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Development and Validation of UV Spectrophotometric Method for the Determination of Latest Anti-Fungal Drug Oteseconazole in Pharmaceutical Dosage Form

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Abstract

A simple, precise, and accurate UV spectrophotometric method was developed and validated for the estimation of Oteseconazole in tablet dosage form. The analysis was performed at a wavelength of 223.3 nm, and the method followed Beer-Lambert's law in the concentration range of 2.50 -15.00 µg/mL with a regression equation of y = 0.14434x + 0.00186, and a high correlation coefficient (R^2 = 0.99986). The slope was found to be 0.14434, indicating good sensitivity. The method demonstrated excellent accuracy, with a percentage recovery ranging from 99.9 % to 101.1 %. Precision studies showed low % RSD values for both intraday (0.61%) and interday (0.48 %) precision, confirming the method's reliability. Robustness was assessed by varying the detection wavelength (± 5 nm), and the method showed recovery values of 100.0 % to 100.2 %. The forced degradation study of the UV method for OTEZ involved subjecting the sample to various stress conditions, including acid, alkali, peroxide, reduction, thermal, photolytic, and hydrolytic treatments.

Keywords: Oteseconazole; Validation; Ultraviolet Spectroscopy; Method Development

Abbreviations

OTZE: Oteseconazole; RVVC: Recurrent Vulvovaginal Candidiasis; CYP51: Cytochrome P450 14α-sterol demethylase; CDR1: Calcein-Dependent Resistance 1; MDR1: Multidrug Resistance Protein 1; HPLC: High-Performance Liquid Chromatography; LC-MS/MS: Liquid Chromatography-Tandem Mass Spectrometry; UV: Ultraviolet; ICH: International Conference on Harmonization; RSD: Relative Standard Deviation.

Introduction

The chemical name for OTEZ (R)-2- (2,4-difluorophenyl)-1,1-difluoro-3- (1H-tetrazol-1-yl)-1- (5- (4-(2,2,2-trifluoroethoxy)phenyl)pyridin-2-yl)propan-2-ol [1]. It has the molecular formula $C_{23}H_{16}F_7N_5O_2$ a molecular weight of 527.39 g/mol [2]. OTEZ is an azole antifungal used to reduce the risk of recurrent vulvovaginal candidiasis (RVVC) by inhibiting the growth of common vaginal yeast [3]. It works by blocking CYP51 (14α-demethylase), an enzyme involved in the production of ergosterol, a key component of fungal cell membranes [4]. Inhibiting this enzyme disrupts the integrity of the fungal cell membrane, leading to cell death. OTEZ also causes the accumulation of toxic 14-methylated sterols, further promoting fungal death. The drug's selectivity for fungal CYP51 helps minimize off-target effects in humans, as its tetrazole metal-binding

group reduces its affinity for the human isoenzyme [5]. In vitro studies indicate that some resistance to OTEZ may arise due to upregulation of efflux pumps (CDR1, MDR1) or changes in the CYP51 target enzyme. Despite this, OTEZ shows activity against Candida species resistant to fluconazole, including Candida albicans, Candida glabrata, and others associated with RVVC [6]. Pharmacokinetically, OTEZ has dose-proportional absorption, with a time-to-peak concentration (Tmax) of 5 to 10 hours. Its bio-availability is influenced by diet, with higher absorption following high-fat, high-calorie meals. A literature review reveals that OTEZ has been primarily analyzed using advanced techniques like high-performance liquid chromatography (HPLC) [7] and liquid chromatography-mass spectrometry (LC-MS/MS) [8] in biological matrices, including human plasma. This study focuses on developing and validating a UV spectrophotometric method to quantify oteseconazole in pharmaceutical formulations, driven by the need for a simpler, accurate, and accessible analytical technique. Oteseconazole has previously been analyzed using advanced methods such as HPLC and LC-MS/MS, which, while effective, are costly, time-consuming, and require sophisticated equipment and expertise-limiting their availability in many laboratories. In contrast, UV spectrophotometry offers a cost-effective, straightforward, and rapid alternative suitable for routine analysis. Given oteseconazole's importance in antifungal therapy, establishing a validated UV spectrophotometric method broadens accessibility for quality control settings, especially in resource-limited environments. This study aims to create a reliable method that adheres to International Conference on Harmonization (ICH) guidelines for accuracy, precision, linearity, and robustness, providing a practical tool for the quality assessment and stability testing of oteseconazole in pharmaceutical formulations. By filling a gap in the available methods, this validated approach contributes an economical and efficient solution for oteseconazole quantification in routine pharmaceutical analysis. The structure of Oteseconazole is shown in Figure 1.

