

Development and validation of different UV-spectrophotometric methods for the estimation of fluconazole in bulk and in solid dosage form

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A simple, sensitive and accurate UV-spectrophotometric method has been developed for the determination of an antifungal drug, fluconazole (FLZ), in raw material and in tablets. The drug shows maximum absorption at 261 nm in selected four different simulated media, namely gastric fluid simulant (HCl), vaginal fluid simulant (VFS), phosphate buffer (PB) and phosphate buffer saline (PBS) at pH 1.5, 4.2, 6.8 and 7.4 respectively. Beer's law is obeyed in the concentration range 10-100 µg/mL of drug. The limits of detection have been calculated for different media, such as HCl, VFS, PB and PBS and are found to be 2.24, 1.49, 1.42 and 1.19 µg/mL, whereas the limits of quantification are 6.82, 4.50, 4.29 and 3.63 µg/mL correspondingly. The methods have been successfully applied to the determination of FLZ in tablets and bulk drug. Results are validated statistically as per ICH guidelines. It is found that the excipients present in the commercial formulation do not interfere with the methods and hence the UV-method permits a rapid and economical quantification of drug in bulk and in tablet dosage form.

Keywords: Assay, Fluconazole, UV-Spectrophotometric estimation, Simulated body fluids, Vaginal fluid simulant

Fluconazole¹(FLZ) chemically known as 2-(2, 4-difluorophenyl)-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 antifungal agent and recommended for the treatment and prophylaxis of disseminated and deep organ candidiasis². 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-densitometry³, spectrofluorimetry⁴, IR-spectroscopic⁵, UV-spectrophotometric⁶⁻¹⁰, microbiological method¹¹⁻¹³, gas liquid chromatography,^{14,15} (GLC), high performance liquid chromatography (HPLC) for biological fluid¹⁶⁻¹⁸, and high performance liquid chromatography (HPLC) for pharmaceutical dosage forms¹⁹⁻²³.

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 in different simulated buffer media i.e. gastric fluid (hydrochloric acid) (pH 1.5), vaginal fluid simulant (pH 4.2), phosphate buffer (pH 6.8) and phosphate buffer saline (pH 7.4). This is a simple, precise and

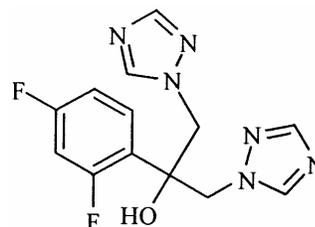


Fig. 1—Chemical structure of fluconazole

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accurate analytical method for the estimation of drug in bulk and different dosage form. The application of proposed method is to calculate the drug in different release experiments of simulated body fluids like HCl (gastric fluid simulant), VFS (vaginal fluid simulant), PB (phosphate buffer) and PBS (phosphate buffer saline) and in routine quality control analysis. The results are analyzed and validated statistically and by recovery studies.

Materials and Methods

Apparatus

A double beam UV-vis spectrophotometer (PharmaSpec-1700, Shimadzu, Japan) connected to computer that was loaded with spectral bandwidth of 1 nm and wave length accuracy of ± 0.3 nm with a pair of 1 cm matched quartz cells. All weights were taken on electronic balance (Vibra, DJ- 150S-S, Shinko Denshi, Japan).

Materials

Pure sample of fluconazole was obtained from Ranbaxy, Gurgaon, India. Tablets of brand name Fungid™ (Batch no. - SBT-910241) containing 150 mg of fluconazole, were procured from a local pharmacy. Double distilled water was used as the solvent for the experiment. Hydrochloric acid, lactic acid, acetic acid, sodium chloride, potassium hydroxide, calcium hydroxide, urea, glucose, glycerol, disodium hydrogen phosphate, and potassium dihydrogen phosphate were purchased from Qualigens (Fisher), Mumbai, India. Bovine serum albumin (BSA) was purchased from Himedia, Mumbai, India. All the above chemicals were of analytical grade.

Preparation of standard solutions and calibration curves

Standard solutions of fluconazole were prepared by dissolving 100 mg of the standard drug separately in different medium such as HCl, VFS, PB and PBS, having pH 1.5, 4.2, 6.8 and 7.4 respectively, and diluted up to 100 mL by respective media to obtain a stock solution of final concentration 1000 $\mu\text{g/mL}$.

Aliquots (0.1 – 1.0 mL) of stock solution of fluconazole were transferred into a series of 10 mL volumetric flasks and volume was made up to the mark with different buffers to produce the concentration range 10-100 $\mu\text{g/mL}$. The absorbance of each solution was measured at 261 nm against the respective medium as blank. The calibration curves were prepared by plotting graph between absorbance and concentration, (Fig. 2).

