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# Development and Validation of Reverse Phase High Performance Liquid Chromatographic Method for Estimation of Ketoconazole in Tablet Dosage Form

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## ABSTRACT

This research manuscript describes simple, sensitive, accurate, precise and repeatable reverse phase high performance liquid chromatography method for the estimation of Ketoconazole in tablet dosage form. The sample was analyzed by reverse phase ACE 5 C18 column (150 mm × 4.6 mm i.d, 5 µm particle size) as stationary phase; methanol: acidic water [91:9, v/v] pH 3.0 as a mobile phase at a flow rate of 0.85 ml/min. Quantification was achieved with Photo diode array detector at 243.0 nm. The retention time for Ketoconazole was found to be 2.764 min. The linearity was obtained in the concentration range of 5-40 µg/ml for Ketoconazole. The method was successfully applied to tablet because no chromatographic interferences from formulation excipients were found. The method retained its accuracy and precision when the standard addition technique was applied.

**Keywords:** Ketoconazole, RP-HPLC, Method validation.

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# INTRODUCTION

Ketoconazole is a synthetic, imidazole-derived antifungal medication used primarily to treat fungal infections[1] . Chemically it is 1-Acetyl-4-[4-[[(2RS, 4SR)-2-(2, 4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl) 1, 3-dioxolan-4-yl] methoxy] phenyl] piperazine (figure 1)[2] Ketoconazole is a official in Indian pharmacopeia (IP)[3], British pharmacopeia (BP)[4], United states Pharmacopeia (USP)[5], European Pharmacopeia [EP][6]. IP, BP and USP describe HPLC , titration methods for its estimation. EP describe HPLC method for its estimation. Literature survey reveals that HPLC [7-8] methods for estimation of Ketoconazole in the plasma, liver, lung and adrenal of the rat and also in human plasma. Literature survey also reveals HPLC[9-12] methods for estimation of Ketoconazole with other drug combination and UV[13] second derivative method for estimation of Ketoconazole in emulsion also in tablet. In literature survey there is no any UV first derivative and HPLC method for estimation of Ketoconazole alone in tablet dosage form. The present communication describes simple, sensitive, rapid, accurate, precise and cost effective spectrophotometric method based on first order derivative for estimation of Ketoconazole in tablet dosage form.

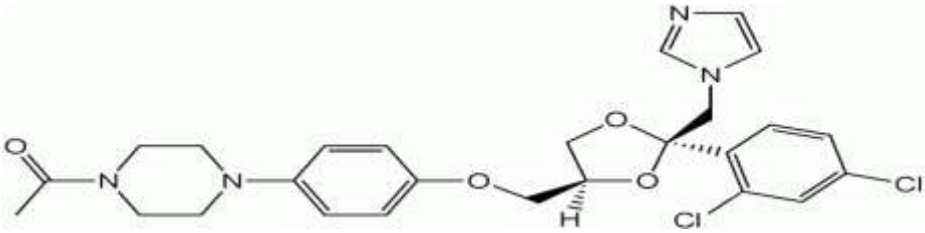


**Figure 1 – Chemical Structure of Ketoconazole**

# MATERIALS AND METHODS

## Apparatus

The chromatography was performed on a Shimadzu (Japan) RP-HPLC instrument (LC-2010$C_{HT}$) equipped with Photo Diode Array (PDA) detector and LC-solution software, ACE $C_{18}$ column (150 mm × 4.6 mm id, 5µm particle size) was used as stationary phase. Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic cleaner (Frontline FS 4, Mumbai, India), Digital pH meter (LI 712 pH analyzer, Elico Ltd., Ahmedabad) were used in the study.