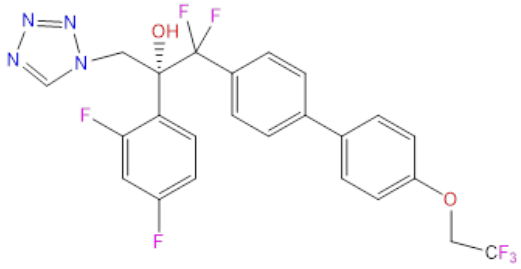

Figure 1: Chemical Structure of OTEZ.

Experimentation

Instrument: A double beam UV-1700 spectrophotometer containing two matched quartz cells with a one cm light path was taken for measuring of absorbance of OTEZ (0.1 mg sensitivity) balance was used for weighing. Ultra Sonicator bath Model no - 91250, PCI Ltd., Mumbai was used in this present study.

Chemicals and Reagents: All solvents and chemicals utilized in this study were of analytical grade and obtained from Merck Specialties Pvt. Ltd. and Rankem Private Limited. The working stndard was kind gift from Shree Icon Pharmaceutical laboatories, Vijayawada, A.P.

Selection of Solvent: Numerous trials were conducted to determine the appropriate solvent system for dissolving OTEZ. Solvents such as acetonitrile, methanol, and distilled water were tested based on the drug's solubility profile. As OTEZ was found to be soluble in acetonitrile, it had been selected as the solubilizing agent for method development.

Selection of Detection Wavelength: To determine the optimum λmax of OTEZ, 10 µg/mL of the OTEZ solution was prepared and scanned in the Ultra Violet wavelength range of 200 - 400 nm. It was observed that the drug showed maximum absorbance at 223.3 nm which was chosen as the detection wavelength for the estimation of OTEZ.

Standard Preparation Solution: The pure drug of 10 mg was weighed and transferred into a 100 mL volumetric flask. The drug was dissolved completely in Acetonitrile and made up to the final volume with the same solvent to get a stock solution of concentration 100 µg/mL. Aliquots of standard stock solution were pipetted out 1 mL to 10 mL and diluted suitably with acetonitrile to get the final concentration of standard solutions.

Preparation of Calibration Curve

Selection of Analytical Concentration Range: Accurate aliquots were pipetted out from the standard stock solution into a series of 10 mL volumetric flasks. The volume was made up to the mark with water to obtain a series of dilutions of concentration range, ranging from 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 µg/mL of OTEZ. The absorbance of the above solutions was measured at 223.3 nm and converted to zero-order spectra calibration curve of absorbance against concentration was plotted. The regression equation and correlation coefficient were determined. Beer Lambert's law was obeyed in the concentration range of 2.5-15.0 µg/mL for the method.

Analysis of Marketed Formulation: Weigh 16.47 mg of OTEZ sample and taken in a 100 mL volumetric flask and it was dissolved in acetonitrile and made up to the mark with the same solvent. Then the solution was filtered using Whitman filter paper No.40. From this filtrate, dilute 1mL to 10mL volumetric flask was made with water to obtain the desired concentration (10 µg/mL). These solutions were analyzed in UV and the result was indicated by % assay.

Degradation Studies for Oteseconazole Using UV Spectroscopy: 2.8.1. Preparation of Stock Solution: Accurately weigh and transfer 24.7 mg of Oteseconazole into a 10 mL clean, dry volumetric flask. Add the diluent and sonicate to dissolve the sample completely. Make the volume up to the mark with the same diluent. This solution serves as the stock solution.

