A QBD BASED RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF KETOCONAZOLE

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ABSTRACT
A stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the quantitative estimation of ketoconazole in bulk drug and pharmaceutical dosage forms using the Analytical Quality by Design (AQbD) framework. Critical method parameters were identified through risk assessment and optimized via Design of Experiments (DoE), ensuring robustness, reliability, and regulatory compliance. Chromatographic separation was achieved on an Agilent Zorbax SB-Aq column (250 × 4.6 mm, 5 µm) employing a mobile phase of phosphate buffer and acetonitrile (35:65, v/v) at a flow rate of 0.8 mL/min, with detection at 225 nm. Ketoconazole eluted at approximately 5.47 min within a total runtime of 15 min.
Validation, performed in accordance with ICH Q2(R1) guidelines, confirmed excellent specificity, linearity, precision, accuracy, robustness, sensitivity, and reproducibility. The calibration curve was linear across 80–120 µg/mL with a correlation coefficient (R^2) of 1.000. Precision studies yielded %RSD values below 2%, while recovery ranged between 99.75% and 100.07%, indicating high accuracy. Limits of detection and quantification were 0.31 µg/mL and 0.94 µg/mL, respectively. Robustness evaluation revealed negligible effects of minor variations in pH and detection wavelength.
Forced degradation under acidic, alkaline, oxidative, thermal, and photolytic conditions demonstrated the method's stability-indicating capability. Maximum degradation was observed under oxidative stress (16.14%), followed by thermal (9.34%), photolytic (8.97%), alkaline (6.71%), and acidic (6.09%) conditions, with degradation products well resolved from the analyte peak. The AQbD-based RP-HPLC method is rapid, accurate, economical, and highly robust, making it suitable for routine quality control, stability testing, and regulatory applications in ketoconazole analysis.
Keywords: Ketoconazole; Analytical Quality by Design; RP-HPLC; Design of Experiments; Method Validation; ICH Q2(R1); Stability-Indicating Method; Forced Degradation; Pharmaceutical Analysis; Quality Control.
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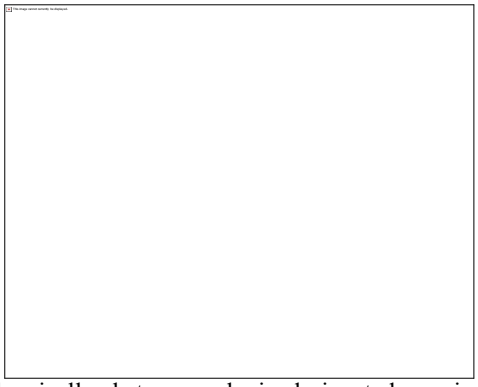
1. INTRODUCTION

Ketoconazole is a synthetic imidazole antifungal agent with broad-spectrum activity against dermatophytes, yeasts, and other pathogenic fungi. Its primary mechanism of action involves inhibition of the cytochrome P450-dependent enzyme lanosterol 14-α-demethylase (CYP51), thereby blocking ergosterol biosynthesis, an essential component of fungal cell membranes. Ergosterol depletion results in altered membrane permeability, leakage of intracellular constituents, and eventual fungal cell death. Consequently, ketoconazole has been widely formulated into tablets, creams, gels, shampoos, ointments, and topical foams for the treatment of candidiasis, dermatophytosis, pityriasis versicolor, seborrheic dermatitis, and dandruff [1–3].



Chemically, ketoconazole is designated as cis-1-acetyl-4-[4-{2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl}methoxyphenyl] piperazine, with a molecular formula of $C_{26}H_{28}Cl_2N_4O_4$ and molecular weight of 531.44 g/mol. It is practically insoluble in water but

exhibits improved solubility under acidic conditions due to protonation of the imidazole ring. The drug possesses pKa values of 2.94 and 6.51, and its chromatographic behaviour is strongly influenced by mobile phase composition and pH, necessitating careful optimization for reliable quantification [4,5].

