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UV SPECTRSCOPIC DETERMINATION OF FLUCONAZOLE BY ENHANCING SOLUBILITY USING GREEN SOLVENTS:AN IMPLICATION

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ABSTRACT

Fluconazole is antifungal agent recommended in the prophylaxis and treatment of infections caused by fungus and yeast. Fluconazole is slightly soluble in water and soluble in ethanol, DMSO, methanol and dimethylformamide. To obviate the usage of organic solvents, enhanced solubility in water of the same was achieved through hydrotropic solubilization technique. Quantitative solubility was inspected through solubilizing the drug in various concentrations of hydrotropes. Sodium salicylate, Sodium acetate, urea, Piperazine, sodium benzoate, sodium citrate and Nicotinamide are a few to mention among various hydrotropic agents. Solubility enhancement ratio was calculated and observed a 10-fold increase than that of in distilled water. A1%,1cm was measured and found to be0.8492. Standard solutions of FNZ were established to obey beer-lamberdt's law in the range of 2.5-90mcg per mL. Ultra Violet Spectroscopy was carried out at 261nm and samples were prepared with a solution of Methanol and 230nm by using 2M urea. The linearity demonstrated a correlation coefficient of 0.999 for Methanol and for 2M urea is 0. 999.The solvents used throughout the experimental work were found to be free from any type of interference from any excipients and method was simple, rapid, precise, accurate and sensitive and can be applied in routine analysis of Fluconazole in single and combined form. Current research work defines a novel approach for usage of eco-friendly solvents in enhancing the solubility of poorly soluble pharmaceutical drug substances and disposal of the solvents are not harmful to the environment.

Keywords: Fluconazole, Hydrotropic solubilization technique, Solubility enhancement ratio, UV-Spectrometric determination

Introduction

Fluconazole is used to treat, manage, prevent, and improve fungus infections, parasitic infection of skin and hair, Inflammatory diseases [1-5]. In the present research work mixed hydrotropic solubilization phenomenon is used to enhance the solubilization of poorly water soluble drugs. Bulk drug samples were identified by the observed IR spectra and the melting points determination. Sodium benzoate, niacinamide and urea are the hydrotropes were selected for the solubility enhancement of drugs. Fluconazole in presence of large concentration of hydrotropes. Marketed fluconazole tablets determined by spectrophotometric analysis using hydrotropic solubilization techniques. Familiarized with the treatment of both systemic and superficial fungal infections in a variety of tissues and also which stops the growth of fungi by preventing them from forming their own protective covering [6-11]. Among its many advantages over other antifungal medications, fluconazole can be taken orally[12-16]. Fluconazole was estimated by two methods they are one employs the simultaneous equation approach, [17-25] while the other the Q-absorption ratio approach [29-33]. The estimation of Fluconazole using green solvents in its dosage form and pure forms using projected approaches is said to be accurate and successful [34-35].

Materials & Methods

A UV-Visible Spectrophotometer (UV-1700 SHIMADZU) with 10mm cells made up of quartz. All the ingredients were weighed on balance (Model Shimadzu AUW-220D).

Using Methanol

Preparation of Standard Stock solution: Standard stock was prepared by dissolving 10mg in 10ml volumetric flask makeup with diluent to get 1000 µg/ml (PPM).

Preparation of Working Standard solution: Pipette out 1 ml from standard stock solution in 10ml volumetric flask makeup with the diluent upto the mark to get concentration of 100µg/ml (PPM).From working standard solution pipette out 1ml in 10ml volumetric flask makeup with the diluent upto mark to get 10µg/ml.

Using 2M Urea

Preparation of Standard Stock solution: Standard stock was prepared by dissolving 10mg in 10ml volumetric flask makeup with diluent to get 1000 µg/ml (PPM).

Preparation of Working Standard solution: Pipette out 1 ml from standard stock solution in 10ml volumetric flask makeup with the diluent upto the mark to get concentration of 100µg/ml (PPM).From working standard solution pipette out 1ml in 10ml volumetric flask makeup with the diluent upto mark to get 10µg/ml.

Determination of Analytical Wavelength

From the standard stock solution 1ml was taken into 10ml volumetric flask and the volume was adjusted to 10ml with Methanol and 2M urea. The resulting solution containing 10µg/ml was scanned between 200-400 nm.