## Reagents and materials

Ketoconazole was kindly supplied as a gift samples from West-coast Pharmaceuticals Ltd, Ahmedabad, Gujarat, India. Ketoconazole tablet was procured from the local pharmacy. Acetonitrile, Methanol, triple distilled water ( Finar Chemicals Ltd., Mumbai, India) used were of HPLC grade. Ortho-phosphoric acid (S.D Fine Chemicals Ltd., Mumbai, India) used were of

AR grade. Nylon 0.45 µm – 47 mm membrane filter (Gelman Laboratory, Mumbai, India) and Whatman filter paper no. 41. (Whatman International Ltd., England) were used in the study.

**Preparation of Acidic Water (pH 3)**

Acidic Water pH 3 was prepared by accurately adding 0.7 mL of Triethyl Amine in 100 mL HPLC grade water and the pH adjusted to 3.0 by diluted ortho-phosphoric acid.

**Preparation of standard stock solutions**

Accurately weighed Ketoconazole (10 mg) and transferred to 100 ml volumetric flask and dissolved in 70 mL methanol. The flask was shaken and volume was made up to mark with methanol to give a solution having concentration 100 µg/mL of ketoconazole.

**Preparation of working standard solutions**

An aliquot of stock solution 1 mL of standard stock solution was transferred in 10mL volumetric flask and diluted to mark with methanol having concentration (10µg/mL).

**Preparation of Sample Solution**

Twenty tablets were weighed and powdered. The powder equivalent to 10 mg of Ketoconazole was transferred to 100 ml volumetric flask. Diluent (Methanol 70 mL) was added and sonicated for 25 min and volume was made up to the mark with methanol. The solution was filtered through Whatman filter paper No. 41. aliquot of 0.1 mL was taken in to a 10 ml volumetric flask and the volume was adjusted up to mark with methanol to get a final concentration (10 µg/mL) of Ketoconazole.

**Chromatographic Condition**

Stationary phase: $C_{18}$ column (150 mm x 4.6 mm id., 5 µm).

Mobile phase: Methanol: Acidic water pH 3.0 [91:9, v/v]

Flow rate: 0.85 mL/min

Injection volume: 20 µL

Temperature: 40 °C

Detection: At 243 nm using PDA detector

**Method development**

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for Ketoconazole was obtained with a mobile phase Methanol: Acidic water pH 3.0 [91:9, v/v] at a flow rate of 0.85 mL/min to get better reproducibility and repeatability. Quantification was carried out at 243 nm based on peak area. Complete resolution of the peaks with clear baseline was obtained (Figure. 6.). System suitability test parameters for Ketoconazole for the proposed method are reported in Table .1

