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Original Article

Ultra-High Performance Liquid Chromatography Method Validation for Micro-dissolution Analysis of Ketoconazole in Alginate-Acacia Gum Composite Capsules

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Abstract: Ketoconazole, a BCS Class 2 antifungal drug, has good absorption but suffers from low solubility and poor bioavailability. To address this, a natural polymer-based matrix of alginate and acacia gum was formulated to enhance dissolution. This study aimed to validate a Ultra High Performance Liquid Chromatography method for analyzing ketoconazole content in micro-dissolution testing of capsule formulations. The method employed a C18 column with a mobile phase of acetonitrile:WFI (0.15% TEA, pH 3.5; 50:50) at 1 mL/min and detection at 265 nm. Validation demonstrated compliance with selectivity, linearity (r = 0.9996), sensitivity (LoD 6.51 µg/mL; LoQ 19.74 µg/mL), accuracy (99.6–101.3%), and precision (<2%, except one interday result at 2.08%). Micro-dissolution testing showed sustained ketoconazole release, exceeding 100% within 240 minutes. Data modeling (performed using DDSolver) indicated comparable fit for the Higuchi (R^2_adj = 0.96) and Korsmeyer (R^2_adj = 0.93) models, suggesting diffusion-controlled release of ketokonazole. The validated UHPLC method is suitable for micro-dissolution studies, and the alginate-acacia gum formulation effectively modulates drug release.

Keywords: ketoconazole capsule, Ultra High Performance Liquid Chromatography, micro-dissolution, alginate-acacia gum composite matrix

1. INTRODUCTION

The pharmaceutical industry continuously seeks innovative approaches to improve drug delivery systems, particularly for compounds with low solubility and poor absorption characteristics. Ketoconazole, a broad-spectrum antifungal agent from the imidazole class, is classified as a Class 2 drug in the biopharmaceutical classification system (BCS) due to its low water solubility and high permeability. Ketoconazole is also known to exhibit strong pH-dependent solubility [1]. This presents significant challenges in achieving optimal therapeutic outcomes [2], [3], [4]. The poor solubility of ketoconazole is a major obstacle in both systemic and local treatments for fungal infections. Its low aqueous solubility (0.017 mg/mL at 25ºC) limits its bioavailability, leading to inadequate drug absorption in the gastrointestinal tract. This condition can reduce therapeutic effectiveness and increase the risk of drug resistance [5]. Furthermore, the poor solubility affects the release of the active pharmaceutical ingredient (API) from dosage forms, resulting in suboptimal concentrations at the site of infection and ultimately diminishing treatment success [6].

To enhance the solubility of ketoconazole, various methods have been explored in several studies. One effective approach is the formulation of nanoemulsions, which significantly improve the solubility and bioavailability of ketoconazole. In the previous study, nanoemulsion was developed using surfactants such as Tween 80 and ethanol, resulting in a formulation that achieved 86.33% drug release within five hours, indicating enhanced solubility [7]. Another study employed spontaneous emulsification to produce a stable nanoemulsion by optimizing surfactant and oil

concentrations, which also demonstrated better penetration ability compared to conventional formulations [7]. Additionally, co-crystal formation with co-formers has shown promising potential in significantly improving the solubility of ketoconazole by modifying the physicochemical properties of the drug and improving molecular interactions with aqueous environments, leading to better dissolution rates [8]. Furthermore, natural polymer-based systems have emerged as another potential strategy. Alginate and acacia gum are natural polymers known for their biocompatibility and ability to form hydrogels, which can modulate drug release rates. The incorporation of ketoconazole into an alginate-acacia gum composite matrix represents a promising strategy to enhance the dissolution profile and therapeutic efficacy of this drug [9].

In the recent study, to demonstrate the increment in drug liberation from the matrix, we performed micro-dissolution testing. The development of micro-dissolution techniques is expected to reduce the use of media and reagents. The development of such study will align with Sustainable Development Goal (SDG) 12. By developing efficient and effective drug formulations, this research aims to promote sustainable practices in pharmaceutical development, ultimately leading to improved health outcomes and reduced environmental impact. This comprehensive approach will provide insights into the drug release profile of ketoconazole from the alginate-acacia gum composite matrix and contribute to the understanding of its potential therapeutic applications [10]. This work became the new contribution in directing the quality control activity toward greener and safer for environment.

Since the research employed new matrix and new dissolution approach, we required validated analytical method. The common method for ketoconazole assay and dissolution test in the compendia (either United States Pharmacopeia and Farmakope Indonesia) is High Performance Liquid Chromatography (HPLC). Both compendia do not compensate the procedure for micro-dissolution testing of our new formulation ketoconazole in an alginate-acacia gum composite matrix in capsule dosage form. Therefore, this study offered another unconventionality by using Ultra High Performance Liquid Chromatography (HPLC) method to monitor the micro-dissolution performance of ketoconazole in such composite matrix. This technique will reduce the analysis time with better resolution, and low solvent consumption [11]. Furthermore, only a few studies have utilized UHPLC for the micro-dissolution method of ketoconazole. Furthermore, this study will not only advance the field of pharmaceutical analysis but also contribute to ongoing efforts to enhance drug delivery systems for better patient care [12].