Analytical methods are indispensable in pharmaceutical development for assay, impurity profiling, dissolution testing, stability evaluation, and routine quality control. Reverse-phase high-performance liquid chromatography (RP-HPLC) remains the technique of choice due to its sensitivity, specificity, reproducibility, and ability to resolve analytes from degradation products and excipients. Several RP-HPLC methods have been reported for ketoconazole estimation in biological matrices and pharmaceutical formulations; however, many suffer from extended run times, complex mobile phases, inadequate robustness, or limited stability-indicating capability [6–9].

Regulatory agencies such as the International Council for Harmonisation (ICH), United States Food and Drug Administration (USFDA), and European Medicines Agency (EMA) emphasize the need for scientifically sound, robust, and lifecycle-compliant analytical procedures. Conventional "One Factor at a Time" (OFAT) approaches often fail to capture variable interactions, resulting in methods with poor robustness. In contrast, Analytical Quality by Design (AQbD) provides a systematic framework involving definition of the Analytical Target Profile (ATP), risk assessment, identification of Critical Method Parameters (CMPs), evaluation of Critical Analytical Attributes (CAAs), and optimization using Design of Experiments (DoE). This strategy establishes a design space that ensures method reliability under minor variations [10–14].

Stability-indicating methods, as mandated by ICH Q1A(R2), require forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic conditions to confirm specificity and resolve degradation products. Despite existing reports, few studies have integrated AQbD principles with comprehensive forced degradation and full validation per ICH Q2(R1). Therefore, the present investigation was undertaken to develop and validate an AQbD-based, stability-indicating RP-HPLC method for ketoconazole quantification in bulk drug and pharmaceutical formulations.

The present study is expected to provide a scientifically optimized and regulatory-compliant analytical method for the estimation of ketoconazole using the Analytical Quality by Design (AQbD) approach. By integrating systematic risk assessment, Design of Experiments (DoE), and comprehensive validation in accordance with ICH Q2(R1) guidelines, the developed RP-HPLC method aims to achieve high specificity, precision, accuracy, sensitivity, robustness, and reproducibility while ensuring efficient separation of ketoconazole from its degradation products under various stress conditions. The optimized analytical method is anticipated to reduce method variability, improve lifecycle management, and provide a rapid, reliable, and stability-indicating procedure suitable for routine quality control, stability studies, dissolution testing, and regulatory evaluation of ketoconazole in bulk drug substances and pharmaceutical dosage forms.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Ketoconazole reference standard (purity ≥99.5%) was kindly supplied as a gift sample by Yarrow Chem Products Pvt. Ltd., Mumbai, India. HPLC-grade acetonitrile and potassium dihydrogen phosphate (KH_2PO_4) were procured from Qualigens, Thermo Fisher Scientific India Pvt. Ltd., Mumbai, India. Potassium hydroxide, hydrochloric acid, sodium hydroxide, and hydrogen peroxide (30%, v/v) of analytical reagent (AR) grade were obtained from Thomas Baker Chemicals Pvt. Ltd., Mumbai, India. Ultrapure water was prepared using an in-house Milli-Q purification system (Millipore, USA). All solvents and chemicals used throughout the study were of HPLC or analytical reagent grade [15,16].

2.2 Instrumentation

Chromatographic analysis was performed using an Agilent 1260 Infinity II High-Performance Liquid Chromatography (HPLC) system (Agilent Technologies, USA) equipped with a quaternary gradient pump (G7111A), online degasser, autosampler (G7129A), thermostatted column compartment, and diode array detector (DAD, G7115A). Data acquisition and processing were carried out using Agilent OpenLAB CDS software. Chromatographic separation was achieved on an Agilent Zorbax SB-Aq C18 analytical column (250 × 4.6 mm, 5 µm particle size). An analytical balance (Shimadzu, Japan), digital pH meter (Mettler Toledo, Switzerland), ultrasonic bath, membrane filtration assembly (0.45 µm nylon filters), hot air oven, UV photostability chamber, and laboratory centrifuge were used during the investigation [17].