Estimation of fluconazole in bulk and in tablets

For the analysis of drug in bulk, accurately weighed 100 mg drug was dissolved in 100 mL of buffer solution in a volumetric flask. After suitable dilution, the absorbances of final contents were recorded against the respective blanks at 261 nm. For the analysis of tablets, 20 tablets of fluconazole (150 mg) were grounded to fine powder and mixed thoroughly. A quantity of powder equivalent to 100 mg of the drug was transferred into a 100 mL volumetric flask and dissolved separately with the HCl, VFS, PB and PBS medium by sonication at 30 min. The solutions were filtered through 0.22 μm membrane filter. The membrane was washed with the same media. The washing was added to the filtrate and the final volume was made up to 100 mL. After suitable dilution, the absorbances of final solutions, corresponding to the 50 $\mu\text{g/mL}$, were recorded at 261 nm against the respective media as blank.

Method validation

The methods were validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures^{24,25}.

Recovery studies

Pure drug at three levels was added to a fixed amount of drug in tablet powder and the total amount was determined to calculate the percentage recovery of the pure drug.

Specificity

The specificity of the methods was evaluated by interaction study obtained from scans (UV) of the standard drug solution, sample solution (of tablet) and placebo tablet matrix solution (these matrices

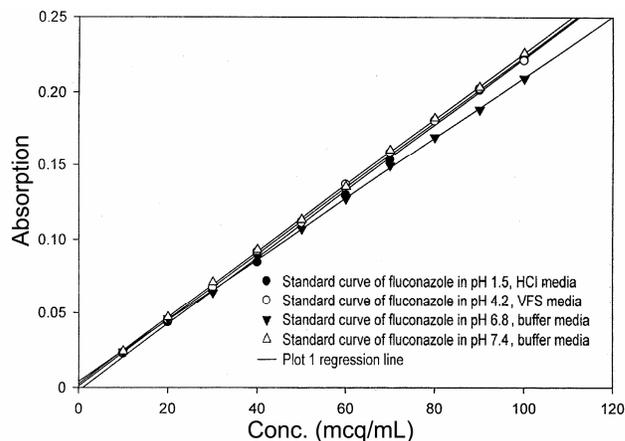


Fig. 2— Calibration curve of standard drug in different simulated body fluid media

solutions were prepared in a manner similar to the sample solution using placebo tablet matrix instead of FLZ tablets). This UV-method was found to be specific for FLZ, as none of the excipients interfered with the calculation of FLZ [Figs 3(a-d)].

Linearity

The absorbances of the standard solutions, in different media at 10-100 $\mu\text{g/mL}$ range were measured at 261 nm. Calibration curves were constructed by plotting average absorbance versus concentrations. Linearity was determined by regression equations for all media solutions.

Accuracy (by standard addition method)

Accuracy can be analyzed by percentage recovery of added standard drug solutions to fixed concentration of sample solutions. For stock solutions, an accurately weighted amount of standard and sample tablet powder equivalent to 100 mg of drug was transferred separately into 100 mL volumetric flasks to get 1000 $\mu\text{g/mL}$ in HCl, VFS, PB and PBS buffer medium respectively.

In a separate dilution three different 10 mL volumetric flasks each having 0.1 mL (10 μg) of sample stock solution were added with 0.1 (10 μg), 0.2 (20 μg), and 0.3 (30 μg) mL of standard stock solution to get final concentration of 10, 20, 30 $\mu\text{g/mL}$ (50, 100 and 150% respectively) after diluting with buffer medium HCl. The same were repeated with diluting media VFS, PB and PBS. All solutions were prepared in triplicate and assayed for percentage recoveries of added standards fluconazole. The accuracy was reported as percentage recovery by the assay of known added amount of analyte in the sample.

Precision

Repeatability was calculated by analyzing three independent FLZ standard solutions (10, 20, 30 $\mu\text{g/mL}$), in triplicate, in different media. The intermediate precision was evaluated on three independent FLZ standard solutions (10 $\mu\text{g/mL}$) on same day and also on three consecutive days. The precision was expressed as the standard deviation, percentage relative standard deviation (coefficient of variation) and confidence interval of each mean.

Limit of detection and limits of quantification

Limit of detection (LOD) and limit of quantification (LOQ) are based on the slope of the calibration curves and standard deviation of y - intercepts of regression lines.

Results and Discussion

The method was validated according to the guidelines of International Conference on Harmonization (ICH). The proposed UV-spectrophotometric method was found to be specific and selective for analysis of FLZ in bulk and in tablets, where no interference was observed at 261 nm by the excipients of tablet, when compared with standard and sample FLZ solution. The absorption spectra of fluconazole in different pH solutions of HCL, VFS, PB and PBS are shown in Figs 3(a-d).