2. MATERIALS AND METHODS

2.1. Equipment and Materials

The equipment used in this study were UHPLC, 0.45 nm micro-filter syringe, 1 cc syringe, vials, UHPLC column, measuring cylinders, beakers, volumetric flasks, drop pipettes, volumetric pipettes and pro-pipettes, stirring rods, spatulas, analytical balance, weighing paper, alumunium foil, filter membrane (0.45 nm filter paper) and vacuum, sonicator, pH meter, hot plate, multiple stirrer, magnetic stirrer, micropipette (100–1000 μL) with micro tips, 1.5 mL microtubes, and vortex mixer.

The materials used in this study include raw ketoconazole (PT Kimia Farma Bandung), ketoconazole in alginate-acacia gum composite matrix (produced in the laboratory), capsule shells (size 3) (PT Kapsulindo Nusantara), methanol (Merck), acetonitrile (JT Baker), triethanolamine (Bratachem), orthophosphoric acid (JT Baker), sodium hydroxide (Merck), hydrochloric acid (Merck), water for injection (Ika Pharma), and distilled water.

2.2. *Research Procedure*

2.2.1. Preparation of ketoconazole beads

The beads preparation procedure was adopted from [13], [14] with the appearance as depicted in Figure 1. The first step in preparing ketoconazole beads was to create a polymer mixture consisting of alginate and acacia gum. One gram of alginate was weighed and dissolved in 100 mL

of distilled water. The distilled water was heated on a hot plate, and once it reached boiling point, the alginate was added and stirred using a magnetic stirrer. The same procedure was followed to prepare the acacia gum polymer: 1 gram of acacia gum was weighed and dissolved in 100 mL of distilled water, which was heated on a hot plate. Once boiling, the acacia gum was added and stirred with a magnetic stirrer. A 5% ketoconazole solution was prepared by weighing 5 grams of ketoconazole and dissolving it in 100 mL of solvent, then stirred using a magnetic stirrer.

The next step was to prepare the polymer mixture with a ratio of alginate to acacia gum (75:25) by weighing 15 grams of alginate and 5 grams of acacia gum, then stirring the mixture using a magnetic stirrer. After that, 10 mL of ketoconazole was added to the polymer mixture, and the solution was left to stand for approximately one hour. A $CaCl_2$ solution was prepared by weighing 37.5 grams of $CaCl_2$ and dissolving it in distilled water. The polymer and ketoconazole mixture was then dropped into the $CaCl_2$ solution. Once the beads were formed, they were subjected to characterization. After characterization, 100 mg of the beads were placed into capsules.

Figure 1. Appearance of the ketoconazole beads from the previous research [14]

2.2.2. Preparation of the mobile phase

The mobile phase was prepared by combining acetonitrile and WFI containing 0.15% TEA, adjusted to pH 3.5 (50:50) using orthophosphoric acid. The mobile phase was then filtered using a 0.45 μm membrane filter.

2.2.3. Preparation of stock solution for validation

The stock solution of ketoconazole raw material was prepared by weighing an amount equivalent to 10 mg or by weighing 50 mg of ketoconazole raw material, then diluting it with 100 mL of methanol to obtain a final concentration of 500 μg/mL. The mobile phase was used to further dilute the stock solution into various concentrations.

2.2.4. Preparation of micro-dissolution test medium

A total of 42.5 mL of concentrated HCl was taken and diluted with distilled water to obtain a final volume of 500 mL. The pH was checked using a pH meter to ensure it reached pH 1.2; if the pH was not within the desired range, it was adjusted using NaOH. From the prepared stock solution, 90 mL was taken for the micro-dissolution test.

2.2.5. Chromatographic conditions

For analysis, UHPLC was used with reversed phase C18 column (Phenomenex Luna: 250 x 4.6 mm, 5 μm). The detector was UV detector at 265 nm for ketoconazole analysis. The sample (20 μL) was introduced into the column by autosampler system with flow rate of 1 mL/min. in the evaluation of ketoconazole, both in the alginate-acacia gum composite matrix and in its conventional form.

2.2.6. Micro-dissolution test

Weighed 100 mg of beads, equivalent to 20 mg of ketoconazole. Dissolved the sample in 90 mL of HCl medium at pH 1.2, which served as the micro-dissolution medium. Performed stirring using a magnetic stirrer at a speed of 100 rpm. At 0, 15, 30, 60, 90, 120, 150, 180, and 240 minutes, withdrew 500 μL of the sample and transferred it into a 1.5 mL microtube. After each sampling, replaced the removed volume with 500 μL of HCl at pH 1.2.

The sample was then transferred into a 5 mL volumetric flask and diluted with acetonitrile up to the mark to achieve a 10x dilution factor. The sample was vortexed for approximately 30 seconds and then filtered using a 0.45 μm syringe filter before being transferred into a vial. The concentration of ketoconazole was determined using a validated UHPLC analytical method. The data obtained were processed using Microsoft Excel, and micro-dissolution data modeling was performed using DDSolver.