Acid Degradation: Pipette 1 mL of the stock solution into a 10 mL volumetric flask. Add 1 mL of 1N HCl to the flask. Heat the solution at 60°C for 1 hour, then neutralize it with 1N NaOH. Make the volume up to 10 mL using the diluent. Filter the solution using 0.22-micron syringe filters, and the resultant solution can be analyzed using UV spectroscopy.

Alkali Degradation: Pipette 1 mL of the stock solution into a 10 mL volumetric flask. Add 1 mL of 1N NaOH to the flask. Heat the solution at 60°C for 1 hour, then neutralize it with 1N HCl. Make the volume up to 10 mL using the diluent. Filter the solution using 0.22-micron syringe filters, and analyze the solution using UV spectroscopy.

Peroxide Degradation: Pipette 1 mL of the stock solution into a 10 mL vacuum flask. Add 1 mL of 10% w/v hydrogen peroxide and make the volume up to the mark with diluent. Heat the flask at 60°C for 1 hour, then allow it to cool to room temperature for 15 minutes. Filter the solution using 0.22-micron syringe filters, and analyze using UV spectroscopy.

Reduction Degradation: Pipette 1 mL of the stock solution into a 10 mL vacuum flask. Add 1 mL of 10 % w/v sodium bisulfite and make the volume up to the mark with diluent. Heat the flask at 60°C for 1 hour, then allow it to cool to room temperature for 15 minutes. Filter the solution using 0.22-micron syringe filters, and analyze using UV spectroscopy.

Hydrolysis Degradation: Pipette 1 mL of the stock solution into a 10 mL vacuum flask. Add 1 mL of HPLC grade water and make the volume up to the required mark with diluent. Heat the solution at 60°C for 1 hour, then allow it to cool for 15 minutes at room temperature. Filter the solution using 0.22-micron syringe filters, and analyze using UV spectroscopy.

Photolytic Degradation: Place the Oteseconazole sample in a photo-stability chamber for 3 hours. After exposure, take the sample, dilute with the appropriate amount of diluent, and analyze using UV spectroscopy.

Thermal Degradation: Place the Oteseconazole sample in a petridish and heat in a hot air oven at 105°C for 3 hours. After the heating process, dilute the sample with diluent, and analyze using UV spectroscopy.

Results and Discussions

Method Optimization

Various solvents, including distilled water, methanol, and acetonitrile, were tested for the solubility of OTEZ at a concentration of 10 µg/mL. OTEZ was found to be both soluble and stable in acetonitrile (ACN) mixture for at least 24 hours at room temperature. Therefore, the ACN mixture was selected for wavelength detection and the preparation of standard and working concentrations.

Method Validation

To validate the proposed method for the pharmaceutical formulation, an assay was performed on 150 mg capsules of OTEZ at the working concentration, with an analysis conducted at 223.3 nm. The UV spectrophotometric method was validated by ICH Q2 (R1) guidelines [9-11], covering parameters such as specificity, linearity, range, accuracy, precision, LOD, LOQ, robustness, and system suitability testing.

Linearity & Range: Fresh aliquots were prepared from standard stock solution ranging from 2.5-15.0 µg/mL and the absorbance values of each concentration were recorded at 223.3 nm for zero order using acetonitrile as blank. The drug shows linearity between 2.5-15.0 µg/mL for the method. The correlation co efficient was found to be 0.99986 for the method.

Precision: Precision of the method was demonstrated by intra-day and inter-day variation studies. In intra-day variation study, six solutions of 10µg/mL were prepared and analyzed three times in a day and the respective absorbances were noted. The results were indicated by % RSD. In the inter-day variation study, six solutions of 10 µg/mL were prepared and analyzed three times for three consecutive days and the respective absorbances were noted. The results were indicated by % RSD. The % RSD for intraday and inter day precision of OTEZ in method was found to be less than 2. According to ICH guidelines, the % RSD should less than 2 (within the acceptance criteria).

