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A Comprehensive Study of RP-HPLC Method Development for Bedaquiline: Leveraging Quality by Design for Optimal Separation, Identification, and Quantification in Anti-Tuberculosis Drug Analysis

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Abstract:

This study presents a simple method to set up and test RP-HPLC for Bedaquiline This drug is used to treat tuberculosis. We used a weQuality by Design (QbD) approach. A strong and fast RP-HPLC method was created to measure Bedaquiline in drugs. It is accurate and specific for both qualitative and quantitative analysis. The method uses a mobile phase with Methanol and 0.1%orthotic Phosphoric Acid. It also employs a C18 column and detects at a wavelength of 242 nm. Using QbD principles for method development helped identify critical method parameters (CMPs). This led to a clearer understanding of variability and made the methods more robust. Validation was carried out per ICH Q2(R1) guidelines. The method showed great linearity (r2 = 0.9999), precision, accuracy, and specificity. The limits of detection (LOD) and quantification (LOQ) were 3 µg/mL and 12 µg/mL, respectively. This optimized method can be reliably used for routine analysis of Bedaquiline.Keywords: *Bedaquiline, RP-HPLC, Quality by Design, Method Validation, Anti-Tuberculosis, Analytical Method Development*

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1. Introduction

Tuberculosis (TB) is a major global health issue. The rise of multidrug-resistant tuberculosis (MDR-TB) makes new treatment strategies urgently needed. In 2022, more than 410,000 people around the world were diagnosed with MDR-TB. This type of TB does not respond to at least isoniazid and rifampicin, the key anti-TB drugs [1]. This trend shows a strong need for new drugs. They should have unique mechanisms and good pharmacokinetic profiles.[1].

Bedaquilinehas emerged as a significant breakthrough in this regard. Bedaquiline was approved by the US Food and Drug Administration in 2012. It is the first drug in the diarylquinoline class. This medication is specifically made to treat pulmonary MDR-TB in adults. Bedaquiline is different from traditional anti-TB drugs. It targets the ATP synthase enzyme in Mycobacterium tuberculosis. This disrupts energy production, which is crucial for the bacteria's survival. This new action method avoids resistance pathways. It also boosts treatment results in combination therapies. [2].

From a pharmacokinetic standpoint, Bedaquiline displays intriguing complexities. It is very lipophilic

and binds over 99% to plasma proteins. Its terminal half-life is about 5.5 months. This is mainly because it distributes deeply in tissues and releases slowly [3]. Metabolism mainly happens through the CYP3A4 enzyme. This process creates a less active N-monodesmethyl metabolite. However, this metabolite still adds to overall drug exposure [4]. Bedaquiline is a Class II drug in the Biopharmaceutics Classification System (BCS). It has low solubility but high permeability. This needs careful planning and tracking to stay bioavailable and stable [5].

Measuring Bedaquiline accurately and reliably in bulk and formulated products is essential. This is important for therapeutic drug monitoring, pharmacokinetic analysis, and stability studies. RP-HPLC is popular for its precision, reproducibility, and flexibility with different analytes [6]. Traditional one-variable-at-a-time (OVAT) methods for developing techniques often miss the mark. They lack the efficiency and strength needed for today's pharmaceutical analysis [7].

The Quality by Design (QbD) approach helps solve these problems. It uses a clear, science-based method. QbD follows the ICH guidelines, especially Q8(R2), Q9, and Q10. Create an Analytical Target Profile (ATP) and find Critical Method Attributes (CMAs). It also looks at Critical Method Parameters (CMPs) through structured experiments. One example is Design of Experiments (DoE) [8]. This method helps everyone understand better. It also keeps things consistent and helps follow regulations during analysis [9].

This study uses the QbD framework to improve the RP-HPLC method. It focuses on analyzing Bedaquiline. It aims for strong performance and effective method validation. Using QbD principles, this research seeks to simplify chromatographic separation and quantification. It also meets current regulatory and operational standards. This highlights the importance of QbD in modern pharmaceutical analytics..

2. Materials and Methods

2.1 Chemicals and Reagents

Bedaquiline was sourced from Megafine pharmaceutical pvt ,ltd ,Nashik. We got HPLC-grade solvents from Himedia Laboratories in Bangalore, India. These included orthotic Phosphoric acid, trifluoroacetic acid (TFA), and distilled water. All chemicals and reagents in this study were analytical grade. They were used without further purification.

2.2 Instrumentation

We used a Waters Alliance e2695 HPLC system for all chromatographic analyses. It had a quaternary gradient pump. It also included a Waters 2998 Photodiode Array (PDA) detector. EMPOWER 2.0 software was used for data collection and processing. This ensured strong control and clear analysis during the study.

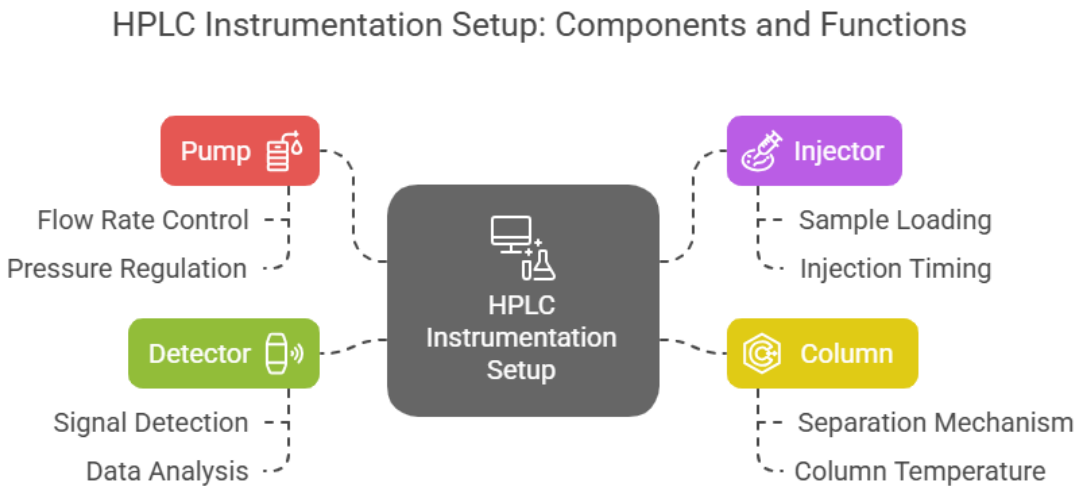


Figure 1. Block diagram of HPLC instrumentation setup

2.3 Chromatographic Conditions

We analyzed Bedaquiline using a reversed-phase high-performance liquid chromatography (RP-HPLC) method. This method ensures the best resolution and consistency. We chose a Symmetry® C18 analytical column (150 mm × 4.6 mm i.d., 3.5 µm particle size; Waters Corp., USA). This column works well with hydrophobic and moderately polar compounds. It provides high selectivity and peak efficiency for many pharmaceutical analytes [10].