2.2.7. System suitability test

System suitability testing was conducted to ensure that the chromatographic system used was capable of providing accurate, precise, and reproducible results. The sample tested was obtained from the micro-dissolution of 100 mg of ketoconazole beads, which were placed into a capsule shell and dissolved in 0.1 N HCl medium at pH 1.2. A 2 mL sample was taken at the 1-hour time point and then diluted with the mobile phase to a final volume of 5 mL. Before analysis, the sample was filtered using a 0.45 μm membrane filter. For system suitability testing, six replicate injections of the ketoconazole bead sample solution were performed.

Several key parameters were evaluated in the system suitability test, including retention time, peak area, tailing factor, number of theoretical plates, and retention factor (k'). Retention time indicated how long it took for the ketoconazole compound to reach the detector after injection; the stability of retention time between injections, marked by a %RSD (relative standard deviation) of <2%, served as an indicator of system stability. Peak area reflected the quantity of the detected compound, and the %RSD of the peak area also had to be <2% to ensure system precision. The tailing factor was used to assess the symmetry of the peak shape in the chromatogram; an ideal value was <2, indicating that the peak was sufficiently symmetrical and did not tail excessively. Theoretical plates represented the efficiency of the column in separating compounds, with an N value ≥2000 indicating an efficient system. Meanwhile, the retention factor (k') provided insight into how long the compound was retained in the column, with a value of k' >2 indicating adequate separation from other compounds.

All parameters met the acceptance criteria if the RSD for retention time and peak area was < 2%, the tailing factor was < 2, theoretical plates were > 2000, and k' was > 2. This indicated that the chromatographic system was stable and suitable for quantitative analysis [15].

2.2.8. Selectivity

The test was carried out by analyzing four types of samples: ketoconazole in an alginate-acacia gum composite matrix in capsule dosage form, ketoconazole, the mobile phase consisting of acetonitrile:WFI with 0.15% TEA at pH 3 (50:50), and the micro-dissolution medium of 0.1 N HCl at pH 1.2. Selectivity was accepted if no interfering peaks were observed at the retention time of ketoconazole, indicating that the analyte could be clearly identified and quantified in the presence of excipients, matrices, or other potential components, as required by [16], [17]. Additionally, representative chromatograms were used to visually demonstrate peak discrimination and confirm the absence of interference.

2.2.9. Linearity

The standard solution was prepared by dissolving 50 mg of ketoconazole standard in 100 mL of methanol, resulting in a concentration of 500 μg/mL. A series of concentrations (10, 50, 100, 150, 200, and 250 μg/mL) was then prepared from the stock solution by taking volumes of 0.2, 1, 2, 3, 4, and 5 mL, respectively, and diluting each with the mobile phase to a final volume of 10 mL. Each of these solutions was transferred into a 1.5 mL microtube. A calibration curve was constructed by

plotting concentration against peak area. A linear regression equation was then obtained. Linearity was considered acceptable if the correlation coefficient (r) was ≥ 0.995 [17], [18].

2.2.10. Accuracy

The standard addition method was used to evaluate accuracy at three levels of standard solutions: 80 ppm, 100 ppm, and 120 ppm (low, medium, and high). The ketoconazole standard was mixed with the sample in a 1:1 ratio and then vortexed. Each level was replicated three times. Recovery was evaluated by comparing the ratio between the total amount in the sample and the concentration of the sample without the standard solution. Accuracy was considered acceptable if the recovery percentage fell within the range of 98-102% [17], [19].

2.2.11. Precision

Precision testing was carried out both intraday and interday by measuring the standard stock solution at concentrations of 80 ppm, 100 ppm, and 120 ppm (standard + micro-dissolution sample). Intraday precision was measured three times per concentration within the same day, while interday precision was measured over three different days. Precision was considered acceptable if the %RSD was ≤ 2.0% [17].

2.2.12. LoD and LoQ

LoD and LoQ were obtained through stepwise dilution of the standard solution (10, 50, 100, 150, 200, and 250 μg/mL). According to [16], [17], LoD was defined as the lowest amount of analyte in a sample that could be detected but not necessarily quantified, while LoQ was the lowest amount that could be quantitatively determined with suitable precision and accuracy. LoD and LoQ were considered acceptable if no interfering peaks were observed at the retention time of ketoconazole in the chromatogram, indicating sufficient selectivity and method suitability at low concentrations.

2.3. Data Analysis

The data obtained from sample measurements using the UHPLC method were processed using Microsoft Excel to calculate the average of area under curve, linear regression equation, correlation coefficient (r), standard deviation (SD), coefficient of variation (%RSD), as well as to construct the calibration curve and calculate the % recovery of the samples. These results were compared with the established validation parameters to determine whether the method met the criteria for accuracy, precision, linearity, and the limits of detection and quantification.

Subsequently, the micro-dissolution data of ketoconazole from the alginate-acacia gum composite capsule formulation in 0.1 N HCl medium (pH 1.2) were analyzed using Microsoft Excel to calculate the percentage of drug release at each time point. The data were then input into DDSolver, an Excel add-in used for modeling drug release kinetics. Modeling was carried out by comparing several kinetic models (such as Higuchi, Korsmeyer, Hixson, and first-order) and selecting the best-fitting model based on statistical parameters such as the coefficient of determination (R^2 and R^2_adj), Akaike Information Criterion (AIC) and a Model Selection Criterion (MSC). The results of this modeling were used to determine the release mechanism of ketoconazole from the matrix system used.

