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Research Article

Stability indicating RP-HPLC-UV method development and validation for estimation of Efinaconazole in bulk drug and pharmaceutical formulation

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Abstract

Efinaconazole is a potent triazole antifungal drug for the topical treatment of onychomycosis. In this comprehensive study, we developed a highly effective and reliable stability-indicating RP-HPLC (Reverse Phase High-Performance Liquid Chromatography) method, alongside two advanced UV-spectrophotometric techniques—zero-order and first-order derivative methods for the accurate estimation of Efinaconazole. Employing the state-of-the-art Shimadzu model 1800 UV spectrophotometer, we ensured precision and reliability in our measurements. The chromatographic separation was expertly achieved on an Enable C18 column in isocratic mode, utilizing a carefully balanced mixture of methanol and 0.01 M potassium dihydrogen phosphate buffer (pH 5.5) in a 90:10 (v/v) ratio. This was conducted at a flow rate of 2 ml/min, with detection at 210 nm, ensuring optimal performance for our analysis. We rigorously subjected Efinaconazole to five distinct stress conditions, meticulously analyzing the resulting degradation products using the RP-HPLC technique. This allowed us to calculate their percentage recovery with high accuracy. The two UV-spectrophotometric methods demonstrated exceptional linearity within the concentration range of 100-500 μg/ml, achieving peak performance with the zero-order method at 261 nm and the first-order derivative method at 270 nm, yielding correlation coefficients of 0.999 and 0.998, respectively. Remarkably, the average recoveries from our recovery studies ranged from 99.44% to 100.42% for the zero-order method and from 99.86% to 100.39% for the first-order derivative method, showcasing the methods' reliability and accuracy. For the RP-HPLC method, we established a linearity range of 25–125 μg/ml, achieving a strong correlation coefficient of 0.998. The retention time for Efinaconazole was consistently recorded at 4.55 min, with recovery rates impressively ranging between 99.8% and 100.08%. This comprehensive validation underscores the robustness and efficacy of our analytical methods for the accurate estimation of Efinaconazole in the treatment of onychomycosis.

Keywords: Efinaconazole; onychomycosis; RP-HPLC; stability indicating

Introduction

Efinaconazole (EFZ) is an innovative antifungal agent, chemically identified as (2R,3R)-2-(2,4-difluorophenyl)-3-(4-methylene-piperidinyl)-1-(1H-1,2,4-triazol-1-yl)-2-butanol, with the molecular formula $C_{18}H_{22}F_2N_4O$ (Figure 1) [1]. As the first triazole antifungal approved for topical use, EFZ provides a powerful solution for toenail onychomycosis, a condition that can significantly impact quality of life. This groundbreaking medication works by effectively inhibiting the enzyme lanosterol 14-demethylase, which disrupts the production of ergosterol, a critical component of the fungal cell wall. As a result, 14α-methyl sterols accumulate while ergosterol levels decline, leading to both fungistatic and fungicidal effects that combat fungal infections at their source. Notably, EFZ is designed to be easily absorbed by keratin; yet, its low binding affinity allows for increased availability of the active drug at the site of nail infection, thereby maximizing therapeutic outcomes. Specifically targeting fungal infections, EFZ does not impact bacterial pathogens, ensuring a focused approach to treatment.

Marketed under the brand name Jublia and produced by Valeant Pharmaceuticals in North America. EFZ is now approved for use across the United States, Canada, and Japan as a convenient 10% topical solution for onychomycosis. With its solubility in organic solvents, limited solubility in methanol and chloroform, a pKa value of 12.7, and classification as a BCS Class II compound [2-4]. EFZ establishes itself as a cutting-edge therapeutic option that delivers effective results for patients struggling with fungal nail infections.

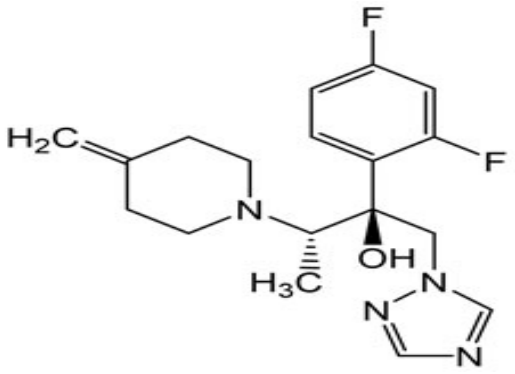

Figure. 1. Chemical structure of EFZ.

The literature survey highlights that EFZ has been effectively analyzed using LC/MS and HPLC techniques in both ex vivo human nail permeation studies [5-6] and evaluations of anti-mycotic activity [7]. Despite this, only a handful of methods exist for estimating EFZ in biological samples, and remarkably, no dedicated UV-Visible method has yet been developed. Currently, there is only one liquid chromatographic method available for estimating it in pure drugs and pharmaceutical formulations. The existing HPLC method employs a phosphate buffer and acetonitrile mixture in a 20:80 ratio as the mobile phase, with a concentration range of 50-150 μg/ml and a detection wavelength of 210 nm.

However, our anticipated methods promise to be not only rapid and straightforward but also incredibly sensitive, accurate, and cost-effective, making them ideal for routine analysis of pharmaceutical formulations. Moreover, the newly developed HPLC method significantly reduces retention time and expands the concentration range compared to previously reported methods, enhancing its effectiveness in analyzing formulations containing minute doses. Importantly, all developed methods will adhere to the stringent validation standards set forth by the International Conference on Harmonization (ICH), ensuring their reliability and precision in pharmacological applications.

Material

Reagents and Chemicals

The EFZ pure drug was strategically procured as a complimentary sample from Mythri Drugs Private Limited in Telangana. We sourced high-quality methanol (HPLC grade) from the reputable Sigma-Aldrich Chemicals Company in Mumbai, India, ensuring optimal purity for our experiments. Essential reagents, including potassium dihydrogen phosphate, potassium hydroxide, and HPLC-grade water (Millipore), were acquired from RAKS Pharma Pvt Ltd in Ahmedabad, India, further ensuring the reliability of our results. We also secured triethanolamine (HPLC grade), glacial acetic acid, and hydrochloric acid (AR grade) from the distinguished Qualigens Company in Bangalore, India. Additionally, we obtained the 10% EFZ topical solution, branded as Jublia and manufactured by Valeant Pharmaceuticals in North America, to enhance the relevance of our study to clinical applications. For the filtration of the sample solution, we utilized 0.45 μm nylon 66 membrane filters from Sigma-Aldrich Chemicals Company, guaranteeing precision and clarity in our analysis.