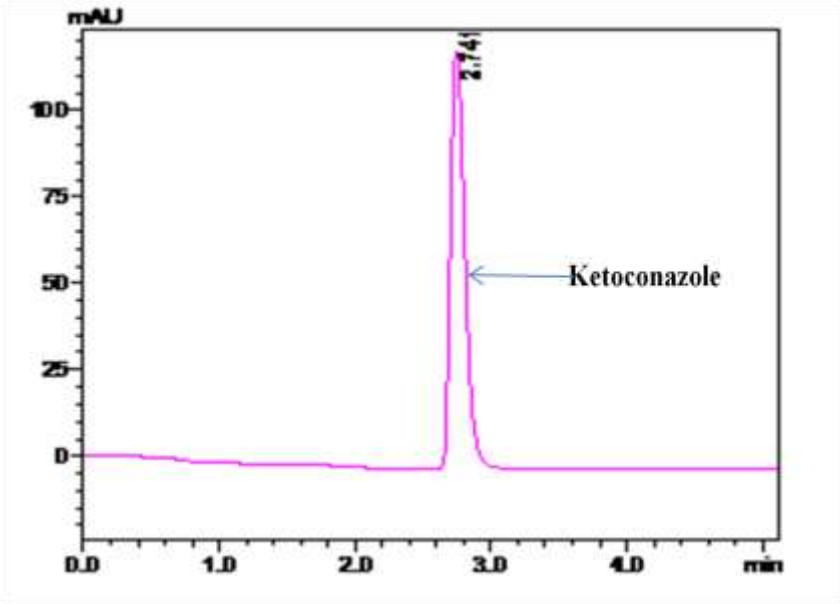


**Figure: 6.Chromatogram of sample solution of Ketoconazole (10 µg/mL) at 243 nm**

**Table 1: System Suitability Parameters of Chromatogram for Ketoconazole**

| Parameters | Ketoconazole ± RSD (n = 6) |
|---|---|
| Retention time (min) | 2.764 ± 0.1461 |
| Tailing factor | 1.459 ± 0.1048 |
| Theoretical plates | 3545 ± 0.4308 |

**Method Validation**

The method was validated in compliance with ICH guidelines.

**Calibration Curve (Linearity)**

Calibration curves were constructed by plotting peak areas Vs concentrations of Ketoconazole and the regression equations were calculated. The calibration curves were plotted over the concentration range 5-40 µg/mL Ketoconazole. Accurately measured mix Standard working solutions of Ketoconazole (0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 mL) were transferred to a series of 10 mL of volumetric flasks and diluted to the mark with mobile phase. Aliquots (20 µL) of each solution were injected under the Chromatographic conditions described above.

**Accuracy (% Recovery)**

The accuracy of the method was determined by calculating recoveries of Ketoconazole by the standard addition method. Known amounts of standard solution of Ketoconazole added at 50 %, 100 % and 150 % levels to pre quantified sample solutions of Ketoconazole.

**Method Precision**

Precision of the method was determined by performing interday variation and intraday variation (% RSD). Intra- day precision (% RSD) was assessed by analyzing standard drug solutions

within the calibration range, three times on the same day. Inter-day precision (% RSD) was assessed by analyzing drug solutions within the calibration range on three different days over a period of 7 days.

**Intermediate Precision (Reproducibility)**

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of sample solutions of Ketoconazole (20, 25, and 30 µg /mL) . The results were reported in terms of relative standard deviation (% RSD).

**Limit of Detection and Limit of Quantification**

LOD and the LOQ of the drug were calculated using the following equations as per International Conference on Harmonization (ICH)[14] guidelines.

LOD = 3.3 × σ/S , LOQ = 10 × σ/S

Where σ = the standard deviation of the response S = Slope of calibration curve.

**ANALYSIS OF KETOCONAZOLE IN TABLET DOSAGE FORM**

The response of the sample solution was measured at 243 nm under the chromatographic condition mentioned above for the quantification of Ketoconazole. The amounts of Ketoconazole present in sample solution were determined by applying values of the peak area to the regression equations of the calibration curve.

## RESULTS AND DISCUSSION

**Linearity**

Linear correlation was obtained between peak area Vs concentrations of Ketoconazole in the concentration range of 5-40 µg/ml. Regression parameters are mentioned in Table and the calibration curves of this drug at 243 nm are shown in Figure 4 .

