Development and Validation of a Stability-Indicating RP-HPLC Method for Estimation of Oteseconazole in Pharmaceutical Dosage Form

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Abstract- A robust, accurate, and stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method was successfully developed and validated for the estimation of Oteseconazole in pharmaceutical dosage form. Chromatographic separation was achieved using a C18 column with a mobile phase consisting of Acetonitrile and KH_2PO_4 buffer (60:40 v/v), at a flow rate of 1.0 mL/min, and detection at 254 nm. The method was validated in accordance with ICH Q2(R1) guidelines for parameters including specificity, linearity (10–60 µg/mL), precision (%RSD < 2%), accuracy (recovery 98.4%–101.2%), robustness, LOD (1.2 µg/mL), and LOQ (3.5 µg/mL). Forced degradation studies under acidic, alkaline, oxidative, thermal, photolytic, and hydrolytic conditions confirmed the method's capability to distinguish between the drug and its degradation products, establishing its stability-indicating nature. This validated method is suitable for routine quality control and stability testing of Oteseconazole in bulk and formulation.

Keywords: Oteseconazole, RP-HPLC, Stability-Indicating, Validation, Forced Degradation, ICH Guidelines

1.INTRODUCTION

Oteseconazole is a novel tetrazole antifungal agent used in the treatment of recurrent vulvovaginal candidiasis. Due to its clinical significance and increasing use, the need for a validated, stability-indicating analytical method is critical for ensuring drug quality and efficacy.

Reverse-phase high-performance liquid chromatography (RP-HPLC) is widely used in pharmaceutical analysis due to its robustness, reproducibility, and precision. However, no comprehensive, stability-indicating HPLC method has yet been reported for Oteseconazole in literature. Hence, the present study was aimed at developing a sensitive, specific, and accurate RP-HPLC method for its estimation and validating the method following ICH Q2(R1) guidelines.

1. Chemical Properties of Oteseconazole-

- IUPAC Name: (2R,3S)-2-(2,4-Difluorophenyl)-3-(5-fluoro-1H-1,2,4-triazol-1-yl)-1-(1H-1,2,4-triazol-1-yl) butan-2-ol
- Molecular Formula: $C_{16}H_{17}F_3N_6O$
- Molecular Weight: 366.34 g/mol
- Solubility: Soluble in methanol and acetonitrile

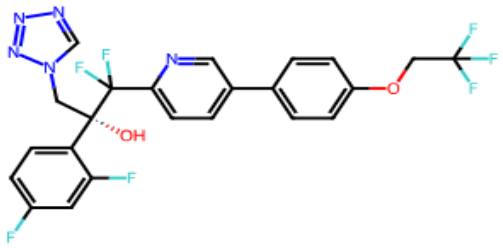


Fig 1- Structure of Oteseconazole

2. MATERIALS AND METHODS

Instrumentation

• HPLC System: Waters e2695 or equivalent

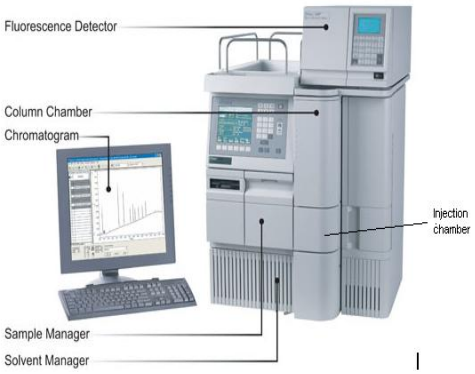


Fig 2- HPLC System

• Detector: PDA or UV detector
• Column: BDS C18 (250 mm × 4.6 mm, 5 µm)
• Software: Empower or equivalent
• Weighing balance, pH meter, sonicator used as required

3. REAGENTS AND CHEMICALS

- Oteseconazole standard (API) – from a certified supplier
- Methanol (HPLC grade), Acetonitrile (HPLC grade), Water (Milli-Q), Triethylamine, OPA
- Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide for degradation studies
-Mobile phase: Acetonitrile: KH_2PO_4(60:40 v/v)

Table:-1 Chromatographic Conditions

Parameter	Specification
Column	C18 (250 × 4.6 mm, 5 µm)
Mobile Phase	Acetonitrile: KH_2(60:40v/v)
Flow Rate	1.0 mL/min
Detection Wavelength	254 nm
Injection Volume	20 µL
Run Time	10 minutes
Retention Time (Rt)	~3.25 minutes

4. PREPARATION OF STANDARD AND SAMPLE SOLUTIONS

Standard Stock Solution:
10 mg of Oteseconazole was weighed and dissolved in methanol to make 100 mL (100 µg/mL). Further dilutions were prepared to get concentrations ranging from 10–60 µg/mL.

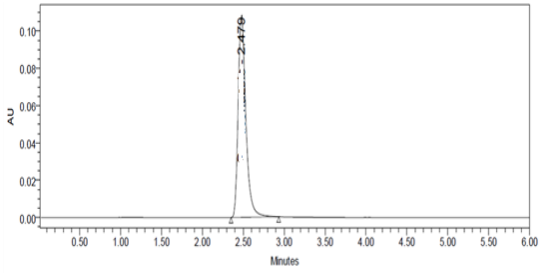


Fig 3- Standard Chromatogram

Sample Solution:
Equivalent to 10 mg of Oteseconazole from the pharmaceutical formulation was dissolved and filtered for analysis.