Instrumentation

The analysis was conducted using the highly advanced UV-Visible Spectrophotometer, specifically the Shimadzu model 1800 twin-beam UV spectrum analyzer, renowned for its precision and reliability. For the HPLC system, we utilized a top-of-the-line Shimadzu model, equipped with the efficient SPD-20AD UV detector, a robust isocratic pump LC-20 AD, and a highly effective loop injector. Separation was expertly achieved at room temperature (25 °C) using an Enable C18 column, which features a superior particle size of 5 μm, a generous length of 250 mm, and an internal diameter of 4.6 mm, ensuring optimal performance and resolution. The accuracy of weighing was maintained using the dependable Shimadzu electronic balance model BL 220H. For data analysis, we utilized the sophisticated UV Probe software (version 2.0), complemented by the powerful Lab Solutions software for HPLC, which enabled comprehensive data processing and analysis. This combination of high-quality instruments and software underlines the rigor and reliability of our analytical approach.

Methods

UV Spectroscopic method

Preparation of standard stock solution of EFZ

To prepare a stock solution of EFZ, 100 mg of pure drug was meticulously weighed and dissolved in 0.1 M HCl within a volumetric flask. The solution was then brought to the desired volume with the same solvent, resulting in a precise concentration of 1000 μg/ml. This careful preparation ensures the reliability and effectiveness of the stock solution for subsequent applications.

Preparation of working solution

Starting with the primary stock solution, we meticulously prepared a series of precise dilutions by pipetting 0.1, 0.2, 0.3, 0.4, and 0.5 ml into five separate 10 ml volumetric flasks. To achieve the desired concentrations of 100, 200, 300, 400, and 500 μg/ml, we carefully brought each flask up to volume using 0.1 M HCl. This systematic approach ensured accuracy and reliability in our dilutions, setting a solid foundation for our subsequent analysis.

Preparation of sample solution of EFZ (Marketed Formulation)

From the formulation, a volume equivalent to 100 mg of EFZ was weighed accurately and taken in a volumetric flask. 30 ml of 0.1 M HCl was surplused and the end volume was made up to 100 ml using the same solvent to get 1 mg/ml solution. The mixture was then sonicated for 10 min and filtered. From this primary stock solution, further dilutions were made to obtain a concentration of 100 to 500 μg/ml using 0.1 M HCl solution.

RP-HPLC Method

Chromatographic conditions

The composition of the mobile phase was optimized to consist of 90% methanol and 10% potassium dihydrogen phosphate at a concentration of 0.01 M and a pH of 5.5. To ensure high quality and performance, the mobile phase was sonicated for 20 min and then filtered using nylon membrane filters with a pore size of 0.45 μm. For the analytical method, a high-performance Enable C18 column was used, which featured a particle size of 5 μm, a length of 250 mm, and an internal diameter of 4.6 mm. The column was equilibrated with the solvent system for 30 min before sample injection. The solvent was pumped through the column at a flow rate of 2.0 mL/min, and UV detection was performed at a wavelength of 210 nm, with a total run time of 7 min. All analyses were conducted at a controlled room temperature of 25 °C, using an injection volume of 20 μl. This carefully designed methodology ensures both accuracy and reliability in the results obtained.

Preparation of standard stock solution

A carefully measured 100 mg of EFZ was transferred into a 100 ml volumetric flask, ensuring precision in our process. This was subsequently dissolved in 75 ml of mobile phase, and the volume

was carefully adjusted to the mark, resulting in a potent stock solution with a concentration of 1000 µg/mL (Stock A). This method guarantees an accurate and reliable foundation for further analysis.

Preparation of working solution

A stock B solution was expertly prepared by carefully pipetting 50 ml from stock A and diluting it to a total volume of 100 ml with the mobile phase, resulting in a precise concentration of 500 µg/ml. This stock B solution then served as the foundation for generating five distinct concentrations. By accurately pipetting 0.5 ml, 1 ml, 1.5 ml, 2 ml, and 2.5 ml into separate 10 ml volumetric flasks and diluting them with the mobile phase, we achieved target concentrations of 25, 50, 75, 100, and 125 µg/ml.

Sample solution preparation of EFZ (Marketed Formulation)

A precise 100 mg dose of EFZ was carefully weighed from its formulation and transferred to a volumetric flask. To ensure complete dissolution, it was first combined with 75 ml of the mobile phase and sonicated. The volume was then accurately adjusted to the mark with additional mobile phase, and the solution was filtered, resulting in a concentrated stock solution of 1000 µg/ml (Stock A). From this high-concentration stock, a series of deliberate dilutions were meticulously prepared to create final concentrations of 25, 50, 75, 100, and 125 µg/ml, each dilution carefully filtered to maintain integrity and reliability.

Forced degradation studies

Comprehensive degradation studies were carried out under five distinct conditions: acidic, basic, oxidative, photolytic, and thermal degradation. This rigorous approach ensures a thorough understanding of the degradation mechanisms involved [8-10].

Acid hydrolysis

To carry out the acid hydrolysis effectively, 50 mg of EFZ was precisely measured and dissolved in 0.1 N HCl. The volume was carefully adjusted to 50 ml with the same solvent, ensuring optimal conditions for the reaction. The solution was then heated for a full 3 h at 80 °C in a round-bottom flask equipped with an air condenser, allowing for thorough hydrolysis. Once the time was complete, the solution was cooled to room temperature, after which it was diluted with the mobile phase to a concentration of 75 µg/ml. This final solution was then injected into the chromatographic system, setting the stage for accurate analysis.

