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Article

Implementation of the QbD Approach to the Analytical Method Development and Validation for the Estimation of the Treprostinil Injection Dosage Form by RP-HPLC

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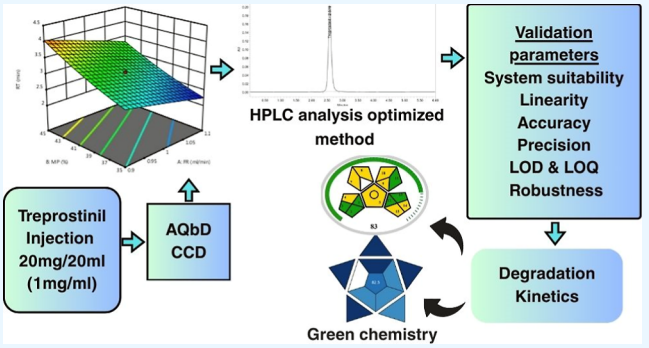
ABSTRACT: Quality by design (QbD) is a predictable quality tool that ensures that products meet predefined specifications. QbD involves understanding dependent variables, key factors, and their interactions, assessed through designed experiments analyzing specific responses. The central composite experimental design describes the interrelationships between the flow rate, buffer, and column temperature at different levels, with observed responses including retention time, theoretical plates, and tailing factor analyzed using Design-Expert software. A stability-indicating reverse-phase high-performance chromatography (RP-HPLC) technique was developed and validated for the quantitative estimation of treprostinil, assessing its stability under various forced degradation conditions. Using the optimized method, successful separation of treprostinil was achieved with an Agilent HPLC system with a photodiode array detector and an Express C_{18} column (5 μm particle size, $L \times$ I.D. = 15 cm × 4.6 mm), maintained at 31.4 °C. The mobile phase, a buffer (0.01N KH_2PO_4) and diluent in a ratio of 36.35:63.35 (v/v), was used at a flow rate of 1.04 mL/min. Detection was performed at 276.0 nm, with treprostinil eluting at 2.579 min within a run time of 6.0 min. Treprostinil peak purity values across all degradation conditions confirmed homogeneity, establishing this method as stability-indicating. The proposed method was accurate, precise (relative standard deviation (% RSD) = 0.4%), linear, and robust (% RSD < 2%). The green analytical procedure index method yielded a total score of 83, classifying it as environmentally friendly, with a similar score obtained using the BAGI and GAPI tools. The developed AQbD-based RP-HPLC method is suitable for the assay of treprostinil in regular production batches and stability samples of treprostinil injection.



INTRODUCTION

Treprostinil is indicated for the treatment of pulmonary arterial hypertension (PAH) (WHO Group 1) and is associated with interstitial lung disease that causes progressive lung tissue scarring.[1] It belongs to a class of drugs known as prostacyclin analogues, which work by dilating (widening) the pulmonary blood vessels to reduce pressure and improve blood flow. This improves cardiac efficiency and exercise capacity and alleviates symptoms such as shortness of breath and fatigue. Treprostinil is recognized for considerably improving symptoms, exercise capacity, and overall survival in patients with PAH. Its versatility, effectiveness in combination therapy, and ability to reduce hospitalizations make it an essential treatment option for managing this complex, life-threatening condition. The IUPAC name of treprostinil (Figure 1) is [(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1Hbenz[f]inden-5-yl] oxy] acetic acid.

Despite its high cost, the role of treprostinil in managing chronic, life-threatening diseases such as PAH translates into long-term cost savings by potentially reducing hospitalizations.

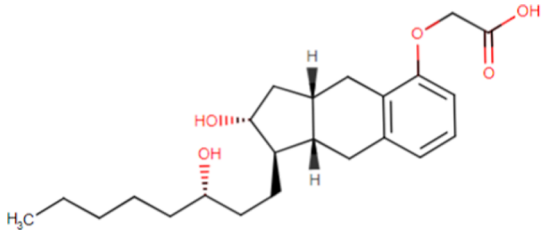


Figure 1. Chemical structure of treprostinil.

