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DEVELOPMENT AND VALIDATION OF AREA UNDER CURVE METHOD FOR THE ESTIMATION OF EFINACONAZOLE IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS

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Abstract: A simple analytical method has been developed for the quantification of Efinaconazole by UV spectrophotometer. In the present work area under curve (AUC) method has been used. The stock solution was prepared by taking 0.1 M HCl as a solvent. In the AUC technique, Efinaconazole has maximum absorbance at 261nm. Hence the area of Efinaconazole was taken in the detection range of 251-271 nm (± 10 nm). Calibration curve was linear over the range of 100-500 µg/ml and with mean recovery of 100.02%. The developed technique was validated according to ICH norms. Thus the proposed method can be successfully applied for the assessment of Efinaconazole in routine analysis.

Index Terms **- Area under curve, Efinaconazole, Spectrophotometric, Validation, ICH guidelines**

I. INTRODUCTION

Efinaconazole is a first triazole antifungal prescribed for topical toenail onycho-mycosis treatment in pediatrics. Efinaconazole is designated chemically as (2R, 3R)-2-(2, 4-Difluorophenyl)-3-(4-methylene-1-piperidinyl)-1-(1H-1, 2, 4-triazol-1-yl)-2-butanol with molecular formula $C_{18}H_{22}F_2N_4O$[1]. It blocks the ergosterol anabolic enzyme fungal lanosterol 14-demethylase. The accumulation of 14α-methyl sterols and lack of ergosterol in the cell wall is liable for the fungi static and fungicidal activity of Efinaconazole. It is shown in vitro to be subsequently adsorbed to keratin. Because of EFZ's low keratin binding, the availability of free drug to the nail infected area will be increased[2, 3].

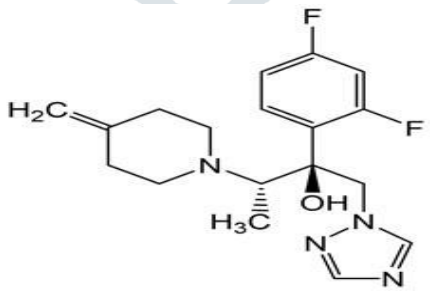

Fig:1. Chemical Structure of Efinaconazole.

Literature survey discloses that Efinaconazole was assayed by LC/MS[4], Liquid chromatographic technique in ex-vivo human nail permeation study samples[5] and in anti mycotic activity[6]. Likewise very few approaches has been reported for the evaluation of Efinaconazole by HPLC but till date no UV spectrophotometric method was reported for the determination of Efinaconazole. In this study, attempt has been made to develop and validate fundamental and economical UV technique for estimation of Efinaconazole.

II. EXPERIMENTAL

2.1 Reagents and Chemicals:

Efinaconazole was procured as a free sample from Mythri drugs private ltd. Telangana. Efinaconazole topical preparation was prepared in-house. Analytical-grade reagents were used for experimental work.

2.2 Instrumentation:

The instrument used was Shimadzu model 1800 UV-Visible twin beam spectrum analyser with a spectral band width of 1 ± 0.2 nm, wavelength accuracy of ± 0.3 nm and a set of quartz cuvettes having 1 cm path length was used.

III. MATERIALS AND METHODS

3.1 Standard stock solution preparation of Efinaconazole:

Efinaconazole stock solution was made by precisely weighing 100 mg of pure sample into a 100 ml volumetric flask and liquefy it in 0.1 M HCl and the volume was made up by using same solvent to get a concentration of 1000 microgram per ml. From this primary stock, further dilutions were formed to attain 100, 200, 300, 400 and 500 µg/ml using 0.1 M HCl solution.

3.2 Preparation of sample solution of Efinaconazole:

An accurate amount of 100 mg of Efinaconazole was weighed and taken in volumetric flask. 30 ml of 0.1 M HCl was surplused and mixed thoroughly and the end volume was adjusted to hundred ml by adding up the same solvent to get 1mg/ml solution. The mixture was then sonicated for 10 minutes. From this primary stock solution, further dilutions were prepared to attain 100 to 500 µg/ml using 0.1 M HCl solution.

IV. AREA UNDER CURVE

This method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths such as λ1 and λ2 representing start and end point of curve region. The area under curve between the two wavelengths (λ1 and λ2) was calculated using UV probe software. In this study Efinaconazole unveiled extreme absorbance at 261 nm hence, area is integrated between detecting spectral range from 251 to 271 nm[7, 8].