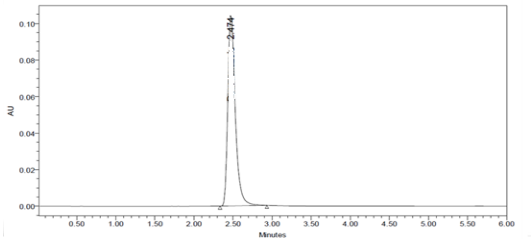
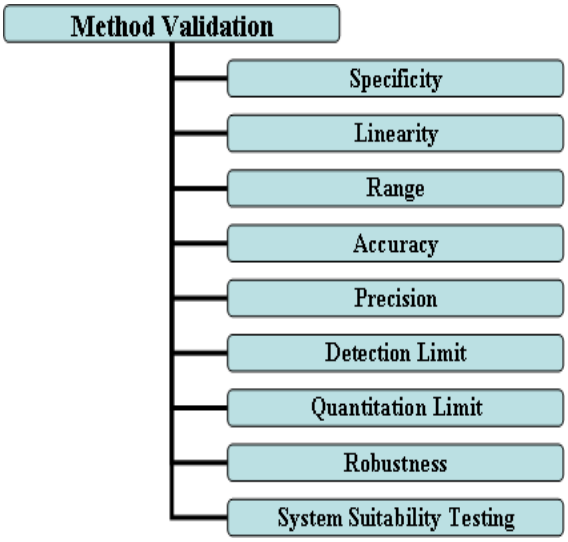


Fig 4-Sample Chromatogram

5. METHOD VALIDATION PARAMETERS



The developed RP-HPLC method for the estimation of Oteseconazole in pharmaceutical dosage form was validated according to ICH Q2(R1) guidelines for the following parameters:

1. System Suitability-
System suitability tests were carried out by injecting the standard solution multiple times and evaluating parameters such as retention time and peak area. The %RSD for both parameters was found to be within acceptable limits (<2%), indicating the system's reproducibility and performance consistency.

- %RSD of retention time and peak area: <2%
- Retention time (Rt): ~3.25 minutes

2. Linearity
Linearity was evaluated by preparing standard solutions of Oteseconazole at concentrations ranging from 10–60 µg/mL. Each concentration was injected into the HPLC system, and a calibration curve was plotted using peak area versus concentration.

- Linearity range: 10–60 µg/mL

- Regression Equation: y = mx + c
- Correlation coefficient (r^2): 0.9996
- Result: The method showed excellent linearity in the specified range.

3. Accuracy (Recovery Studies)

Accuracy was determined through recovery studies performed by spiking known amounts of Oteseconazole into the sample matrix at 80%, 100%, and 120% of the target concentration. The results confirmed the accuracy of the method.

- Recovery range: 98.4% – 101.2%
- Result: Recovery results were within the acceptable range (98%–102%), indicating high accuracy.

4. Precision

Precision was evaluated in terms of repeatability (intra-day) by injecting six replicates of the same concentration. The %RSD of peak areas was calculated.

- %RSD: <1.2%
- Result: Precision of the method was well within the acceptable limits (%RSD <2%).

5. Robustness

Robustness was assessed by making deliberate minor variations in chromatographic conditions, including changes in flow rate (±0.1 mL/min), detection wavelength (±2 nm), and pH of the mobile phase (±0.2). The method remained unaffected by these changes.

- Result: The method was robust under the tested conditions, showing minimal changes in retention time and peak area.

6. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated based on the standard deviation of the response and slope of the calibration curve.

- LOD: 1.2 µg/mL
- LOQ: 3.5 µg/mL
- Result: The method demonstrated sufficient sensitivity to detect and quantify low concentrations of Oteseconazole.

7. Specificity

Specificity was evaluated by analyzing the sample in the presence of excipients and performing forced degradation studies. The method could distinguish Oteseconazole from its degradation products and excipients without interference at the retention time.

- Result: No interference from excipients or degradation products was observed. The method is specific and stability-indicating.