Although expensive, its ability to reduce long-term healthcare costs, minimize hospitalization, and improve patient produc-

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tivity makes it economically valuable. Since treprostinil injection is an orphan drug with a high price, an analytical quality by design (AQbD) approach was used to develop and validate a low-cost, simple, and highly sensitive reverse-phase high-performance liquid chromatography (RP-HPLC) method for estimating treprostinil in treprostinil injection (20 mg/20 mL).

The literature review reveals existing methods for the estimation of treprostinil (active) in bulk using high-performance liquid chromatography. However, conventional methods are tedious, requiring multiple runs, with a high risk of failure in real-time applications. Existing methods also lack robustness in assessing the stability under varying environmental conditions. Inaccurate estimations of degradation can lead to inconsistent dosing or therapeutic failures, emphasizing the need for reliable methods. Regulatory agencies, such as the United States Food and Drug Administration (FDA) and European Medicines Agency, increasingly advocate for QbD to ensure product quality and safety. Implementing QbD for treprostinil not only enhances the consistency and reliability of the product but also aligns with regulatory expectations for modern pharmaceutical manufacturing, ultimately streamlining the approval process and ensuring patient safety.[26,27]

Currently, no publicly available methods exist for the quantitative determination of the treprostinil assay (20 mg/20 mL) using a QbD approach, degradation kinetics, and green analytical chemistry. The developed method enables drug estimation in injection formulation for routine batch testing in manufacturing. It can be used to test the drug concentration in each production lot and ensure consistency across different production batches. This method can also be implemented in stability studies to monitor any changes in the drug concentration over time under various storage conditions, confirming the stability of the drug in the final formulation.

The QbD approach is a powerful analytical tool used during the development of the method development stage. AQbD is a methodical approach that begins with pre-established objectives, emphasizing the comprehension of the product and process through quality risk management and research.[1,2] In line with FDA guidance Q8(R2) on pharmaceutical development, a three-stage approach was implemented: stage 1 (Method Design) is the method validation phase involved defining method requirements, conditions, and vital limitations; stage 2 (Method Qualification) confirmed the ability of the method to meet its intended purpose; and stage 3 (Continued Method Verification) ensured ongoing reliability during routine use.[3]

At its core, the AQbD approach necessitates defining the analytical target profile (ATP) before selecting an analytical technology. The primary goal is to identify critical method attributes (CMAs), including material characteristics, instrument-related factors, operational elements, and method variables that considerably impact analytical performance. This is achieved through risk assessments and screening studies, followed by optimization utilizing experimental models to enhance the efficiency of the method.[1,4−6]

For HPLC techniques, high-risk CMPs include material properties, instrument settings, mobile phase components (such as buffer type, concentration, pH, elution method, and organic modifier), column specifications and preparation, and the expertise of the analyst.[7]

The essential stages in developing an analytical method using AQbD principles are as follows: initiating the project → conducting a literature review and initial risk assessment → identifying ATP, CMAs, and risk assessment → optimizing and developing the method using Design of Experiments → establishing the method operable design region, control plan, and risk assessment → validating the AQbD method → ongoing method monitoring.[8−10]

The central composite design (CCD) was selected for the optimization of the drug estimation method, owing to its efficiency and statistical robustness in modeling nonlinear correlations, exploring the entire design space, and identifying optimal conditions with minimal experiments. Its ability to manage multiple factors, model quadratic effects, and offer flexibility makes it superior to other experimental designs, especially for complex pharmaceutical optimization. The resource efficiency, flexibility, and ability to capture complex interactions make CCD a preferred method for ensuring precision and quality in the development of analytical techniques for injectable formulations. While alternative designs, such as full factorial or Box−Behnken, could be considered, they may not offer the same balance of efficiency, flexibility, and robustness.[11−15]