2.3 Analytical Quality by Design (AQbD) Strategy

The analytical method was developed following the principles of Analytical Quality by Design (AQbD). The Analytical Target Profile (ATP) was established to develop a rapid, precise, accurate, stability-indicating RP-HPLC method suitable for routine quality control analysis of ketoconazole in bulk drug and pharmaceutical formulations. Risk assessment identified Critical Method Parameters (CMPs) affecting Critical Analytical Attributes (CAAs). CMPs included mobile phase composition, buffer strength, pH of the aqueous phase, and flow rate, while retention time, theoretical plate count, peak

symmetry, and peak purity were considered CAAs. Design of Experiments (DoE) methodology was employed using Design-Expert® software (Stat-Ease Inc., USA) to evaluate variable interactions. Response surface methodology was utilized to optimize chromatographic conditions and establish a robust analytical design space [18–20].

2.4 Preparation of Buffer Solution

Phosphate buffer was prepared by dissolving potassium dihydrogen phosphate in purified water. The pH was adjusted to 8.0 ± 0.05 using potassium hydroxide solution, filtered through a 0.45 µm membrane, and sonicated for degassing [21].

2.5 Preparation of Standard Solution

Accurately weighed 10 mg of ketoconazole reference standard was dissolved in acetonitrile to prepare a primary stock solution (1000 µg/mL). A working standard solution (100 µg/mL) was prepared by diluting 1.0 mL of stock to 10 mL with mobile phase [22].

2.6 Selection of Detection Wavelength

The working standard solution was scanned between 190–400 nm using the DAD detector. Based on maximum absorbance and minimal baseline interference, 225 nm was selected as the optimum analytical wavelength [23].

2.7 Chromatographic Conditions

Chromatography was performed on an Agilent Zorbax SB-Aq column (250 × 4.6 mm, 5 µm) at 30°C. The mobile phase consisted of phosphate buffer and acetonitrile (35:65, v/v), filtered and degassed prior to use. Flow rate was 0.8 mL/min, injection volume 10 µL, detection wavelength 225 nm, and total run time 15 min. Ketoconazole eluted at ~5.47 min [24].

2.8 Method Validation

Validation was performed according to ICH Q2(R1) guidelines [25].

System suitability: Six replicate injections of 100 µg/mL standard were analyzed for retention time, theoretical plates, peak asymmetry, and %RSD [26].

Specificity: Chromatograms of blank, mobile phase, placebo, and ketoconazole standard were compared to confirm absence of interference [27].

Linearity: Calibration standards (80–120 µg/mL) were injected in triplicate; regression analysis confirmed linearity [28].

Accuracy: Recovery studies at 80%, 100%, and 120% levels were performed in triplicate [29].

Precision: Intra-day and inter-day precision were assessed with replicate injections [30].

LOD/LOQ: Determined based on signal-to-noise ratios of 3:1 and 10:1 [31].

Robustness: Evaluated by deliberate variations in buffer pH (7.9–8.1) and wavelength (223–227 nm) [32].

2.9 Forced Degradation Studies

Stability-indicating capability was assessed per ICH Q1A(R2) [33]. Ketoconazole was subjected to:

Acidic degradation: 0.1 N HCl treatment.

Alkaline degradation: 0.1 N NaOH treatment.

Oxidative degradation: Exposure to 30% H_2O_2.

Thermal degradation: Heating at 120°C for 3 h.

Photolytic degradation: UV exposure at 254 nm for 5 h.

Samples were neutralized, diluted, filtered, and analyzed. Degradation percentage and peak purity confirmed stability-indicating nature [34–36].

2.10 Statistical Analysis

Data were analysed using Design-Expert® software for DoE and Microsoft Excel® for statistical calculations. Acceptance criteria were established according to ICH Q2(R1) [25].

3. RESULTS AND DISCUSSION

3.1 Analytical Quality by Design (AQbD)-Based Method Development and Optimization

Analytical Quality by Design (AQbD) principles were employed to develop a robust, reliable, and reproducible RP-HPLC method for the determination of ketoconazole. Unlike the conventional trial-and-error approach, AQbD emphasizes systematic understanding of the analytical process through risk assessment and statistically designed experiments. Initially, the Analytical Target Profile (ATP) was established to obtain a rapid, stability-indicating chromatographic method with acceptable system suitability, excellent peak symmetry, short retention time, high theoretical plate count, and reproducible analytical performance.

