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PHARMAHEALTHSCIENCES

**International Journal of Pharma Research and Health Sciences**

**Available online at www.pharmahealthsciences.net**

## Original Article

# UPLC Method Development and Validation for Terconazole in Active Ingredient

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| A R T I C L E   I N F O | A B S T R A C T |
|---|---|

The objective of the study was to develop UPLC method for the determination of purity of Terconazole in API and its validation. Ultra performance liquid chromatoghaphy is a better technique than HPLC in terms of performance and speed, so it was selected. The method was developed using Acetonitrile and Sodium dihydrogen ortho phosphate and Acquity BEH C18 (50×2.1mm, 1.7μ) as a stationary phase at a flow rate of 0.25ml/min. Validation was done by linearity, precision, and robustness studies. The precision was found to be within the limits. The linearity studies indicated the drug obeys Beer's law and revealed the specified range of linearity for drug was between 80μg/ml and 120μg/ml. The robustness was observed from the insignificant variation in the analysis by changes in flow rate, mobile phase ratio, wavelength, column oven temperature and pH. Forced Degradation study revealed the drug degraded initially by the fluence of acid, alkali, and peroxide. Solution stability study showed the drug was not stable for more than 2 h at 25°C but stable at 5°C.

**Key words:** Terconazole , Antifungal activity, Acquity BEH C 18, method development and validation

## 1. INTRODUCTION

Quantitative chemical analysis [1, 2] is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Drug analysis is the base for the determination of the product. Every year numbers of drugs are introduced into the market. Also quality is important in every product or service, but it is of more importance in

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medicine as it involves life. The results of typical quantitative analysis can be computed from two measurements. One is the mass or volume of sample to be analyzed and second is the measurement of some quantity that is proportional to the amount of analyte in that sample and normally completes the analysis.

In analytical chemistry [3], it is of prime importance to gain information about the qualitative and quantitative composition of substances and chemical species, that is, to find out what a substance is composed of and exactly how much.

In qualitative analysis, information regarding the presence or absence of one or more compounds of the same suffices. In quantitative analysis, quantity of the substance can be measured. In instrumental analysis, a physical property of a substance is measured to determine its chemical composition. Modern analytical chemistry is classified based on different principles, but most frequently they are divided into two large classes. [4, 5]

## 2. MATERIALS AND METHOD

**Method Development**

**UPLC Instrumentation & Conditions:**

The UPLC system employed was **HITACHI L2130** with D Elite 2000 Software with Isocratic with UV-Visible Detector (L-2400), 25 mg of Terconazole standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.2 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

**The standard & sample stock solutions** were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Terconazole, so that the same wave number can be utilized in UPLC UV detector for estimating the

Terconazole. While scanning the Terconazole solution we observed the maxima at 236 nm.

## MOBILE PHASE PREPARATION

The mobile phase used in this analysis consists of a mixture of Buffer (triethylamine & pH adjusted to 2.5 with glacial acetic acid) and Acetronitrile in a ratio of 75 : 25. 250 ml of this buffer solution was added and properly mixed with 750 ml of acetronitrile and a homogenous solution is achieved. This mobile phase was filled and sonicated for 15 minutes before using in the experiment

**Sample & Standard Preparation For The Analysis**
25 mg of Terconazole standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase.

Further dilution was done by transferring 0.2 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase. [6-8]

The sample was analysed by UPLC by using the above method and a very nicely resolved peak has been obtained at a Retention Time of about 4.39 min. The respective chromatogram is attached in the following page.
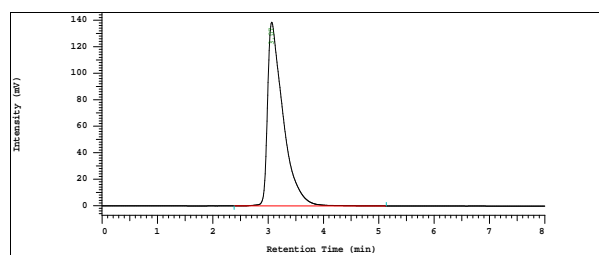


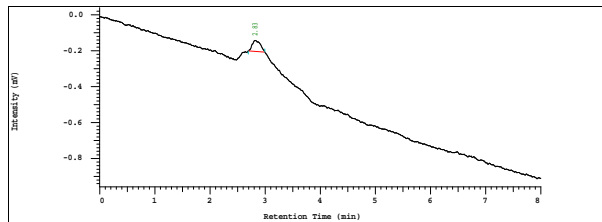**Fig 1: UPLC spectrum of Terconazole (20 ppm) in optimized conditions (RT 3.07 min.)**
**Peak results**

| Sr no | Name | RT | Area |
|-------|------|-----|------|
| 1 | Terconazole | 3.07 | 3046971 |

**Optimization of chromatographic conditions:**

The chromatographic conditions were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc.