Following these principles, this study sought to develop and refine an HPLC method for treprostinil quantification based on QbD guidelines. The optimized method was validated in accordance with ICH Q2 (R2) recommendations before implementation.[16]

As environmental regulations tighten, pharmaceutical companies are required to comply with rigorous standards on waste disposal, emissions, and chemical usage. By adopting sustainable methods that score well on these indices, pharmaceutical companies can ensure that their analytical procedures align with both regulatory requirements and sustainability goals. To assess the environmental impact, green analytical procedure index (GAPI) and blue applicability grade index (BAGI) tools were implemented.

The GAPI and BAGI indices provide a valuable framework for assessing and improving the environmental footprint of analytical methods in pharmaceutical research. By promoting green chemistry principles, reducing hazardous waste, improving energy efficiency, and fostering innovation, these scoring systems remarkably contribute to sustainable pharmaceutical practices. This shift not only minimizes the environmental impact of the industry but also creates long-term economic and social benefits, positioning the pharmaceutical sector as a leader in environmentally responsible practices. The GAPI green chemistry tool and the BAGI blue applicability green index tool were used throughout this study from the sample collection and cleanup to final quantification via the instrument to assess the greenness of the method.[17−23]

Following the development of analytical methods using the QbD methodology for quantifying treprostinil in API/pharmaceutical dosage forms (injection) and incorporating stability studies, the suggested chromatographic technique differs from that in the literature.

MATERIALS AND METHODS

Chemicals and Reagents. Treprostinil was obtained as a gifted sample from Dr. Reddy's Laboratories. Sodium potassium dihydrogen phosphate and HPLC-grade methanol were purchased from Merck.

Instrumentation. The study was conducted with an Agilent HPLC instrument with a PDA detector and controlled by Empower software version 2. Additional equipment

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ACS Omega 2025, 10, 17827−17835

Table 1. Design Summary of CCD

design summary					
file version: DX 13.0.5.0 study type: response surface design type: central composite design (CCD) design model: quadratic			ATP: robustness, CQA: retention time, area, theoretical plates, and asymmetry runs: 20		
CMPs	unit	type	subtype	min.	max.
temperature of the column	0^C	numeric	continuous	24.9546	35.0454
flow rate	mL/min	numeric	continuous	0.831821	1.16818
% buffer ratio	%	numeric	continuous	31.591	48.409

included an analytical balance (Mettler Toledo) and an ultrasonicator. The chromatographic separation was performed using an Agilent Express C_{18} column (5 μm particle size, $L \times$ I.D. = 15 cm × 4.6 mm).

Preparation of the Diluent. A 50:50 (v/v) methanol−water mixture was prepared based on the solubility of the drug.

Preparation of 0.01 N Potassium Dihydrogen Phosphate Buffer (pH 6.8). To prepare the buffer, 1.36 g of KH_2PO_4 was dissolved in 1000 mL of HPLC-grade water. The solution was then refluxed in a sonicator for 10−15 min, filtered through a 0.45 μm nylon filter, and its pH was adjusted to 4.8 with orthophosphoric acid.

Preparation of the Mobile Phase. The mobile phase was prepared by mixing buffer (0.01N KH_2PO_4) and the diluent in a 36.35:63.35 (v/v) ratio based on the optimized method conditions.

Preparation of the Standard Stock Solution. A precisely weighed 10 mg portion of treprostinil was transferred to a 50 mL volumetric flask. Subsequently, 10 mL of the diluent was introduced and agitated for 10 min in a sonicator, and the final volume was adjusted using the same diluent. The mixture was filtered through a 0.45 μm filter, and 0.5 mL of the filtrate was diluted to 10 mL with the diluent, yielding a final concentration of 20 μg/mL treprostinil.

