

Original Research Article

Spectrophotometric Methods for the Determination of Ketoconazole in Pharmaceutical Dosage Forms

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Abstract

Purpose: To develop simple, sensitive and economical methods for the determination of (KZ) in both pure samples and pharmaceutical formulations.

Methods: The first method (A) was based on the oxidation of the studied drug by a known excess of cerium (IV) as an oxidizing agent and subsequent determination of unreacted oxidant by reacting it with indigo carmine (IN) dye. The second method (B) involved potassium permanganate as oxidant and methylene blue (MB) as dye. Spectrophotometry was used to determine the residual amount of the dyes by measuring the absorbance of the two dyes at 610 and 660 nm, respectively. Comparison was made with the British Pharmacopoeia 1998 method.

Results: Linear calibration graphs were obtained at 4 - 30 and 5 - 35 µg/ml for the two methods at 610 and 660 nm, respectively. The regression equation was $Y = 0.01X + 0.133$, ($R^2 = 0.996$, $RSD = 78\%$ and limit of detection (LOD) of µg/ml for method A. For the second method (B), it was $Y = 0.008X + 0.039$, ($R^2 = 0.995$, $RSD = 0.95\%$ and $LOD = 2.3$ µg/ml. The proposed methods were successfully applied to the determination of KZ in pharmaceutical formulations.

Conclusion: The results demonstrate that the methods are as accurate and reproducible as official methods.

Keywords: Ketoconazole, Spectrophotometry, Cerium, Permanganate, Indigo carmine, Methylene blue.

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INTRODUCTION

Ketoconazole (Fig 1) is chemically known as cis-1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-imidazole-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl] piperazine.

The drug is a highly effective broad spectrum antifungal agent [1]. Different methods have been reported for its determination such as spectrophotometry [2-5], chromatography [6-8] and electrochemistry [9,10]. The oxidation of ketoconazole with cerium (Ce (IV)) has not been reported in literature before, therefore, a

comprehensive investigation on the reaction conditions was found to be necessary.

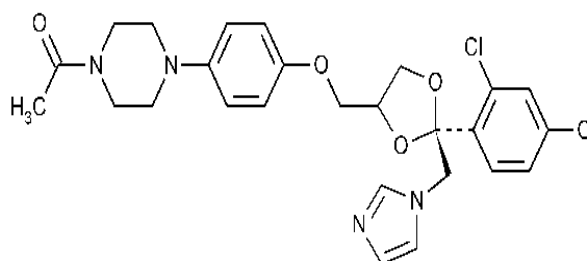


Fig 1: Structure of ketoconazole

Standard chromatographic methods of ketoconazole generic assay require elaborate and sophisticated instrumentation and are of low sampling frequency.

Most of the spectrophotometric methods used for ketoconazole assay are based on either its oxidation or complexation with metal ions. Some of these methods require heating, extraction, long time for maximum color development and lengthy procedure as well as lacking sensitivity, specificity and wide dynamic ranges. The present method is simpler as it is based on indirect measurement of the drug concentration by using oxidation reaction of the drug using excess amount of the oxidant, then the remaining amount of the oxidant decolorizes certain dyes and the residual amount of the dye is determined by measuring its absorbance [11].

EXPERIMENTAL

Apparatus

A double-beam optical system SpectroScan 80D UV-VIS spectrophotometer with spectral bandwidth of 2 nm, wavelength accuracy of ± 0.3 nm (with automatic wavelength correction), wavelength range (190 - 1100 nm), wavelength reproducibility of ± 0.2 nm and a pair of 1-cm matched quartz cells was used to measure absorbance of the resulting solution.

Reagents and solutions

All chemicals used were of analytical reagent grade and the solvents were spectroscopic grade. Double distilled water was used wherever required. Commercial tablets Nizoral® and Funazole® labeled to contain 200 mg ketoconazole per tablet was used.

The stock solution of 250 $\mu\text{g/ml}$ KZ was prepared in 1M H_2SO_4 solution and was used to prepare different standard solutions. An aqueous solution of IN (Aldrich; 200 $\mu\text{g/ml}$) and MB (Merck; 100 $\mu\text{g/ml}$) was prepared by dissolving the appropriate weight of the dye in a very small volume of water and then made up to 100 ml in a calibrated flask.

The stock solutions of dyes were allowed to stand at room temperature for a few weeks without any significant decay. A stock solution of 1.0 $\mu\text{g/ml}$ Ce(IV) (Aldrich) was prepared by dissolving the appropriate weight in 0.5 M H_2SO_4 solution.

This solution was then standardized using sodium oxalate and stored in a dark.

A stock solution of KMnO_4 solution (600 $\mu\text{g/ml}$) was prepared by dissolving appropriate amount in 200 ml of water. The mixture is warmed for 30 min, cooled to room temperature then the final volume was adjusted to 250 ml.

Optimisation of experimental conditions

The optimum conditions for color development in each method were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species for methods A and B. In a series of experiments, the volumes of both dyes were varied using the constant concentrations of both KZ and the selected oxidants, the results revealed that the optimum volumes of both indigo carmine and methylene Blue dyes were 2.5 and 2 ml of the given concentrations respectively. For Methods A and B, the effect of time on the absorbance values were investigated, the obtained results show no appreciable change after 8 min, so it decided to measure the absorbance after 10 min from preparing the mixtures.