Accuracy (Recovery Studies): Accuracy of the developed method was confirmed by performing recovery studies at three different concentration ranges 50 %, 100 %, 150 % each one in triplicate and the accuracy was indicated by % recovery. The % RSD for accuracy of OTEZ in the method was found to be less than 2. The % recovery was in the range of 100.5. According to ICH guidelines the statistical results were within the acceptance range.

Ruggedness: Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective absorbances were noted. The results were indicated by % RSD. The % RSD values for ruggedness of three replicates of OTEZ at a concentration of 10 µg/mL was found to be within the acceptance limits.

Robustness: Robustness of the method was determined by carrying out the analysis at two different wavelengths (±5nm). The respective absorbances were noted and the results were indicated by % RSD. The % RSD values were

found to be within the acceptance criteria.

Discussion

The ultraviolet spectra of OTEZ were scanned in the region between 200-400 nm. The spectra of OTEZ at different concentrations were absorbed maximum at 223.3 nm, which was selected as the detection wavelength which is shown in Figure 2. The response of the OTEZ was found to be linear in the concentration range of 2.5-15.0 µg/mL with a good correlation coefficient of r2=0.99986 (y = 0.14434 x + 0.00186). Figure 3 shows the OTEZ linearity calibration curve. Table 2 lists the Linearity of OTEZ of the proposed UV method. The inter-day and intra-day precision of OTEZ is tabulated in Table 3. The % RSD was less than 2 in all precision results cases which indicates that the method was precise. In this recovery, study accuracy was carried out by using a standard addition method at three different concentration levels (50 %, 100 %, and 150 %). The mean percentage recovery at each level should be 99.9 - 101.1 %. All the results are well within the acceptance criteria and results indicate that the method is accurate. Results are displayed in Table 4. Ruggedness was performed to check the reproducibility which showed the % RSD less than 2 which indicates that the method was rugged. The robustness of the method was evaluated by analyzing samples at two wavelengths (±5 nm), with the resulting absorbance values expressed as % RSD these values fell within the acceptance criteria which is shown in Table 5. The developed method was eventually applied for the quantification of OTEZ in capsules. The mean % assay values were found to be 99.8 %. The amount of the drug in the capsule sample was in good agreement with the label claim of the formulation. The assay results are shown in Table 1. The forced degradation study of OTEZ was conducted using UV spectroscopy under various stress conditions to evaluate stability. In the control sample, no degradation was observed (0 %). Under acidic conditions, OTEZ showed 12.4 % degradation, while alkaline conditions caused 11.5% degradation. Oxidative degradation with peroxide resulted in the highest degradation (14.1%), and reduction conditions led to a modest 4.2 % degradation. Thermal and photolytic stresses resulted in 4.4 % and 2.2 % degradation, respectively. Hydrolysis caused minimal degradation (1.5%), indicating that OTEZ exhibits significant stability, especially under photolytic and hydrolytic conditions. Results are tabulated in Table 6 and summary of validated parameters are shown in Table 7.

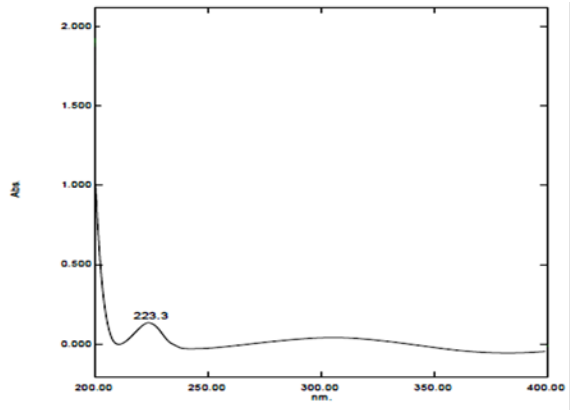

Figure 2: UV spectrum of OTEZ.