Method Development and Optimization Using the QbD Approach (Experimental Design). *Identification of the Analytical Target Profile, Critical Method Attributes, and Risk Assessment.* ATP was established through a literature review and risk assessment to determine the fundamental factors influencing treprostinil quantification via HPLC. The chosen critical method parameters were related to different parameters of HPLC (i.e., column temperature, flow rate, and buffer ratio).

Method Development and Optimization Using Central Composite Design. The method optimization employed CCD, which is commonly used for second-order models. Three key factors, buffer concentration, flow rate, and column temperature, were optimized across three levels (high, mid, and low) within specific ranges: 30%−50% 0.01 N potassium dihydrogen phosphate, a 27.00−33.00 °C column temperature, and a 0.9−1.10 mL/min flow rate. Contour and 3D surface plots were generated to illustrate the impact of each parameter on critical quality attributes (CQAs) such as peak area, theoretical plates, retention time, and asymmetry. A desirability function was employed to predict these CQAs under the optimized conditions. The detailed experimental design for treprostinil injection is presented in Tables 1 and 2, as well as Tables S1−S3.

Risk Assessment and Control Strategy. To assess its efficacy and durability, the optimized approach was examined for critical material attributes. Consequently, the method's performance and parameters were assessed for robustness under a number of specifically selected conditions. Minor adjustments to crucial parameters, including flow rate, column temperature, and percentage of the buffer phase, were made to assess the robustness of the method.

Table 2. Characteristics of the Solution Obtained from the Optimized Method

flow rate	buffer concentration	column temperature	retention time	tailing factor	theoretical plates
1.03591	36.33488	31.4616	2.568	1.1	3045.65

Chromatographic Conditions. Treprostinil was tested in pharmaceutical formulations and degradation investigations using a reverse-phase Agilent Express C_{18} column (5 μm particle size, $L \times$ I.D. = 15 cm × 4.6 mm). The column temperature was maintained at 31.4 °C. The mobile phase, consisting of the buffer (0.01N KH_2PO_4) and diluent in a ratio of 36.35:63.35 (v/v), was used at a flow rate of 1.04 mL/min. The analysis was performed utilizing a PDA detector at 276.0 nm, with 10.0 μL injections of both the reference material and sample solutions.

Method Validation. The RP-HPLC method for treprostinil injection was verified for linearity, precision, accuracy, and limits of quantification and detection, as per the ICH Q2 (R2) guidelines.

System Suitability Parameters. System suitability was evaluated by injecting standard treprostinil solutions (20 ppm) six times and assessing parameters such as peak tailing, USP plate count, and resolution. The relative standard deviation (% RSD) of the peak area for these injections was required to remain below 2%.

Specificity. Specificity was confirmed by verifying that no interfering peaks were observed in placebo and blank samples at the drug retention times, thus validating the specificity of the method.

Linearity. Treprostinil (10 mg) was precisely weighed and then placed in a 50 mL volumetric flask. After adding 50 mL of the diluent and sonicating for 20 min, the flask was filled with a 50:50 v/v water: methanol mixture to create a standard stock solution (200 ppm treprostinil). The linearity of the procedure was determined by examining successive dilutions of the treprostinil sodium stock solution between 5 and 30 ppm.

Accuracy. Accuracy was assessed by analyzing treprostinil sodium solutions spiked with 50%, 100%, and 150% standard treprostinil. Recovery and % RSD were calculated to verify the accuracy of the data.

Precision. Precision was evaluated through repeatability (intraday variation) and intermediate (interday variation) precisions, as per ICH guidelines, using six replicates for both interday and intraday assessments.

Robustness. Robustness was evaluated by intentionally adjusting the temperature, mobile phase ratio, and flow rate. The following parameters were used for the injection of

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ACS Omega 2025, 10, 17827−17835

samples in duplicate: temperature (23 and 33 °C), mobile phase ratio (±5% v/v), and flow rate (0.9 and 1.1 mL/min).