Base hydrolysis (alkaline)

The acid hydrolysis procedure was executed by effectively dissolving the drug in a precise 0.1 N NaOH solution. Following this, a carefully measured volume of the solution was pipetted and diluted with the mobile phase to create a robust concentration of 75 µg/ml. This preparation was then injected into the chromatographic system, ensuring optimal results.

Oxidative degradation

Oxidation was expertly conducted using a 6% hydrogen peroxide (H_2O_2) solution, following the established procedure for acid hydrolysis. Afterward, the optimal volume was precisely diluted with the mobile phase to achieve a concentration of 75 µg/ml, which was then then injected into the chromatographic system.

Photolytic degradation

To thoroughly examine the impact of UV light, we strategically placed 50 mg of EFZ on a watch glass and subjected it to direct sunlight for a full 12 h. Following this exposure, the drug was meticulously dissolved and diluted to an exact concentration of 75 µg/ml, which was then injected into the liquid chromatographic system for detailed analysis.

Thermal degradation

To investigate the impact of temperature on EFZ, we measured 50 mg of the substance on a watch glass and subjected it to a controlled environment in a hot air oven set at 80 °C for a precise duration of 3 h. After this heating period, the sample was allowed to cool to room temperature, ensuring an accurate assessment of its properties. Following this, we prepared a dilution to achieve a targeted concentration of 75 µg/ml, which was then injected into a reverse-phase high-performance liquid chromatography (RP-HPLC) system for thorough analysis.

Analytical method validation

The proposed technique was validated following ICH-Q2B guidelines. Different validation parameters were used to validate the method [11].

Linearity and Range

The linearity of an analytical method is a crucial attribute that ensures results are directly proportional to the concentration of analytes within a specified range. This property enables precise measurements, either instantly or through well-defined mathematical adjustments. In our study utilizing UV methods, we rigorously determined linearity by employing a minimum of five distinct concentrations of the standard stock solution of EFZ, spanning from 100 to 500 µg/ml. We plotted the concentrations against their corresponding absorbance values, providing a clear demonstration of the method's linearity and reliability. Similarly, in the RP-HPLC method, we prepared five different concentrations of EFZ from the standard stock solution, ranging from 25 to 125 µg/ml. These samples were injected into the HPLC system, further validating the robustness and precision of our analytical approach.

Selectivity

A robust comparison-based approach was implemented to guarantee the absence of any interference and to firmly establish the selectivity of the method. This was achieved through the thorough analysis of chromatograms from both blank and placebo samples, ensuring our results are reliable and precise.

Accuracy

Accuracy is a crucial indicator of the precision of an analytical procedure. In this study, we evaluated accuracy at three distinct concentration levels: 80%, 100%, and 120%. By comparing the measured concentrations to the concentrations that were intentionally added, we calculated the percentage of recovery. This robust analysis not only highlights the efficiency of the method but also demonstrates the reliability of substance recovery by examining the correlation between the amounts added and measured.

Precision

Precision is a critical measure of reliability in analytical methods, reflecting the consistency of results when the same method is applied to multiple samplings of a homogeneous sample. It is categorized into two key types: intraday and interday precision. Intraday precision, determined by calculating the relative standard deviation (RSD) of results obtained within a single day, underscores the method's reliability on a short-term basis. In contrast, interday precision evaluates the RSD of results gathered over multiple days, highlighting the method's consistency over time.

Sensitivity

The sensitivity of a method is evaluated by determining the limits of detection (LOD) and quantification (LOQ). LOD refers to the smallest amount of analyte that can be reliably identified in a sample, while LOQ indicates the smallest amount of analyte that can be measured accurately and precisely. Following the comprehensive guidelines set forth by the International Conference on Harmonisation (ICH), we determined the LOD and LOQ using robust statistical methods. By analyzing

the variation in response and the slope of the corresponding standardization curve, we established the LOD and LOQ values. This process allows us to quantify the method's sensitivity for detecting and measuring analytes in various samples.

σ = standard variance (deviation)

$$LOD = 3.3*\sigma/ Slope$$

$$LOQ = 10*\sigma/ Slope$$

Ruggedness

Ruggedness is defined as the reliability of test results across different laboratories and analysts under standard conditions. This study compellingly demonstrates the determination of EFZ using innovative methods, carried out by two distinct analysts within comparable laboratory settings. This approach not only underscores the robustness of the findings but also highlights the reproducibility essential for trustworthy results in scientific research.

Robustness

The robustness of the analytical technique was rigorously assessed by deliberately adjusting the chromatographic parameters across a wide array of conditions. To thoroughly evaluate the stability of the developed method, we made precise modifications to critical parameters, including detection wavelength, flow rate, and pH. This comprehensive approach not only demonstrates the method's reliability but also underscores its adaptability in varied analytical scenarios.

System suitability

System suitability tests play a critical role in confirming that the chromatographic system delivers the necessary resolution and reproducibility for accurate analysis. In this instance, we conducted a rigorous system suitability test by injecting six replicates of a standard EFZ solution. We meticulously assessed essential parameters in the resulting chromatogram, including retention time, theoretical plates, and asymmetric factors, to ensure optimal performance and reliability of the method.

Results

Linearity

In this study, we employed zero-order and first-order derivative methods to prepare five serial dilutions of standard stock solutions of EFZ, ranging from 100 to 500 µg/ml. These dilutions allowed us to plot concentration versus absorbance graphs, clearly illustrating the relationship between the two. For the RP-HPLC method, we prepared five serial dilutions of the standard stock solution, ranging from 25 to 125 µg/ml, which were injected into the HPLC system. The resulting data demonstrated a strong linearity in the analytical method, as the peak area proved to be directly proportional to the analyte concentration in the sample. To quantify this relationship, we employed a regression line to calculate the regression coefficient (R^2), intercept, and slope, as summarized in Tables 1-3.