3. RESULTS AND DISCUSSION

3.1. Chromatographic conditions

A chromatographic method was developed to detect drugs in supersaturation studies with pH shift using 0.1 N HCl medium at pH 1.2. This chromatographic method employed a reversed-phase mode with a C-18 (nonpolar) column as the stationary phase due to its high stability, good reproducibility, and wide availability. Ketoconazole was a weakly basic drug with pKa values of 2.94 and 6.51. The mobile phase used in this study successfully produced good peak symmetry and resolution while minimizing tailing issues, with acceptable system suitability parameters.

The selection of the mobile phase consisting of a mixture of acetonitrile and WFI with 0.15% TEA at pH 3.3 (50:50) was based on acetonitrile's efficiency as an organic solvent in enhancing the

elution of nonpolar compounds, as well as its low viscosity and minimal backpressure. The addition of triethylamine (TEA) served to neutralize interactions between weak bases such as ketoconazole and free silanol groups on the stationary phase, thereby reducing peak tailing. The pH was adjusted to 3.3 to remain below the pKa of ketoconazole, ensuring that the drug stayed in its protonated form and remained soluble in the mobile phase, allowing for more stable and controlled separation.

3.2. System suitability test

The system suitability test (SST) was a critical initial step in chromatographic analysis to ensure that the instrument system including the column, pump, detector, and mobile phase operated optimally before sample injection. SST aimed to evaluate the performance of the chromatographic system to ensure it produced accurate, precise, stable, and reproducible analytical results. The parameters tested in SST included retention time, peak area, tailing factor, number of theoretical plates, and retention factor (k') [15]. The values of all parameters in the system suitability test met the acceptable criteria. SST results were presented in Table 1.

The retention time showed a %RSD of 0.18%, which was well below the established limit (<2%), indicating system stability and reproducibility of retention time across injections. The peak area ratio also showed a %RSD of 0.09%, demonstrating high consistency in the detector's response to the target compound. The peak symmetry (tailing factor) was 1.20, which was below the maximum limit of 2. This indicated that the chromatographic peak was symmetrical and did not exhibit significant tailing, which was crucial for accurate quantification. The number of theoretical plates (N) was 2557.17, exceeding the minimum requirement of 2000. This value reflected the high efficiency of the column in separating the target compound. The retention factor (k') was 5.15, indicating that the compound was sufficiently retained in the column to allow for good separation, yet still within an efficient analytical time frame. A k' value greater than 2 indicated that ketoconazole was neither eluted too early nor too late, ensuring good resolution.

Table 1. Results of system suitability test sample microdissolution of ketoconazole beads in capsule dosage form using a mobile phase composed of acetonitrile:WFI containing 0.15% TEA at pH 3.5 (50:50)

System Parameters	Acceptance Criteria	Results
Retention time (minute)	RSD < 2%	0.18%
The ratio of peak area	RSD < 2%	0.09%
Tailing factor	< 2	1.20
Theoretical plates	>2000	2557.17
Retention factor (k')	>2	5.15

The primary function of the system suitability test (SST) was to ensure the reliability of the chromatographic system before it was used for sample analysis, thereby guaranteeing accurate and precise results. SST also played a crucial role in preventing systematic errors that could affect data validity, maintaining consistency between injections within a single analytical session, and detecting potential technical issues such as column degradation, unstable pump performance, or non-homogeneous mobile phase mixing. If SST was not performed, several consequences could arise, such as the analytical data becoming invalid due to unverified system performance. Additionally, chromatographic peaks could become asymmetric, exhibit tailing, or even split, which complicated integration and concentration calculations. Retention time instability could also occur, increasing the risk of compound misidentification and leading to poor injection-to-injection precision, as indicated by high %RSD values that failed to meet validation criteria. This could result in misinterpretation of the microdissolution profile, which might actually have stemmed from an unsuitable system rather than issues with the product formulation itself.

3.3 Selectivity

The results of the selectivity test demonstrated that the UHPLC method used was capable of specifically detecting the ketoconazole peak without interference from other components in the system. The evaluation was carried out by comparing chromatograms from four separately analyzed sample types: ketoconazole in the alginate-acacia gum composite matrix capsule formulation, ketoconazole standard, the mobile phase consisting of acetonitrile:WFI with 0.15% TEA at pH 3 (50:50), and the microdissolution medium of 0.1 N HCl at pH 1.2.

Based on the chromatogram results shown in Figure 2, the ketoconazole peak was clearly detected at a retention time of approximately 3-4 minutes, with no significant peaks observed at the same retention time in the media, mobile phase, or excipient samples. This confirmed that there was no overlap between the ketoconazole peak and other components, indicating that the method met the selectivity criteria outlined in the [17] guidelines. Therefore, the UHPLC method used in this study was considered selective for detecting ketoconazole in the bead matrix formulation without significant interference from solvents or media, and could be reliably applied in microdissolution analysis.

