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Spectrophotometric estimation of Fluconazole in pure drug and pharmaceutical formulation

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Abstract— A simple, sensitive, rapid and accurate spectrophotometric method was developed in ultraviolet region for the estimation of Fluconazole in pure drug and pharmaceutical formulation. The drug has an absorption maximum at 260 nm and obeys Beer's Lambert law in the concentration range of 2-10 µg/ml with correlation coefficient of 0.9999 in solvent. Excellent recovery proved that the method was sufficiently accurate. There is no interference from any common pharmaceutical additives and diluents. Results of the analysis were validated by recovery studies according to ICH Q2B guidelines

. . **Keywords —** Fluconazole; methanol Spectrophotometry; Validation; Pharmaceutical formulations

INTRODUCTION

Fluconazole (FLZ) [1] chemically known as 2-(2, 4-diflurophenyl)-1, 3-bis (1H-1, 2, 4-triazol-1-yl)-2-propanol (fig. 1) is a synthetic triazole derivative antifungal agent that has been found to be effective against a wide range of systemic and superficial fungal infections. This drug is a broad spectrum anti-fungal agent and recommended for the treatment and prophylaxis of disseminated and deep organ candidiasis[2]. 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.[3] fluconazole inhibits the fungal cytochrome P450 enzyme 14α-demethylase. Mammalian demethylase activity is much less sensitive to fluconazole than fungal demethylase. This inhibition prevents the conversion of lanosterol to ergosterol, an essential component of the fungal cytoplasmic membrane, and subsequent accumulation of 14α-methyl sterols.[4] Fluconazole is primarily fungistatic; however, it may be fungicidal against certain organisms in a dose-dependent manner, specifically Cryptococcus.[5] Fluconazole is active against the following microorganisms namely Blastomyces dermatitidis Candida spp. (except C. krusei and C. glabrata) [6] Fungal resistance to drugs in the 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).[7] 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 in this way will confer 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 [7] The full spectrum of fungal susptibility and resistance to fluconazole can be found in the TOKU-E's product data sheet [8] Fluconazole is a drug indicated for the treatment and prophylaxis arrhythmia if used concurrently with other drugs that prolong the QT interval. Berberine has been shown to exert synergistic effects with fluconazole even in drug-resistant Candida albicans infections.[15]

of fungal infections where other antifungals have failed or are not tolerated (e.g., due to adverse effects), including. Candidias is caused by susceptible strains of Candida Tinea corporis, tinea cruris or tinea pedis [9] . Adverse drug reactions associated with fluconazole therapy include:[9] Common (≥1% of patients): rash, headache, dizziness, nausea, vomiting, abdominal pain, diarrhea, and/or elevated liver enzymes. Fluconazole is secreted in human milk at concentrations similar to plasma. Therefore, the use of fluconazole in lactating mothers is not recommended.[10] Some people are allergic to azoles, so those allergic to other azole drugs might be allergic to fluconazole.[11] That is, some azole drugs have adverse side-effects. Some azole drugs may disrupt estrogen production in pregnancy, affecting pregnancy outcome. [12] Fluconazole taken at a dose of 150 mg is in FDA pregnancy category C. However, high doses (400 mg to 800 mg a day) have been associated with a rare and distinct set of birth defects in infants. If taken at these doses, the pregnancy category is changed from category C to category D. Pregnancy category D means there is positive evidence of human fetal risk based on human data. In some cases the potential benefits from use of the drug in pregnant women with serious or life-threatening conditions may be acceptable despite its risks. Fluconazole should not be taken during pregnancy or if one could become pregnant during treatment without first consulting a doctor.[13] FDA is now saying that treatment with chronic, high doses (400–800 mg/day) of Diflucan (fluconazole) during the first trimester of pregnancy may be associated with a rare and distinct set of birth defects in infants[14]

Fluconazole is an inhibitor of the human cytochrome P450 system, particularly the isozyme CYP2C9 (CYP3A4 to lesser extent). In theory, therefore, fluconazole decreases the metabolism and increases the concentration of any drug metabolised. by these enzymes. In addition, its potential effect on QT interval increases the risk of cardiac