V. ANALYTICAL METHOD VALIDATION

The method validation parameters were used to validate the method[9].

5.1 Linearity:

This method obeys the Beer-Lambert's law in the linearity range of 100-500 µg/ml. (Fig. 2-6 and Table No.1). Calibration curve was furnished in Fig. 7.

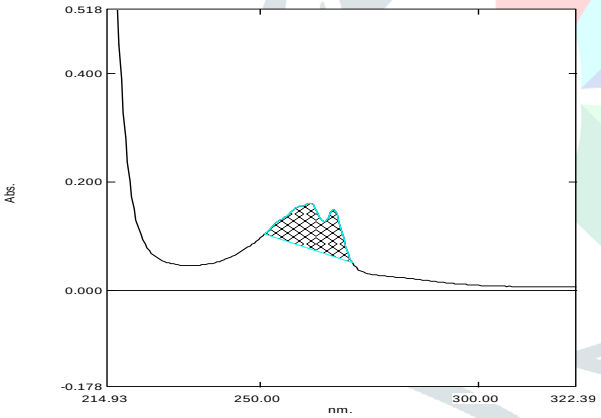

Fig: 2. AUC spectrum of Efinaconazole in 100 µg/mL solution.

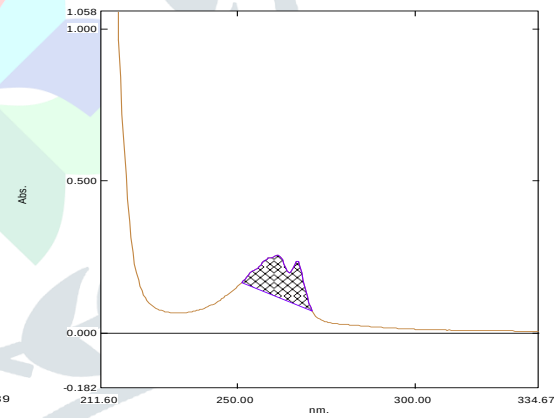

Fig: 3. AUC spectrum of Efinaconazole in 200 µg/mL solution.

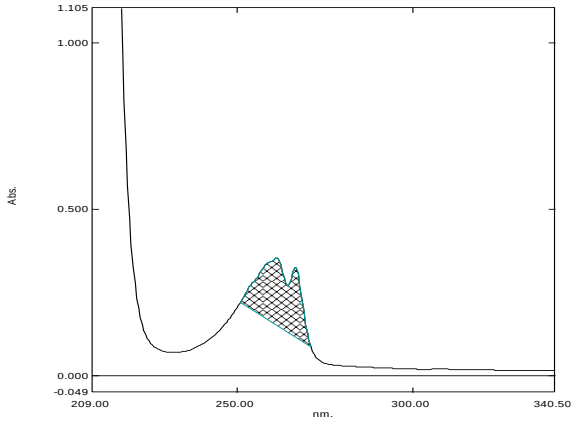

Fig: 4. AUC spectrum of Efinaconazole in 300 µg/mL solution

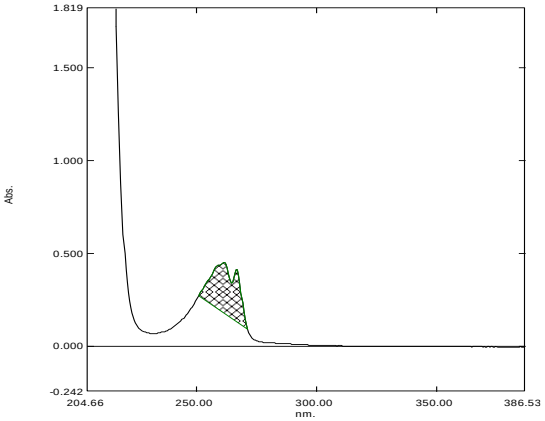

Fig: 5. AUC spectrum of Efinaconazole in 400 µg/mL solution.