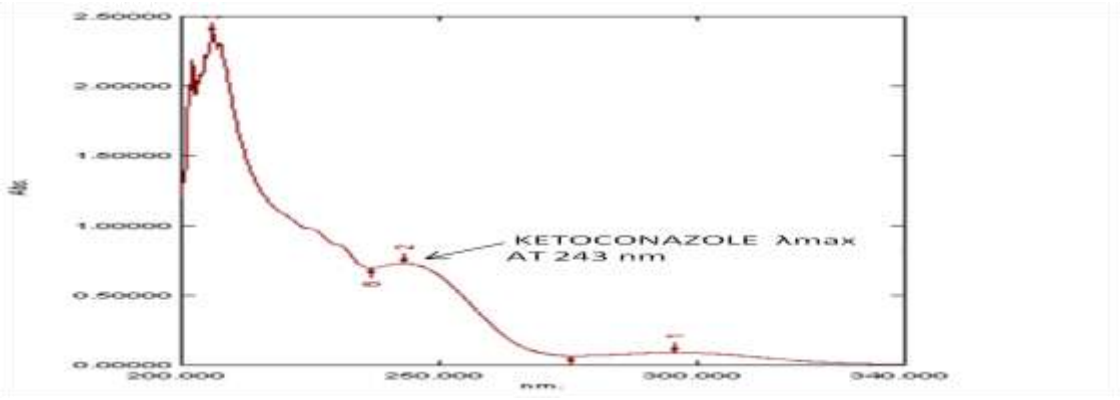


**Figure: 3 UV Spectrum of Ketoconazole at 243 nm**

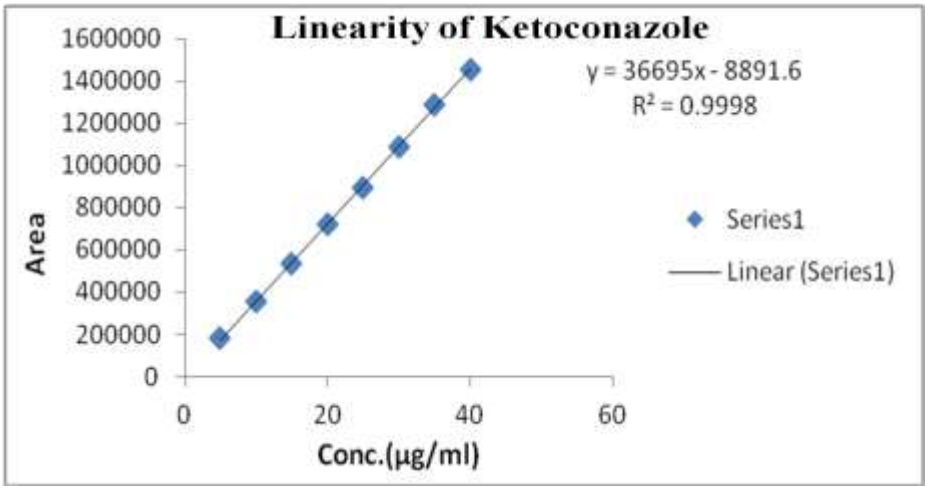


**Figure: 4 Calibration curve of Ketoconazole at 243 nm**

**Method Precision**

The RSD value for Ketoconazole was found to be 1.098 % respectively. The RSD value was found to be < 2%, which indicates that the proposed method is repeatable.

**Intermediate Precision (Reproducibility)**

The low RSD values of interday and intraday for Ketoconazole, respectively, reveal that the proposed method is precise.

**LOD & LOQ**

LOD value for Ketoconazole was found to be 0.056 µg/ml and LOQ value for Ketoconazole was found to be 0.149 µg/ml (Table 4). These data show that the proposed method is sensitive for the determination of Ketoconazole.

**Table 4: Regression Analysis Data and Summary of Validation Parameter for the proposed Method**

| **Parameters** | | **RP-HPLC method Ketoconazole** |
|---|---|---|
| Detection wavelength(nm) | | 243 nm |
| Concentration range (µg/mL) | | 5-40 |
| Regression equation Y= mx + c | | Y = 36695x – 8891.6 |
| Correlation coefficient | | 0.9998 |
| Repeatability (% RSD[a], n = 6) | | 1.098 |
| Precision | Intraday (n=3) | 0.097 – 0.150 |
| (%RSD[a]) | Interday (n=3) | 0.161 – 0.183 |
| LOD[b](µg/mL) | | 0.056 |
| LOQ[c](µg/mL) | | 0.149 |
| % Recovery (Accuracy, n= 3) | | 100.03 ± 0.656 |
| % Assay ± SD[d] (n = 6) | | 99.91 ± 0.58 |

RSD[a] = Relative standard deviation, LOD[b] = Limit of detection, LOQ[c] = Limit of quantification, S. D[d] = Standard deviation

**Accuracy**

The recovery experiment was performed by the standard addition method. The recovery obtained was 100.03 ± 0.656 % for Ketoconazole (Table 2). The low value of standard deviation indicates that the proposed method is accurate. Results of recovery studies are shown in Table 2.