Fluconazole can Be synthesized from a halogenated acetophenone derivative.[16] Literature survey has revealed various methods for estimation of FLZ in topical (creams, lotions), oral (tablets, capsules, syrups, solutions), eye drops, biological fluids (intravenous) and in other pharmaceutical formulations,such as TLC-densitometry[17] ,spectrofluorimetry [18] IR-spectroscopic [19],UV-spectrophotometric [20-24] microbiologicalmethod [25-26], gas liquid chromatography [27-28] (GLC), high performance liquid chromatography (HPLC) for biological fluid[29-31], and high performance liquid chromatography (HPLC) for pharmaceutical dosage forms[32-37].

The aim of this work is to establish the conditions for quantitative determination of FLZ from the different simulated body fluid media and in solid dosage form and to define essential parameters required for identification. The literature survey does not reveal any UV-spectrophotometric methods together for the determination of the drug in bulk and in pharmaceuticals, in different simulated, physiologic body fluids like gastric, vaginal, topical and blood serum. This paper reports a study on the development of a new validated UV-spectrophotometric method for the quantitative determination of FLZ in bulk and solid dosage form

EXPERIMENTAL SECTION

Fluconazole working standard (purity, 99.80%) used from Cadila Healthcare Ltd., Ahmedabad ,India. Fluconazole tablets were obtained from Cadila Healthcare Ltd., Ahmedabad, India. Each tablet was labeled contain 4 mg Of Fluconazole. All other reagents used were of analytical reagent grade supplied by Spectrochem, India. Spectral and absorbance measurements were made on a UV-Visible spectrophotometer 1700 Shimadzu Limited with 10mm matched pair ofquartz cell and spectral band width of ±2nm.

Selection of solvent

The ideal property of a solvent should be that the drug should be completely soluble in the solvent used . The drug should be stable in the solvent used and should be economical and volatile. After suitable literature survey, practical experience and taking above factors intoconsideration the suitable solvents selected was methanol

Selection of Method and Wavelength

For estimation of Fluconazole single-wavelength spectrophotometric method employing 260 nm analytical wavelength was used

Standard solutions and calibration curve

Accurately weighed 10mg of Fluconazole is transferred into a 100ml volumetric flask and dissolved in 30ml of methanol. It was then sonicated for 10 minutes, and made up to the mark with methanol to give a stock solution having 100 μg/ml concentration. For calibration curve, serial dilutions were made for Fluconazole in the range of 2, 4, 6, 8, and 10μg/ml concentrations were prepared by diluting the stock solution with methanol. The absorbance values of above solutions were measured in the wavelength at λ max 260 nm against methanol as blank and calibration curve was prepared. It obeyed beer's law in these concentration ranges

Sample preparation for tablet analysis

To determine the content of Fluconazole in conventional tablets (label claim: 4mg Fluconazole per tablet), twenty tablets were weighed, their mean weight was determined and they were finely powdered and powder equivalent to 10 mg of Fluconazole was weighed and transferred into a 10 ml volumetric flask containing 10 ml methanol, sonicated for 10 min and the resulting sample solution was then filtered through Whatmann filter paper (No. 41). The filtrate was further diluted to obtain the final concentration of 100μg/ml. Appropriate dilutions of Fluconazole were scanned over the range of 400-200 nm and the absorbance at wavelength 260 nm was measured. From calibration curve the final drug concentration in tablet was calculated.

Method Validation

Method validation was performed in terms of specificity and selectivity, precision and accuracy,linearity and stability ICH Q2B, 1996 [38].

Precision and Accuracy

Accuracy of analysis was determined by performing recovery studies by spiking different concentrations of pure drug in the pre analyzed tablet samples within the analytical concentration range of the proposed method at three different set at level of 50%, 100% and 150%.Precision was calculated for inter day and for intraday. The data obtained shows that method is sufficiently precise. Precision is calculated as % Relative Standard Deviation.

Linearity and Stability

The response for Fluconazole was linear in the concentration range of 2- 10μg/ml withcoefficient of correlation r2 = 0.9999 for pure drug. Problems of stability are usually encounteredwith these compounds.

