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SIMULTANEOUS ESTIMATION OF DELAMANID AND BEDAQUILINE IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORMS BY RP-HPLC

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ABSTRACT

A simple, accurate, precise, and economical RP-HPLC method was developed and validated for the simultaneous estimation of Delamanid and Bedaquiline in bulk drug and pharmaceutical dosage forms. Chromatographic separation was achieved using a Kromasil C18 column (150 × 4.6 mm, 5.0 µm) with a mobile phase consisting of 0.01 N ammonium acetate buffer and acetonitrile in the ratio of 60:40 % v/v. The mobile phase was pumped at a flow rate of 1.0 mL/min, and the column temperature was maintained at 30°C. The ammonium acetate buffer was prepared by adding 0.1% acetic acid and adjusting the pH to 4.0. The optimized detection wavelength was 220 nm. The retention times of Delamanid and Bedaquiline were found to be 2.249 and 2.970 min, respectively. The percentage RSD for Delamanid and Bedaquiline was found to be 1.4% and 0.5%, respectively, indicating good precision. The percentage recovery was found to be 99.69% for Delamanid and 99.90% for Bedaquiline. The LOD and LOQ values obtained from the regression equations were 0.10 and 0.30 for Delamanid and 0.25 and 0.74 for Bedaquiline, respectively. The assay results were found to be 99.52% for Delamanid and 99.95% for Bedaquiline. The regression equations were y = 19629x + 3362.1 for Delamanid and y = 23316x + 9810.4 for Bedaquiline. The developed method provided short retention times and reduced total analysis time, making it simple, rapid, economical, and suitable for routine quality control analysis of Delamanid and Bedaquiline in pharmaceutical industries.

Keywords: *Delamanid, Bedaquiline, RP-HPLC, Simultaneous Estimation, Method Validation, Pharmaceutical Dosage Form.*

1. INTRODUCTION

The quality, safety, and efficacy of pharmaceutical products are essential for ensuring their therapeutic effectiveness and patient safety. Consequently, quality assurance and quality control play a vital role in the development, manufacture, and regulatory evaluation of pharmaceutical formulations. Reliable analytical methods are therefore required for the identification, quantification, and assessment of drug substances and their dosage forms. The quality of analytical results is strongly influenced by the performance, selectivity, accuracy, precision, robustness, and reproducibility of the analytical method employed. Hence, the development and validation of simple, rapid, economical, and reliable analytical methods are important for routine quality control and regulatory compliance [1].

Pharmaceutical analysis involves several analytical techniques, including spectrophotometric, electrochemical, titrimetric, and chromatographic methods. Among these, high-performance liquid chromatography (HPLC) has become one of the most widely employed techniques because of its versatility, sensitivity, precision, and ability to separate and quantify multiple components in complex pharmaceutical matrices. HPLC provides efficient separation based on differences in the distribution of analytes between the stationary and mobile phases and is particularly useful for the analysis of compounds with diverse physicochemical properties [2-3].

Reverse-phase high-performance liquid chromatography (RP-HPLC), which employs a non-polar stationary phase and a relatively polar mobile phase, is the predominant mode used for pharmaceutical analysis. Its advantages include high resolution, reproducible retention, flexible mobile-phase selection, and compatibility

with various detection systems. The performance of an HPLC method depends on several factors, including column chemistry, mobile-phase composition, pH, flow rate, temperature, detection wavelength, and injection volume. Optimization of these parameters is essential for achieving satisfactory resolution within a short analysis time [4].

Therefore, the development of a robust and validated RP-HPLC method is highly valuable for the simultaneous estimation of pharmaceutical compounds in bulk drug substances and dosage forms, supporting efficient routine quality control and ensuring consistent product quality.