**Table 2: Recovery Data for the proposed method**

| Drug | Level | Amount of sample taken (µg/mL) | Amount of standard spiked (%) | Mean% Recovery ± SD |
|---|---|---|---|---|
| Ketoconazole | I | 10 | 50 % | 101.11 ± 1.10 |
| | II | 10 | 100 % | 99.16 ± 0.330 |
| | III | 10 | 150 % | 99.74 ± 0.537 |
| Mean% Recovery ± SD | | | | 100.03 ± 0.656 |

**Analysis of Ketoconazole in tablet dosage form**

The proposed validated method was successfully applied to determine Ketoconazole in tablet dosage form. The result obtained for Ketoconazole was comparable with the corresponding labelled amounts (figure 5 ) (Table 3)

**Table 3: Analysis of Ketoconazole in tablet by proposed method ( n = 6)**

| Sample no. | Label claim (%) | Amount found (%) | % Label claim |
|---|---|---|---|
| 1 | 200 | 199 | 99.5 |
| 2 | 200 | 201 | 100.5 |
| 3 | 200 | 198 | 99 |
| 4 | 200 | 200 | 100 |
| 5 | 200 | 201 | 100.5 |
| 6 | 200 | 200 | 100 |
| Mean | | 199 | 99.91 |
| SD | | 0.00116 | 0.5845 |
| %RSD | | 0.5829 | 0.5850 |

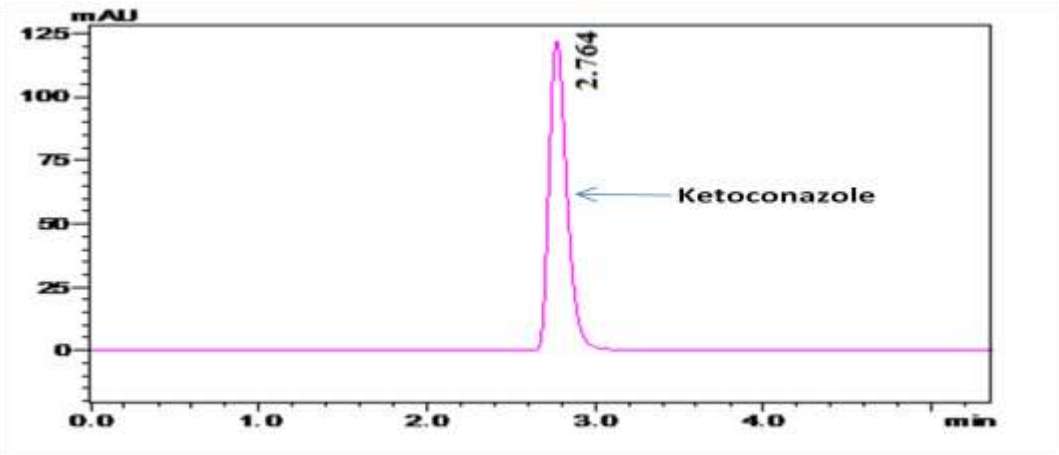


**Figure.: 5 Chromatogram of Standard Solution of Ketoconazole (10 µg/mL) at 243 nm**

## CONCLUSION

A simple, sensitive, repeatable and specific RP-HPLC method has been developed for the estimation of Ketoconazole using a PDA detector. The method was validated for accuracy, precision, linearity, specificity, LOD & LOQ and robustness. In this proposed method the linearity is observed in the concentration range of 5-40 µg/ml for Ketoconazole with co-efficient of correlation, ($R^2$) = 0.9998. The result of the analysis of Ketoconazole tablet by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the Ketoconazole in pharmaceutical dosage form without any interference of excipients.

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