RESULTS AND DISCUSSION

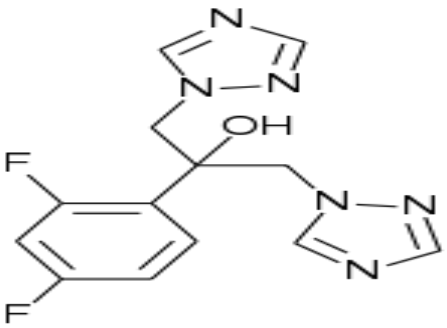
Standard calibration curve for Fluconazole covering the range 2-10μg/ml, prepared by serial dilution with methanol for pure drug and tablet formulation were developed and validated. The procedure was adopted as per designed protocol, based on ICH Q2B guidelines [38].. The calibration curve was obtained by plotting absorbance vs analyte concentration. The slope and

intercept of the calibration line was determined by linear regression.

Selectivity and specificity

The drug Fluconazole in the formulation was well identified under this condition. No interference observed in nine different samples of Fluconazole. Fig.2 showed a linear relationship between the absorbance and the concentration, with correlation coefficient and percentage estimated with standard deviation of 0.9999, 99.75 ± 1.20, respectively. The results are shown in Table 1 and 2.

Fig ;1 The structure of Fluconazole



Systematic (IUPAC) name OF Fluconazole IS

2-(2,4-difluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)propan-2-ol

Fig; 2 CALIBRATION CURVE OF FLUCONAZOLE

SHOWING LINEARITY RELATION SHIP

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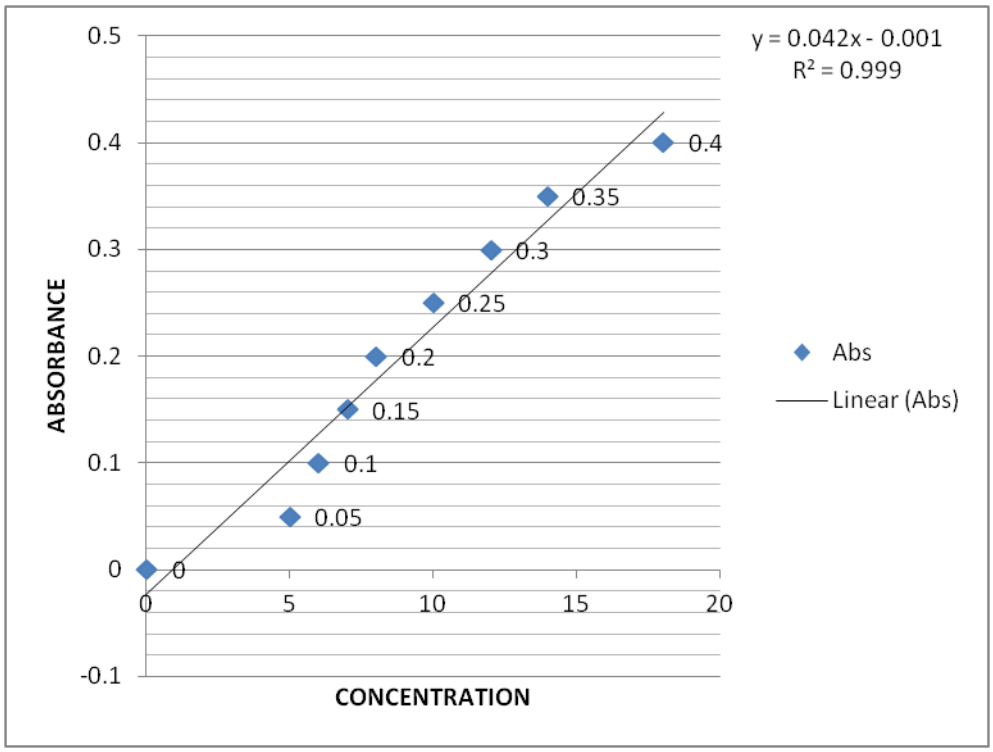

Table 1: Linearity regression data for Fluconazole

Parameters	Value of Fluconazole
λ max nm	260
Beer's law limit (µg/ml)	2-10 (µg/ml)
Correlation coefficient	0.9999
Regression equation (Y*)	Abc=A+B*C
Slope(B)	0.0427
Intercept (A)	-0.001

Table 2: Results of analysis of laboratory samples

Label claim (mg/tab)	% Concentration estimated* (Mean ± % R.S.D.)
4 mg/tab	99.75 ± 1.20

***Average of nine determinations; R.S.D., Relative Standard Deviation*

Recovery

As shown in Table 3 excellent recoveries were made at each added concentration.