The average λ_{max} is found to be 261 nm. A standard calibration curve of the drug was constructed by plotting absorbance versus concentration. Linear absorbance versus concentration gives regression equations $Y = 0.0022X - 0.0021$, $Y = 0.0022X + 0.0013$, $Y = 0.0022X + 0.0025$ and $Y = 0.0022X + 0.0025$ with a correlation coefficient (r^2) of 0.9990, 0.9996, 0.9996 and 0.9997 respectively for the drug in different buffer systems HCL, VFS, PB and PBS. The above linear regression equations with a correlation coefficients (r^2) indicate a good linearity between absorbance and concentration in the range of 10-100 $\mu\text{g/mL}$ (Table 1).

The accuracy of the proposed method by standard addition method was determined for tablet and the mean recovery ($n=3$) is found to be 101.36 ± 0.56 , 106.29 ± 1.78 , 100.74 ± 2.08 , and 104.44 ± 0.15 in different types of buffer like HCl, VFS, PB and PBS

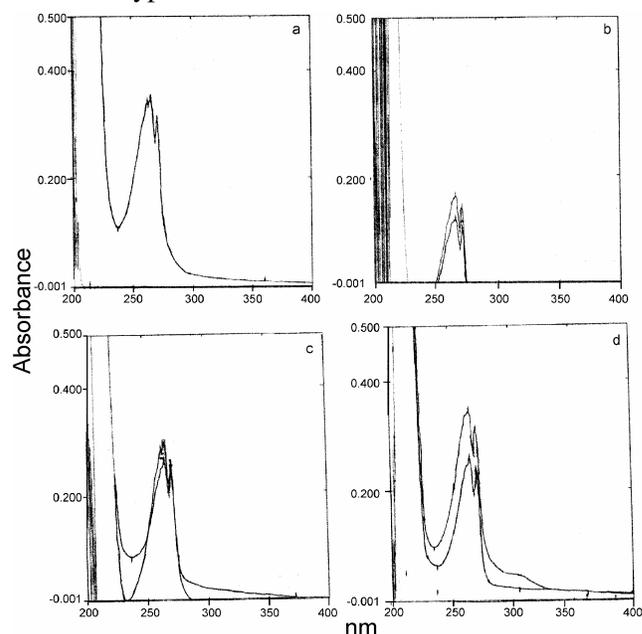


Fig. 3— UV-scans of standard drug, tablet drug and placebo tablet matrix in (a) Media 1, (b) Media 2, (c) Media 3 and (d) Media 4 at pH 1.5, 4.2, 6.8 and 7.4 respectively

respectively (Table 2) which was in the near agreement with the labeled amount and thus indicates the accuracy of the method. The standard and sample solutions were stable for 48 h (Table 3).

Results of assay on tablet solid dosage form of fluconazole by proposed UV-method are reported in Table 4. The assay results obtained by the different proposed methods are found to be 108.83 ± 1.72 ,

109.73 ± 0.91 , 109.62 ± 1.46 and 105.54 ± 3.27 respectively which is in acceptable agreement with the pharmacopoeial limit. The assay results of proposed UV-methods when compared using Student t-test do not reveal significant difference between the experimental values obtained for the standard drug and sample drug analysis by the two methods ($P > 0.05$), (Table 4).

Table 1— Regression analysis and system suitability parameters for the quantification of fluconazole from UV-spectrophotometer in different media

Parameter	Media 1	Media 2	Media 3	Media 4
Linearity range, $\mu\text{g/mL}$	10-100	10-100	10-100	10-100
Regression equation	$Y=0.0022X - 0.0021$	$Y=0.0022X+0.0013$	$Y=0.0021X +0.0041$	$Y=0.0022X +0.0025$
Correlation coefficient(r^2)	0.9990	0.9996	0.9996	0.9998
Molar absorptivity (ϵ), L/mol/cm	0.745×10^3	0.705×10^3	0.723×10^3	0.736×10^3
Sandell ¹⁸ sensitivity $\mu\text{g.cm}^2/0.001 \text{ abs unit}$	0.410	0.434	0.423	0.416
95% confidence interval for slope	< 0.0001	< 0.0001	< 0.0001	< 0.0001
95% confidence interval for intercept	0.2025	0.2420	0.0014	0.0127
Repeatability ^a (% RSD)	0.98	0.76	0.87	0.92
Intermediate precision ^b (%RSD)	1.00	0.82	1.20	1.10
LOD, $\mu\text{g/mL}$	2.24	1.49	1.42	1.19
LOQ, $\mu\text{g/mL}$	6.82	4.50	4.29	3.63

Media 1— Gastric fluid simulant (GFS), $pH = 1.5$

Media 2— Vaginal fluid simulant (VFS), $pH = 4.2$

Media 3— Phosphate buffer (PB), $pH = 6.8$

Media 4— Phosphate buffer saline (PBS), $pH = 7.4$

^aResidual standard deviation (RSD) of 6 independent determination in a day.