Preparation of calibration curve

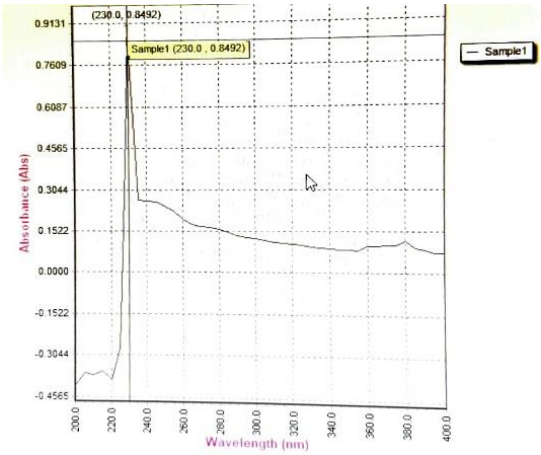
A serial dilutions are made concentration of serial dilutions are made concentration of 2.5,5,7.5,10,12.5,15,17.5,20 µg/ml by using Methanol.By using 2M Urea serial dilutions are made 20,30,40,50,60,70,80,90µg/ml.

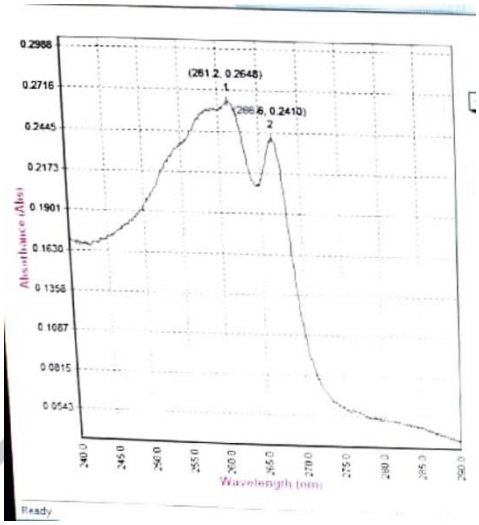
UV Method Validation

1.Linearity and Range :The linearity of the response was verified at 2 to 12µg/ml

concentrations. The calibration curve was obtained by plotting the absorbance against concentration data and was treated by linear regression analysis. The equation of the calibration curve for Fluconazole was obtained.

2.Precision:The correctness of the method was determined by performing the recovery study. Each solution was repeated three times and the recovery in the form of percentage was calculated. The precision was calculated as intra-day and inter-day variation studies.





3.Detection Limit and Quantification Limit:

LOD = 3.3×residual standard deviation/ Slope

LOQ = 10×residual standard deviation /Slope

4.Recovery Study: Accuracy was performed by recovery of drug at three different levels as 80, 100, and 120% of Fluconazole standard concentration. The samples for the study were prepared in a before mentioned procedure for separate percent levels. The resulting solutions were subjected to analysis for recovery studies.

5.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.

Results and discusions

Analytical wavelength

Fig:1Maximum absorbance is observed at 261nm in Methanol (towards right) and 230nm in 2M

urea (towards left)

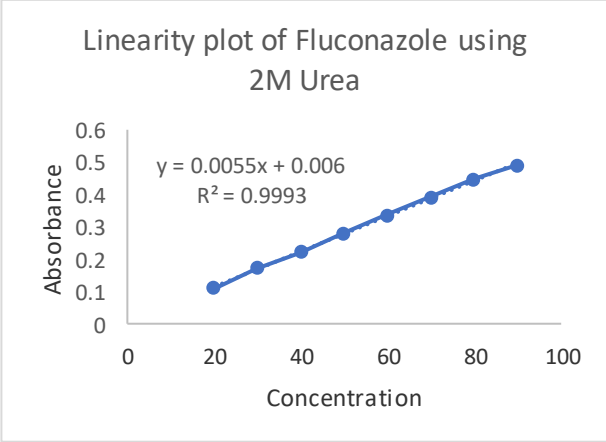
Calibration Curve

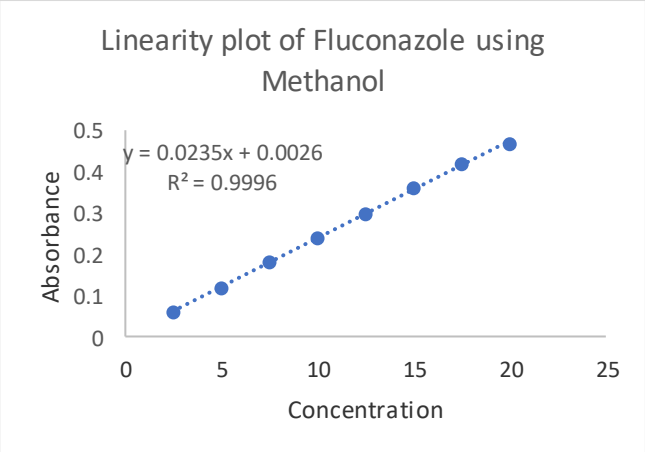
The results of absorbance for all the prepared concentrations were plotted i.e. Concentration vs. Absorbance the method observed to be linear over the prepared concentration range with the standard equation y=0.0235x+0.0026 and Regression value was obtained 0.9996 using Methanol as solvent and