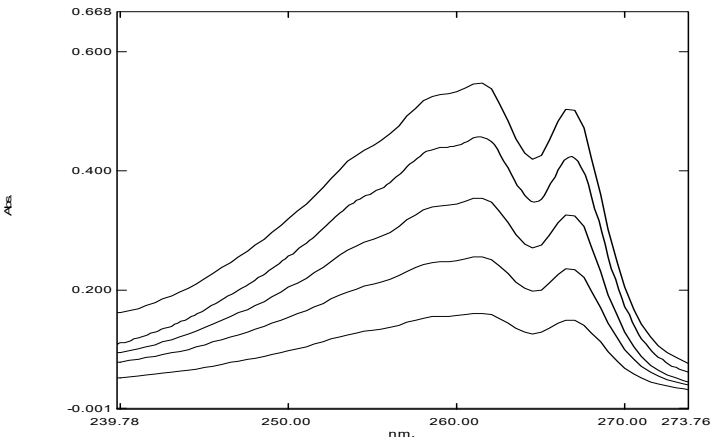

Figure 2. Zero order derivative spectrum of EFZ at 261 nm.

The UV overlay spectrum and calibration curves for both methods have been meticulously illustrated in Figures 2-5. Additionally, the RP-HPLC chromatogram and its corresponding calibration curves are presented in Figures 6-11, providing a clear visual

representation of our findings. The chromatograms of the blank and placebo samples further validate our results and are included in Figures 12 and 13. This comprehensive approach strengthens our conclusions and underscores the reliability of the methods employed.

Table 1. Result of calibration curve of EFZ at 261 nm by zero-order derivative method.

Concentration (µg/ml)	Absorbance	%RSD
100	0.162 ±0.001414	0.8729
200	0.2555±0.002168	0.8485
300	0.3536 ±0.001211	0.3401
400	0.4565 ±0.001871	0.4098
500	0.546 ±0.003162	0.5791

Mean ± Std. Deviation (n=6)

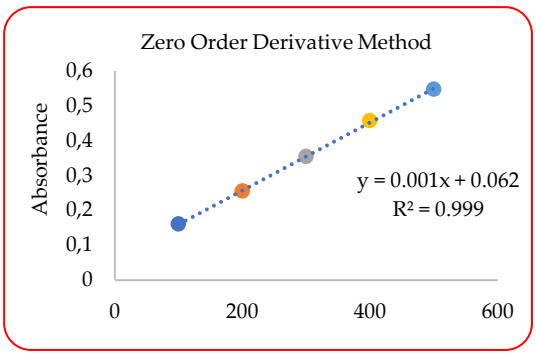

Figure 3. Calibration curve of EFZ at 261 nm by Zero order derivative method.

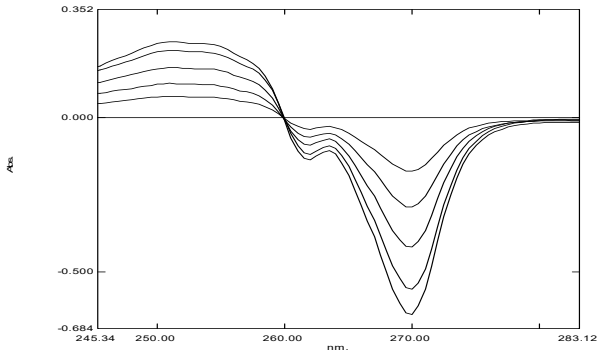

Figure 4. First-order derivative spectrum of EFZ at 270 nm.

Table 2. Result of calibration curve of EFZ at 270 nm by first-order derivative method.

Concentration (µg/ml)	Absorbance	%RSD
100	0.1718±0.001472	0.8566
200	0.2926±0.00216	0.7381
300	0.383±0.00253	0.6605
400	0.5036±0.004412	0.8759
500	0.5942±0.003061	0.5151

Mean ± Std. Deviation(n=6)

Table 3. Result of calibration curve of EFZ at 210 nm by RP-HPLC method.

Sl. No	Concentration (µg/ml)	%RSD
1	25	1765552
2	50	2663496
3	75	3598542
4	100	4612566
5	125	5715044

(n=6)

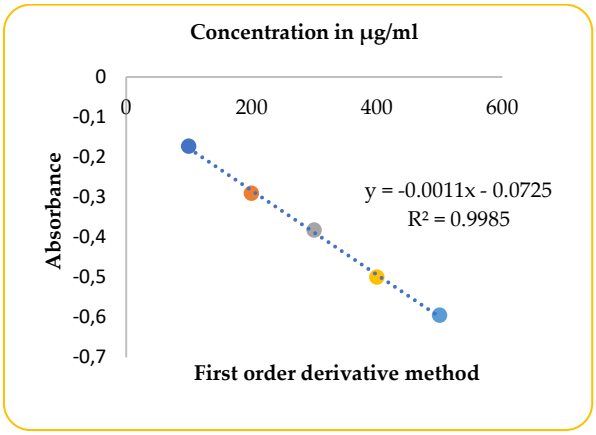

Figure 5. Calibration curve of EFZ at 270 nm by first-order derivative method.

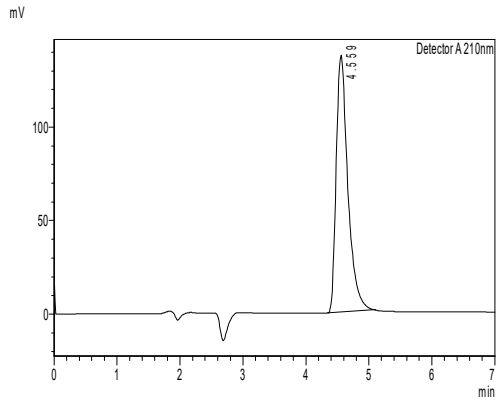

Figure 6. Chromatogram of 25 mcg EFZ at 210 nm.

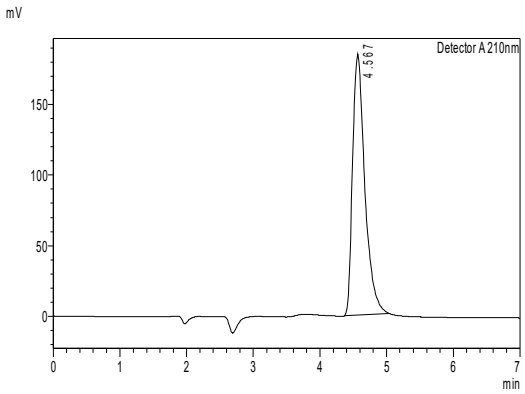

Figure 7. Chromatogram of 50 mcg EFZ at 210 nm.