The mobile phase used a mix of HPLC-grade methanol and 0.1% orthotic phosphoric acid. This blend was optimized to improve elution strength and peak shape. It aimed to reduce tailing and boost signal intensity. TFA is a volatile organic acid. It helps reduce ionization of key parts. It also stabilizes peak shapes in acidic conditions. This leads to better reproducibility and improved separation performance [11].

The system ran in isocratic mode at a flow rate of 1.0 mL/min. This kept the pressure stable and the elution profiles consistent. We used a fixed injection volume of 10 µL for all analyses. This amount strikes a good balance between sensitivity and sample use. Detection was carried out at 242 nm using a photodiode array (PDA) detector. This wavelength is the λ_max of Bedaquiline. It offers high sensitivity and selectivity. This ensures precise measurement in both bulk and formulated dosage forms [12].

Column temperature was maintained at ambient conditions, and the total run time per injection was approximately 10 minutes, enabling efficient sample throughput. All solvents were degassed and filtered through a 0.22 µm membrane filter prior to use to eliminate particulate contamination and air bubbles, which could otherwise affect column performance and detection stability.

The column temperature stayed at room temperature. Each injection took about 10 minutes, which

allowed for efficient sample processing. All solvents were degassed and filtered using a 0.22 µm membrane filter. This step removed particles and air bubbles. Doing this helped ensure better column performance and stable detection [13].

2.4 Preparation of Calibration Standards

We began by making a primary stock solution. This helps us create a reliable calibration curve for Bedaquiline. We measured the right amount of the active pharmaceutical ingredient (API). Then, we mixed it with the mobile phase. This gave us a final concentration of 1.2 mg/mL. The mobile phase was a mix of HPLC-grade methanol and 0.1% orthotic phosphoric acid. This ensured the analyte dissolved completely and worked well with the chromatographic system.

This stock solution was sonicated for about 10 minutes to mix it well. Then, it was filtered through a 0.22 µm syringe filter. This step removed any undissolved particles or contaminants that might affect chromatographic performance. The filtered solution was serially diluted with the same mobile phase. This created calibration standards with concentrations from 30 µg/mL to 180 µg/mL. We chose this concentration range based on expected analyte levels in dosage forms. It also matches the detector's linear dynamic range for Bedaquiline at 242 nm.

We made each calibration standard fresh. Then, we analyzed it using the best chromatographic conditions from Section 2.3. For each concentration point, we did three injections (n=3). This checked the repeatability of the injections and made the data more reliable. We recorded the peak areas with EMPOWER 2.0 software. Then, we plotted the average peak area values against their concentrations. This helped us create the calibration curve.

We checked if the method is linear. We did this by calculating the regression equation and the correlation coefficient (R^2). This confirmed that our quantitative estimates are statistically valid. The method is linear if the R^2 value is over 0.999. This shows little deviation from the best-fit line. Accurate results are crucial for quality control in pharmaceuticals and pharmacokinetic studies [14].

All calibration procedures met regulatory standards for method validation. They followed ICH Q2(R1) guidelines. These guidelines stress the need for accuracy, precision, and linearity in method development.

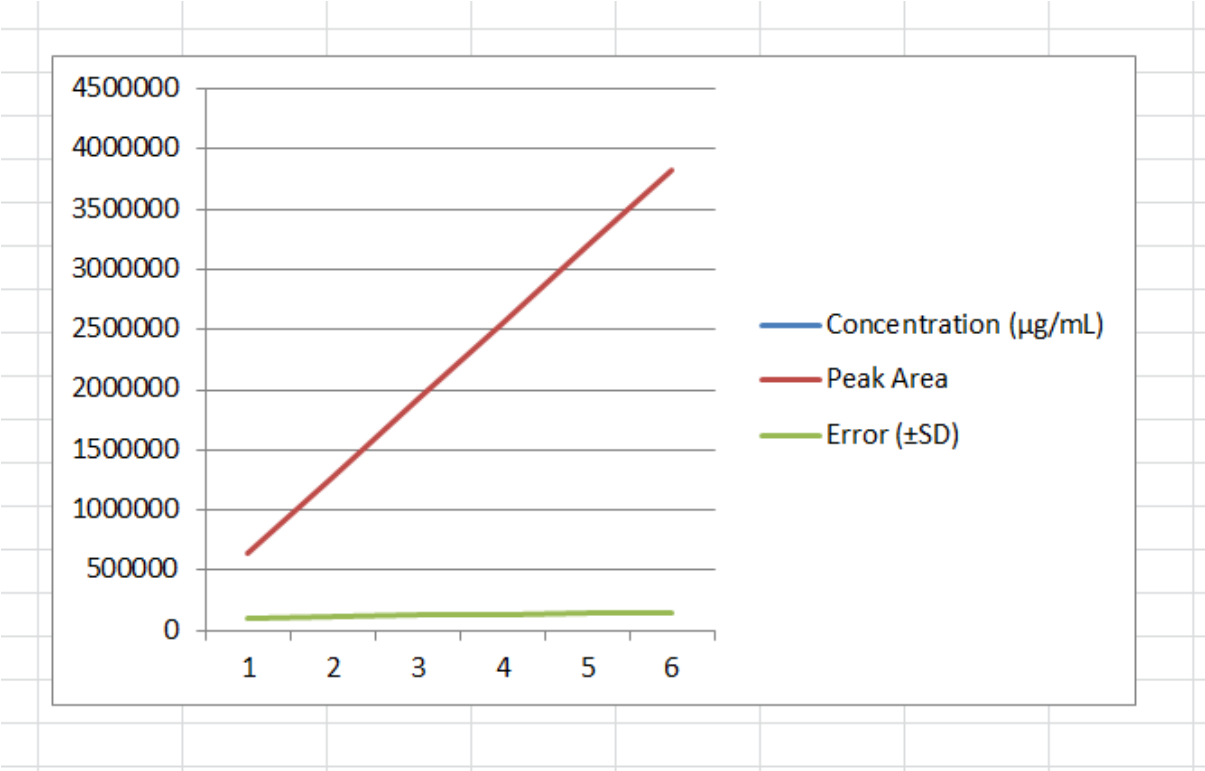


Figure 2. Calibration graph for Bedaquiline

2.5 Quality by Design (QbD) Framework

The Quality by Design (QbD) framework improved the RP-HPLC method for Bedaquiline. This method follows ICH guidelines Q8(R2), Q9, and Q10. It focuses on creating quality from the beginning, not just testing the final product. [13].