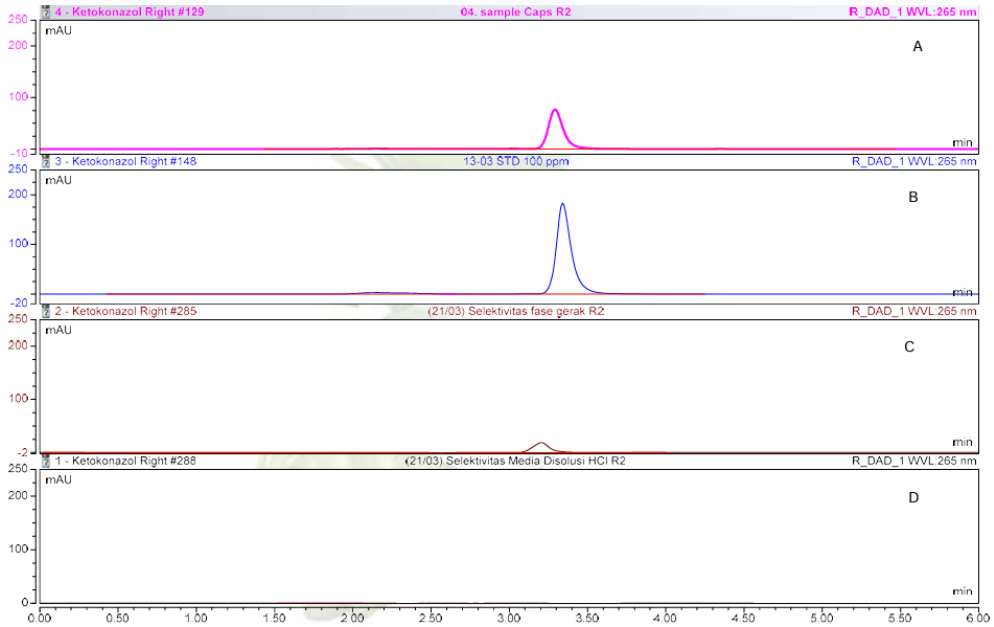

Figure 2. Selectivity evaluation of ketoconazole in alginate-acacia gum composite matrix capsule formulation (A), ketoconazole (B), mobile phase of acetonitrile:WFI with 0.15% TEA at pH 3 (50:50) (C), and micro-dissolution medium of 0.1 N HCl at pH 1.2 (D).

3.4 Linearity

The obtained calibration curve demonstrated a linear relationship between ketoconazole concentration and peak area, as shown in Figure 3. The resulting regression equation was y = 0.1753x - 0.2898, with a correlation coefficient (r) of 0.9993. This result met the accepted criterion of r ≥ 0.995 [17], [18]. This indicated that the validated UHPLC method had excellent capability to provide consistent and proportional results in response to variations in ketoconazole concentration within the tested range.

Conceptually, linearity was one of the key parameters in method validation, as it reflected the extent to which the analytical system's response (in this case, peak area) was directly proportional to the concentration of the analyte in the sample. A linear calibration curve indicated that the method was suitable for quantifying ketoconazole with high precision and accuracy within the specified concentration range. Therefore, the linearity results reinforced the validity of the UHPLC method as a reliable analytical approach for evaluating the micro-dissolution of ketoconazole from capsule formulations based on an alginate-acacia gum composite matrix.

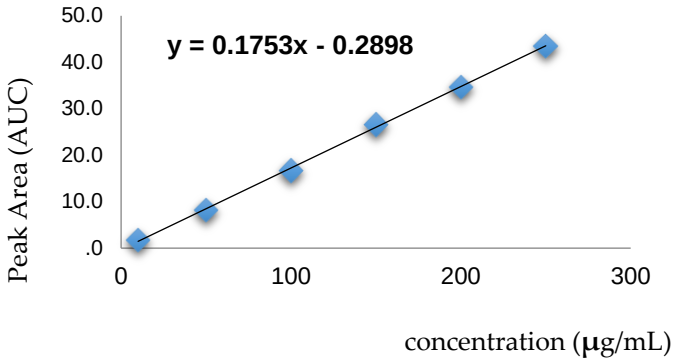

Figure 3. Linear plot between ketoconazole standard concentration and area under curve of chromatogram in linearity testing

3.5. Accuracy

Based on the test results summarized in Table 2, at low (80 ppm), medium (100 ppm), and high (120 ppm) concentration levels, the obtained recovery values ranged from 99.58% to 101.28%, indicating that nearly all of the added ketoconazole could be recovered by the method. The UHPLC method used demonstrated excellent accuracy performance across all tested concentration levels.

All recovery results not only matched the targeted values but also fell within the acceptable range of 98%-102% for analytes at a concentration of 100 µg/mL [17], [19]. The consistency and uniformity of the measurements at each concentration level indicated that the method provided accurate quantification at both low and high levels. This served as an important indicator that the UHPLC method was suitable for microdissolution testing of ketoconazole in the developed alginate-acacia gum matrix based capsule formulation.

