



DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF OXICONAZOLE IN BULK AND FORMULATION

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ABSTRACT:

UV spectrophotometric method was developed and validated for quantitative estimation of Oxiconazole. Oxiconazole is soluble in acetonitrile, methanol and mixture of methanol and water in ratio of 30:70. Oxiconazole was dissolved in the mixture of methanol and water (30:70) and the resulting solution was scanned in UV range (200-400nm). The λ_{\max} was found to be 215nm. The concentration range for analysis was 5.5-7.5 $\mu\text{g/ml}$. The developed method was validated for linearity, accuracy, precision, robustness, LOD and LOQ. Linearity was obtained in the range of 5.5-7.5 $\mu\text{g/ml}$ and the regression coefficient was found to be 0.9943. LOD and LOQ were found to be $\mu\text{g/ml}$ and $\mu\text{g/ml}$, respectively. The method was reproducible with accuracy. Hence, can be used for routine analysis of Oxiconazole.

KEYWORDS:

Oxiconazole, methanol, UV Spectrophotometer, Method development and Validation

INTRODUCTION:

Oxiconazole is topical antifungal agent used in the treatment of tinea corporis, tinea cruris and tinea pedis.^{[1],[2]} It is available in market in form of cream. Its antifungal activity is primarily due to inhibition of ergosterol biosynthesis. Ergosterol is a vital component for cell membrane integrity. Molecular formula of Oxiconazole is $\text{C}_{18}\text{H}_{13}\text{Cl}_4\text{N}_3\text{O}$ and its molecular weight is 429.12g/mol. IUPAC name of Oxiconazole is (z)-1-(2,4-dichlorophenyl)-N-[(2,4-dichlorophenyl)methoxy]-2-imidazol-1-ylethanamine. Oxiconazole is practically insoluble in water and freely soluble in methanol^{[3],[4],[5]}.

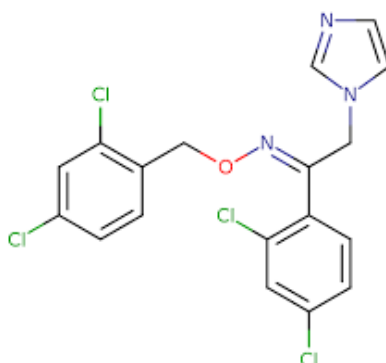


Figure 1: Chemical structure of Oxiconazole

In the literature survey it was found that no UV spectrophotometric analytical method was reported for the analysis of Oxiconazole. Although validated methods for estimation of Oxiconazole by HPLC were reported⁶. This research work introduces UV spectrophotometric method for estimation of Oxiconazole.

MATERIALS AND METHODS:

Chemicals and reagents:

Oxiconazole was obtained as free gift sample from Harman Finochem Pvt. Ltd. Methanol and water were used of HPLC grade. ZodermTM cream was purchased from local market.

Instruments:

Analytical balance (Aczet CY224C) and UV-Visible double beam spectrophotometer (Labman LMSP-UV1900) instruments were used.

Method:

Preparation of standard stock solution:

Accurately weighed 10mg of Oxiconazole was transferred in 10ml volumetric flask. Sufficient quantity of mixture of methanol :water (30:70) was added to dissolve the Oxiconazole. The volume was made upto mark using same solvent (Conc. Of Oxiconazole: 1000µg/ml).

1ml of above solution was transferred into 10ml volumetric flask and volume was made upto mark using mixture of methanol:water (30:70) (Conc. Of Oxiconazole: 100µg/ml)

Working Standard solution:

0.75ml of above solution was transferred into 10ml volumetric flask. Volume was made upto mark using methanol:water (30:70) as solvent. (Conc. Of Oxiconazole: 7.5µg/ml).

Preparation of sample solution:

Transfer the cream (ZodermTM) equivalent to 10mg of Oxiconazole into 10ml volumetric flask. Sufficient quantity of mixture of methanol:water (30:70) was added to dissolve the Oxiconazole. The volume was made upto mark using the same solvent. 1ml of above solution was transferred into 10ml volumetric flask and volume was made upto mark using mixture of methanol:water (30:70). 0.75ml of above solution was transferred into 10ml volumetric flask. Volume was made upto mark using methanol:water (30:70) as solvent. (Conc. Of Oxiconazole: 7.5µg/ml).

1.Method Validation:

The developed method was validated as per ICH guidelines.^{[7],[8],[9]} The parameters assessed were specificity, linearity, range, accuracy, precision (repeatability), system suitability, LOD & LOQ.