The process started with defining the Analytical Target Profile (ATP). This profile clearly stated the goals of the method. This study aimed to accurately and precisely quantify Bedaquiline in pharmaceutical dosage forms. It focused on achieving strong sensitivity, good resolution, and efficient run time.

1. **QbD approach for HPLC method optimization**

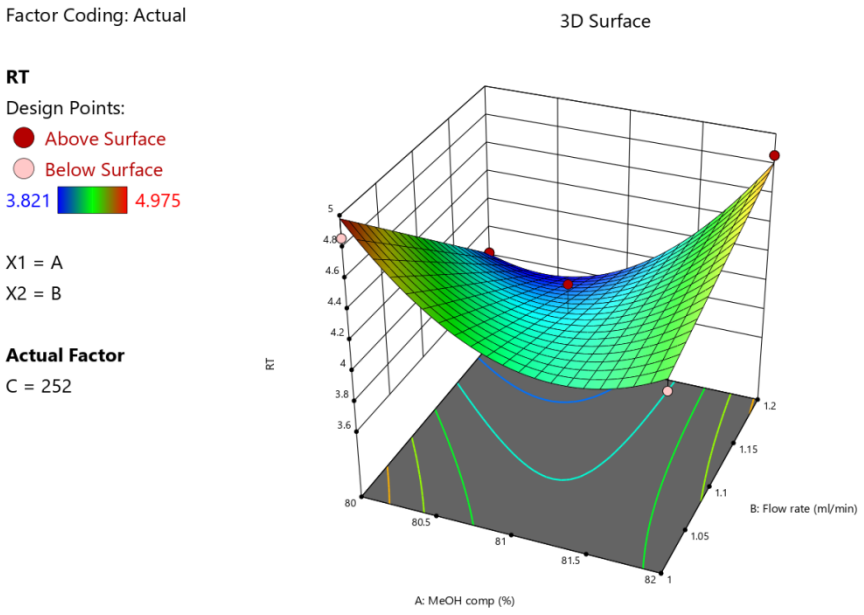
The seventeen runs were used to analyse the response surface study type, central composition design, and quadratic design model. (Table 1)

Table 1: Optimization of parameters for analysis of Rifapentine

Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4
	A: MeOH comp (%)	B: Flow rate (mL/min)	C: Wavelength(nm)	RT	PA	TP	TF
1	80	1.1	253	4.892	1806.38	8164	1.25
2	81	1.2	251	4.195	1630.53	8028	1.12

3	81	1	251	4.331	1956.95	8693	1.06
4	80	1.2	252	3.821	1655.63	7675	1.17
5	81	1.1	252	4.084	1781.92	8121	1.36
6	81	1.1	252	4.067	1775.74	8259	1.16
7	81	1.1	252	4.065	1773.12	8260	1.16
8	81	1.1	252	4.066	1776.66	8262	1.26
9	82	1	252	4.342	1961.08	8526	1.06
10	80	1.1	251	4.516	1841.31	8023	1.02
11	80	1	252	4.86	1978.34	8695	1.14
12	81	1	253	4.781	2044.15	8417	1.11
13	81	1.2	253	3.889	1873.32	7834	1.25
14	82	1.2	252	4.86	1858.42	8421	1.16
15	82	1.1	253	4.785	1956.63	8249	1.13
16	82	1.1	251	4.975	1945.8	8546	1.16
17	81	1.1	252	4.28	1972.14	8193	1.27

The equation retention time (for actual values) RT1=4.08 + 0.1091 x A- 0.1936 x B + 0.0412 x C + 0.3893 x A x B - 0.1415 x A x C - 0.1890 B x C + 0.4221 x A^2 + 0.2504 x C^2 concluded that, as mobile phase MeOH increases, flow rate decreases, and the value of RT is increased. The p-values < 0.0001 indicate that the analytical model was significant. The signal-to-noise (S/N) ratio of 15.075, greater than 4, indicates a sufficient signal. Therefore, we interpret that this mode can help navigate the design space. (Figure no.3)