1.1 HPLC Instrumentation, Method Development and Validation

HPLC instrumentation consists of several essential components, including the solvent delivery system, degassing unit, sample injector, analytical column, mobile phase, and detector. Based on the mechanism of eluent displacement, HPLC pumps are generally classified as syringe pumps and reciprocating piston pumps. Modern HPLC systems are also equipped with online degassing units to remove dissolved gases from the mobile phase, thereby minimizing baseline fluctuations and improving detector performance. Mobile phases should be properly filtered and degassed by techniques such as vacuum degassing, membrane filtration, helium purging, sonication, or combinations thereof [5].

The sample introduction system enables reproducible injection of samples into the pressurized mobile-phase stream. Modern injector valves and autosamplers allow accurate sample introduction with minimal flow disturbance and band broadening. The analytical column is considered the heart of the HPLC system because its stationary-phase characteristics largely determine separation efficiency and selectivity. Important column parameters include particle size, particle shape, pore size, surface area, carbon loading, and end-capping. Silica-based C18 and C8 columns are widely employed in reversed-phase HPLC because of their excellent reproducibility and broad applicability [6].

The mobile phase plays a critical role in chromatographic separation. Its composition, pH, solvent strength, ionic strength, and compatibility with the analyte must be carefully optimized. The detector converts the separated analyte into a measurable signal. UV-visible detectors are commonly used in pharmaceutical analysis because of their sensitivity, simplicity, and suitability for compounds containing chromophores. Other detectors include fluorescence, refractive index, electrochemical, mass spectrometric, and infrared detectors.

1.2 HPLC Method Development

Analytical method development involves systematic selection and optimization of chromatographic conditions to achieve adequate separation, sensitivity, accuracy, precision, and reproducibility. The major steps include selection of the HPLC method and initial system, selection of initial conditions, selectivity optimization, system parameter optimization, and method validation. During development, the physicochemical properties of the analyte, solubility, pKa, stability, sample matrix, detection characteristics, and available literature should be considered. Parameters such as stationary phase, mobile-phase composition, pH, flow rate, column temperature, injection volume, and detection wavelength are optimized to obtain satisfactory retention and resolution within an acceptable analysis time [7-8].

1.3 Stability-Indicating Method

A stability-indicating analytical method is capable of accurately measuring the active pharmaceutical ingredient in the presence of degradation products, process impurities, excipients, and other potential interfering substances. Such methods are particularly important for stability studies because they provide reliable information regarding drug degradation and product quality during storage. Forced degradation studies under conditions such as acidic, alkaline, oxidative, thermal, and photolytic stress are commonly performed to establish degradation pathways and demonstrate the specificity of the analytical procedure [9-10].

1.4 Analytical Method Validation

Analytical method validation provides documented evidence that an analytical procedure is suitable for its intended purpose. Important validation characteristics include system suitability, specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ). System suitability confirms that the chromatographic system performs adequately before sample analysis. Linearity establishes the relationship between analyte concentration and detector response. Precision evaluates the closeness of agreement among repeated measurements and includes repeatability, intermediate precision, and reproducibility. Accuracy is generally established through recovery studies, while specificity demonstrates the ability of the method to distinguish the analyte from other components [11-15].

Robustness evaluates the ability of the method to remain unaffected by small, deliberate changes in analytical parameters such as flow rate, pH, mobile-phase composition, and column temperature. LOD represents the lowest concentration that can be detected, whereas LOQ represents the lowest concentration that can be quantitatively determined with suitable accuracy and precision. The commonly used equations are $LOD = 3.3\sigma/S$ and $LOQ = 10\sigma/S$, where σ is the standard deviation of the response and S is the slope of the calibration curve. Properly developed and validated HPLC methods therefore provide reliable, accurate, precise, and reproducible results and are essential for routine pharmaceutical quality control [16-17].