2.Linearity:

0.55, 0.6, 0.65, 0.7 & 0.75 ml of standard stock solution (Conc. of Oxiconazole=100µg/ml) were transferred to 10ml volumetric flask separately & the volume was made up to the mark with methanol:water (30:70) to obtain concentration of 5.5, 6, 6.5, 7 & 7.5µg/ml of Oxiconazole. The solutions were prepared and analyzed at wavelength 215nm and absorbances were noted. Calibration curve of Oxiconazole was constructed by plotting the absorbance v/s conc. of Oxiconazole. The correlation coefficient (r^2) of least square linear regression for Oxiconazole was calculated.

3.Range:

The range of analytical method was decided for Oxiconazole (5.5-7.5µg/ml).

4.Accuracy:

The accuracy was determined by calculating % recovery of Oxiconazole. It was carried out by adding known amounts of analyte corresponding to three concentration levels 80, 100& 120% & results were expressed as % recovery.

5.Precision:

The precision of analytical method was studied by performing repeatability. Repeatability studies were carried out by estimating responses of working standard solution (Conc. of Oxiconazole: 7.5µg/ml) for 5 times. The results were reported in terms of percentage relative standard deviation (% RSD).

Robustness:

By deliberate change in wavelength that is 213nm and 217nm of concentration 7.5µg/ml of Oxiconazole was analyzed, in the same environmental condition.

Limit of Detection (LOD):

LOD is the lowest amount of analyte in sample that can be easily detected but not necessarily quantified. LOD was calculated by following formula.

$$\text{LOD} = 3.3 \cdot \sigma / S$$

Whereas, σ = Standard deviation and S= Slope of regression coefficient.

Limit of Quantification (LOQ):

LOQ is the lowest amount of analyte in sample that can be easily detected and quantified with suitable precision and accuracy. LOD was calculated by following formula.

$$\text{LOD} = 10 \cdot \sigma / S$$

Whereas, σ = Standard deviation and S= Slope of regression coefficient.

Assay:

Assay of cream (Sample solution) was also calculated.

RESULTS AND DISCUSSION:

The absorption spectrum shows λ_{max} of Oxiconazole at 215nm.

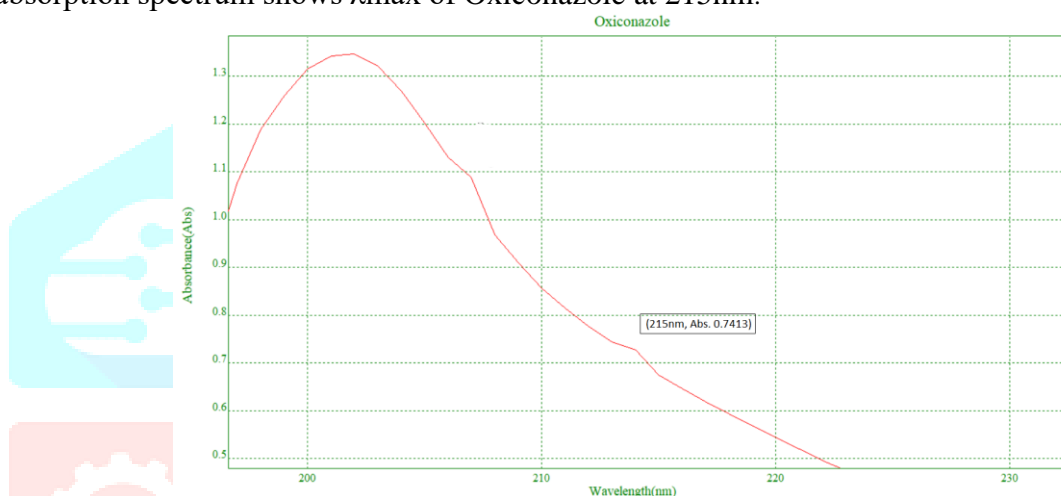


Figure 1: Absorption spectrum of Oxiconazole

Method Validation:

1.Lineariry:

The absorbance is proportional to the concentration and linear in the range of 5.5-7.5 $\mu\text{g/ml}$ (Tab: 1).The value of r^2 was 0.9943 which is well within acceptance limit ($r^2 < 1$).