Table 3: Recovery data for Fluconazole

Level added (%)	Recovery (%)*	RSD %
50	98. 88	0.85
100	100.43	1.15
150	99.67	1.22

** Mean of three determinations*

Precision

Precision evaluated through intraday and inter day of the pure drug from solvent are presented in Tables 4 and 5, respectively.

Table 4: Results of intraday precision of Fluconazole

Parameter	% Drug estimated* (Mean ± %R.S.D.)		
	6 µg/ml	8 µg/ml	10 µg/ml
Morning	98.30±0.68	99.90±0.48	99.45±0.17
Afternoon	99.92±0.83	99.62±0.23	101.02±0.61
Evening	99.62±1.20	100.02±1.32	99.22±1.03

**Average of nine determinations; R. S. D., Relative Standard Deviation*

Table 5: Results of interday precision of Fluconazole

parameter	% Drug estimated* (Mean ± %R.S.D.)		
	6 µg/ml	8 µg/ml	10 g/ml
Day 1	100.30±1.08	99.90±1.18	99.45±0.99
Day 2	101.02±1.33	101.02±0.96	98.02±0.98
Day 3	100.72±0.62	99.72±0.92	99.62±0.73

**Average of nine determinations; R. S. D., Relative Standard Deviation*

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

The LOD determined as the amount of drug and LOQ was determined as the lowes tconcentration for drug shown in Table 6.

Table 6: Limit of detection and limit of quantitation for drug in solvent

LOD (µg/ml)	LOQ (µg/ml)
10.46	31.70

CONCLUSION

. A spectrophotometric method for quantifying Fluconazole in tablet has been developed and validated. The method is selective, precise, accurate and linear over the concentration range

studied. The method is simple and suitable for the determination of Fluconazole in formulation without in terference from excipients or from common degradation products, suggesting its application in IPQC and pharmacokinetic studies

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REFERENCES

[1] Klavs F, *Analytical Profiles of Drug and Excipients* (Academic Press, An Imprint of Elsevier, New Jersey), 27(1987) 67-112.

[2] Martidale, The Extra Pharmacopoeia, edited by JEF Renolds (Royal Pharmaceutical Soc., Londan), 1990, 404-406.

[3] MP Biomedicals

[4] Pfizer Australia Pty Ltd. Diflucan (Australian Approved Product Information). West Ryde (NSW): Pfizer Australia; 2004.

[5] Longley, Nicky; Muzoora, Conrad; Taseera, Kabanda; Mwesigye,James; Rwebembera, Joselyne; Chakera, Ali; Wall, Emma; Andia, Irene et al. (2008). "Dose Response Effect of High-Dose Fluconazole for HIV-Associated Cryptococcal Meningitis in Southwestern Uganda". *Clinical Infectious Diseases* 47 (12): 1556–1561. DOI:10.1086/593194. ISSN 1058-4838. PMID 18990067.

[6] Sweetman S, editor. Martindale: The complete drug reference. 34th ed. London: Pharmaceutical Press; 2004. ISBN 0-85369-550-4

[7] Bennett J.E. (2011). Chapter 57. Antifungal Agents. In L.L. Brunton, B.A. Chabner, B.C. Knollmann (Eds), Goodman & Gilman's The Pharmacological Basis of Therapeutics, 12e. Retrieved May 22, 2012 from http://www.accessmedicine.com.ezp1.lib.umn.edu/content.aspx?aID=16678770.

