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Original Research Article

UV spectrophotometric method development and validation for estimation of ketoconazole in bulk and pharmaceutical dosage form

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ABSTRACT

Purpose: For the determination of ketoconazole in bulk and pharmaceutical dosage form in accordance with ICH guidelines, a new, economical, precise, sensitive, linear, accurate, and quick UV-Spectrophotometric approach has been developed in Methylene chloride.
Materials and Methods: The UV-visible spectrum of Ketoconazole was examined to determine its maximum absorption wavelength that is λ max at 255.2 nm. Linearity, accuracy, precision and robustness tested for this procedure.
Results: Ketoconazole was found to have a maximum absorbance at 255.2 nm. The regression coefficient for the concentration range of 5–25 μg/ml. Ketoconazole. LOD and LOQ were determined to be 0.0225 and 0.75 μg/ml, respectively. The procedure was successfully used on ketoconazole in commercial formulation, and the outcomes were in good agreement with label claims.
Conclusion: Depending on the results, the given method can be successfully applied of Ketoconazole in bulk and Pharmaceutical dosage form.

1. Introduction

A broad-spectrum antifungal drug called ketoconazole is used to treat or stop fungal infections. It is a white crystalline powder that is an imidazole derivative. It is miscible in strong bases and poorly soluble in water.[1] Since ketoconazole has a high level of permeability and insufficient solubility in aqueous environments, it is classified as a class II medication under the Biopharmaceutical Classification System (BCS). It is used to treat or prevent fungal infections, including those of the skin, nails, scalp, and GI tract as well as thrush and GI infections. There are formulations of ketoconazole for oral pills, cream, and dandruff shampoo.[2]

Cis-1-acetyl- 4 -[4 -[2- (2, 4-dichlorophenyl)-2-(1H-imidazole-1-ylmethyl)-1,3-dioxolon-4-yl] methoxy piperazine, often known as Ketoconazole (KC) and the Structural formula is shown Figure 1.

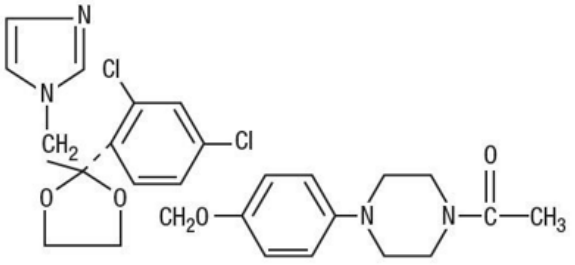

Fig. 1: Chemical structure of Ketoconazole

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An azole derivative known as ketoconazole works by inhibiting sterol 14-demethylase, a microsomal cytochrome P450 dependent enzyme system necessary for the organisation of fungal cell membranes.[3] Ketoconazole affects the function of membrane-bound enzymes and fungal cell membranes, and it prevents the conversion of lanosterol into ergosterol. By increasing membrane permeability and allowing for the release of small ions, amino acids, and proteins from the fungus, ketoconazole causes cell death. Ketoconazole has a wide range of antifungal action. The goal of this research is to create an innovative, quick, accurate, reproducible, and time-saving technique and validate it in accordance with the International Council for Harmonization guidelines.[4]

2. Materials and Methods

2.1. Materials

Ketoconazole was a gift sample obtained from Aarti Drugs Limited, Mumbai. Ketoconazole (NIZORAL 200) tablets were purchased from a local pharmacy. All of the other chemicals and reagents used were of analytical grade and obtained from Sigma Chemicals in Mumbai.

2.2. Instruments

For all absorbance measurements, a UV visible double beam spectrometer [Systronics 2201] and Shimadzu 1800-UV spectrophotometer with 1cm quartz cuvettes were used. All weights were taken on analytical balance (Shimadzu AY220).

2.3. Method development

2.3.1. Selection of solvent

The solubility of ketoconazole in methylene chloride was studied and the UV spectra of the drug were recorded. The drug's absorbance value was higher at its maximum when methylene chloride served as a solvent. Methylene chloride was decided upon as a solvent for more study due to its reduced price.

2.3.2. Preparation of standard stock solution

100 mg Ketoconazole was accurately weighed and dissolved in 100 ml Methylene chloride to prepare a solution of 1000 μg/ml concentration. Pipette out 10ml from the previous stock solution and dilute to 100 ml to prepare a solution of 100 μg/ml concentration. Further, 0.5ml of solution was diluted to 10ml using Methylene chloride to obtain 5 μg/ml working standard solutions. All determinations were conducted in triplicate.[5–9]

2.3.3. Preparation of sample stock solution

The contents of 20 tablets were weighed and combined in a mortar and pestle. 10 mg of weighed ketoconazole powder was added to a volumetric flask with 5 ml of Methylene chloride, then make up the volume to 10 ml (Conc.1000μg/ml). Pipette 1 ml of the sample stock solution was transferred to 10 ml volumetric flask and dilute up to the mark with the solvent(Conc.100μg/ml).

2.3.4. Determination of maximum absorption

10 mg/ml Ketoconazole was scanned over a range of 200-400 nm to determine lmax of Ketoconazole using Methylene chloride as blank. Hence the maximum absorption was found to be at 255.2 nm.

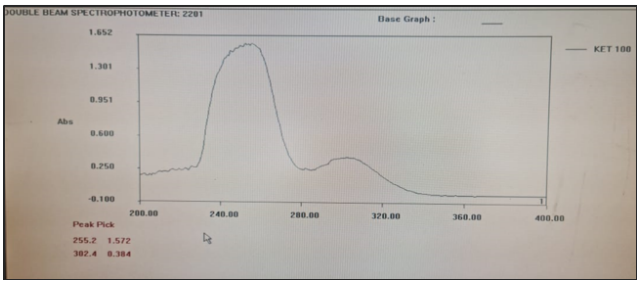

Fig. 2:

3. Method Validation

The process validation of the proposed method was developed as per the guidelines of the International Conference on Harmonization under section Q2 (R1).