y=0.0055x+0.006 and Regression value obtained was 0.9993 using 2M Urea as solvent. From the calibration data obtained it was found that the regression coefficient was less than 1 which is within the limits.

Linearity

Table 1: Grid lines showing Fluconazole using Methanol as solvent





Concentration	Absorbance
2.5	0.0597
5	0.1194
7.5	0.1791
10	0.2390
12.5	0.2987
15	0.3584
17.5	0.4181
20	0.4673

Figure 2: Linear graph plot exhibiting regression equation in two solvents

Table 2: Grid lines showing Fluconazole using 2M Urea as solvent

Concentration	Absorbance
20	0.112
30	0.911
40	0.224
50	0.281
60	0.336
70	0.411
80	0.448
90	0.492

Precision: The % RSD values found to be less than 2 that indicate this method precise for the determination of the pure form.

%RSD =(SD of measurement/mean value of measurement) X 100.

Table 3: Table showing method as well as intermediate precision data using Methanol as solvent

Concentration (µg/ml)	Intra-day	Inter-day
10	0.2343	0.2414
10	0.2313	0.2412
10	0.2321	0.2432
10	0.2314	0.2411
10	0.2324	0.2441
10	0.2354	0.2452
Average	0.2328	0.2426
Standard deviation	0.0016	0.0017
%RSD	0.0071	0.0073

Table 4: Table showing method as well as intermediate precision data using using 2M Urea as solvent

Concentration (µg/ml)	Intra-day	Inter-day
10	0.1412	0.1223
10	0.1421	0.1221
10	0.1432	0.1241
10	0.1423	0.1231
10	0.1443	0.1243
10	0.1451	0.1251
Average	0.1430	0.1245
Standard deviation	0.0014	0.0124
%RSD	1.1423	1.8648

Accuracy (Recovery Study): Accuracy was performed by the recovery in percent. The recovery performed at various extents like 50, 100 and 150% of Fluconazole standard concentration. Three samples were prepared for each recovery level. The analysis of the solution was performed and the recovery in percent was calculated from the calibration curve. The percent recovery for Fluconazole was 98.15 and RSD was 0.0071 using Methanol and by using 2M urea 99.07 ad RSD 1.1423 which is less than 2, which shows that the method has good reproducibility.

Detection Limit and Quantification Limit: (LOD & LOQ) Limit of detection is the minimal amount of analyte which can be detected and quantification limit is the lowest possible concentration that can be quantified the values of detection limit and quantification limit were found to be 0.0965µg/ml & 0.02926µg/ml respectively using methanol and by using 2M urea 0.07531 µg/ml and 0.03412 µg/ml.

Specificity: Specificity is a property of any method to correctly measure the response of analyte in the presence of all other ingredients. These results were applied against the standard Fluconazole and tablet formulations. Excipients of tablet have no any interference with analyte,

which shows that the method has good specificity.

Ruggedness

Table 5: Table showing ruggedness data(Analyst-1) using Methanol as solvent

S.No.	Analyst	Result	Mean	%Assay	%RSD
1	Analyst-1	0.2543	0.2528	98.62	0.0061
2	Analyst-2	0.2815	0.2881	99.51	0.0223

Table 6: Table showing ruggedness data(Analyst-1) using 2M urea

S.No	Analyst	Result	Mean	%Assay	%RSD
1	Analyst-1	0.3141	0.3123	98.34	0.0811
2	Analyst-2	0.3121	0.3132	99.91	0.0231

Conclusion

A suitable Ultra Violet Spectroscopic method was designed and validation was performed as per ICH guidelines for the estimation of Fluconazole in dosage formulations. It was shown above that, the proposed method was linear, selective, specific and cost effective proving the dependability of the method. Hence, the proposed method was successfully applied to routine analysis of Fluconazole in various dosage formulations.

Conflict of Interest

Authors declare no conflict if interest

Acknowledgement

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