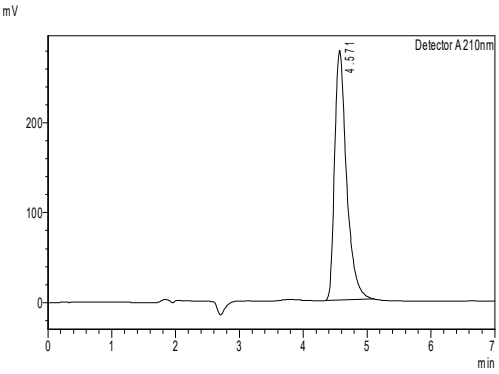

Figure 8. Chromatogram of 75 mcg EFZ at 210 nm.

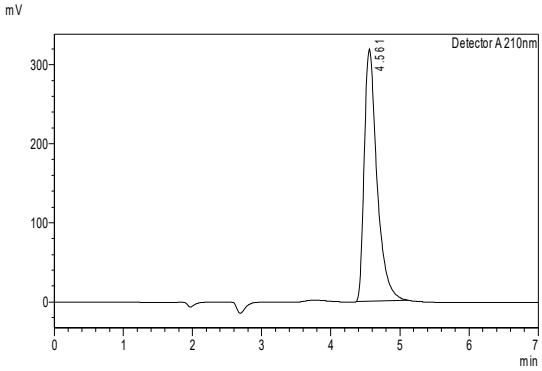

Figure 9. Chromatogram of 100 mcg EFZ at 210 nm.

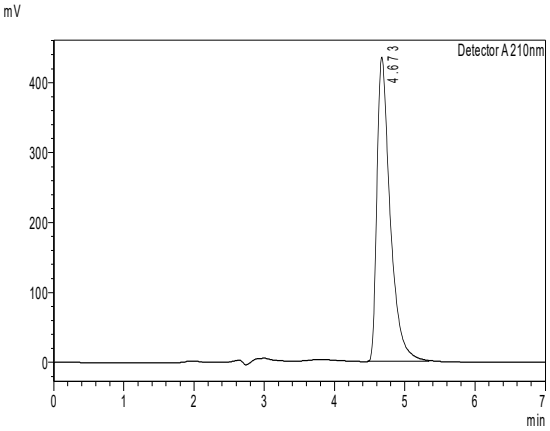

Figure 10. Chromatogram of 125 mcg EFZ at 210 nm.

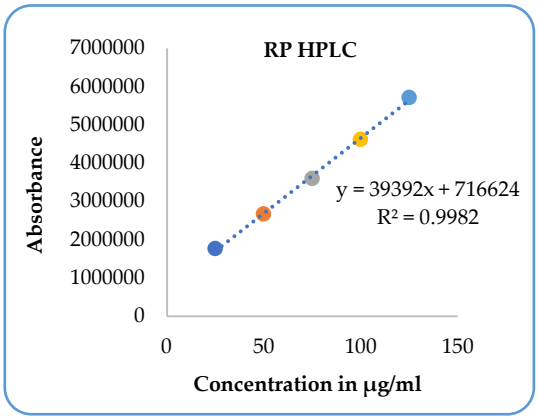

Figure 11. Calibration curve for EFZ at 210 nm by RP-HPLC method.

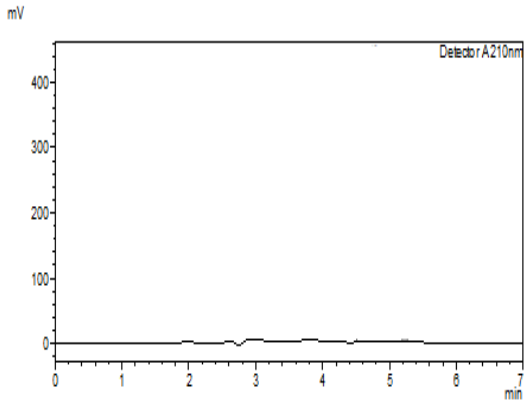

Figure 12. Chromatogram of Blank.

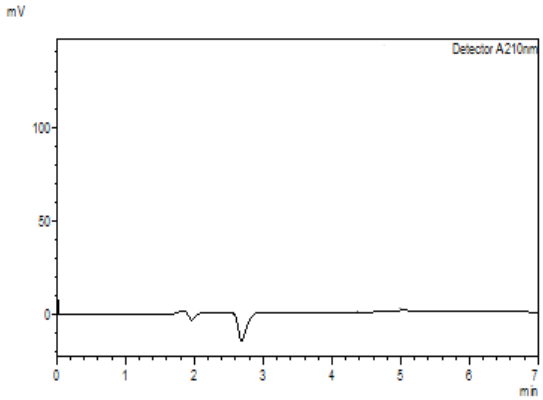

Figure 13. Chromatogram of Placebo.

Accuracy

Accuracy is rigorously evaluated across three distinct concentration levels, with results expressed as percentage recovery, standard deviation, and relative standard deviation (RSD) (Table 4-6). The compelling statistical validation data for accuracy determination using UV and RP-HPLC methods are comprehensively outlined in Table 7, underscoring the reliability of these analytical techniques.

Table 4. Accuracy of EFZ for zero order derivative method.

Level of % recovery	Amount present (µg/ml)	Amount of standard drug added (µg/ml)	Total amount recovered (µg/ml)	% Recovery
80%	200	160	358.2	99.5
	200	160	356.9	99.1
	200	160	359	99.72
100%	200	200	399.6	99.9
	200	200	400	100
	200	200	401.5	100.3
120%	200	240	438	99.5
	200	240	442	100.4
	200	240	446	101.36

Table 5. Accuracy of EFZ for first order derivative method.