**Table 1: Summary of Process Optimization**

| Column Used | Mobile Phase | Flow Rate | Wave length | Observation | Result |
|---|---|---|---|---|---|
| Waters C$_{18}$, 5μm, 25cmx4.6mm i.d. | Methanol : Water = 70 : 30 | 1.0 ml/min | 236 Nm | Peak broken at the end | Method rejected |
| Waters C$_{18}$, 5μm, 25cmx4.6mm i.d. | Methanol : Water = 60 :40 | 0.8 ml/min | 236 Nm | Very low Response | Method rejected |
| Develosil C$_{18}$, 5μm, 15cmx4.6mm i.d. | Methanol: water = 50 : 50 | 1.0 ml/min | 236 Nm | Splitting of peak | Method rejected |
| Develosil C$_{18}$, 5μm, 15cmx4.6mm i.d. | ACN: acetate buffer = 70 : 30 | 1.0 ml/ min | 236 Nm | Did not observe any peak | Method rejected |
| Develosil C$_{18}$, 5μm, 15cmx4.6mm i.d. | ACN : phosphate buffer = 60:40 | 1.0 ml/min | 236 Nm | Broad Peak & Rt was very late | Method rejected |
| Develosil C$_{18}$, 5μm, 15cmx4.6mm i.d. | ACN : Phosphate buffer = 75:25 | 1.0 ml/min | 236 Nm | Good sharp peak | Method accepted |



**Fig 2 : Chromatogram for Blank**
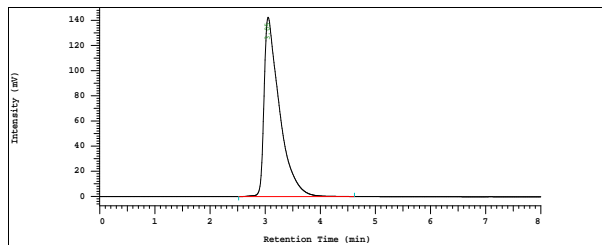


**Fig 3 : UPLC spectrum of Terconazole (20 ppm) in optimized conditions (RT 3.05 min.)**

**Forced degradation studies:**

Following protocol was strictly adhered to for forced degradation of Terconazole Active Pharmaceutical Ingredient (API). The API (Terconazole) was subjected

to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body. This is one type of accelerated stability studies that helps us determining the fate of the drug that is likely to happen after along time storage, within a very short time as compare to the real time or long term stability testing. The various degradation pathways studied are acid hydrolysis, basic hydrolysis, thermal degradation and oxidative degradation. [9, 10]

**1. Acid hydrolysis:** An accurately weighed 25 mg. of pure drug was transferred to a clean & dry 25 ml volumetric flask. To which 0.1 N Hydrochloric acid was added & make up to the mark & kept for 24 hrs. from that 0.2 ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase, then injected into the UPLC system against a blank of HCl ( after all optimized conditions )
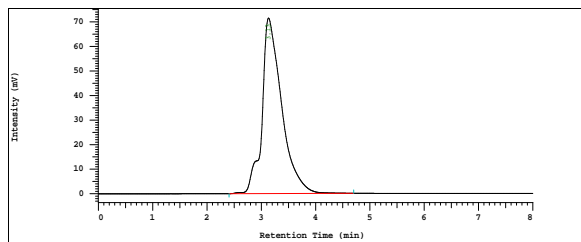


**Fig 2: Chromatogram showing degradation peak 0.1 N HCl**

**2. Basic hydrolysis**

An accurately weighed 25 mg. of pure drug was transferred to a clean & dry 25 ml volumetric flask. To which 0.1 N Sodium hydroxide was added & make up to the mark & kept for 24 hrs. from that 0.2 ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase, then injected into the UPLC system against a blank of . NaOH ( after all optimized conditions )
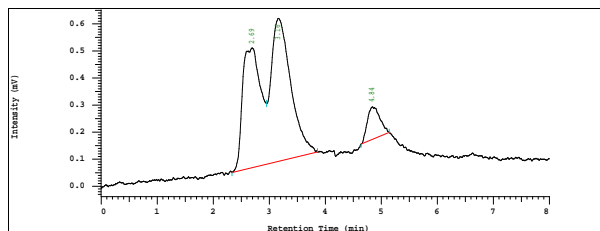
**Fig 4: Chromatogram showing degradation peak in 0.1 N NaOH**

## Method validation:

### Accuracy : *Recovery study:*

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of TERCONAZOLE were taken and added to the pre-analyzed formulation of concentration 10µg/ml. From

| Conc. in µg/ml | AUC n=6 |
|---|---|
| 0 | 0 |
| 10 | 1190634 |
| 20 | 2750684 |
| 30 | 4067931 |
| 40 | 5068743 |
| 50 | 6115263 |

that percentage recovery values were calculated.[11] The results were shown in table-2.