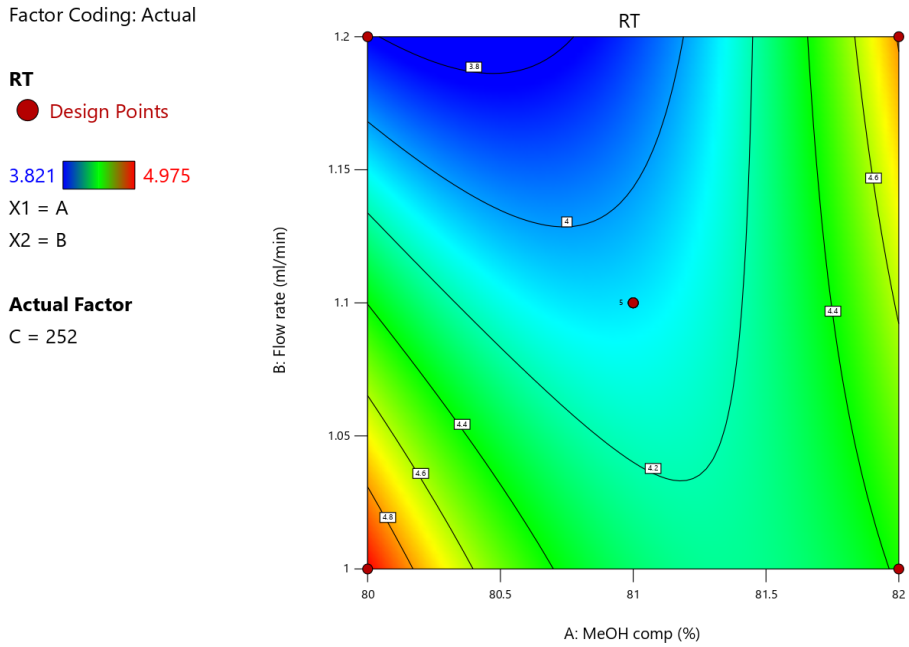
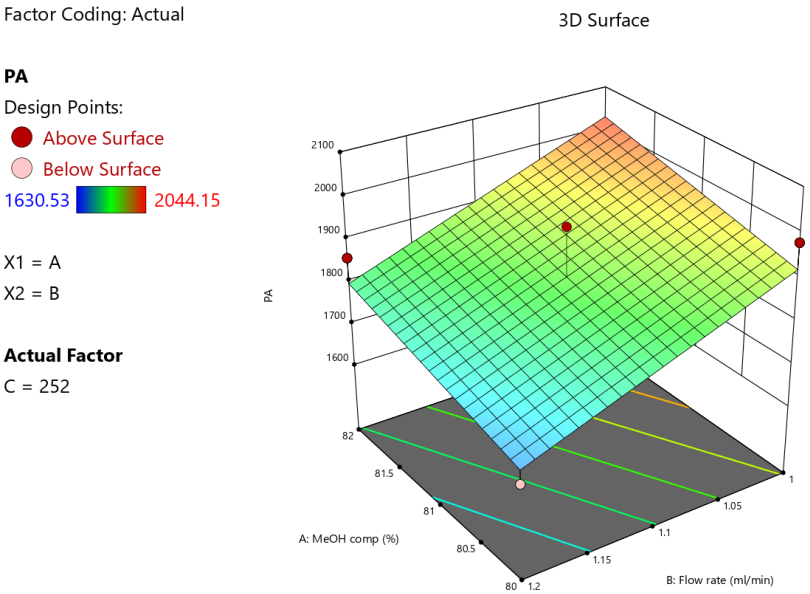


Figure no. 3: a) The effect of an independent factor on the retention time of a 3D sensitive surface. (b). Analysis of the independent factor's impact on retention time using a contour plot.

The equation peak asymmetry (for actual values) PA = 1858.12 + 55.03 x A - 115.33 x B + 38.24 x C interpreted that as mobile phase (A) increases (+55.03) with β2 (-115.33) flow rate decreases, the peak asymmetry increases. The model F-value of 7.72 indicated that the model was significant; additionally, p-values < 0.0033 also indicated that the analytical model was significant. The signal-to-noise ratio of 8.958 indicates sufficient signal. Hence, the parameter was also optimised to direct the design space. (Figure no. 4)



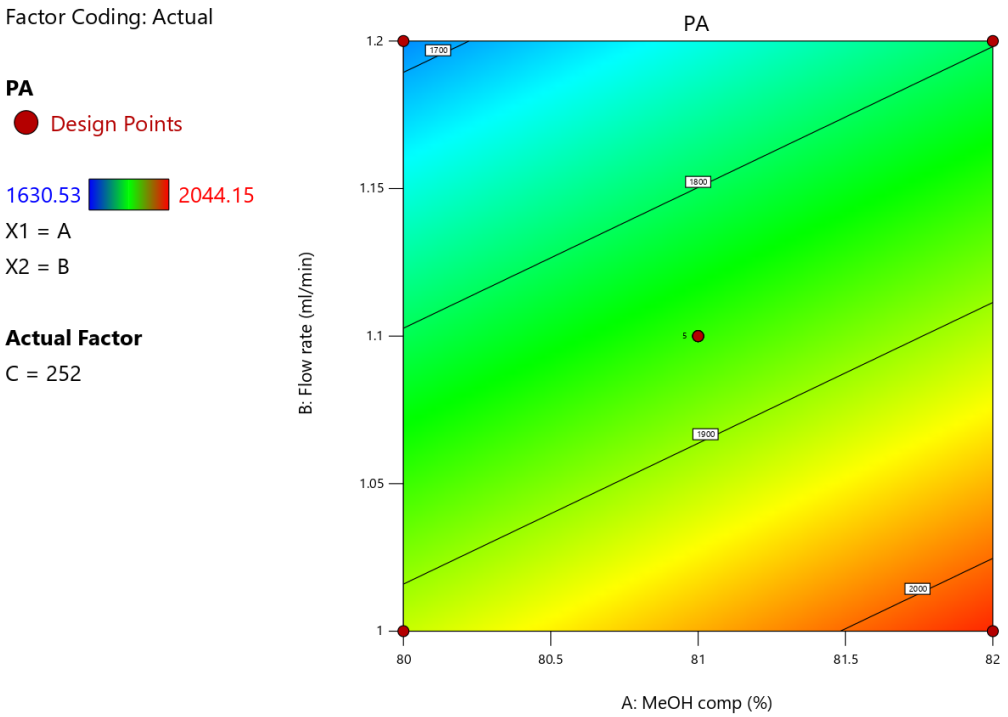


Figure no. 4: a) The effect of an independent factor on the peak area of a 3D sensitive surface. (b). Analysis of the independent factor's impact on peak area using a contour plot.

Moreover, the equation TP= 8256.82 + 148.13 x A -296.62 x B - 78.25 x C + 228.75 A x B - 109.50 x A x C + 20.50 x B x C determined that mobile phase β1 positive coefficient (A, +148.13) increases, the β2 negative coefficient (B) decreased, and the value of TP is increased.