Table 1: Linearity of Oxiconazole

Concentration ($\mu\text{g/ml}$)	Absorbance
5.5	0.4051
6	0.5038
6.5	0.5595
7	0.6523
7.5	0.7413

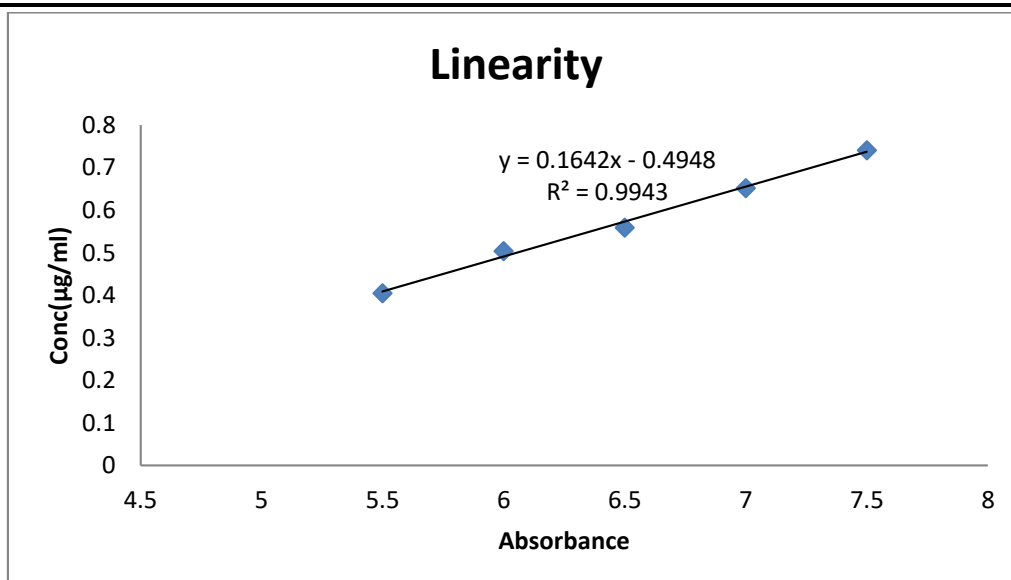


Figure 2: Linearity curve of Oxiconazole

2.Range:

The range is from 84-115% of test concentration. (Oxiconazole: 5.5-7.5µg/ml)

3.Accuracy:

The percentage recoveries of the results indicate that the recoveries are well within acceptance range (RSD<2), therefore method is accurate.

Standard absorbance: 0.7413

Table 2: Accuracy Results of Oxiconazole

% Level	Reps	Spiked conc. (µg/ml)	Absorbance	Amount Recovered (µg/ml)	% Recovery	Average	Std. deviation	% RSD
80	1	6	0.601	6.08	101.34	101.51	0.23	0.23
	2		0.602	6.09	101.51			
	3		0.603	6.10	101.67			
100	1	7.5	0.7413	7.5	100	100.03	0.07	0.07
	2		0.7413	7.5	100			
	3		0.7421	7.50	100.1			
120	1	9	0.901	9.11	101.28	101.47	0.07	0.07
	2		0.905	9.15	101.73			
	3		0.902	9.12	101.39			

4.Precision :

The % RSD < 2 values obtained shows that method developed is precise. Absorbance of standard solution were recorded (Conc. of Oxiconazole=7.5µg/ml)

Table 3: Precision Results of Oxiconazole

Reps	Absorbance
1	0.7413
2	0.742
3	0.7413
4	0.7411
5	0.742
Average	0.74154
STDEV	0.000494975
RSD	0.06

5. Robustness:

Deliberate changes were made in the wavelength keeping other operational parameters same and effect on the results were observed. (Table 4)

Table 4: Result of Robustness of Oxiconazole

Maximum wavelength (nm)	213	217
Absorbance	0.721	0.765
	0.723	0.763
	0.720	0.766
Average	0.722	0.764
STDEV	0.000707	0.000707
% RSD	0.097937	0.092473

6. LOD:

LOD of Oxiconazole was found to be 1.156 µg/ml.

7. LOQ:

LOQ of Oxiconazole was found to be 3.504 µg/ml.

Assay:

In this the amount of drug present in the marketed formulation that is Zoderm™ cream was calculated. It was found to be 99.78% of labeled claim. (Absorbance: 0.7397).

Summary of Validation Parameters:**Table 5: Summary of Validation Parameters**

Parameters	Values
Maximum wavelength (nm)	215
Range (µg/ml)	5.5-7.5
Regression equation	$y = 0.164x - 0.494$
Slope	0.164
Intercept	0.494
Regression coefficient (r^2)	0.994
Repeatability(n=5) % RSD	0.0
LOD (µg/ml)	1.15
LOQ (µg/ml)	3.5

CONCLUSION:

This research work was focused to develop a UV-spectrophotometric method for Oxiconazole in bulk and in formulation. The developed method was validated as per ICH guidelines in which mixture of methanol and water in ratio of 30:70 was used as a solvent. The results showed that the method is accurate, reproducible, reliable, robust and economic. Hence, this method can be used for routine analysis of Oxiconazole in bulk and in formulation.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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