Table :- 2 Results Validation Data Table

Parameter	Result
Linearity range	10–60 µg/mL
Regression Equation	y = mx + c
Correlation Coefficient	0.9996
Precision	%RSD < 1.2
Recovery	98.4% – 101.2%
LOD	1.2 µg/mL
LOQ	3.5 µg/mL
Robustness	Method robust under minor changes
Specificity	No interference

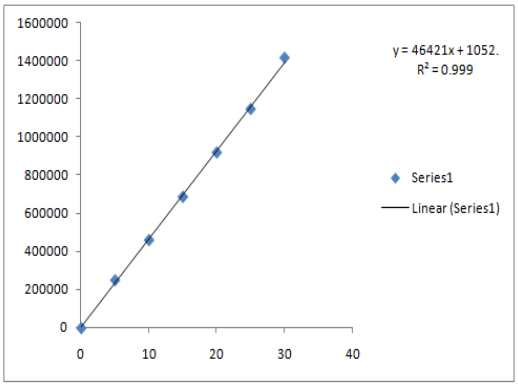


Fig 5- Linearity Plot

6. FORCED DEGRADATION STUDIES

Table :- 3 Degradation Data of Oteseconazole

S.No.	Degradation Condition	%Drug Un-Degraded	% Drug Degraded
1	Acid(1N HCl, 60°C, 30 min)	93.90	6.10
2	Alkali(1N NaOH, 60°C, 30 min)	97.75	2.25
3	Oxidation(20% H_2O_2, 60°C)	96.26	3.74
4	Thermal(80°C, 6 hrs)	97.10	2.90
5	UV	98.85	1.15
6	Water	99.77	0.23

7. CHROMATOGRAMS AND FIGURES

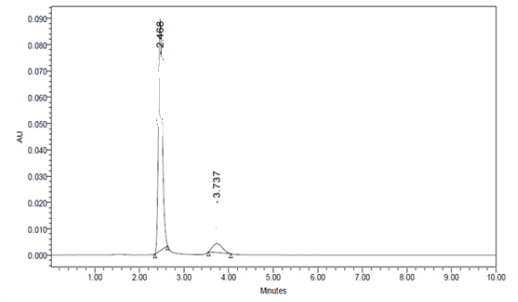


Figure 6- Acid degradation chromatogram

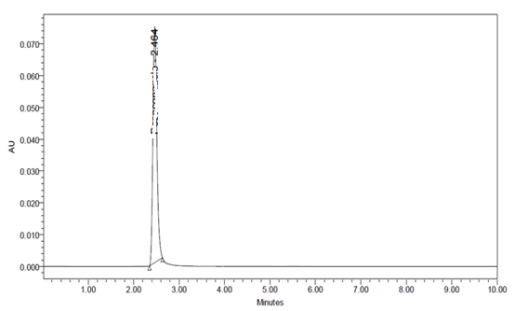


Fig 7-- Base degradation chromatogram

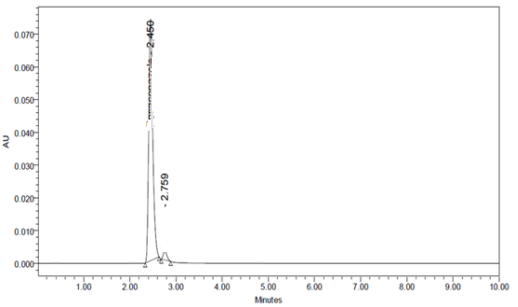


Fig 8- Peroxide degradation chromatogram

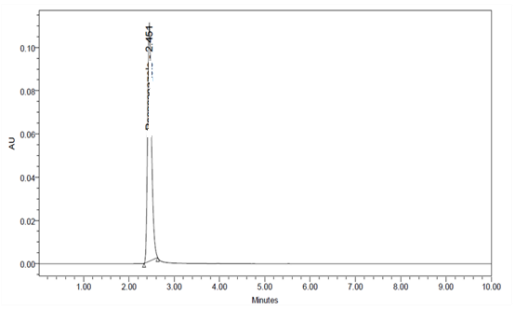


Fig 9- Thermal degradation chromatogram

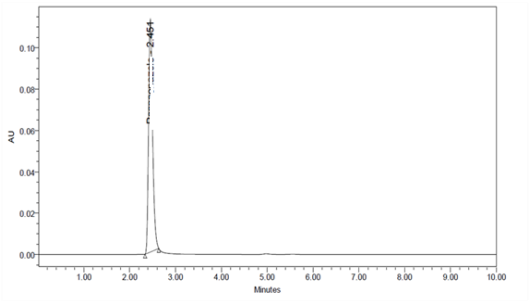


Fig 10 - UV degradation chromatogram

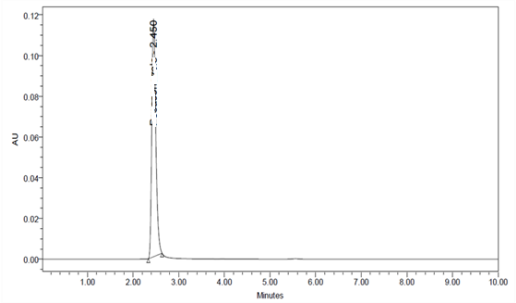


Fig 11- Water degradation chromatogram

8. DISCUSSION

The developed RP-HPLC method is simple, rapid, and suitable for routine analysis of Oteseconazole. Degradation studies confirm its stability-indicating power. The method validation met all ICH criteria, ensuring accuracy, precision, and reproducibility. Forced degradation results help in understanding the intrinsic stability of the drug.

9. CONCLUSION

A validated, stability-indicating RP-HPLC method was successfully developed for the estimation of Oteseconazole. The method is accurate, precise, robust, and specific. It can be used for routine quality control and stability testing of the pharmaceutical formulation.

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