The model F-value of 33.45 indicated that the model was significant; moreover, p-values < 0.0001 also indicated that the analytical model terms were significant. The S/N ratio of 21.657 indicates a sufficient signal. Hence, this parameter was also optimised to navigate the design space. (Figure 5)

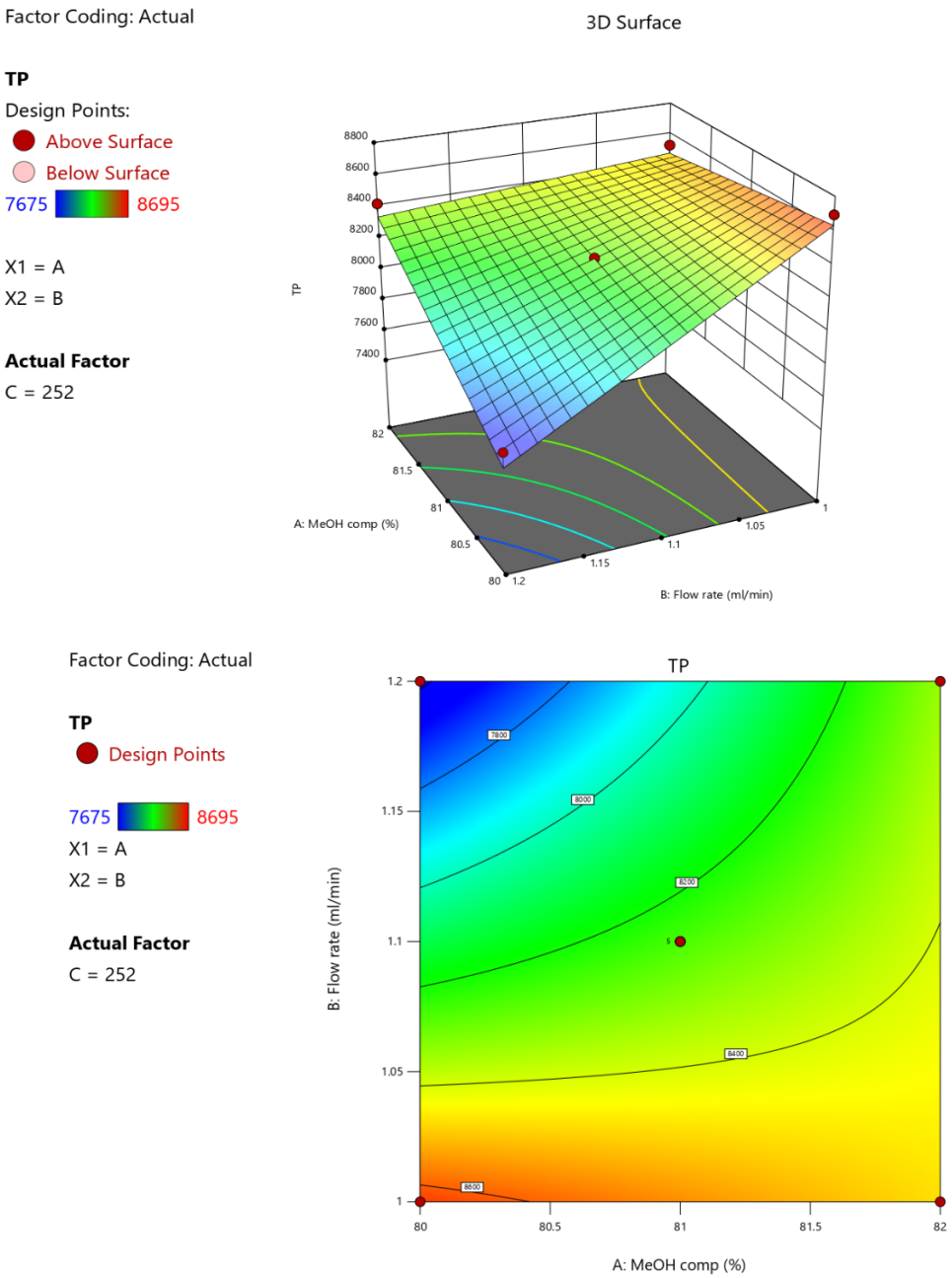


Figure no. 5: a) The effect of an independent factor on the theoretical plate of a 3D sensitive surface. (b). Analysis of the independent factor's impact on theoretical plate using a contour plot.

The equation for TF = + 1.24 - 0.0087 x A + 0.0413 x B + 0.0475 x C + 0.0175 x A x B - 0.0650 x A x C + 0.0200 x B x C - 0.0523 x A^2 - 0.0573 x B^2 - 0.0497 x C^2 resulted in the mobile phase coefficient decreased as flow rate and wavelength increased, and the TF increased. The model F-value of 2.33 implies the model was not significant relative to the noise. There is a 13.86% chance that an F-value

this large could occur due to noise. (Figure 4); however, the S/N ratio of 4.433 indicates a sufficient signal. Hence, the parameter was also optimised to navigate the design space. (Figure 6).

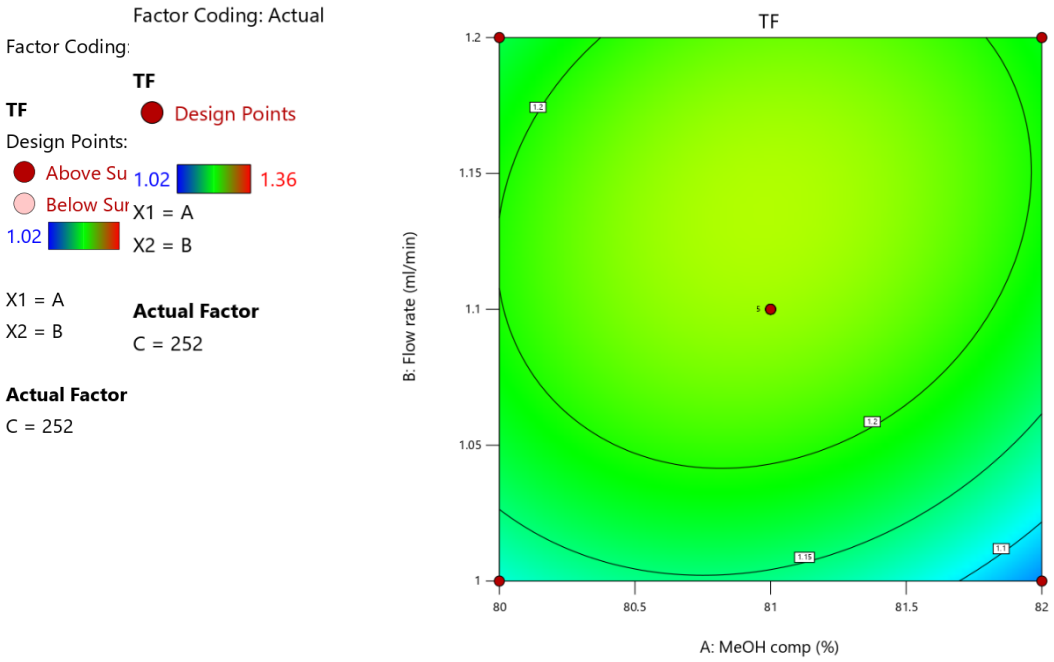


Figure no. 6: a) The effect of an independent factor on the tailing factor of a 3D sensitive surface. (b). Analysis of the independent factor's impact on tailing factor using a contour plot.