A preliminary risk assessment was performed to identify the Critical Method Parameters (CMPs) that could significantly influence the Critical Analytical Attributes (CAAs). Mobile phase composition, buffer strength, pH of the aqueous phase, and flow rate were identified as high-risk variables affecting chromatographic separation, whereas column temperature, detection wavelength, and injection volume exhibited comparatively lower risk when maintained within controlled limits.

The identified CMPs were optimized using Design of Experiments (DoE) through Design-Expert® software. Experimental responses included retention time, theoretical plates, peak asymmetry, and peak purity. Statistical evaluation demonstrated that buffer strength and flow rate exerted the greatest influence on chromatographic performance, whereas mobile phase composition moderately affected retention behaviour. The generated

response surface and contour plots facilitated identification of the optimum chromatographic region where all analytical responses simultaneously satisfied the predefined ATP.

The optimized chromatographic conditions consisted of an Agilent Zorbax SB-Aq (250 × 4.6 mm, 5 µm) analytical column with a mobile phase comprising phosphate buffer and acetonitrile (35:65, v/v), delivered at a flow rate of 0.8 mL min^{-1} with UV detection at 225 nm. These conditions provided excellent chromatographic separation with a symmetrical ketoconazole peak, minimal peak tailing, and a retention time of approximately 5.47 min.

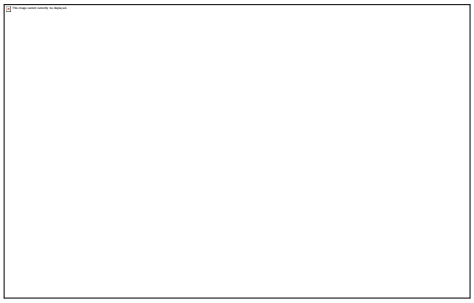


Figure 1. Design of Experiments (DoE) optimization profile for ketoconazole.

The desirability function generated by the software indicated that the optimized conditions were well within the analytical design space, confirming robustness and minimizing the likelihood of method failure during routine quality control analysis.

3.2 Chromatographic Method Optimization

Several chromatographic variables were systematically investigated during method development to achieve adequate retention, excellent peak symmetry, and acceptable system suitability. Different stationary phases, mobile phase compositions, buffer strengths, pH values, and flow rates were evaluated.

Initially, chromatographic separation was attempted using different proportions of phosphate buffer and acetonitrile. Lower organic solvent concentrations resulted in prolonged retention times, whereas higher acetonitrile content produced insufficient separation and broader chromatographic peaks. Optimization through AQbD established that a mobile phase consisting of phosphate buffer and acetonitrile (35:65, v/v) produced optimum chromatographic performance.

Similarly, buffer pH significantly influenced peak symmetry because ketoconazole is a weakly basic compound with pKa values near physiological pH. Adjustment of buffer pH to approximately 8.0 minimized secondary interactions with residual silanol groups on the stationary phase and significantly improved peak shape.

The optimized chromatographic conditions produced a sharp, symmetrical ketoconazole peak with baseline resolution and excellent reproducibility. No interfering peaks originating from the diluent, mobile phase, or excipients were observed, demonstrating adequate specificity of the developed analytical procedure.

Chromatographic Characteristics

Parameter	Optimized Condition
Column	Agilent Zorbax SB-Aq (250 × 4.6 mm, 5 µm)
Mobile phase	Phosphate buffer : Acetonitrile (35:65, v/v)
Flow rate	0.8 mL min^{-1}
Detection wavelength	225 nm
Injection volume	10 µL
Column temperature	30°C
Run time	15 min
Retention time	5.47 min

The optimized method required less than six minutes for complete elution of ketoconazole while maintaining excellent chromatographic efficiency, making it highly suitable for routine pharmaceutical quality control laboratories.