Level of % recovery	Amount present (µg/ml)	Amount of standard drug added (µg/ml)	Total amount recovered (µg/ml)	% Recovery
80%	100	80	178.9	99.38
	100	80	181	100.5
	100	80	182.4	101.3
100%	100	100	201.3	100.6
	100	100	200	100
	100	100	198	99
120%	100	120	222	100.9
	100	120	219.2	99.6
	100	120	218	99.09

Table 6. Accuracy of EFZ for RP-HPLC method.

Level of % recovery	Amount present (µg/ml)	Amount of standard drug added (µg/ml)	Total amount recovered (µg/ml)	% Recovery
80%	50	40	89.1	99
	50	40	91.2	101.3
	50	40	91.5	101.5
100%	50	50	100.5	100.5
	50	50	99.7	99.7
	50	50	101.2	101.2
120%	50	60	111	100.9
	50	60	109.6	99.63
	50	60	110	100

Table 7. Statistical validation data for accuracy determination.

Level of % recovery	Level of % recovery	Mean*	Standard Deviation*	Co-efficient of Variation*	Standard Error*
Zero order derivative	80%	99.44	0.3143	0.0031	0.1815
	100%	100.06	0.2081	0.0021	0.1202
	120%	100.42	0.9301	0.0093	0.5370
First order derivative	80%	100.39	0.9644	0.0096	0.3936
	100%	99.86	0.8083	0.0081	0.3299
	120%	100.22	0.9853	0.0098	0.4022
RP-HPLC	80%	100.08	0.3011	0.0030	0.1228
	100%	100.02	0.1755	0.0017	0.0716
	120%	99.8	0.1915	0.0019	0.0782

*n = 3

Evaluation of the commercially available formulation

The observed wavelengths from both the UV methods and the retention times of the drug samples, as extracted from the chromatograms, remained completely consistent. This consistency compellingly demonstrates that there are no significant interactions between the drug and the additional components present in the commercial formulations. Furthermore, the calculated percent relative standard deviation (RSD) value serves as a robust quantitative measure of the method's precision, effectively confirming its suitability for the essential testing of EFZ in marketed preparations. These results not only bolster the reliability and validity of the analytical approach but also decisively affirm its practical application in the quality control and assessment of EFZ-containing products available on the market. The data is systematically presented in Tables 8 to 10.

Precision

When multiple samples of a homogeneous material were collected under controlled conditions, we defined the precision of our method as the consistency of the measurements obtained. To quantify this precision, we utilized the relative standard deviation (RSD), which is a widely recognized metric in the field. Impressively, the percent RSD value for the precision results of EFZ was well within the acceptable limits, demonstrating the reliability of our method. Detailed statistical validation data supporting these precision results can be found in Table 11.

Table 8. Assay results of marketed formulation by UV-Spectrophotometric methods.

SI. No.	Zero order derivative method			First order derivative method		
	Amount present (mg/ml)	**Amount obtained (mg/ml)**	**Amount obtained (%)**	**Amount present (mg/ml)**	**Amount obtained (mg/ml)**	**Amount obtained (%)**
1	100	99.6	99.6	100	100.9	100.9
2	100	100.05	100.05	100	100.10	100.10
3	100	99.5	99.5	100	99.8	99.8
4	100	99.3	99.3	100	99.7	99.7
5	100	99.8	99.8	100	99.05	99.05
6	100	100.01	100.01	100	99.04	99.04

Table 9. Assay results of marketed formulation by RP-HPLC method.

SI. No.	Amount present (mg/ml)	Amount obtained (mg/ml)	Amount obtained (%)
1	100	99.09	99.09
2	100	100.7	100.7
3	100	99.5	99.5
4	100	100.12	100.12
5	100	100.67	100.67
6	100	100.33	100.33

Table 10. Statistical validation data for marketed formulation.

Method	Components	Mean*	Standard Deviation*	Co-efficient of Variation*	Standard Error*
Zero order	EFZ	99.71	0.0941	0.00094	0.0385
First order	EFZ	99.76	0.1091	0.0011	0.0447
RP-HPLC	EFZ	100.06	0.1019	0.0010	0.0417

*n = 6

Table 11. Statistical validation data for precision.

Method	Components	Precision	Mean*	Standard Deviation*	Co-efficient of Variation*	Standard Error*
Zero order		Intra day	100.05	0.3385	0.0033	0.1387
		Inter day	100.03	0.1255	0.0012	0.0515
First order		Intra day	100.15	0.6606	0.0066	0.2707
	EFZ	Inter day	100.13	0.6196	0.0062	0.2539
RP-HPLC		Intra day	100.03	0.5804	0.0058	0.2370
		Inter day	100.24	0.4939	0.0049	0.2017

*n = 6

Ruggedness

Ruggedness is a critical parameter that assesses the reliability of results in the face of varying external factors, including analysts, laboratories, instruments, reagents, and testing days. By employing a standard solution of EFZ with two different analysts, we rigorously evaluate this parameter. The findings regarding ruggedness are summarized in Table 12, showcasing the consistency and robustness of our results.

Robustness

To rigorously assess the stability of the technique, a thorough study was undertaken, incorporating strategic modifications to essential parameters, specifically flow rate and detection wavelength. The flow rate was intentionally varied to 1.8 ml/min and 2.2 ml/min, with a standard flow rate of 2.0 ml/min. Similarly, the detection wavelength was adjusted to 208 nm and 212 nm, while the operational wavelength remained at an optimal 210 nm. The results of this robustness evaluation, highlighting the technique's reliability, are meticulously detailed in Table 13.

Table 12. Ruggedness results for variations in analyst.

Method Parameter	Retention Time*	Tailing factor*
Analyst	4.559	1.458
Analyst 01	4.557	1.456
Analyst 02	4.561	1.460

*n = 6

Table 13. Robustness result for variations in flow rate and wavelength (nm).

Method Parameter - Flow Rate				
(ml/min)	**Level**	**Retention Time**	**Tailing factor**	**% RSD**
1.8	-0.2	4.557	1.456	0.0310
2.0	0	4.559	1.458	0
2.2	+0.2	4.561	1.460	0.0310
Method parameter- Wavelength				
Detection (nm)	**Level**	**Retention time**	**Tailing factor**	**% RSD**
208	-0.2	4.569	1.456	0.1549
210	0	4.571	1.458	0
212	+0.2	4.573	1.460	0.2168

System suitability parameters

The thorough evaluation of system suitability parameters for all three methods—two UV methods and one RP-HPLC method-is detailed in Tables 14 and 15. The results clearly demonstrate that the developed system exhibits exceptional precision, reproducibility, and suitability for its intended analytical purpose, establishing a robust foundation for further sample analysis.