Limit of Detection. Three standard stock solutions were pipetted into 10 mL volumetric flasks in 0.25 mL aliquots and diluted. From these solutions, 0.3 mL of the treprostinil solutions was transferred to 10 mL volumetric flasks and diluted. The LOD was calculated using the formula LOD = 3.3 × σ/s (where σ is the standard deviation and s is the slope of the calibration curve).

Limit of Quantification. The LOQ was determined similarly using 0.9 mL of treprostinil solutions. It was calculated using the formula LOQ = 10 × σ/s.

FORCED DEGRADATION STUDIES

Oxidation. First, 1 mL of 20% hydrogen peroxide (H_2O_2) was mixed with 1 mL of a treprostinil stock solution for the oxidation experiments. To test the stability of the sample, the solution was diluted to 20 ppm, and 10 μL was injected into the HPLC system after 30 min at 60 °C.

Acid Degradation Analysis. A mixture of 1 mL of the treprostinil stock solution and 1 mL of 2N hydrochloric acid was heated at 60 °C for 30 min. The resulting mixture was then diluted to a 20 ppm solution. A 10 μL sample was injected into the system, and the chromatogram was observed to evaluate the stability of the sample.

Base Degradation Analysis. A combination of 1 mL of the treprostinil stock solution and 1 mL of 2N sodium hydroxide was heated at 60 °C for 30 min. The resulting mixture was then diluted to a 20 ppm solution. A 10 μL sample was injected into the system, and a chromatogram was observed to evaluate the stability of the sample.

Thermal Degradation Analysis. The standard drug solution was exposed to 105 °C for 6 h in an oven to examine thermal degradation. The resulting solution was diluted to 20 ppm for HPLC analysis. A 10 μL sample was injected into the system, and the chromatogram was analyzed to evaluate the stability of the sample.

Light Sensitivity Analysis. The photochemical stability of the drug was examined by exposing a 200 ppm solution to UV light in a UV chamber for 7 days or in a 200 W/min photostability chamber. The resulting solution was diluted to 20 ppm for the HPLC analysis. A 10 μL sample was introduced into the system, and the chromatogram was analyzed to assess the stability of the sample.

Neutral-Degradation Analysis. The neutral stress test involved heat-testing treprostinil in water at 60 °C for 6 h. The resultant solution was diluted to 20 ppm for HPLC analysis. A 10 μL sample was introduced into the system, and the chromatogram was recorded to assess the stability of the sample.

Green Assessment of the Developed Method via the GAPI. The GAPI was used to assess the environmental impact of the method. The semiquantitative GAPI tool consists of five pentagrams representing the following: (1) the sampling procedure, (2) sample preparation, (3) chemicals and reagents, (4) instrumentation, and (5) the general procedure. It offers sufficient information to evaluate and quantify the environmental effects connected to every stage of an analytical approach, from sampling to final instrumental analysis. The tool uses three primary colors—green, yellow, and red—indicating low, medium, and high impact, respectively.

BAGI. The applicability of the method was assessed based on several factors, including the type of analysis, number of analytes that are simultaneously determined, analytical technique, required analytical instrumentation, number of samples, sample preparation, number of samples analyzed per hour, reagents and materials used, preconcentration requirements, automation degree, and amount of sample.

RESULTS AND DISCUSSION

Design of the Experiment (Optimized Method and Development). A factorial design was conducted with Design-Expert software v11 (CCD) to observe the effect of three independent variables—flow rate (FR, mL/min), organic ratio of the mobile phase (MP, %), and temperature (T, °C)—on three responses: retention period (RT), tailing factor (TF), and theoretical plates (TPs). These parameters were optimized for the proposed method. Several characteristics within the design space were further optimized by using the CCD. Figures 2, 3, and 4 illustrate the 3D response surface plots for retention duration, TPs, and TF. Figure 5 depicts the final optimized solution.