3.3 Specificity

Specificity is one of the most critical validation parameters because it demonstrates the capability of an analytical method to accurately quantify the analyte in the presence of formulation excipients, degradation products, or other potential interferences.

Specificity was evaluated by injecting blank solution, working standard solution, and linearity samples under identical chromatographic conditions. The chromatograms revealed no interfering peaks at the retention time corresponding to ketoconazole (5.47 min). The baseline remained stable throughout the analytical run, and complete peak separation was achieved.

The overlay chromatogram further demonstrated identical retention characteristics across different concentration levels without alteration in peak shape or retention behaviour.

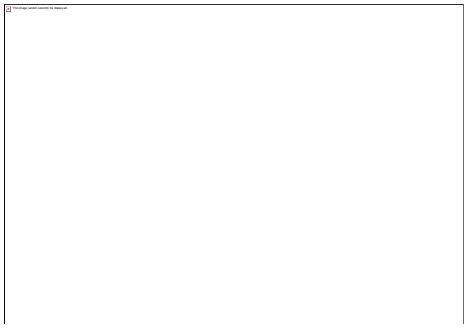


Figure 2. Representative chromatograms showing blank, ketoconazole standard, and overlay chromatograms.

The absence of interfering peaks confirmed excellent specificity of the developed RP-HPLC method. Furthermore, the diode array detector demonstrated satisfactory peak purity, indicating that the ketoconazole peak was free from co-eluting impurities or degradation products under optimized chromatographic conditions.

3.4 System Suitability

System suitability testing was performed before sample analysis to verify the performance of the chromatographic system. Six replicate injections of the ketoconazole working standard (100 µg mL^{-1}) were analysed.

The chromatographic system exhibited highly reproducible retention time, consistent peak area, excellent theoretical plate count, acceptable peak asymmetry, and satisfactory peak purity. The percentage relative standard deviation (%RSD) for peak area was significantly below the acceptance limit of 2%, indicating excellent repeatability of the chromatographic system.

Table 1. System suitability parameters for ketoconazole

Parameter	Result	Acceptance Criteria
Retention time (min)	5.47	Consistent
Peak asymmetry	1.12	≤2.0
Theoretical plates	11,388	>2000
Peak purity	1.00	Pass
Instrument precision (%RSD)	0.22	≤2.0
Method precision (%RSD)	1.26	≤2.0
Intermediate precision (%RSD)	0.84	≤2.0

The theoretical plate count exceeded 11,000, indicating excellent column efficiency and high chromatographic resolution. The asymmetry factor remained close to unity, confirming symmetrical peak shape with minimal tailing.

Low %RSD values for instrument precision (0.22%), method precision (1.26%), and intermediate precision (0.84%) demonstrated outstanding repeatability and reproducibility of the analytical procedure. These observations indicate that the developed chromatographic method is sufficiently robust for routine quantitative estimation of ketoconazole in pharmaceutical formulations.

Overall, AQbD-based optimization successfully established a chromatographic method capable of delivering consistent analytical performance while satisfying all predefined analytical quality attributes. The optimized method provides rapid analysis, excellent specificity, high chromatographic efficiency, and reproducible results, thereby meeting current ICH recommendations for analytical method development and validation.

3.5 Linearity

The linearity of the developed RP-HPLC method was evaluated by analysing ketoconazole standard solutions at five concentration levels ranging from 80 to 120 µg mL^{-1}. Each concentration was injected in triplicate under optimized chromatographic conditions. The calibration curve was constructed by plotting peak area against corresponding concentration.

The chromatographic responses increased proportionally with increasing drug concentration, indicating excellent detector response throughout the investigated concentration range. Linear regression analysis produced an excellent correlation coefficient (R^2 = 1.0000), confirming a direct linear relationship between concentration and chromatographic response.