2. AIM, OBJECTIVES AND PLAN OF WORK

The present study was aimed at developing a simple, accurate, precise, sensitive, selective, reproducible, rapid, and stability-indicating HPLC method for the simultaneous estimation of Bedaquiline and Delamanid in bulk drug substances and pharmaceutical dosage forms. The developed analytical method was intended to be validated according to ICH guidelines and subsequently applied for the simultaneous estimation of both drugs in pharmaceutical formulations. Bedaquiline is a diarylquinoline antimycobacterial agent used in combination with other antibacterial drugs for the treatment of tuberculosis caused by drug-resistant Mycobacterium tuberculosis. It is marketed under the brand name Sirturo and has the molecular formula C32H31BrN2O2 with an average molecular weight of 555.505 g/mol. Bedaquiline is a solid powder, practically insoluble in water, with a predicted logP of 7.13 and predicted pKa values of 13.61 and 8.91. Its mechanism of action involves inhibition of mycobacterial ATP synthase through binding to subunit c, thereby interfering with energy generation in M. tuberculosis. Bedaquiline is highly protein bound (approximately 99.9%), has a volume of distribution of about 164 L, and undergoes extensive oxidative metabolism, predominantly through CYP3A4, forming the N-monodesmethyl metabolite M2. It is mainly eliminated through feces and has a prolonged terminal half-life of approximately 5.5 months. Delamanid is a nitro-dihydro-imidazooxazole derivative and antimycobacterial drug used as part of an appropriate combination regimen for pulmonary multidrug-resistant tuberculosis. It is marketed under the brand name Deltyba and has the molecular formula C25H25F3N4O6 with an average molecular weight of 534.492 g/mol. Delamanid occurs as a solid powder, has limited aqueous solubility, and possesses a predicted logP of 6.14 and a predicted pKa of 5.51. It is a prodrug that undergoes biotransformation through the mycobacterial F420-dependent coenzyme system, including the deazaflavin-dependent nitroreductase Rv3547, producing active metabolites that inhibit mycolic acid biosynthesis and exert antimycobacterial activity against both growing and non-growing M. tuberculosis. Delamanid exhibits high protein binding (≥99.5%), a large apparent volume of distribution of approximately 2,100 L, and reaches steady-state plasma concentrations within 10–14 days. It is predominantly metabolized by albumin and, to a lesser extent, by CYP3A4, with additional contributions from CYP1A1, CYP2D6, and CYP2E1. The drug is primarily eliminated through feces, with less than 5% excreted in urine, and has an elimination half-life of approximately 30–38 hours. Considering the therapeutic importance of these drugs and their use in combination regimens for drug-resistant tuberculosis, development of a reliable stability-indicating HPLC method for their simultaneous estimation is important for routine quality control and assessment of pharmaceutical formulations.

3. MATERIALS AND METHODS

3. 1. Materials

Bedaquiline and delamanid reference standards and their combined pharmaceutical dosage form were used for the study. HPLC-grade acetonitrile, methanol, potassium dihydrogen orthophosphate, orthophosphoric acid, and distilled/milli-Q water were procured from Rankem, India. Hydrogen peroxide, hydrochloric acid, and sodium hydroxide were used for forced-degradation studies.

3. 2. Instruments

The analysis was performed using a Waters HPLC 2695 system equipped with a quaternary pump, autosampler, and photodiode-array (PDA) detector, operated using Empower 2 software. A PG Instruments T60 UV-visible spectrophotometer with matched quartz cells was used for spectrophotometric measurements. A Denver electronic balance, pH meter, and ultrasonicator (BVK Enterprises, India) were also used.

3. 3. Preparation of Solutions

Acetonitrile:water (50:50, v/v) was selected as the diluent based on the solubility of the drugs. Standard stock solutions were prepared separately by accurately weighing 10 mg of delamanid and 20 mg of bedaquiline into 50-mL volumetric flasks, dissolving with diluent using sonication, and making up to volume to obtain concentrations of 200 and 400 µg/mL, respectively. Working standard solutions were prepared by appropriate dilution to obtain final concentrations of 20 µg/mL delamanid and 40 µg/mL bedaquiline.