3.6. Precision

Intraday precision was assessed by performing three measurements on the same day for each concentration. The results showed that the %RSD (Relative Standard Deviation) ranged from 0.11% to 1.09%, which met the criteria set by [17]. The intraday precision results were presented in Table 2. This indicated that the UHPLC method used demonstrated high consistency in producing results within a short time interval.

Table 2. Intraday and interday precision along with the accuracy of microdissolution sample (1:1)

Concentration of ketoconazole (ppm)	Intraday precision		Interday precision		Accuracy
	Mean± SD	%RSD	Mean± SD	%RSD	Recovery (%)
80	106.82±0.12	0.11	113.84±1.89	1.64	101.28
100	116.72±1.28	1.09	119.33±2.48	2.08	99.58
120	123.67±0.55	0.45	123.31±1.89	1.53	100.02

Interday precision was evaluated over three different days under similar laboratory conditions and instrumentation for each concentration. The results showed %RSD values ranging from 1.53% to 2.08%, as listed in Table 2. The precision value for the 100 ppm concentration slightly exceeded the ideal threshold of 2.0%; however, it was generally still acceptable, as this fluctuation might have been attributed to daily variable factors such as laboratory room temperature, mobile phase stability, and UHPLC system performance during the testing period.

These results indicated that although a slight increase in variability was observed in the interday measurements, the method still demonstrated a good level of reproducibility. This suggested that the UHPLC method validated in this study was reliable for determining ketoconazole content both in the short term (same day) and over different days (interday), making

it suitable for use in micro-dissolution testing for both quality control and formulation development purposes.

3.7. Micro-dissolution Profile

The results of the micro-dissolution test of ketoconazole in the alginate-acacia gum composite matrix in capsule dosage form showed a progressive and sustained release of the active ingredient throughout the 240-minute observation period, as presented in Figure 4. The initial release at 15 minutes indicated that the average dissolved ketoconazole reached approximately 39%, then increased to nearly 48% at 60 minutes. A significant rise was observed at 120 minutes, with the release reaching up to 77.06%. The release continued to increase at 180 and 240 minutes, even exceeding 100% in several replicates. The micro-dissolution profile curve was shown in Figure 4. Release values above 100% were most likely due to inaccuracies during the sampling or dilution process, such as pipetting variations or inconsistencies in media replacement during sampling. Nevertheless, these values could still be interpreted as the maximum release of the active compound from the capsule matrix.

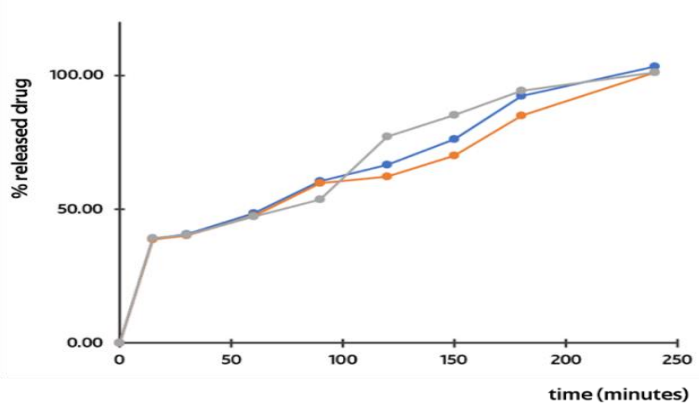

Figure 4. Micro-dissolution profile curve of ketoconazole in 0.1 N HCl at pH 1.2 as the medium (obtained from 3 replicates)

This release profile illustrated that the drug delivery system based on an alginate-acacia gum composite matrix was capable of efficiently releasing ketoconazole in an acidic medium of 0.1 N HCl at pH 1.2. This aligned with the characteristics of ketoconazole as a weakly basic compound with pKa values of 2.94 and 6.51, which exhibited increased solubility at low pH. The use of natural matrices such as alginate and acacia gum not only helped maintain formulation stability but also functioned as a controlled release system that could modulate dissolution in a sustained manner.

When the dissolution profile was examined based on official/compendial methods, particularly as stated in the United States Pharmacopeia (USP), ketoconazole typically showed more than 80% release within 30 minutes when tested using a conventional dissolution system with a 900 mL medium volume and a paddle apparatus at 75 rpm. In contrast, this study employed a micro-dissolution system with only 90 mL of medium and stirring at 100 rpm, which naturally created a different dissolution environment. Although the ketoconazole release at 30 minutes in this micro-dissolution method did not reach 80%, the consistent and significantly increasing release trend in the subsequent time points indicated that this micro-dissolution system still represented a valid drug release profile.

The difference in profiles between the micro-dissolution method and the compendial method was understandable due to factors such as medium volume, system scale, and the properties of the formulation matrix. Nevertheless, considering the efficiency in solvent usage and the system's ability to mimic small-scale biological conditions, the micro-dissolution method used in this study was regarded as a valid alternative for evaluating new formulations, particularly during the early

development stage. Moreover, these results supported the effectiveness of the previously validated UHPLC system in accurately detecting ketoconazole at various testing time points.