Table 14. Statistical data of EFZ by UV-Spectrophotometric methods.

Parameter	Zero order derivative	Parameter
Wavelength (nm)	261	270
Linear range (μg/ml)	100-500	100-500
Molar absorptivity (liter, $mole^{-1}$ $cm^{-1)}$	0.0012	0.0014
Slope (m)	0.001	0.001
Intercept (c)	0.062	0.072
Correlation co-efficient (r^2)	0.999	0.998
Range of % RSD	0.2239 – 1.5886	0.8566 – 0.5151
Limit of Detection (μg/ml)	4.6669	4.8574
Limit of Quantitation (μg/ml)	14.1421	14.7196

Table 15. Statistical data of EFZ at 210 nm by RP–HPLC method.

Parameter	RP–HPLC method
Wavelength (nm)	210
Linear range (µg/ml)	25-125
Slope (m)	39392
Intercept (c)	716624
Correlation co-efficient (r^2)	0.998
Limit of Detection (µg/ml)	1.69
Limit of Quantitation (µg/ml)	5.15
Retention time (min)	4.571
Tailing factor	1.458
Theoretical plate	3068

Degradation studies

The acidic degradation analysis revealed the formation of two significant deterioration products, appearing at retention times of 4.158 min and 4.998 min. Notably, no peak was detected at the standard retention time, decisively indicating that complete degradation occurred during the acid hydrolysis process. The representative chromatogram showcasing the acid degradation of EFZ is presented in Figure 14, further illustrating the robustness of these findings.

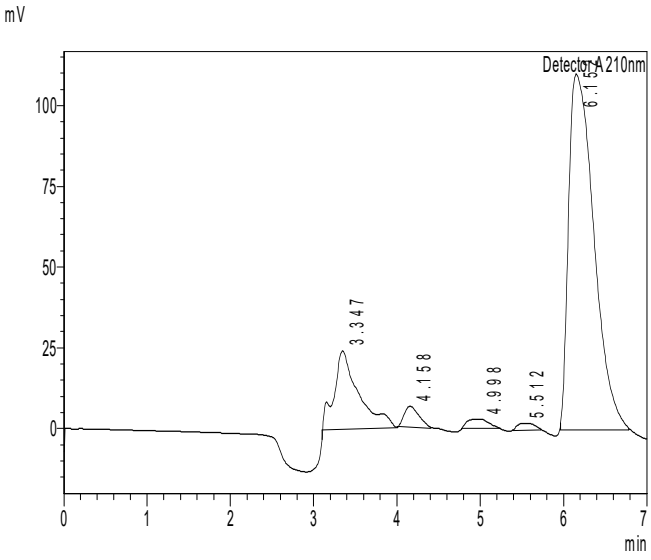

Figure 14. Representative chromatogram of acid-degraded EFZ.

On alkali degradation, the result states high degradation of EFZ. However, in base hydrolysis, it has the same RT with 66.53% deterioration. The chromatographic picture of EFZ is furnished in Figure 15.

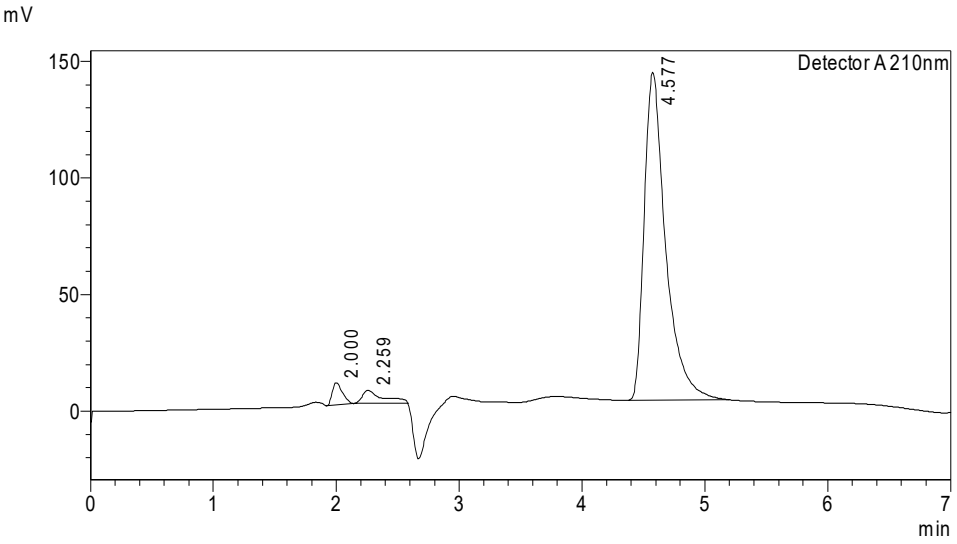

Figure 15. Representative chromatogram of alkali-degraded EFZ.

In oxidative degradation, an impressive 98.14% complete degradation is achieved, leading to the formation of a degradation product at 4.5 min that closely resembles the standard EFZ. The accompanying chromatogram in Figure 16 vividly illustrates this remarkable transformation.

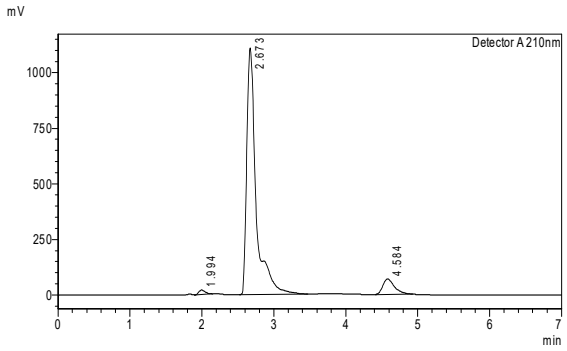

Figure 16. Representative chromatogram of peroxide-mediated degraded EFZ.