For sample preparation, ten tablets were weighed and powdered, and an amount equivalent to one tablet was transferred to a volumetric flask. Acetonitrile was added and the mixture was sonicated, followed by dilution with the selected diluent. The solution was filtered through a 0.45-µm membrane filter and further diluted to obtain concentrations equivalent to 20 µg/mL of delamanid and 40 µg/mL of bedaquiline.

3. 4. Preparation of Mobile-Phase Buffer

A 0.1 N potassium dihydrogen orthophosphate buffer was prepared by dissolving 1.36 g of potassium dihydrogen orthophosphate in approximately 900 mL of milli-Q water. The solution was sonicated and degassed, diluted to 1000 mL with water, and treated with 1 mL of triethylamine. The pH was adjusted to 3.8 using dilute orthophosphoric acid.

3. 5. Method Validation

The developed HPLC method was validated in accordance with applicable ICH guidelines for system suitability, specificity, precision, linearity, accuracy, robustness, limit of detection (LOD), limit of quantification (LOQ), and stability-indicating capability.

System Suitability

Standard solutions containing 20 µg/mL delamanid and 40 µg/mL bedaquiline were injected six times. Retention time, peak area, theoretical plate count, peak tailing, and resolution were evaluated. The percentage relative standard deviation (%RSD) of peak areas was required to be not more than 2%.

Specificity

Specificity was assessed by injecting blank, standard, and sample solutions. The chromatograms were examined for any interfering peaks at the retention times of delamanid and bedaquiline.

Precision

Method precision was evaluated by analyzing the standard solution containing 20 µg/mL delamanid and 40 µg/mL bedaquiline. The precision was expressed as %RSD of the assay results.

Linearity

Linearity was evaluated using standard solutions corresponding to 25–150% of the working concentration. The concentrations used were 5–30 µg/mL for delamanid and 10–60 µg/mL for bedaquiline. Calibration curves were constructed by plotting peak area against concentration, and the regression equation and correlation coefficient were determined.

Accuracy

Accuracy was assessed by the standard-addition method at 50%, 100%, and 150% levels. Known quantities of delamanid and bedaquiline standards were spiked into the sample solution and analyzed. Percentage recovery was calculated, with an acceptance range of 98.0–102.0%.

Robustness

Robustness was evaluated by introducing deliberate variations in chromatographic conditions, including flow rate, mobile-phase composition, and column temperature. Flow rates of 0.9 and 1.1 mL/min and temperatures of 25 and 35°C were evaluated. The effect of these changes on system suitability and assay results was assessed.

LOD and LOQ

LOD and LOQ were evaluated by serial dilution of standard solutions to obtain low-concentration solutions of delamanid and bedaquiline. The corresponding chromatographic responses were evaluated to establish the detection and quantification limits of the method.

6. Forced-Degradation Studies

Forced-degradation studies were performed to establish the stability-indicating capability of the developed method. Standard drug solutions were subjected to acid, alkali, oxidative, thermal, photolytic, and neutral degradation conditions.

For **oxidative degradation**, standard solutions were treated with 20% hydrogen peroxide and maintained at 60°C for 30 min.

For **acid degradation**, the drug solutions were treated with 2 N hydrochloric acid and refluxed at 60°C for 30 min.

For **alkali degradation**, the drug solutions were treated with 2 N sodium hydroxide and refluxed at 60°C for 30 min.

For **thermal degradation**, the drug solutions were exposed to dry heat at 105°C for 1 h.

For **photolytic degradation**, the drug solutions were exposed to UV radiation in a photostability chamber for 1 day (approximately 200 Wh/m²).

For **neutral degradation**, the drug solutions were refluxed in water at 60°C for 1 h.

Following stress treatment, the solutions were appropriately diluted to obtain concentrations of 20 µg/mL delamanid and 40 µg/mL bedaquiline. A 10-µL aliquot was injected into the HPLC system, and the chromatograms were evaluated for degradation and the presence of additional peaks. The developed method was considered stability-indicating based on its ability to resolve the drug peaks from their degradation products.

