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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF FLUCONAZOLE IN SOFT GELATIN CAPSULE

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ABSTRACT: A simple, accurate, cost effective and reproducible spectrophotometric method has been developed for the estimation of Fluconazole in soft gelatin capsule dosage form. For UV spectrophotometric method, maximum absorption was found at λmax 266 nm. The percentage recovery of Fluconazole ranged from (99.76 ± 0.57) in capsule dosage form. The developed method was validated as per ICH guidelines with respect to linearity, accuracy (recovery), precision and specificity. Beers law was obeyed in the concentration range of 200 – 800 µg /ml having line equation y = 0.0018x - 0.0216 with correlation coefficient of 0.9996. By treating the data statistically and by recovery study, results of study were validated.

KEYWORDS: UV/Visible spectrophotometric method, Fluconazole, Soft gelatin capsule.

INTRODUCTION : Chemically Fluconazole is 2-(2,4-difluorophenyl)- 1,3-*bis*(1*H*-1,2,4-triazol-1-yl)propan-2-ol (fig. 1).[1,2] Fluconazole is a triazole antifungal drug used in the treatment and prevention of superficial and systemic fungal infections. Fluconazole inhibits the fungal cytochrome P450 enzyme 14α-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. The literature survey reveals that Fluconazole was analyzed by TLC, HPLC and Spectrophotometry [3-11] methods alone and in combination with another drugs.

. **Fig. 1: Chemical Structure of Fluconazole**

Analytical chemistry has been important since the early days of chemistry, providing methods for determining which elements and chemicals are present in the world around us. Modern analytical chemistry is dominated by instrumental analysis. Many analytical Chemists focus on UV Spectrophotometer by which analytical method may be developed and helpful for routine analysis in quality control laboratories and to establish quality assurance parameters. Literature surveys reveals that there in no single estimation of this drug has not been reported in soft gelatin capsule formulation. Thus the present study was undertaken to develop and validate a simple, sensitive, accurate, precise and reproducible U.V method for Fluconazole.

MATERIALS AND METHOD:

Apparatus: Instruments used were UV-Visible double beam spectrophotometer (UV-1800, SHIMADZU Limited, Japan) with 1cm matched quartz cells, Micropipette of Variable volume 10- 1000 µL (Gene Pete Co.) and Digital balance (Shimadzu).

Reagents & Materials: Fluconazole pure drug and marketed formulation(Soft Gelatin capsule) was obtained from GLPL(Gujrat Liqui Pharmaceuticals Ltd.), Waghodia, Gujrat as gift sample with 99.99% w/w assay value and was used without further purification. All chemicals and reagents used were of analytical grade.

METHOD:

Preparation of Stock Solution: Take 100 mg of Fluconazole and add 80 ml of 0.1N HCl. Sonicate it for 10 minutes and makeup the volume up to 100 ml with 0.1N HCl. From this prepare various solutions in the range of 200 – 800 µg/ml and take the absorbance at 266 nm. And prepare calibration curve.

Preparation of Sample Solution: Mix the content of 20 capsules and calculate the average weight of one capsule. When sonicated for 30 minutes highest amount of extraction was achived. So now in average weight of one capsule add 80 ml of 0.1N HCl, and add 80 ml of 0.1N HCl Sonicate it for 30 minutes. Filter it through Whattman filter paper #41 and make up the volume up to 100 ml with 0.1N HCl and make appropriate dilution and calculate the amount from calibration.

Method Validation [12,13]**:** Various methods for analysis of Fluconazole in soft gelatin capsule were performed and the method was validated according to ICH Q2B guidelines.

Linearity: The aliquots of concentration ranging 100-1000 µg/mL were prepared in triplicate, but linearity was found to be between 200 – 800 µg/ml concentrations. The linearity was calculated by the least square regression method.

Precision and Accuracy: The precision of an analytical procedure express the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed conditions. The precision of the assay was determined by repeatability (intraday) and intermediate precision (inter-day) and reported as RSD % for a statistically significant number of replicate measurements. The intermediate precision was studied by comparing the assays on two different days and the results were presented as the standard deviation and RSD %. Accuracy is the percent of analyte recovered by assay from a known added amount. Data from triplicate trials over two different concentration levels (400µg/mL and 500µg/mL) covering the linearity range were obtained.

LOD and LOQ: LOD (k= 3.3) and LOQ (k= 10) of the method were established according to ICH definitions. LOD and LOQ of method are reported in Table no 1. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations- LOD = 3.3 S/M; LOQ = 10 S/M Where S is the standard deviation of the absorbance of the sample and M is the slope of the calibrations curve. The limit of quantification (LOQ) is defined as the lowest concentration of analyte that can be estimated with an acceptable limit of accuracy and precision. The values of LOD and LOQ are given in Table 1.