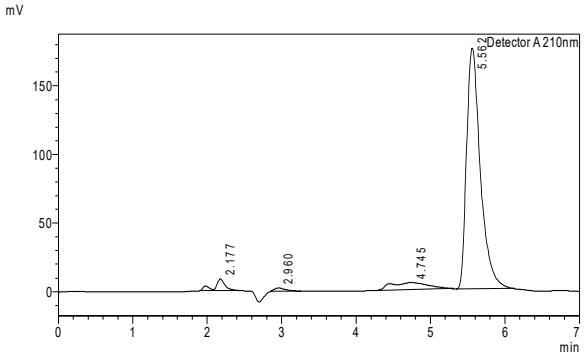

Figure 17. Representative chromatogram of photolytically degraded EFZ.

The results of the photolysis experiment revealed a striking degradation of EFZ at 4.7 min, with an impressive degradation rate of 81.78%. This significant finding is illustrated in Figure 17, underscoring the rapid breakdown of EFZ under the tested conditions.

The results of the thermal degradation study revealed significant degradation of EFZ, with a notable 46.78% reduction observed after just 4.7 min of thermal exposure. This highlights the compound's vulnerability to thermal conditions. For a clear illustration of these findings, please refer to the chromatogram presented in Figure 18.

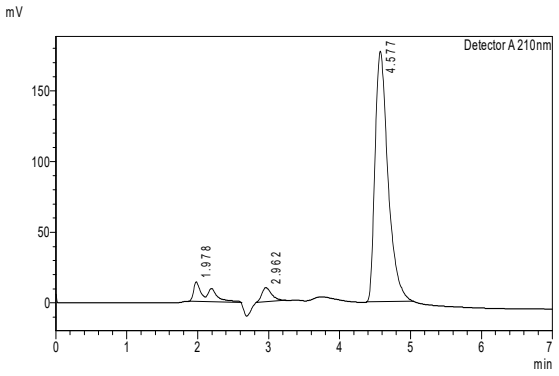

Figure 18. Representative chromatogram of thermally degraded EFZ.

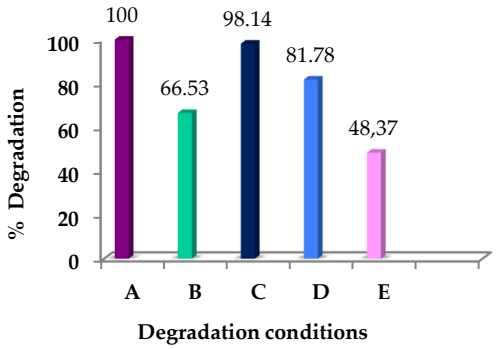

Figure 19. Bar graph of Percentage degradation.

The percentage degradation data of EFZ, subjected to various degradation conditions, is thoroughly presented in Table 16. Complementing this data, Figure 19 offers a clear and impactful bar graph illustrating the percentage degradation, highlighting the significant effects of these conditions.

Table 16. Percentage degradation of EFZ and its peak purity index.

Sr. No.	Degradation condition	Percentage of degradation	Percentage purity of EFZ	Remarks
A	Acid 0.1 N Hcl / 80 °C / 3 h	100 %	No peak was observed at its RT	Complete degradation
B	Base 0.1 N NaOH / 80 °C / 3 h	66.53 %	33.46 %	High degradation
C	Oxidation / 3% H 2O2/ 80 °C / 3 h	98.14 %	1.85 %	Complete degradation
D	Sunlight / 12 h	81.78 %	18.21 %	High degradation
E	Thermal / 80 °C / 3 h	48.37 %	51.62 %	Moderate degradation

Discussion

All the methods developed for the determination of EFZ have undergone rigorous validation, demonstrating clarity, cost-effectiveness, and exceptional precision. The robustness analysis confirmed

that the retention time, theoretical plates, and tailing factor all fall well within satisfactory limits. Furthermore, the results from accelerated stability studies provide a comprehensive evaluation of EFZ stability under a range of ICH-recommended conditions. As a result, this study presents significant advantages over existing methodologies. In previously established methods, acetonitrile was used as the solvent, presenting challenges due to its higher cost and the complex procedures required for preparing the mobile phase. In sharp contrast, our innovative method utilizes methanol, which not only reduces costs but also simplifies the preparation process. Remarkably, our developed method achieves a retention time of under 5 min, with an overall run time of just 7 min at a wavelength of 210 nm, across a concentration range of 25 to 125 μg/ml. This efficiency makes it both rapid and reliable, far surpassing the previous method, which required 15 min of runtime and a retention time of 5.5 min over a broader concentration range of 50 to 150 μg/ml. Moreover, our study thoroughly investigated degradation under five different stress conditions, reinforcing the robustness and reliability of the developed analytical method. The proposed UV spectrophotometric techniques-specifically, the zero-order derivative and first-order derivative methods are not only straightforward but also remarkably efficient and economical. Fully validated in accordance with ICH guidelines, these methods stand out as superior options for estimating EFZ, providing exceptional recovery, precision, and linearity.

Conclusion

The results from the UV-spectrophotometric and stability-indicating HPLC techniques for determining EFZ in bulk drugs and pharmaceutical formulations are truly impressive! These methods are not only exceptionally reliable and sensitive, but they also deliver outstanding accuracy and precision. Our recovery experiments yielded remarkable results, with recoveries nearing 100%. The percentage RSD for precision is comfortably within acceptable limits, reinforcing the trustworthiness of these techniques. What's more, the RP-HPLC method excels in providing excellent resolution in an impressively quick analysis time, making it incredibly efficient. Its straightforward design ensures that even laboratories without access to complex instruments like LC-NMR and GC-MS can implement it effectively. These developed UV and stability-indicating HPLC techniques are truly invaluable for routine quality control of EFZ in bulk drugs and pharmaceutical formulations. They guarantee accuracy and reliability, empowering professionals across the industry to enhance their work with confidence.

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Authors contribution

All the authors have contributed equally.

Declaration of interest

The authors declare no conflict of interest.

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