Thus, examining all the results, we have concluded that the surface sensitivity of BBD indicates that the experimental conditions were matched to linear and quadratic equations using multiple regression Next, we identified Critical Method Parameters (CMPs). These are the key operational variables that can impact method performance. We chose several parameters.

Factors include:

- Mobile phase composition
- Flow rate
- Detection wavelength
- Column temperature
- Injection volume

This was based on our experience, early tests, and scientific reasoning.

We used a Design of Experiments (DoE) strategy. This helped us check and enhance the method's strength and performance. This statistical method lets us check many variables at once. We can see how they interact. This helps us find the best conditions. We use fewer experiments than the usual trial-and-error method. Full factorial and central composite designs are often used in these studies. They help outline a design space where the method works well [14].

Also, we did a risk assessment using an Ishikawa (fishbone) diagram. This tool shows possible sources of method variability.

We looked at different areas to find and manage errors or drift. These areas included:

- Instrument performance
- Column selection
- Reagent quality
- Analyst technique
- Environmental factors

Integrating these QbD principles optimized the analytical method's performance. It also met regulatory expectations for method lifecycle management. This made the method stronger, easier to repeat, and better for regular quality control [15].

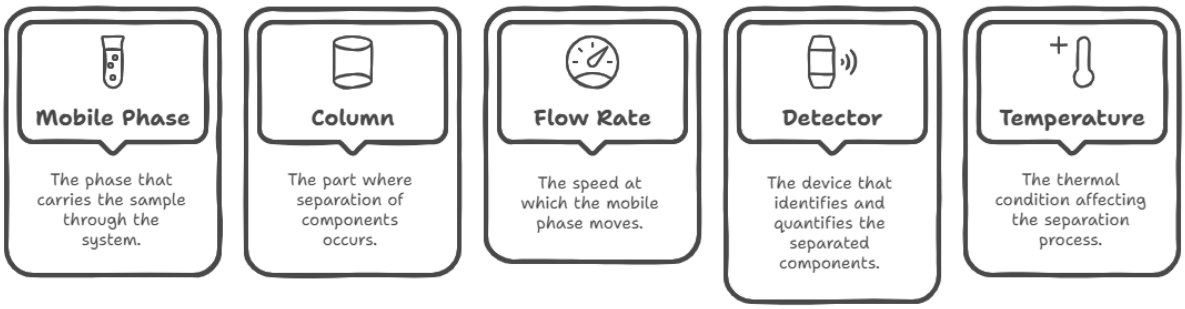


Figure 7. Ishikawa diagram for method variability

3. Method Development Using QbD and Box-Behnken Design

3.1 Critical Method Parameters (CMPs)

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In the Quality by Design (QbD) approach, it's key to find and evaluate Critical Method Parameters (CMPs). This step helps create a strong and dependable analytical method. CMPs are the key variables that affect a method's Critical Quality Attributes (CQAs). These include peak resolution, retention time, tailing factor, and sensitivity [16].

In this study, we found three main CMPs. We based this on preliminary trials, existing literature, and scientific reasons.

Flow Rate: The flow rate, ranging from 0.9 to 1.1 mL/min, is key. It affects both the analyte retention time and the overall chromatographic behavior. Small changes can cause big differences in elution time and peak symmetry. This can impact how accurate the measurements are [17].

Mobile Phase Composition: The methanol and 0.1% orthotic phosphoric acid amount in the mobile phase changed from 45% to 55% (v/v). Its percentage changes the elution strength of the mobile phase. Changes in composition can affect retention, resolution, and selectivity. This is important when analyzing compounds with different polarity profiles, like Bedaquiline [18].

Detection Wavelength: We explored the best detection wavelength from 237 to 247 nm. This range includes the λ_max of Bedaquiline. Accurate wavelength selection ensures maximal absorbance and

specificity while minimizing background noise. Small changes in wavelength can greatly affect peak height and area. This is because many pharmaceutical compounds have a chromophoric nature [19]

.3.2 Design of Experiments (DoE)

Factors include:

Mobile phase composition

Flow rate

Detection wavelength

Column temperature

Injection volume

This was based on our experience, early tests, and scientific reasoning.

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- Environmental factors

Integrating these QbD principles optimized the analytical method's performance. It also met regulatory expectations for method lifecycle management. This made the method stronger, easier to repeat, and better for regular quality control [15].

3.3 Box-Behnken Design (BBD) in QbD Approach

We used a Box-Behnken Design (BBD) to improve our method development in the QbD framework. BBD is a response surface methodology (RSM). It helps evaluate the relationship between several variables and responses. It does this with fewer experimental runs than full factorial designs. It helps optimize three or more parameters at once. This also cuts down on the number of trials needed.

In this study, we used BBD to improve these critical method parameters (CMPs):

- Flow rate (0.9, 1.0, 1.1 mL/min)
- 0.1% orthotic phosphoric acid (45%, 50%, 55%)
- Detection wavelength (237, 242, 247 nm)

The experiment had 15 runs, with three center points. It aimed to study how the chosen variables interacted. The study focused on three main chromatographic responses: retention time, peak area, and tailing factor.

ANOVA showed that flow rate and mobile phase composition significantly interact. This affects both retention time and peak shape. The response surface plots showed the best conditions. These matched the final optimized method: a flow rate of 1.0 mL/min, 50% 0.1% orthotic phosphoric acid, and a wavelength of 242 nm.

Using BBD in the QbD framework improved the RP-HPLC method's strength and reliability. It also cut down the trial-and-error time during optimization.

4. Method Validation

Method validation followed the International Council for Harmonisation (ICH) Q2(R1) guidelines. These guidelines detail key rules for evaluating analytical methods in the pharmaceutical industry. We examined each validation trait:

- System suitability
- Linearity
- Accuracy
- Precision
- Specificity
- Detection limit
- Quantitation limit
- Robustness
- Range

This ensured the reliability and reproducibility of the RP-HPLC method for Bedaquiline

4.1 System Suitability

Before routine analyses, we did system suitability testing. This checked the performance integrity of the chromatographic system. A standard solution of Bedaquiline, at 120 µg/mL, was injected six times. This was done under optimized chromatographic conditions.