3. RESULTS AND DISCUSSION

3. 1 Selection of Detection Wavelength

The UV spectra of delamanid and bedaquiline were evaluated to identify a suitable wavelength for simultaneous detection. Based on the spectral response of both drugs, **220 nm** was selected as the optimized detection wavelength for the chromatographic analysis.

3.2 Method Development and Optimization

The chromatographic method was developed by systematically varying the mobile-phase composition, organic modifier, stationary phase, and chromatographic conditions. Several trials were performed to obtain satisfactory peak shape, resolution, retention time, and system suitability.

In the first trial, methanol:0.1% orthophosphoric acid (50:50, v/v) was used as the mobile phase with an Ascantis C18 column. Both drugs were eluted; however, peak fronting was observed. In the second trial, acetonitrile:0.1% orthophosphoric acid (50:50, v/v) was used with the same column. Although both compounds were eluted within a short runtime, splitting of the bedaquiline peak was observed. In the third trial, the column was changed to a Discovery C18 column; however, broad peaks and peak splitting persisted. In the fourth trial, a Kromasil C18 column was employed with acetonitrile:0.1% orthophosphoric acid (50:50, v/v). Both drugs were eluted, but asymmetry of the bedaquiline peak was observed.

Based on these observations, the chromatographic conditions were further optimized using a **Kromasil C18 column (4.6 × 150 mm, 5 µm)** with **0.01 N potassium dihydrogen phosphate buffer:acetonitrile (60:40, v/v)** as the mobile phase. The flow rate was maintained at 1.0 mL/min, the column temperature at 30°C, detection wavelength at 220 nm, injection volume at 10 µL, and total runtime at 6 min. Acetonitrile:water (50:50, v/v) was used as the diluent.

Under the optimized conditions, delamanid and bedaquiline were eluted at approximately **2.24 and 2.97 min**, respectively, with satisfactory peak symmetry, theoretical plate count, and resolution. The method therefore provided rapid and efficient simultaneous separation of both drugs and was selected for further validation.

Optimized Chromatographic Conditions

Table 01: Optimized RP-HPLC chromatographic conditions for simultaneous estimation of Delamanid and Bedaquiline

Parameter	Optimized condition
Column	Kromasil C18, 4.6 × 150 mm, 5 µm
Mobile phase	0.01 N KH_2PO_4 buffer:acetonitrile (60:40, v/v)
Flow rate	1.0 mL/min
Detection wavelength	220 nm
Column temperature	30°C
Injection volume	10 µL
Runtime	6 min
Diluent	Acetonitrile:water (50:50, v/v)
Delamanid retention time	~2.24 min
Bedaquiline retention time	~2.97 min

3.3 System Suitability

System suitability was evaluated by six replicate injections of the standard solution containing 20 µg/mL delamanid and 40 µg/mL bedaquiline. The retention times, theoretical plate counts, tailing factors, and resolution were satisfactory. The mean retention times were approximately 2.25 min for delamanid and 2.98 min for bedaquiline. The theoretical plate counts were greater than 6800 for delamanid and 12,000 for bedaquiline, while the tailing factors were below 1.5. Resolution between the two peaks was approximately 6.3–6.4. The %RSD of peak areas was within the prescribed limit of 2.0%, confirming the suitability of the chromatographic system.

3.4 Specificity

Specificity was established by comparing chromatograms of the blank, placebo, standard, and sample solutions. No significant interfering peaks were observed at the retention times of delamanid and bedaquiline. The method was therefore considered specific for the simultaneous determination of both drugs in the pharmaceutical formulation.

3.5 Linearity

The linearity of the method was evaluated over the concentration ranges of **5–30 µg/mL for delamanid** and **10–60 µg/mL for bedaquiline**. The calibration curves showed a strong linear relationship between concentration and peak area.