Further analysis of the micro-dissolution data using kinetic modeling indicated that both the Higuchi and Korsmeyer models provided a good fit for describing the drug release profile from the formulation. The Higuchi model yielded an adjusted coefficient of determination (R^2_adj) of 0.96, Akaike Information Criterion (AIC) value of 54.07, and Model Selection Criterion (MSC) value of 2.35. These values suggested a strong correlation between the experimental data and the Higuchi model.

Similarly, modeling with the Korsmeyer model using DDSolver resulted in an n value of 0.34, which was below 0.45, indicating that the release of ketoconazole followed a Fickian diffusion mechanism. This suggested that the drug release occurred primarily through diffusion within the polymer matrix. The findings from the Korsmeyer model supported the same release mechanism proposed by the Higuchi model, reinforcing that diffusion was the dominant mechanism of ketoconazole release in this system [20].

Based on the calculation results, the Higuchi and Korsmeyer models showed no significant differences and were considered comparable. Therefore, the Korsmeyer model was deemed more appropriate for describing diffusion-based drug release in this study. The Higuchi and Korsmeyer models both described drug release from solid dosage forms, such as polymeric gel matrices, as a diffusion-controlled process dependent on the square root of time. This indicated that the amount of drug released was directly proportional to the square root of the dissolution time. This mechanism theoretically aligned with solid matrix-based formulations, where the active pharmaceutical ingredient was released primarily through the movement of drug molecules from within the matrix into the micro-dissolution medium via diffusion pathways [20].

In this formulation, the combination of sodium alginate and gum acacia formed a hydrophilic gel matrix that swelled upon contact with the micro-dissolution medium, creating hydrated channels through which drug molecules could gradually diffuse. Although ketoconazole was a relatively hydrophobic compound, its incorporation within a hydrophilic polymeric matrix allowed for gradual and controlled release via diffusion through aqueous channels. This matrix-based system facilitated the diffusion of ketoconazole by improving its wettability and dispersion, without relying on erosion or polymer degradation.

Therefore, although both models demonstrated a good statistical fit, the Korsmeyer model offered a more versatile mathematical description of the diffusion controlled release observed in this hydrophilic matrix system, even for a poorly water-soluble drug like ketoconazole.

3.8. LoQ and LoQ

The obtained LoD (Limit of Detection) result was 6.51 µg/mL, which could still be reliably detected. The LoQ (Limit of Quantification) for ketoconazole was 19.74 µg/mL, indicating the lowest analyte concentration that could be quantified with acceptable accuracy and precision [21]. The relatively high LoD and LoQ values were attributed to the fact that the concentrations used in this study were also relatively high. Therefore, in this research, the validation parameters for LoD and LoQ were deemed unnecessary, as they were not relevant to the analytical objectives. According to [17], the determination of LoD and LoQ was generally required for analytical methods used in impurity testing, degradation product analysis, or for quantifying compounds at very low concentrations. Meanwhile, the primary objective of this study was to evaluate the micro-dissolution profile and the performance of the chromatographic system in detecting ketoconazole from bead formulations, not for trace analysis or quantification at low active compound concentrations.

However, LoD and LoQ testing was still carried out in the micro-dissolution study because, during the initial stage of drug release, the dissolved concentration of ketoconazole was very low. Therefore, it was essential to ensure that the analytical method was capable of detecting (LoD) and accurately measuring (LoQ) the amount of drug released. In this study, the validation of LoD and LoQ ensured that the data at each time point particularly during the early sampling stages could

still be measured accurately, thereby ensuring that the resulting micro-dissolution profile was valid and representative.

4. CONCLUSION

The UHPLC method with the optimized isocratic system met the validation criteria, including selectivity, linearity, accuracy, precision, limit of detection, and limit of quantification, in accordance with the ICH guideline. This method was proven to be validated and could be used for the analysis of ketoconazole in an alginate-acacia gum composite matrix. The micro-dissolution test results showed that the release of ketoconazole occurred gradually, exceeding 100% within 240 minutes, with the Higuchi model identified as the best-fit kinetic model, indicating that the release mechanism followed diffusion through the polymer matrix.

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References

[1] H. Yu *et al.*, "Enhancing Solubility and Dissolution Rate of Antifungal Drug Ketoconazole through Crystal Engineering," *Pharmaceuticals*, vol. 16, no. 10, p. 1349, Oct. 2023, doi: 10.3390/ph16101349.

[2] A. L. Viçosa *et al.*, "Bioequivalence studies and sugar-based excipients effects on the properties of new generic ketoconazole tablets formulations and stability evaluation by using direct compression method," *Pharm Dev Technol*, vol. 14, no. 5, pp. 530–539, 2009, doi: 10.1080/10837450902832877.

[3] A. Górniak, B. Karolewicz, H. Czopor-Irzabek, and O. Gładysz, "A physicochemical and dissolution study of ketoconazole-pluronic F127 solid dispersions," *Farmacia*, vol. 64, pp. 244–251, Apr. 2016.

[4] A. Rahayu, D. A. C. Rosyida, and I. Nuraini, "Formulation and Optimization of Nanostructured Lipid Carriers (NLC) Ketoconazole using Full Factorial Design," *Medical Sains : Jurnal Ilmiah Kefarmasian*, vol. 7, no. 3, pp. 561–570, Aug. 2022, doi: 10.37874/ms.v7i3.448.