^bResidual standard deviation (RSD) of 9 independent determinations .

(Three independent samples per day for 3 days) .

Table 2— Accuracy test results in different simulated media for fluconazole in bulk and in solid dosage form by UV-spectrophotometer

Media type	Sample conc. $\mu\text{g/mL}$	Conc. of added standard ^a $\mu\text{g/mL}$	Percentage recovery \pm SD	Mean percentage recovery \pm SD
Media 1 ($pH = 1.5$)	10	10	101.53 ± 0.61	101.36 ± 0.56
	10	20	101.56 ± 0.40	
	10	30	100.99 ± 0.68	
Media 2 ($pH = 4.2$)	10	10	106.14 ± 3.22	106.29 ± 1.78
	10	20	106.37 ± 1.07	
	10	30	106.37 ± 1.07	
Media 3 ($pH = 6.8$)	10	10	98.80 ± 1.38	100.74 ± 2.08
	10	20	98.78 ± 3.67	
	10	30	104.64 ± 1.19	
Media 4 ($pH = 7.4$)	10	10	105.68 ± 2.28	104.44 ± 0.15
	10	20	104.80 ± 3.17	
	10	30	102.84 ± 1.14	

SD— Standard deviation (n=3).

^aConcentration of standard addition was done at 50, 100 and 150% respectively (The added 20 μg of standard drug solution was equivalent to 100 %).

Table 3— Stability of the standard and sample solutions of fluconazole in different media by UV methods

Media	Time interval h	UV method			
		Standard solution		Sample solution	
		Recovery (n=3), %	Difference, %	Recovery (n=3), %	Difference, %
Media 1 (pH = 1.5)	0	98.20	0.00	102.52	0.00
	24	98.20	0.00	105.79	3.27
	48	100.02	1.82	106.57	0.21
Media 2 (pH = 4.2)	0	100.18	0.00	107.91	0.00
	24	104.27	4.09	107.91	0.00
	48	104.28	0.00	108.36	0.45
Media 3 (pH = 6.8)	0	99.91	0.00	106.57	0.00
	24	96.10	3.81	106.98	0.41
	48	98.00	1.91	107.00	0.02
Media 4 (pH = 7.4)	0	97.72	0.00	105.00	0.00
	24	100.46	2.74	107.00	2.00
	48	104.09	3.64	107.82	0.82

Table 4— Assay results of marketed fluconazole (Fungid™-150) dosage forms by UV- method

Releasing media	Drug in standard solution \pm SD	Drug in dosage form solution \pm SD
Media 1 (HCl, pH = 1.5)	98.76 \pm 1.39	108.83 \pm 1.72
Media 2 (VFS, pH = 4.2)	99.73 \pm 0.53	109.73 \pm 0.91
Media 3 (PB, pH = 6.8)	98.00 \pm 4.76	109.62 \pm 1.46
Media 4 (PBS, pH = 7.4)	100.46 \pm 0.53	105.54 \pm 3.27

SD– Standard deviation (n=3).

The repeatability (% RSD or percentage relative standard deviation) and intermediate precision (% RSD) were observed for analysis of three independent in replicate samples. The repeatability values are found to be 0.98, 0.76, 0.87 and 0.92 in HCl buffer, VFS buffer, PB buffer and PBS buffer respectively and the intermediate precision (% RSD) for selected sample in three consecutive different days are found to be 1.0 in HCl buffer, 0.82 in VFS buffer, 1.20 in PB buffer and 1.10 in PBS buffer respectively. The low values of both percentage relative standard deviation and intermediate precision confirm the high degree of precision and accuracy of the proposed method.

The LOD and LOQ are found to be 2.24 and 6.82 $\mu\text{g/mL}$ in hydrochloric acid buffer, 1.49 and 4.50 $\mu\text{g/mL}$ in vaginal fluid simulant, 1.42 and 4.29 $\mu\text{g/mL}$ in phosphate buffer, and 1.19 and 3.63 $\mu\text{g/mL}$ in phosphate buffer saline respectively.

Conclusion

The method is found to be very simple, rapid, precise, accurate and sensitive. The validated UV-method can be used for the drug analysis in routine for bulk and solid dosage forms, at four different types of pH media.

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