Table 1: Analytical validation parameters:

Parameters	Fluconazole
Wavelength	266 nm
Range	200 – 800 µg mL^{-1}
Linearity	0.9996
Intercept	0.0216
Slope	0.0018
Accuracy	99.76 ± 0.57
Intra day precision	% RSD < 2
Inter day precision	% RSD < 2
LOD	20.47 µg mL^{-1}
LOQ	68.23 µg mL^{-1}
Reproducibility	% RSD < 2

Table 2: Results of the marketed fluconazole soft gelatin capsule:

Drug	**Labeled (mg/cap)**	**Amt Found (mg/cap)**	**% Labeled claim**
FLUCONAZOLE	150	149.7	99.80±1.24

RESULTS AND DISCUSSION: In the start of the method development for this drug, different solvents were tested such as water, methanol, 0.1N HCl and 0.1N NaOH. Due to greater solubility and reproducible readings of maximum absorbance in 0.1N HCL, it was selected for further study. By serial dilution of standard stock of Fluconazole (1000µg/ml), the different dilutions of standard drug with concentration 200-800 µg/ml were prepared

and calibration curve was prepared by plotting graph between absorbance and concentration (µg/ml) (Fig. 3). The results of linearity are presented in table 1. The data was statistically validated by means of least square regression method. Calibration curve data were constructed in the range of the concentrations of 10µg/ml to 100µg/ml. Beer's law was obeyed over this concentration range. The regression equation was found to be y = 0.0018x - 0.0216 with correlation coefficient of 0.9996. The stock solutions and working standards were made in 0.1N HCL. Calibration curve as presented in Fig. 2**.**

Figure 2: Zero order curves for Fluconazole.

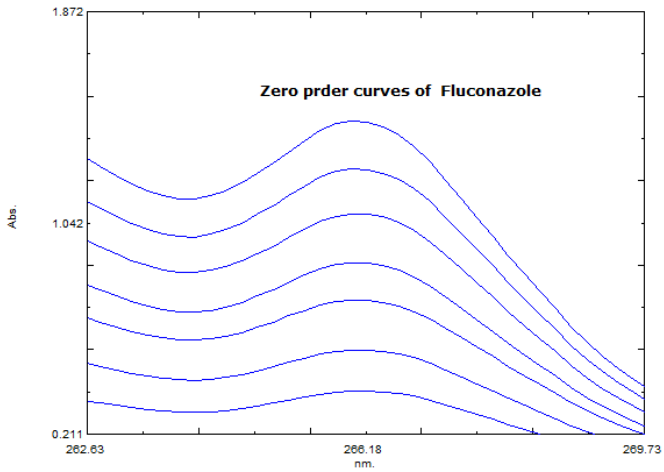
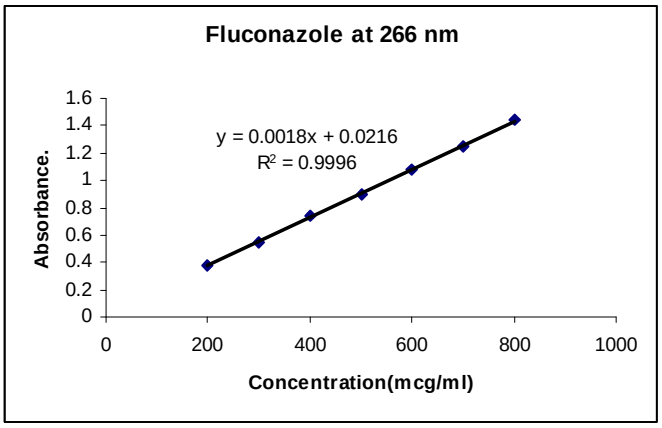


Figure 3: Zero order Linearity plot for Fluconazole at 266 nm.



CONCLUSION: The developed UV spectrophotometric method is simple, précised, accurate and reproducible for the estimation of Fluconazole in soft gelatin capsule formulation. Limit of detection was found to be 20.47 and the Limit of quantification to be 68.23. The calibration curves showed linearity between the absorbance and

concentration (200-800µg/ml) and correlation coefficient was found to be 0.9996. The percentage recovery was found to be 99.76 ± 0.57. The proposed method will be suitable for the analysis of Fluconazole in Soft gelatin capsule pharmaceutical formulation.

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