Table 2. Linearity data of Ketoconazole

Concentration (µg/mL)	Mean Peak Area
80	5,164,344 ± 8,950
90	5,813,842 ± 10,214
100	6,467,463 ± 11,325
110	7,108,693 ± 12,106
120	7,740,262 ± 13,018

Regression equation:

y = mx + c

Correlation coefficient (R^2):

1.0000

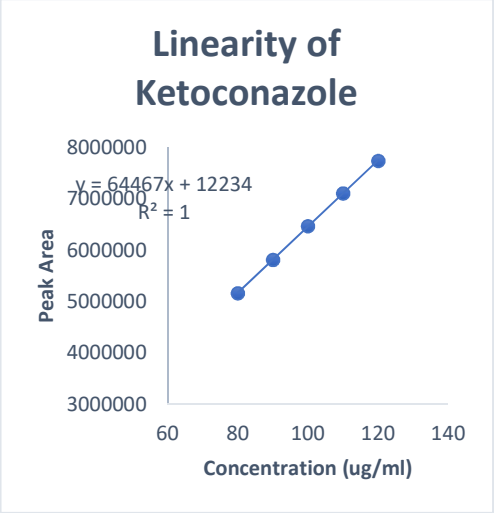


Figure 3. Calibration curve of Ketoconazole

The calibration curve exhibited excellent linearity without significant deviation from the regression line. The coefficient of determination (R^2 = 1.0000) exceeded the acceptance criterion recommended by ICH Q2(R1), confirming the suitability of the method for quantitative estimation of ketoconazole within the selected analytical range. The proportional increase in peak area further

demonstrates excellent detector sensitivity and reliable quantitative performance.

3.6 Accuracy

Method accuracy was evaluated by recovery studies using the standard addition technique at 80%, 100%, and 120% concentration levels. Each concentration level was analyzed in triplicate, and percentage recovery was calculated.

Table 3. Accuracy (Recovery) Study

Level	Mean Recovery (%)	SD	%RSD
80%	99.75	0.37	0.37
100%	100.07	0.30	0.30
120%	99.96	0.17	0.17

The recovery values ranged between 99.75% and 100.07%, demonstrating excellent analytical accuracy. The %RSD values obtained at all concentration levels were well below the acceptable limit of 2%, indicating excellent repeatability of recovery measurements.

According to ICH Q2(R1), recovery values within 98–102% are considered acceptable for assay methods. Therefore, the obtained recovery results confirm that the developed RP-HPLC method accurately quantifies ketoconazole without interference from formulation excipients or analytical procedures.

The low standard deviation further indicates minimal experimental variability, reflecting excellent analytical precision during sample preparation and chromatographic analysis.

3.7 Precision

Precision evaluates the closeness of agreement among independent analytical measurements obtained under identical conditions. Precision of the developed RP-HPLC method was assessed in terms of instrument precision, repeatability (method precision), and intermediate precision.

3.7.1 Instrument Precision

Instrument precision was determined by six consecutive injections of ketoconazole standard solution under identical chromatographic conditions.

The extremely low %RSD (0.22%) indicates excellent injector reproducibility and detector stability throughout the analytical sequence.

3.7.2 Method Precision (Repeatability)

Method precision was evaluated by preparing six independent sample solutions containing ketoconazole and analysing them under optimized chromatographic conditions.

The obtained %RSD of 1.26% demonstrates excellent repeatability of the analytical procedure and satisfies the acceptance criterion specified by ICH guidelines.

3.7.3 Intermediate Precision

Intermediate precision was assessed by repeating the analysis on a different day by another analyst using the same chromatographic system.

The %RSD value remained below 2% (i.e. 0.84%), confirming excellent reproducibility of the analytical method under normal laboratory variations.

Overall, instrument precision, repeatability, and intermediate precision collectively demonstrate that the developed RP-HPLC method possesses excellent analytical precision suitable for routine pharmaceutical quality control.

3.8 Limit of Detection (LOD) and Limit of Quantification (LOQ)

Method sensitivity was evaluated by determining the Limit of Detection (LOD) and Limit of Quantification (LOQ) using signal-to-noise ratio criteria recommended by ICH Q2(R1).

The developed RP-HPLC method demonstrated excellent analytical sensitivity with an LOD of 0.31 µg/mL and an LOQ of 0.94 µg/mL.

