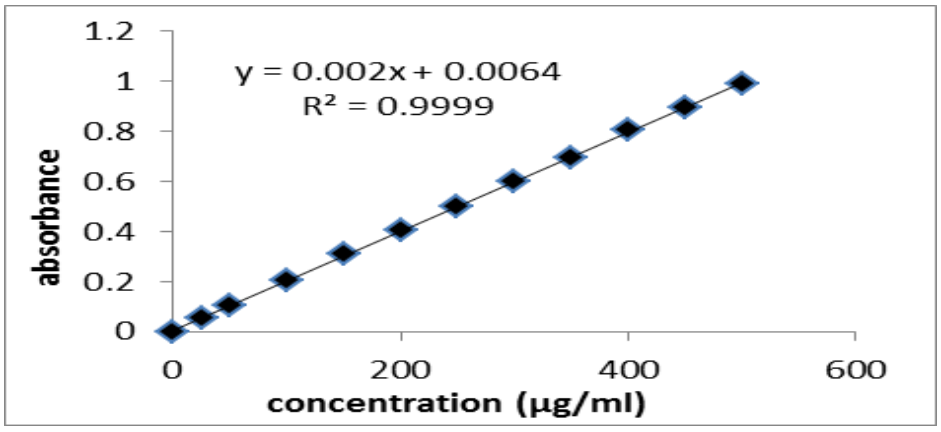

Fig. 3.2.1. – Standard plot of Fluconazole in water

3.3. Precision

The precision of an analytical procedure is the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed condition.[19] The %RSD value was found to be < 2% for intraday and interday precision.

Table 3.3.1: Intraday precision (n=3)

Conc. (µg/ml)	Mean Abs	S.D.	%RSD
200	0.403	0.00351	0.870
250	0.500	0.003	0.600
300	0.602	0.00379	0.629
350	0.697	0.00289	0.414

Table 3.3.2: Interday precision (n=12)

Conc. (µg/ml)	Mean Abs	S.D.	%RSD
200	0.403	0.00624	1.55
250	0.500	0.00981	1.96
300	0.598	0.01034	1.73
350	0.696	0.01187	1.70

3.4 Accuracy

Accuracy is the closeness of the obtained value with respect to true value. The % recovery was found to be in range of 98 to 99 % and % RSD value was found to be ≤ 2%.

Table 3.4.1: Accuracy (n=9)

Conc. (µg/ml)	Mean Abs ± SD	% RSD	Measured Conc. (µg/ml) ± SD	% Recovery
200	0.401 ± 0.008	2.01	197.67 ± 4.04	98.83
250	0.501 ± 0.009	1.97	247.50 ± 4.92	99.00
300	0.597 ± 0.011	1.87	296.00 ± 4.92	98.67
350	0.697 ± 0.012	1.71	345.67 ± 5.97	98.76

3.5 Robustness

Robustness is the ability of method to resist small change in method parameter. The % recovery after altering λmax by ± 1nm was found to be within 98 to 100 percent range.

Table 3.5.1: Robustness (n=3)

Conc. (µg/ml)	Mean Abs ±SD	% Recovery
200	0.396 ± 0.00255	98.21
250	0.491 ± 0.00325	98.20
300	0.589 ± 0.00382	98.22
350	0.683 ± 0.00545	98.25
400	0.789 ± 0.00615	98.18

3.6 LOD and LOQ

LOD (limit of detection) is the lowest amount of analyte in a sample that can be detected but not necessarily quantified as exact value. LOQ (limit of quantification) is lowest amount of analyte in a sample that can be quantified with suitable precision and accuracy.[19]

Table 3.6.1: LOD and LOQ

Slope	Intercept ± SD	LOD (µg/ml)	LOQ (µg/ml)
0.002	0.0064 ± 0.001	1.59	4.82

3.7. CONCLUSION

The proposed method was found to be simple, rapid, selective, precise and non expensive for routine analysis of drug content in the formulation.

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