[8] Spectrum of fungal susptibility and resistance to fluconazole

[9] Rossi S, editor. Australian Medicines Handbook 2006. Adelaide: Australian Medicines Handbook; 2006. ISBN 0-9757919-2-3

[10] Product information from Pfizer Inc

[11] http://aac.asm.org/cgi/reprint/AAC.01500-08v1.pdf

[12] Kragie, Laura; Turner, Stephanie D.; Patten, Christopher J.; Crespi, Charles L.; Stresser, David M. (2002). "Assessing Pregnancy Risks of Azole Antifungals Using a High Throughput Aromatase Inhibition Assay". . PMID 12489563.

[13] Fluconazole, PubMed Health

[14] http://www.drugs.com/fda/diflucan-fluconazole-safety-communication-long-term-during-pregnancy-may-associated-birth-defects-13013.html

[15] Xu, Yi; Wang, Yan; Yan, Lan; Liang, Rong-Mei; Dai, Bao-Di; Tang, Ren-Jie; Gao, Ping-Hui; Jiang, Yuan-Ying (2009). "Proteomic Analysis Reveals a Synergistic Mechanism of Fluconazole and Berberine against Fluconazole-ResistantCandida albicans: Endogenous ROS Augmentation". *Journal of Proteome Research* 8 (11): 5296–5304. DOI:10.1021/pr9005074. ISSN 1535-3893. PMID 19754040. Free Full Text
PMID 19754040. Free Full Text

[16] Richardson, R. K.; 1983, U.S. Patent 4,404,216

[17] Hillis J, Morelli I, Neville D, Fox J & Leary A C, *Chromatogr*, 67 (11/12) (2008) 995-998.

[18] El-Bayoumi A, El-Shanawany A A, El-Sadek M E & Ab dEl-Sattar A, *Spectrosco Lett*, 30(1) (1997) 25-46.

[19] Seleck O F, *Anal Lett*, 29(12) (1996) 2163-2176

[20] Ekiert R J & Krzek J, *Acta Pol Pharm*, 66(1) (2009) 19-24.

[21] Jalali F & Rajabi M J, *Chin J Chem*, 25(9) (2007) 1300-1303.

[22] Aboul- Enein H Y, Goger N G & Turkalp A, *Anal Lett*, 35(2002) 1193-1204.

[23] Goger N G & Aboul-Enein H Y, *Anal Lett*, 34 (2001) 2089-2098.

[24] Sadasivudu P, Shastri N & Sadanandam M, *Int J Chem Tech Res*, 1(4) (2009) 1131-1136.

[25] Sane R T, Fulary A A & Joshi A N, *Indian Drugs*, 31 (1994) 207-210.

[26] Kelly M, *Braj J Pharma Sci*, 45(4) (2009) 693-700.

[27] Hurtado F K, Souza M J, Melo J & Rolim Clarice M B, *Lat Am J Pharm* 27(2) (2008) 224-228.

[28] Wood P R & Tarbit M H, *J Chromatogr*, 383 (1986) 179-186.

[29] Debruyne D,Ryckelynck J P & Bigot M C, *J Pharm Sci*, 77 (1998) 534-535.

[30] Cociglio M, Brandissou S & Alric R, *J Chromatogr B*, 686 (1996) 11-17.

[31] Hosotsubo K K, *J Chromatogr B*, 529 (1990) 223-228.

[32] Inagaki K, Takagi J & Gill M A, *Ther Drug Monit*, 14 (1992) 306-311

[33] Xu W P, Hu B C & Chen L M, *Chin J Pharmac Anal*, 17 (1997) 313-315.

[34] Y whong Z & Xia X P, *Chin J Pharmac Anal*, 20 (2000) 31-35.

[35] Al-Rimawi F, *Jord J Chem*, 4(4) (2009) 357-365.

[36] Limpiti D,Lhieochaiphant D & O-ariyakul N,*CMU*, 5(3) (2006) 341-349

[37] Abdel-Moety E M, Khattab F I, Kelani K M & AbdEl-sattar A, *II Farmac*, 57 (2007) 931-938

[38] International Conference on the Harmonization of Technical Require ments for the Registration Of Pharmaceutical for Human Use (ICH) Q2B 1996. Validation of Analytical Procedures, Methodology

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