The low LOD indicates that trace quantities of ketoconazole can be reliably detected, whereas the low LOQ confirms accurate and precise quantification even at very low concentrations. Such sensitivity makes the method highly suitable for routine quality control, dissolution studies, and stability analysis where low analyte concentrations may be encountered.

3.9 Robustness

Method robustness was investigated by introducing small deliberate variations in chromatographic parameters, including buffer pH and detection wavelength.

Table 4. Robustness Study

Effect of Buffer pH

Buffer pH	RT (min)	Area	%RSD
8.1	5.45	6,480,456	
8.0	5.47	6,467,463	
7.9	5.48	6,451,375	0.23

Effect of Detection Wavelength

Wavelength	RT (min)	Area	%RSD
227 nm	5.47	6,428,334	
225 nm	5.47	6,467,463	
223 nm	5.47	6,469,055	0.36

The chromatographic system exhibited only negligible variation in retention time and peak area following deliberate changes in pH and wavelength. The retention time remained essentially constant (approximately 5.47 min), while theoretical plate count exceeded 11,000 under all conditions.

Peak asymmetry remained close to unity (approximately 1.1), indicating maintenance of excellent chromatographic peak shape despite minor experimental variations.

The %RSD values for peak area under altered chromatographic conditions were significantly below the acceptance limit of 2%, confirming that

the analytical method is sufficiently robust for routine laboratory application.

The robustness study clearly demonstrates that minor variations in analytical parameters do not significantly influence chromatographic performance, thereby validating the reliability and ruggedness of the AQbD-developed RP-HPLC method. The analytical design space established during method optimization successfully ensured consistent chromatographic performance under normal operating variations.

3.10 Forced Degradation Studies

Forced degradation studies were performed according to ICH Q1A(R2) guidelines to establish the stability-indicating capability of the developed AQbD-based RP-HPLC method. Ketoconazole was subjected to acidic, alkaline, oxidative, thermal, and photolytic stress conditions to evaluate its degradation behaviour. The stressed samples were analysed under optimized chromatographic conditions to determine degradation percentage and ensure adequate separation between the parent drug and degradation products.

The developed chromatographic method successfully resolved ketoconazole from all degradation products without any interference, confirming its stability-indicating nature. Peak purity analysis performed using the diode array detector demonstrated that the ketoconazole peak remained spectrally homogeneous under all stress conditions, indicating the absence of co-eluting degradation products.

Figure 4. Representative chromatograms of forced degradation studies (Chromatogram of A-Acid Sample, B-Base Sample, C-Oxidation Sample, D-Dry Heat Sample, E-UV Sample)

The chromatograms revealed complete baseline separation between ketoconazole and degradation products under all applied stress conditions. No interference at the retention time of ketoconazole (5.47 min) was observed, confirming the specificity and stability-indicating capability of the developed RP-HPLC method.

Table 5. Results of Forced Degradation Study

Stress Condition	Peak Area	Assay (%)	Degradation (%)
Control	6,467,463	100.00	—
Acid (0.1 N HCl)	6,079,513	93.91	6.09
Base (0.1 N NaOH)	6,039,800	93.29	6.71
Oxidative (30% H_2O_2)	5,428,984	83.86	16.14
Thermal (120°C, 3 h)	5,813,351	90.66	9.34
Photolytic (254 nm, 5 h)	5,893,036	91.03	8.97

Ketoconazole exhibited varying susceptibility toward different stress conditions. Among all degradation studies, oxidative stress produced the highest degradation (16.14%), indicating that ketoconazole is particularly sensitive to oxidation. The oxidative degradation is attributed to oxidation of the imidazole moiety and the piperazine side chain, leading to structural modifications and reduced chromatographic response.

Thermal degradation resulted in 9.34% degradation after exposure to 120°C for 3 h, suggesting moderate thermal instability of ketoconazole. Elevated temperature may induce molecular rearrangement or cleavage of susceptible functional groups within the molecule.