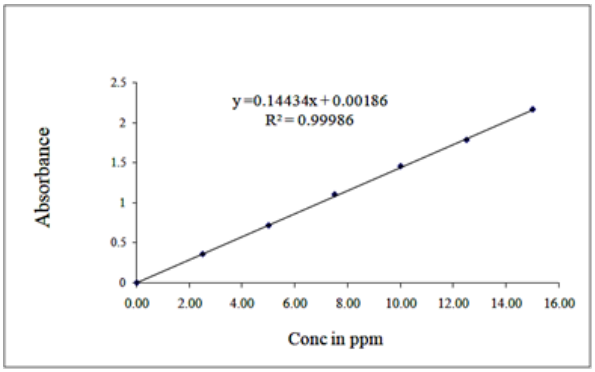

Figure 3: Calibration curve for OTEZ.

Wave Length nm	Label claim (mg)	Standard absorbance	Test absorbance	Amount found (mg/mL)	% recovery
223	150	1.457	1.467	10.08	100.8

Table 1: Results of Analysis of Formulation by UV-Spectrophotometry.

S. No	OTEZ	
	Conc.(µg/mL)	Absorbance
1	2.5	0.358
2	5	0.716
3	7.5	1.104
4	10	1.458
5	12.5	1.787
6	15	2.168
Regression equation	y = 0.14434x + 0.00186	
Slope	0.14434	
Intercept	0.00186	
R^2	0.99986	

Table 2: Linearity of OTEZ.

Name of the drug	S.No	Absorbance		% Assay	
		Intra-day	Inter-day	Intra-day	Inter-day
OTZE	1	1.462	1.445	100	99.8
	2	1.446	1.446		
	3	1.453	1.453		
	4	1.445	1.457		
	5	1.467	1.449		
	6	1.459	1.463		

Table 3: Intra and Inter-Day Precision.

Name of the drug	Amount of µg/mL		% of drug added	% recovered	% Mean Recovery
	LC	Pure drug			
OTZE	150 mg	5	50	99.9	100.5
		10	100	100.6	
		15	150	101.1	

Table 4: Accuracy Data of UV Method.

Parameter	Concentration 20 (µg/mL)	% Assay of OTEZ
Robustness Change in λmax (± 5nm)	λ+ : 228 nm	100
	λ - : 218 nm	100.2

Table 5: Robustness Results.

S.No	Degradation	Absorbance	% degradation
1	Control	1.453	0
2	Acid	1.274	12.4
3	Alkali	1.287	11.5
4	Peroxide	1.248	14.1
5	Reduction	1.392	4.2
6	Thermal	1.389	4.4
7	Photolytic	1.422	2.2
8	Hydrolysis	1.432	1.5

Table 6: Forced degradation results of OTEZ.

Parameter	Results
Beer's law limit (µg/mL)	2.50-15.0
Linear regression equation	y=0.14434x+0.00186
Linearity indicated by correlation coefficient	0.99986
Precision indicated by %RSD	0.18
Intraday precision	0.61
Inter day precision	0.48
Accuracy indicated by % recovery	99.9-101.1%
Robustness indicated by % recovery(W+,W-)	100.0,100.2
Ruggedness indicated by % recovery	99.8

Table 7: Summary of Validation & Optical Characteristics.

Conclusion

The developed UV spectrophotometric method for Oteseconazole (OTEZ) capsules is simple, precise, and highly accurate. Analyzing at 223.3 nm, it adheres to Beer-Lambert's law in the concentration range of 2.50–15.00 µg/mL, with a high correlation coefficient (R2 = 99986), demonstrating strong linearity. Sensitivity is high with a slope of 0.14434, and accuracy is confirmed by recovery rates between 99.9 % and 101.1 %. Precision studies show low % RSD for both intraday (0.61 %) and interday (0.48 %) analyses, ensuring reliability. Robustness was verified by varying the wavelength (±5 nm), yielding consistent recovery (100.0 %–100.2 %). Stress testing confirmed the method's stability, making it suitable for routine quality control of OTEZ formulation

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Conflict of Interest: None declared

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