The % relative standard deviation (%RSD) of the peak area was 0.49%. This is well below the acceptable limit of 2.0%. So, it shows excellent injection precision. The column efficiency was calculated at 2621 theoretical plates. This shows it has good resolving power for the analyte. The tailing factor was found to be 1.05. This shows a symmetrical peak shape with little distortion. All these parameters met the acceptance criteria. This confirms that the method is suitable for reliable routine use [26].Table 1. System Suitability Results for Bedaquiline RP-HPLC Method

Table 2. System Suitability Results for Bedaquiline RP-HPLC Method

System Suitability Parameter	Acceptance Criteria	Observed Result
Capacity Factor (k')	≥ 2	2.27
Precision (%RSD, n = 6)	≤ 2%	0.49%
USP Tailing Factor	≤ 2	1.05 ± 0.008
USP Plate Count (N)	> 2000	2621 ± 5.04
Resolution (Rs)	> 2	3.76

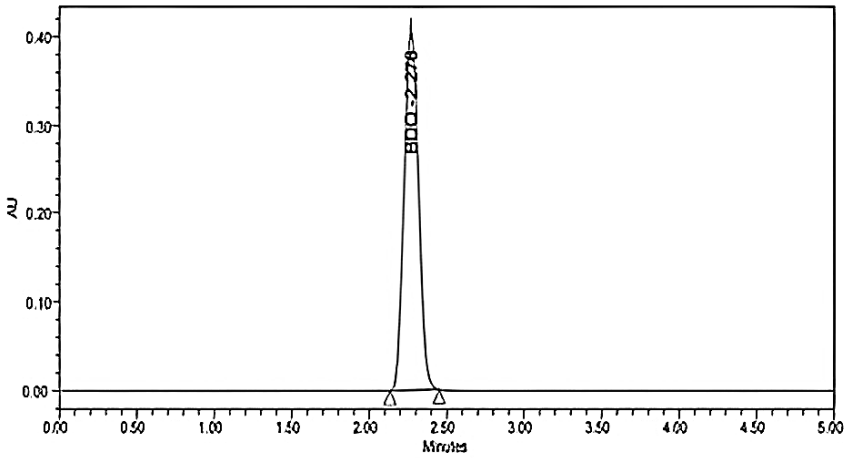

Figure 8 HPLC chromatogram for estimation of system suitability for Bedaquiline

4.2 Linearity

We tested the method's linearity from 30 to 180 µg/mL. This range includes the expected analyte levels in dosage forms. Calibration standards were made as described before. Each level was tested three times.

The calibration curve showed a clear link between analyte concentration and peak area. The regression equation obtained was:

$y = 20325x + 21664$

where y represents the mean peak area and x is the concentration of Bedaquiline in µg/mL. The correlation coefficient (r^2) was 0.99998. This shows a strong linearity and very little deviation from ideal behavior.

The results show that the RP-HPLC method is linear in the specified range.

It helps with tasks such as:

- Content uniformity
- Dosage form analysis
- Pharmacokinetic studies [27]

4.3 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

We determined the sensitivity of the RP-HPLC method. We calculated the Limit of Detection (LOD) and the Limit of Quantitation (LOQ). This was based on the standard deviation of the intercept (σ) and the slope (S) of the calibration curve. These values were derived using the standard ICH-recommended equations::

$LOD = 3.3 \times (\sigma/S)$

$LOQ = 10 \times (\sigma/S)$

Using these formulas on the regression data gave an LOD of 3 µg/mL and an LOQ of 12 µg/mL. The method can accurately detect and measure low levels of Bedaquiline. [28].

4.4 Accuracy

We checked the method's accuracy. We ran recovery studies at three levels: 50%, 100%, and 150% of the target concentration. This target is 120 µg/mL. This means testing at 60, 120, and 180 µg/mL. Each level was prepared in triplicate and analyzed using the developed method.

The percent recovery was between 100.1% and 101.1%. The %RSD stayed below 1%. This shows the method is both accurate and precise. These results show that the method accurately measures Bedaquiline levels in samples. It works well, even with different concentrations. [29]

4.5 Precision

Precision studies checked both repeatability (intraday) and intermediate precision (interday). They analyzed samples at three concentration levels: 60, 120, and 180 µg/mL. For intraday precision, multiple replicates were analyzed within a single day. We repeated the same procedure for interday precision over three days. The conditions remained consistent throughout.

The %RSD values stayed below 1.1% at all levels and times. This shows the method is reproducible. It also confirms that small changes in the environment or time don't affect its performance [30].

4.6 Robustness

We tested robustness by carefully changing key method parameters a little, such as:

- Flow rate: ±10% (0.9 and 1.1 mL/min)
- Mobile phase composition: ±5% variation methanol and 0.1% orthotic phosphoric acid concentration
- Detection wavelength: ±5 nm from the optimized 242 nm

These changes did not significantly affect retention time, peak area, or tailing factor. The chromatographic profiles stayed consistent. The method is reliable and robust, even with small changes in testing conditions [31].

4.7 Specificity

The method's specificity showed when we analyzed samples exposed to light. This simulates how Bedaquiline might break down during photodegradation. The resulting chromatograms were examined for interference at the analyte's retention time.

No overlapping or co-eluting peaks showed up at 2.27 minutes. This time is for Bedaquiline's retention. This shows that the method can measure the drug accurately. It does this even with degradation products or possible excipients. This validates the method's stability-indicating capability [32].