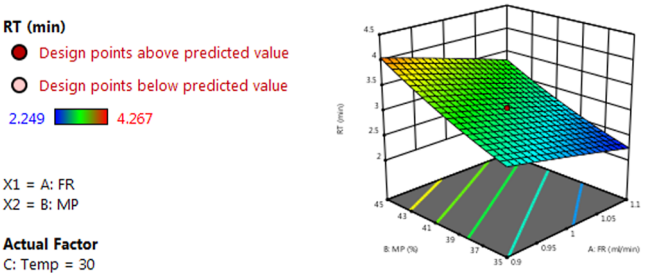

Figure 2. 3D plot for retention time.

Statistical Analysis. ANOVA was used to analyze the QbD data. The predicted R^2 values for all responses R_1 (0.9162), R_2 (0.7210), and R_3 (0.9065) are in reasonable agreement with the adjusted R^2 values of 0.9795, 0.9231, and 0.5720, respectively, showing minimal differences. These models are suitable for navigating the design space. ANOVA results for responses R_1, R_2, and R_3 show model F-values of 193.56, 26.54, and 79.22, respectively, indicating that the models are significant. The p-values of 0.0001, 0.0164, and 0.0001 for the variables are also significant. These models can navigate design space exploration.

The optimized method parameters for RP-HPLC analysis of treprostinil injection are presented in Table 3

Figures 6 and 7 illustrate the blank and optimized chromatogram of treprostinil injection.

METHOD VALIDATION

System Suitability. System suitability testing is a crucial component of method development, ensuring that the chromatographic system operates as intended. This test mainly verifies repeatability and resolution of the analysis. It involved six replicate injections of a standard solution containing treprostinil (20 ppm). Measurements were taken for parameters, such as USP plate count, resolution, and peak tailing. The system suitability test results showed a retention time of 2.58 min, TPs of 3040, TF of 1.10, and % RSD for the six replicate injections within the acceptable range.

Linearity. The linearity of the method was demonstrated by using a proportional correlation between the area response

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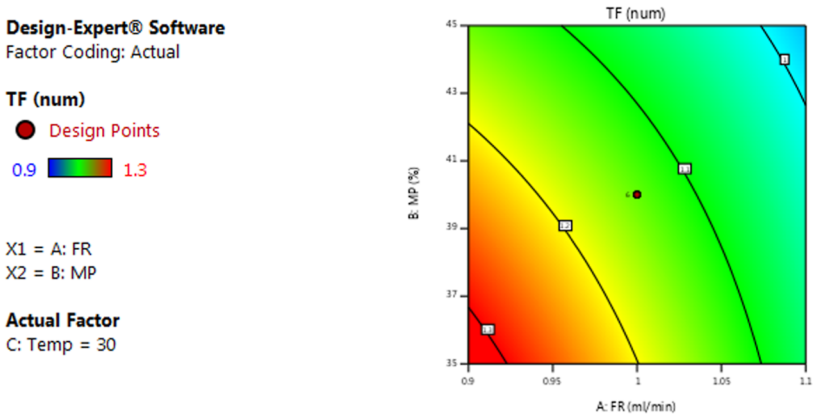

Figure 3. 3D plot for theoretical plates.