[5] S. Rani, Sonu, J. Kaur, and D. M. D. Vaja, "Ketoconazole: A Review of It's Therapeutic Effectiveness in Superficial and Systemic Fungal Infections," *International Journal of Science and Research (IJSR)*, vol. 13, no. 3, pp. 1258–1260, Mar. 2024, doi: 10.21275/SR24318150843.

[6] R. Shankar, V. Tiwari, C. P. Mishra, C. K. Singh, D. Sharma, and S. Jaiswal, "Formulation and Evaluation of Nanoemulsion for Solubility Enhancement of Ketoconazole," *International Journal of Research in Pharmaceutical and Nano Sciences*, vol. 4, no. 6, pp. 365–378, 2015.

[7] E. Ernoviya, M. Masfria, K. R. Sinaga, Indonesia. 2Department of 1Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Sumatera Utara, Medan, and I. Pharmaceutical Technology, Faculty of Pharmacy, University of Sumatera Utara, Medan, "Optimization and evaluation of topical ketoconazole nanoemulsion," *Asian Journal of Pharmaceutical and Clinical Research*, vol. 11, no. 5, pp. 143–146, 2018, doi: 10.22159/ajpcr.2018.v11i5.23540.

[8] S. K. Deore, R. K. Surawase, and A. Maru, "Formulation and Evaluation of O/W Nanoemulsion of Ketoconazole," *Research Journal of Pharmaceutical Dosage Forms and Technology*, vol. 11, no. 4, pp. 269–274, Dec. 2019, doi: 10.5958/0975-4377.2019.00045.4.

[9] V. Annisa, T. N. S. Sulaiman, A. K. Nugroho, and A. E. Nugroho, "A novel formulation of ketoconazole entrapped in alginate with anionic polymer beads for solubility enhancement: Preparation and characterization," *Pharmacia*, vol. 70, pp. 1423–1438, Nov. 2023, doi: 10.3897/pharmacia.70.e108120.

[10] E. Jantratid, N. Janssen, C. Reppas, and J. B. Dressman, "Dissolution media simulating conditions in the proximal human gastrointestinal tract: an update," *Pharm Res*, vol. 25, no. 7, pp. 1663–1676, Jul. 2008, doi: 10.1007/s11095-008-9569-4.

[11] S. Annissa, I. Musfiroh, and L. Indriati, "Perbandingan Metode Analisis Instrumen HPLC dan UHPLC : Article Review," *Farmaka*, vol. 17, no. 3, pp. 189–197, 2019.

[12] U. Nations, "Goal 12: Ensure sustainable consumption and production patterns," United Nations Sustainable Development Goals (UN SDGs).

[13] V. Annisa, T. N. S. Sulaiman, A. K. Nugroho, and A. E. Nugroho, "Pharmacokinetics evaluation of newly formulated beads alginate/gum acacia loaded ketoconazole in rabbit plasma by oral administration," *ADMET and DMPK*, vol. 12, no. 2 SE-Original Scientific Articles, pp. 335–341, Nov. 2023, doi: 10.5599/admet.2042.

[14] V. Annisa, F. A. Lumakso, L. Chabib, and N. A. Deviami, "Validation of Ultra High Performance Liquid Chromatography (UHPLC) Method for Testing the Dissolution of Ketoconazole Gum- Alginate Tablet Matrix," *Jurnal Farmasi Kryonaut*, vol. 4, no. 2, pp. 170–185, 2025.

[15] Bijulibazar, "Draft Guideline on Analytical Method Validation on Non-pharmacopoeial Products for Regulatory Approval," vol. 1, no. 1, 2019.

[16] D. Elder, "Validation of analytical procedures – ICH Q2(R2)," *European Pharmaceutical Review*, vol. 29, no. 1, p. 5, 2024.

[17] ICH, "International commission on harmonisation. ICH Q2 (R2) validation of analytical procedures," *European agency for the evaluation of medicinal products*, vol. 2, no. December 2023, 2023.

[18] SNI 06-6989.20, "Air dan air limbah – Bagian 20 : Cara uji sulfat, SO42- secara turbidimetri," *Badan Standardisasi Nasional*, no. SNI 06-6989.20-2004, pp. 1–5, 2004.

[19] S. A. Ramadhan and I. Musfiroh, "Review Artikel: Verifikasi Metode Analisis Obat," *Farmaka*, vol. 19, pp. 87–92, 2021.

[20] Y. Zhang *et al.*, "DDSolver: An add-in program for modeling and comparison of drug dissolution profiles," *AAPS Journal*, vol. 12, no. 3, pp. 263–271, 2010, doi: 10.1208/s12248-010-9185-1.

[21] N. Amrutiya, M. Madan, and A. Bajaj, "Development and validation of RP-HPLC method for simultaneous estimation of prednicarbate, mupirocin and ketoconazole in topical dosage forms," *Journal of Analytical Chemistry*, vol. 65, no. 11, pp. 1148–1154, 2010, doi: 10.1134/S1061934810110109.