For delamanid, the regression equation was:

y = 19629x + 3362.1

For bedaquiline, the regression equation was:

y = 23316x + 9810.4

The correlation coefficient was **0.999 for both drugs**, demonstrating excellent linearity within the investigated concentration ranges.

3.6 Precision

System Precision

System precision was evaluated using six replicate injections of the standard solution. The %RSD of peak areas was **1.5% for delamanid** and **0.4% for bedaquiline**, which were below the acceptance criterion of 2.0%. The results confirmed satisfactory instrument precision.

Method Precision

Repeatability was assessed by analyzing six independently prepared sample solutions. The %RSD values were **0.3% for delamanid** and **0.2% for bedaquiline**, indicating good repeatability of the analytical method.

Intermediate Precision

Intermediate precision was assessed on a different day using the same analytical procedure. The %RSD values were **0.3% for delamanid** and **0.2% for bedaquiline**. These results demonstrated good reproducibility of the method under different analytical conditions.

3.7 Accuracy

Accuracy was determined by the standard-addition method at 50%, 100%, and 150% concentration levels. Triplicate determinations were performed at each level. The mean percentage recoveries were **99.69% for delamanid** and **99.90% for bedaquiline**, demonstrating satisfactory accuracy of the method and compliance with the acceptance range of 98–102%.

3.8 Sensitivity

The sensitivity of the developed method was determined in terms of the limit of detection (LOD) and limit of quantification (LOQ). The LOD values were **0.10 µg/mL for delamanid** and **0.25 µg/mL for bedaquiline**, while the corresponding LOQ values were **0.29 µg/mL and 0.74 µg/mL**, respectively. These results indicate that the method is sufficiently sensitive for the determination of both drugs at low concentrations.

3.9 Robustness

Robustness was evaluated by introducing deliberate variations in flow rate, mobile-phase composition, and column temperature. The flow rate was varied to 0.9 and 1.1 mL/min, while changes in mobile-phase composition and temperature were also evaluated.

The %RSD values obtained under the different conditions ranged from **0.1–0.9% for delamanid** and **0.1–0.7% for bedaquiline**, all of which were below the acceptance criterion of 2.0%. No significant changes in chromatographic performance were observed. The method was therefore considered robust.

3.10 Assay of Pharmaceutical Formulation

The validated method was applied to the simultaneous assay of delamanid and bedaquiline in the combined pharmaceutical formulation containing **20 mg of delamanid and 40 mg of bedaquiline** per dosage unit.

The mean assay values were **99.52% for delamanid** and **99.95% for bedaquiline**, with %RSD values of **0.3% and 0.2%**, respectively. The results were within the acceptable assay limits, demonstrating the applicability of the developed method for routine quality control analysis of the formulation.

3.11 Forced-Degradation Studies

Forced-degradation studies were performed on the pharmaceutical formulation under acidic, alkaline, oxidative, thermal, photolytic, and neutral conditions. The stressed samples were analyzed using the developed HPLC method to evaluate drug degradation and demonstrate its stability-indicating capability.

Delamanid showed degradation of **6.16% under acidic**, **4.54% under alkaline**, **4.02% under oxidative**, **2.54% under thermal**, **2.06% under photolytic**, and **1.17% under neutral** conditions. The corresponding undegraded drug contents were 93.84%, 95.46%, 95.98%, 97.46%, 97.94%, and 98.83%, respectively.

Bedaquiline showed degradation of **5.45% under acidic**, **4.11% under alkaline**, **3.68% under oxidative**, **2.99% under thermal**, **1.98% under photolytic**, and **0.95% under neutral** conditions. The corresponding undegraded drug contents were 94.55%, 95.89%, 96.32%, 97.01%, 98.02%, and 99.05%, respectively.

Among the stress conditions investigated, both drugs showed comparatively greater degradation under acidic conditions, followed by alkaline and oxidative conditions. The chromatograms demonstrated separation of the drug peaks from degradation products, confirming the stability-indicating capability of the developed method.