Preparation of calibration curves

Method A: Different volumes of standard KZ (250 $\mu\text{g/ml}$) solutions were pipetted into 10 ml volumetric flasks, then 0.5 ml of Ce(IV) (1.0 $\mu\text{g/ml}$) solution was added the mixture was then shaken and kept for 10 min. after that 2.5 ml of IN (200 $\mu\text{g/ml}$) was added, then the solution was made up to the 10.0 ml with distilled water. The absorbance was then measured at 610 nm after 10 min.

Method B: The same method above was performed except that 2 ml KMnO_4 (600 $\mu\text{g/ml}$) and 2.0 ml of MB (100 $\mu\text{g/ml}$) dye solutions were used, the absorbance of the final solutions were measured at 660 nm.

Procedures for drug formulations

An amount of finely ground tablets equivalent to 5.0 mg of KZ was accurately weighed, dissolved in appropriate amount of distilled water and transferred to a 100-ml volumetric flask, the flask was sonicated for about 20 min, finally the volume was made up to the mark. The content was kept aside for 5 min, and filtered using 0.45 μm GHP filter paper. The first (10 ml) portion of the filtrate was discarded and a suitable aliquot was used for the assay as described under general analytical procedure for the proposed

method or using high performance liquid chromatography (HPLC) method.

Statistical analysis

The data were expressed as mean ± SD and analysed statistically using Student t-test with the aid of Microsoft Excel 2003 software. Data were considered significantly different at 95 % confidence limit.

RESULTS

Calibration curves for KZ determination using the proposed methods A and B were constructed by plotting absorbance vs. concentration using the optimized amounts of oxidants and dyes. The intercepts, slopes, and correlation coefficients were calculated using the method of least squares. Beer's law is obeyed over concentration ranges of 5 - 30 µg/ml for (Method A) and 5 - 35 µg/ml for (Method B). The mean molar absorptivity (ϵ), limit of detection (LOD = 3 s/k) and limit of quantitation (LOQ = 10 s/k) were calculated, where s is the standard deviation of replicate determinations in the absence of analyte under the same conditions as sample analysis and k is the slope. The LOD were 1.5 and 2.3 µg/ml using methods A and B respectively, these statistical results are shown in Table 1.

Table 1: Statistical analysis of calibration graphs and analytical data for the determination of ketoconazole using the proposed methods

Parameter	Method A	Method B
Wavelength λ_{max} (nm)	660	610
Regression data		
Slope	0.010	0.008
Intercept	0.133	0.039
Correlation coefficient (r^2)	0.996	0.995
Beer's law limits (µg/ml)	5-30	5-35
Sandell's sensitivity (µg/ml)	0.11	0.13
LOD (µg/ml)	1.5	2.3
LOQ (µg/ml)	4.5	6.9
RSD %	0.78	0.95

Table 2: Comparison between the proposed method and the standard method

Drug	Proposed method		Standard method		t-value
	Amount taken (µg/ml)	Recovery ± RSD %	Amount taken (µg/ml)	Recovery ± RSD %	
Method A	25	97 % ± 8	15	98 % ± 2	0.85
Method B	25	98 % ± 8	50	97 % ± 2	0.56

Tabulated student t-value at 95 % confidence level and 6 degrees of freedom is (2.44)

Application to drug formulation

The suggested method were applied successfully for the determination of ketoconazole in commercial tablets from local market, Statistical comparison of the accuracy and precision of the proposed methods with an HPLC method [12] was performed using student's t-tests at a 95 % confidence level. The t-values did not exceed the theoretical values; there is no significant difference in accuracy or precision between the proposed and the official method as shown in Table 2.

DISCUSSION

Ketoconazole undergoes fast oxidation reaction with strong oxidizing agents. It also shows no absorption band in the visible region which makes it difficult to be determined directly using simple spectrophotometric methods. So we suggest two simple and inexpensive procedures for the determination of KZ in pure and pharmaceutical preparations based on treating KZ solutions with an excess amount of oxidizing agent, then the residual amount of oxidant bleaches certain dye, so that the remaining amount of dye can be determined spectrophotometrically. As a result, a proportional increase in the absorbance for the two dyes is observed with increasing concentration of KZ.

In method A, Ce(IV) in sulfuric acid was used as oxidizing agent and IN was used as a dye; the absorbance was measured at 610 nm. In method B, potassium permanganate was used as oxidizing agent and MB was used as the dye which has a maximum absorption at 660 nm. Preliminary experiments were performed to fix the upper concentrations of the oxidants that could be used to maintain excess amounts. A Ce(IV) concentration of 8.0 µg/ml was found to bleach the color due to 5 µg/ml IN whereas 34.0 µg/ml KMnO₄ was required to destroy the blue color of MB.

For both methods, the absorbance was monitored at 25 °C with time which showed that the oxidation reaction is fast and complete in 5 min, and contact times up to 8 min had no effect on the absorbance of dyes.

CONCLUSION

The developed methods are simple, rapid, and accurate, and also compare well with standard methods.

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