**Table 2: Accuracy Readings**

| Sample ID | Concentration (~g/ml) | | %Recovery of Pure drug | Statistical Analysis |
|---|---|---|---|---|
| | Pure drug | Formulation | | |
| $S_1$ : 80 % | 16 | 20 | 100.21 | Mean= 100.06% |
| $S_2$ : 80 % | 16 | 20 | 99.62 | S.D. = 0.387427 |
| $S_3$ : 80 % | 16 | 20 | 100.35 | % R.S.D.= 0.387195 |
| $S_4$ : 100 % | 20 | 20 | 99.51 | Mean= |
| $S_5$ : 100 % | 20 | 20 | 100.68 | 99.93669% |
| | | | | S.D. = 0.646091 |
| $S_6$ : 100 % | 20 | 20 | 99.62 | % R.S.D.= 0.6465 |
| $S_7$ : 120 % | 24 | 20 | 100.36 | Mean= |
| $S_8$ : 120 % | 24 | 20 | 99.57 | 99.65333% |
| | | | | S.D. = 0.668905 |
| $S_9$ : 120 % | 24 | 20 | 99.03 | % R.S.D. = 0.671232 |

### Precision: *Repeatability*

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of five replicates of a fixed amount of drug. Terconazole(API) The percent

relative standard deviation were calculated for Terconazole are presented in the table-3.

**Table 3: Standard Deviation**

| UPLC Injection Replicates of Terconazole | Area | Retention Time |
|---|---|---|
| Replicate – 1 | 3046971 | 3.11 |
| Replicate – 2 | 3050069 | 3.1 |
| Replicate – 3 | 3041269 | 3.09 |
| Replicate – 4 | 3056984 | 3.06 |
| Replicate – 5 | 3054126 | 3.07 |
| Average | 3049884 | 3.086 |
| Standard Deviation | 6147.003 | 0.020736 |
| % RSD | 0.201549 | 0.671952 |

**Linearity & Range:** The calibration curve showed good linearity in the range of 10 – 60 µg/ml, for Terconazole (API) with correlation coefficient ($r^2$) of 0.994. A typical calibration curve has the regression equation of y = 12436x + 89741 for Terconazole.
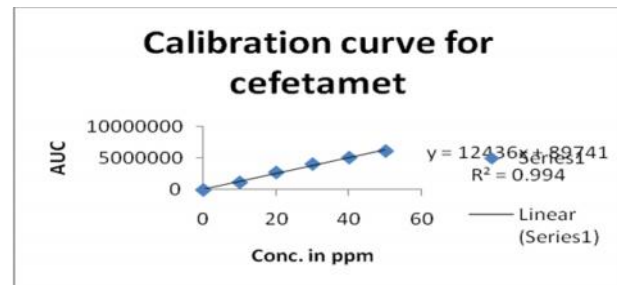


**Fig 5: Calibration curve of .Terconazole (API).**

## Method Robustness:

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature ($\pm 2^0$C), Wavelength of detection (±2nm) & acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table-4, % RSD < 2%) the developed RP-UPLC method for the analysis of Terconazole( API).

**Table 4: Result of method robustness test**

| Change in parameter | % RSD |
|---|---|
| Flow (1.1 ml/min) | 0.36 |
| Flow (0.9 ml/min) | 0.47 |
| Temperature ($27^0$C) | 089 |
| Temperature ($23^0$C) | 0.96 |
| Wavelength of Detection (236 nm) | 0.32 |
| Wavelength of detection (240 nm) | 0.14 |

**LOD & LOQ:**

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.05 & 0.15 µg/ml respectively.

## 3. RESULT & DISCUSSION

To develop a precise, linear, specific & suitable stability indicating RP-UPLC method for analysis of Terconazole, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-UPLC various columns are available, but here $C_{18}$ Develosil ODS HG-5 RP 150mm x 4.6mm 5µm particle size column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl). .

## 4. CONCLUSION

A sensitive & selective RP-UPLC method has been developed & validated for the analysis of Terconazole API. Further the proposed RP-UPLC method has excellent sensitivity, precision and reproducibility.

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