Forced-Degradation Results

Table 02: Results of forced degradation studies of Delamanid and Bedaquiline under different stress conditions

Stress condition	Delamanid degraded (%)	Delamanid undegraded (%)	Bedaquiline degraded (%)	Bedaquiline undegraded (%)
Acid	6.16	93.84	5.45	94.55
Alkali	4.54	95.46	4.11	95.89
Oxidation	4.02	95.98	3.68	96.32
Thermal	2.54	97.46	2.99	97.01
UV	2.06	97.94	1.98	98.02
Water	1.17	98.83	0.95	99.05

6.12 Summary of Validation Results

Table 03: Validation parameters and results for the simultaneous estimation of Delamanid and Bedaquiline

Validation parameter	Delamanid	Bedaquiline	Acceptance criterion
Linearity range (µg/mL)	5–30	10–60	—
Correlation coefficient	0.999	0.999	Close to 1.0
Regression equation	y = 19629x + 3362.1	y = 23316x + 9810.4	—
Mean assay (%)	99.52	99.95	90–110%
Specificity	Specific	Specific	No interference
System precision (%RSD)	1.5	0.4	NMT 2.0%
Method precision (%RSD)	0.3	0.2	NMT 2.0%
Accuracy (% recovery)	99.69	99.90	98–102%
LOD (µg/mL)	0.10	0.25	—
LOQ (µg/mL)	0.29	0.74	—
Robustness (%RSD range)	0.1–0.9	0.1–0.7	NMT 2.0%

The developed RP-HPLC method provided rapid and efficient simultaneous separation of delamanid and bedaquiline using a Kromasil C18 column and a 0.01 N potassium dihydrogen phosphate buffer:acetonitrile mobile phase (60:40, v/v). The short retention times and satisfactory resolution enabled rapid analysis within a 6-min runtime [18-19].

The method demonstrated satisfactory specificity, linearity, precision, accuracy, sensitivity, and robustness. The low %RSD values obtained during precision and robustness studies confirmed the reliability and reproducibility of the method [20-21]. The assay results were close to the labeled claim, indicating its suitability for quantitative analysis of the combined pharmaceutical formulation. Forced-degradation studies demonstrated measurable degradation under different stress conditions while maintaining adequate separation of the drug peaks from degradation products, confirming the stability-indicating nature of the method [22-23].

A rapid, accurate, precise, sensitive, specific, robust, and stability-indicating RP-HPLC method was successfully developed and validated for the simultaneous estimation of delamanid and bedaquiline in bulk and pharmaceutical dosage form. The method showed excellent linearity, satisfactory recovery, low variability, and adequate sensitivity [24-25]. The developed method can therefore be applied for routine quality-control analysis and stability assessment of delamanid and bedaquiline-containing pharmaceutical formulations.

4. CONCLUSION

A simple, rapid, accurate, precise, and robust RP-HPLC method was developed and validated for the simultaneous estimation of delamanid and bedaquiline in bulk drug and pharmaceutical dosage form. The

retention times of delamanid and bedaquiline were 2.249 and 2.970 min, respectively. The method showed good precision, with %RSD values of 1.4% for delamanid and 0.5% for bedaquiline. The mean percentage recoveries were 99.69% and 99.90% for delamanid and bedaquiline, respectively, confirming the accuracy of the method. The LOD and LOQ values were 0.10 and 0.29 µg/mL for delamanid and 0.25 and 0.74 µg/mL for bedaquiline, respectively. The assay results were 99.52% for delamanid and 99.95% for bedaquiline. The calibration curves exhibited good linearity, with regression equations of y = 19629x + 3362.1 for delamanid and y = 23316x + 9810.4 for bedaquiline. The short retention times and 6-min total run time demonstrate that the proposed method is rapid, economical, and suitable for routine quality-control analysis of delamanid and bedaquiline in pharmaceutical formulations.

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CONFLICT OF INTEREST
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AUTHOR CONTRIBUTIONS
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