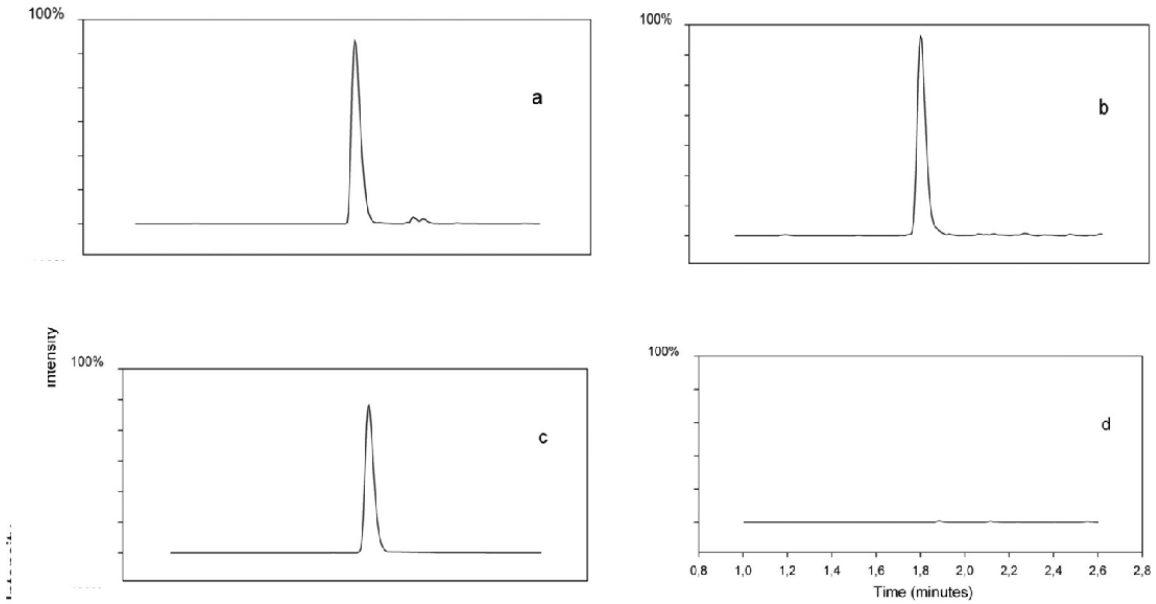

Figure 9. Chromatograms of Bedaquiline standard and photodegraded sample

5. Results and Discussion

The RP-HPLC method for measuring Bedaquilinein medicines is effective and strong. It also meets regulatory standards. Integrating the Quality by Design (QbD) framework in method development was key. It helped optimize performance, understand method variability, and ensure long-term reliability in analysis..

5.1 Method Optimization Outcomes

Under the best conditions, we used a Symmetry C18 column (150 mm × 4.6 mm, 3.5 µm). The mobile phase was a mix of 0.1% OPA and methanol. The flow rate was 1.0 mL/min, and we detected at 242 nm. This method gave a retention time of 2.27 minutes for Bedaquiline. The peaks were clear with no interference from excipients or breakdown products. They showed great symmetry, with a tailing factor of 1.05. This meets all chromatographic quality standards.

A 3^2 full factorial Design of Experiments (DoE) helped find the best conditions. Flow rate and organic phase ratio were checked. They affect retention time, peak area, and tailing factor. The statistical model showed a strong interaction between these parameters. The response surface analysis identified the design space for the best method performance..

5.2 Method Validation Highlights

All validation studies followed ICH Q2(R1) guidelines. They showed that the method is reliable and strong analytically.

System Suitability: Injecting a 120 µg/mL standard solution in six tests gave:

- %RSD of peak area: 0.49%
- Theoretical plates: 2621

- Tailing factor: 1.05. These values are within acceptable limits. This confirms the system is ready for routine analysis.

Linearity: The calibration curve for concentrations from 30 to 180 µg/mL was linear. The regression equation is:

$y = 20325x + 21664$, and $r^2 = 0.99998$

The high correlation coefficient shows a strong link between concentration and peak area.

- LOD and LOQ: These values are found using the standard deviation of the intercept and slope method.

- LOD was determined to be 3 µg/mL
- LOQ was 12 µg/mL

These values show that the method is sensitive and good for detecting low levels of the analyte.

- Accuracy (Recovery Studies): Conducted at 50%, 100%, and 150% of the target level (120 µg/mL). Results are as follows:

- Percent recovery ranged from 100.1% to 101.1%
- %RSD was always < 1.0%. This high precision shows the method accurately quantifies Bedaquiline at different concentrations.

• Precision: Evaluated through both intraday and interday studies:

- %RSD values stayed under 1.1% for all tested concentrations: 60, 120, and 180 µg/mL. This shows the method's repeatability and intermediate precision.

• Robustness: Tested by introducing variations in:

- Flow rate (±10%)
- Mobile phase ratio (±5%)
- Wavelength (±5 nm)

These changes did not affect retention time, peak area, or tailing factor. This confirms the method is robust.

• Specificity: Analyzed using photodegraded samples. No co-eluting or interfering peaks were observed at the retention time of 2.27 minutes. This validates the method's specificity and stability-indicating capability.

6 Discussion

The RP-HPLC method shown here combines strong analysis with QbD-driven reliability. Controlling Critical Method Parameters (CMPs) boosts throughput and shortens run times. It also ensures high reproducibility, precision, and linearity. These features make it perfect for estimating Bedaquiline in bulk drugs and medicines.

The method is strong under changing analytical conditions. Using QbD principles helps meet regulatory standards. It also makes lifecycle management easier. Plus, it allows smooth changes after approval.

7. Conclusion

This study shows the successful creation and testing of a QbD-driven RP-HPLC method. It quantifies Bedaquilinein pharmaceutical formulations. We used QbD principles to optimize the method. This

involved defining the Analytical Target Profile (ATP). We needed to find Critical Method Parameters (CMPs). Then, we used a 3^2 full factorial Design of Experiments (DoE). As a result, we achieved high analytical performance and better understood variability. The final method showed great linearity (r^2 = 0.99998) from 30 to 180 µg/mL. It had a short retention time of 2.27 minutes. The peaks were symmetrical (tailing factor = 1.05) and the theoretical plate count was high at 2621. This indicates strong chromatographic efficiency.

Validation studies showed the method's precision. The intra- and interday %RSD was under 1.1%. It also confirmed accuracy with recovery rates between 100.1% and 101.1%. The sensitivity was good, with LOD at 3 µg/mL and LOQ at 12 µg/mL. The method was strong. Small changes in conditions did not change its performance. The method showed strong specificity. It clearly identified Bedaquiline, even when tested with possible degradation products and excipients. These results show that the method works well for regular quality control. It's also effective for stability studies and meeting pharmaceutical regulations. This approach meets global regulatory standards and supports efficient lifecycle management. It creates a reliable analytical platform for current and future Bedaquiline-based therapies.

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