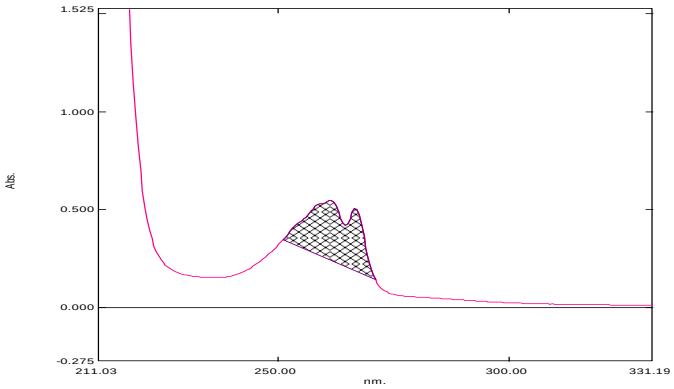

Fig:6. AUC spectrum of Efinaconazole in 500 µg/mL solution.

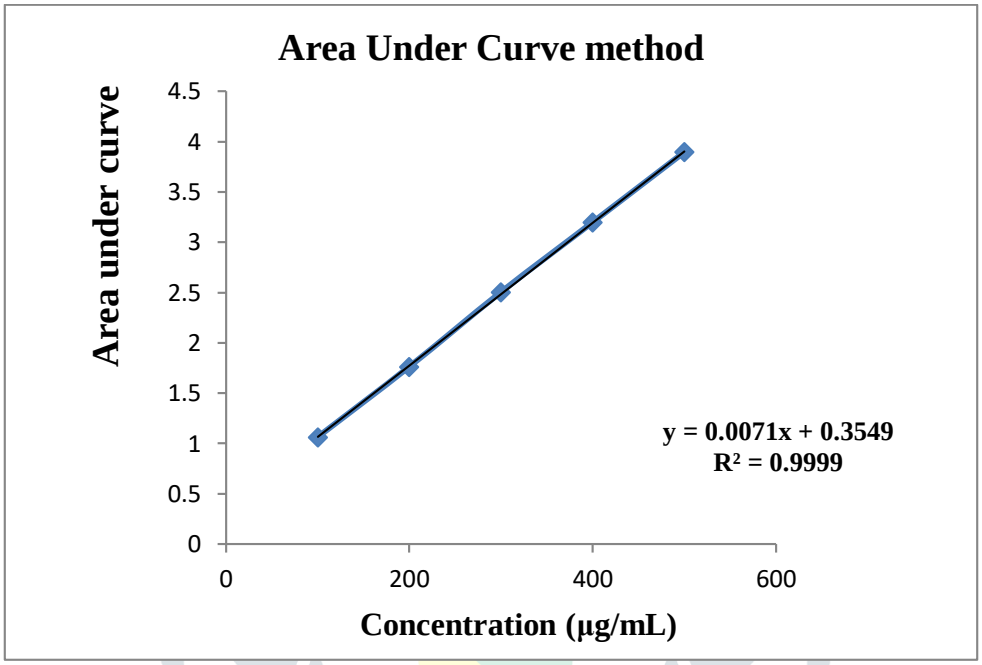

Fig: 7. Calibration curve of Efinaconazole in the wavelength range of 251-271 nm for Area under curve method.

Table. 1: Result of calibration curve for Efinaconazole at 251-271 nm by Area under curve method.

Concentration (µg/mL)	Area Mean ± Std. Deviation(n=6)	%RSD
100	1.0626±0.0034	0.3186
200	1.7618±0.0044	0.2523
300	2.5028±0.0037	0.1504
400	3.1993±0.0019	0.0615
500	3.8945±0.0028	0.0739

5.2 Accuracy:

Recovery studies are executed by standard addition technique (Table No.2, 3). It is demonstrated as the percentage of analyte recovery, standard deviation and RSD. The Statistical validation data for accuracy determination is shown in Table No.4.

Table 2: Accuracy of Efinaconazole.

Level of % recovery	Amount taken from formulation (µg/mL)	Amount of standard drug added (µg/mL)	Total amount recovered (µg/mL)	% Recovery
80%	300	240	538.7	99.75
	300	240	541	100.18
	300	240	541.8	100.33
100%	300	300	600	100
	300	300	601.2	100.2
	300	300	599.1	99.85
120%	300	360	659	99.84
	300	360	658.7	99.8
	300	360	661	100.15

Table 3: Assay results of Formulation.

SI. No.	Amount present (mg/mL)	Amount obtained (mg/mL)	Amount obtained (%)
1	800	798.8	99.85
2	800	803.1	100.38
3	800	799.3	99.91
4	800	802.6	100.3
5	800	798	99.75
6	800	797.9	99.73

Table 4: Statistical validation data for accuracy determination.