4. Result and Discussion

4.1. Linearity

Linearity is defined as an ability of the analytical procedure to obtain test results, which is directly proportional to the concentration of the analyte in the sample. Pipette out 0.5, 1, 1.5, 2, and 2.5ml from the solution of 100 μg/ml and dilute to 10ml with the Methylene chloride. Concentration is 5, 10, 15, 20, 25μg/ml. Taking the absorbance at 255.2 nm and calculate regression coefficient for the range of (5-25 μg/ml).[10–15]

Table 1: Results for linearity

Sr. No.	Concentration (μg/ml)	Absorbance
1.	5	0.128
2.	10	0.228
3.	15	0.335
4.	20	0.421
5.	25	0.531

4.2. Range

Ketoconazole shows linearity in the range of 5-25 μg/ml.

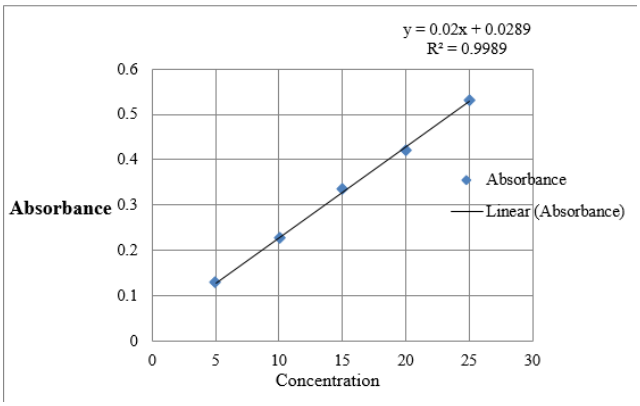

Fig. 3: Calibration curve for Ketoconazole

Table 2: Optimization parameter of pure drug.

Parameter	Method values
Wavelength detection	255.2nm
Beer's range	5-25
Correlation Coefficient	0.99882
Regression coefficient	y = 0.02x + 0.028
Slope	0.02
Intercept	0.028

4.3. Accuracy

The accuracy of the proposed method was estimated by % recovery of the method at the three-level of percentage addition. The % recovery Ketoconazole was found to be 97.5% to 99.25% and was shown in Table 3. The results of the recovery studies undoubtedly demonstrate the accuracy of the proposed method.

Table 3: Parameters for accuracy

Accuracy % Level	Amount Spiked μg/ml	Amount Recovered μg/ml	% recovery with SD
80	8	7.8	97.5
100	10	9.92	99.2
120	12	11.91	99.25

4.3.1. Precision

The closer repeated measurements of the same sample are, the more precise the instrument is. Precision is determined by the coefficient of variation or the standard deviation (spread of data) over the mean. Precision was determined by taking five readings of 20μg/ml concentration intra-day and inter-day. The results were confirmed to be within tolerance, or less than 2% RSD.

The % RSD value for intra-day and inter-day precision is 2% (0.356 & 0.183, respectively), which is within the limit and hence validates the precision parameter.

Table 4: Parameters for intra-day precision

Precision intra-day Concentration (μg/ml)	Absorbance
20	0.421
20	0.422
20	0.421
20	0.423
20	0.425
20	0.422
Mean	0.4223333
SD	0.0015055
%RSD	0.3564827

Table 5: Parameters for Inter- day precision

Precision inter-day Concentration(μg/ml)	Absorbance	
	Day 1	Day 2
20	0.421	0.488
20	0.422	0.487
20	0.421	0.489
20	0.423	0.487
20	0.425	0.488
20	0.422	0.489
Mean	0.422333333	0.488
SD	0.001505545	0.089443
% RSD	0.356482708	0.183284

4.3.2. Limit of detection (LOD)

Limit of detection is defined as the lowest amount of analyte in a sample that can be detected. LOD is based on the standard deviation value from precision and slope of regression coefficient.

Formula for calculating LOD = 3.3*σ/S

Where,

σ= Standard deviation,

S = Slope of regression coefficient

LOD-0.0225 μg/ml

The minimum amount of Ketoconazole to detect was found to be 0.0225 μg/ml. Sensitivity parameter is validated.

4.3.3. Limit of quantification[LOQ]

Limit of quantification is defined as the lowest amount of analyte in the sample that can be quantified. LOQ is calculated by the

Formula; LOQ = 10*σ/S

Where,

S = Slope of regression coefficient

σ = Standard deviation

LOQ-0.75 μg/ml

The minimum amount of Ketoconazole to quantify was found to be 0.75μg/ml Sensitivity parameter is validated.

4.4. Robustness

The robustness of the developed method shows a non-significant influence of the absorption level through the analysis of the ketoconazole solution in Methylene chloride at different wavelengths (± 1 nm). The data of the robustness study were shown in Table 6.

Table 6: Robustness value at concentration of 10 μg/ml.

Robustness Wavelength	Absorbance	Average	SD	% RSD
	1.572			
254nm	1.573	1.572333	0.000577	0.036719
	1.572			
	1.573			
256nm	1.574	1.555667	0.030892	0.036719
	1.52			

5. Conclusion

This newly developed method for using a UV spectrophotometer was deemed to be simple, reliable and selective, providing adequate accuracy, precision with lower detection limits, more precise quantification, and sensitivity. Good recoveries were obtained in all cases, and the consistent agreement with the stated process demonstrated that the proposed method could be used efficiently for Ketoconazole determination.

6. Source of Funding

None.

7. Conflict of Interest

None.

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