Photolytic degradation produced 8.97% degradation following exposure to ultraviolet radiation (254 nm), demonstrating moderate photosensitivity. Therefore, ketoconazole formulations should be protected from prolonged exposure to light during manufacturing, storage, and transportation.

Acidic and alkaline hydrolysis caused comparatively lower degradation of 6.09% and 6.71%, respectively. These findings indicate that ketoconazole possesses relatively good stability under mild hydrolytic conditions compared to oxidative stress.

The degradation percentages obtained in this study fall within the generally accepted range of 5–20% recommended for forced degradation studies, ensuring meaningful assessment of degradation pathways without complete destruction of the analyte.

The developed AQbD-based RP-HPLC method successfully separated degradation products from the parent drug under every stress condition, thereby fulfilling the essential requirement of a stability-indicating analytical procedure.

3.11 Comparison with Previously Reported RP-HPLC Methods

To evaluate the analytical performance of the developed method, it was compared with previously published RP-HPLC methods for ketoconazole estimation.

Table 6. Comparison of the developed AQbD-RP-HPLC method with reported methods

Parameter	Reported Methods	Present Method
Approach	Conventional RP-HPLC	AQbD-based RP-HPLC
Optimization	Trial-and-error	Design of Experiments (DoE)
Validation	Partial	Complete ICH Q2(R1) validation
Stability-indicating study	Limited	Comprehensive forced degradation
Retention time	6–12 min	5.47 min
Total run time	15–25 min	15 min
LOD	0.5–1.5 µg/mL	0.31 µg/mL
LOQ	1–5 µg/mL	0.94 µg/mL
Accuracy	98–102%	99.75–100.07%
Precision (%RSD)	<2%	0.22–1.26%
Robustness	Limited	Excellent

The comparison demonstrates that the present AQbD-based RP-HPLC method offers several advantages over conventional chromatographic methods. The method provides shorter retention time, lower detection limits, improved robustness, systematic optimization, enhanced method understanding, and comprehensive stability-indicating capability.

The implementation of AQbD principles further reduces analytical variability and improves method lifecycle management, making the procedure highly suitable for industrial quality control laboratories and regulatory submissions.

DISCUSSION

The present investigation established a robust AQbD-based RP-HPLC method for ketoconazole, enabling systematic identification and optimization of critical variables through risk assessment and statistical experimentation. The optimized conditions yielded excellent peak symmetry, high efficiency (>11,000 plates), reproducible retention time (5.47 min), and outstanding reliability. Validation according to ICH Q2(R1) confirmed specificity, perfect linearity ($R^2 = 1.0000$), accuracy (99.75–100.07% recovery), precision (%RSD < 2%), sensitivity (LOD 0.31 µg/mL; LOQ 0.94 µg/mL), and robustness. Forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic stress demonstrated the stability-indicating capability of the method, with oxidative degradation as the predominant pathway. Overall, this AQbD-optimized method is simple, rapid, economical, highly sensitive, and fully compliant with regulatory guidelines, making it suitable for routine assay determination, stability testing, quality control, and regulatory evaluation of ketoconazole in bulk and dosage forms.

CONCLUSION

An AQbD-based RP-HPLC method was developed and validated as a stability-indicating assay for ketoconazole in bulk and dosage forms. Defining the Analytical Target Profile, performing risk assessment, and optimizing Critical Method Parameters via Design of Experiments yielded excellent peak symmetry, high efficiency, and reproducible retention (5.47 min). Validation per ICH Q2(R1) confirmed specificity, linearity ($R^2 = 1.0000$), accuracy (99.75–100.07%), precision (%RSD < 2%), sensitivity (LOD 0.31 µg/mL; LOQ 0.94 µg/mL), and robustness. Forced degradation under multiple stress conditions established stability-indicating capability, with oxidative degradation as the major pathway. Compared to conventional methods, this AQbD approach ensures systematic optimization, reduced variability, improved robustness, and regulatory compliance. Overall, the method is simple, rapid, economical, precise, and ideal for routine QC, stability testing, dissolution studies, and regulatory evaluation of ketoconazole.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation**.**

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