Level of % recovery	Mean *	Standard Deviation*	Co-efficient of Variation*	Standard Error*
80%	100.08	0.3011	0.0030	0.1228
100%	100.02	0.1755	0.0017	0.0716
120%	99.8	0.1915	0.0019	0.0782

***n=3**

5.3 Precision:

There are two forms of precision namely interday and intraday. Intraday precision is the data obtained by RSD calculation within a day (Table No.5), Interday precision is the data obtained by RSD calculation on period of days. (Table No.6) and statistical validation data for precision is furnished in Table No.7.

Table 5: Intra-day precision of Efinaconazole.

SI. No.	Amount present (µg/mL)	Amount obtained (µg/mL)	Amount obtained (%)
1	400	400.9	100.22
2	400	398.7	99.67
3	400	402.02	100.50
4	400	398.6	99.65
5	400	404.2	101.05
6	400	403	100.75

PTO

Table 6: Inter-day precision of Efinaconazole.

Amount present in (µg/mL)	Amount obtained (µg/mL)	% Obtained
Day 1		
400	400.60	100.15
400	399.2	99.8
400	400.5	100.12
400	401.1	100.27
400	400.77	100.19
400	399.7	99.92
Day 2		
400	399.2	99.8
400	400.9	100.22
400	402	100.5
400	403.02	100.75
400	398.8	99.7
400	396	99
Day 3		
400	404	101
400	402.8	100.7
400	398	99.5
400	397.4	99.35
400	401.9	100.47
400	396.4	99.1

Table 7: Statistical validation data for precision

Components	Precision	Mean*	Standard Deviation*	Co-efficient of Variation*	Standard Error*
Efinaconazole	Intra day	100.22	0.5711	0.0056	0.2331
	Inter day	100.03	0.5615	0.0056	0.2292

***n = 3**

5.4 Limit of Detection (LOD) and Quantification Limit (LOQ):

The threshold of detection and quantification of the drug was calculated with the standard deviation and slope.

LOD= 3.3* SD/inclination

LOQ= 10* SD/ inclination

*SD= Standard deviation

*Inclination can also be called as slope of the calibration curve.

VI. RESULTS AND DISCUSSION

AUC (Area under Curve) spectra for Efinaconazole was noted in the wavelength of 251 -271 nm. The UV-visible spectroscopic method for the Efinaconazole by area under curve was found to be simple and affordable. The concentrations from 100-500 µg/ml were found to be linear. The regression equations of resulting curves were y = 0.0071x + 0.3549 with correlation coefficient of 0.9999. The values of standard deviation were adequate and the recovery experiments are performed, its recoveries are very much nearer to 100%. The percentage recovery was noted in the range of 99.8 % – 100.08 % which specifies accuracy. The inter-day and intra-day exactness values for precision were found to be 0.5698 and 0.5613 respectively thus the technique is stated as precise. The values of detection along with quantification threshold were 11.1746 µg/ml and 33.8624 µg/ml respectively. The result of the analysis for pharmaceutical preparation by the proposed technique was consistent with the epithet claim. Hereby, this technique can be employed for regular QC analysis of Efinaconazole in pure form and in its final preparations.
(Table No.8)

Table 8: Statistical data of Efinaconazole at 251-271 nm respectively.

Parameter	AUC method
λ_{max} (nm)	251-271
Linear range (µg/mL)	100-500
Molar absorptivity (liter,mole^{-1}cm$^{-1)}$	0.0083
Slope (m)	0.007
Intercept (c)	0.354
Correlation co-efficient (r^2)	0.999
Range of % RSD	0.3186 – 0.0739
Limit of Detection(µg/mL)	11.1746
Limit of Quantitation(µg/mL)	33.8624

VII. CONCLUSION

AUC method was developed for the evaluation of Efinaconazole in bulk medicate and in its final preparations. The suggested technique is straightforward and genuine; this approach is fit for routine assessment of Efinaconazole in pure drug and in its finished preparations. Detection and Quantification limits were reached which reports that the technique is keen. Elevated recoveries and satisfactory % RSD values affirms precision and exactness. Test result reveals that, this approach can be employed for schedule examination of Efinaconazole in bulk drug and formulation development.

VIII. ACKNOWLEDGEMENT

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