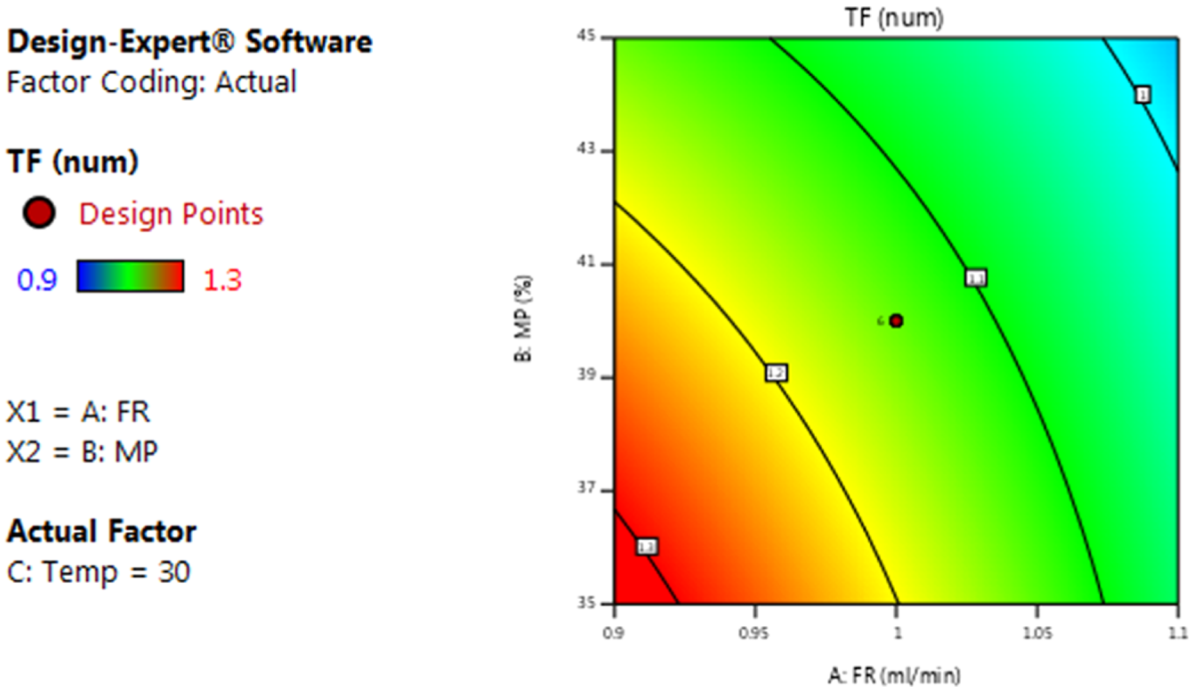

Figure 4. 3D plot for the tailing factor.

and sample concentration over a working concentration range. This ensures that the response is proportional to the concentration. The linear range for treprostinil injection was found to be 5 to 30 ppm. The regression equation of the calibration curve was $y = 3814.7x + 396.55$ with a correlation coefficient R^2 of 0.9999 when the graph was plotted with peak area vs concertation (Table S4 and Figure S1), indicating a linear response across the recommended range (as per acceptance criteria, the correlation coefficient must be more than 0.99).

Accuracy. To assess the accuracy of the approach, treprostinil was added at three different concentration levels (50%, 100%, and 150%). The %mean recoveries were 99.70%, 99.34%, and 99.43%, with a % RSD of 0.62%. Results in Table S5 indicate that the recovery percentages ranged between 99% and 101%, indicating that the developed approach is precise and suitable for standard exploratory analysis of treprostinil.

Precision. Six injections were introduced into the HPLC column. The area response and % RSD of the treprostinil peak are shown in Table S6, indicating an appropriate degree of precision for the HPLC technique. The % RSD for intraday was 0.4%, and for interday, it was 0.3%, both indicating an acceptable level of precision (as per the acceptance criteria, RSD must be less than 2%).

LOD and LOQ. Based on the standard deviation of the slope and intercept, the LOD and LOQ for treprostinil injection were determined to be 0.05 and 0.14 μg/mL, respectively (as per the acceptance criteria, LOD must be $\geq$3 and LOQ must be $\geq$10).

Robustness. The robustness of the treprostinil injection was tested by making small intentional modifications to intrinsic method parameters, such as flow rate, mobile phase, and temperature. The robustness of the method was studied by varying the experimental settings, including the flow rate ($\pm$0.2 mL/min), MP % ($\pm$5%), and column temperature ($\pm$5 °C). The % RSD for peak area was found to be less than two for changes in the flow rate, mobile phase, and temperature. The results are presented in Table S7.

The current method is a novel, precise, sensitive, stable, and cost-effective method compared with previously reported

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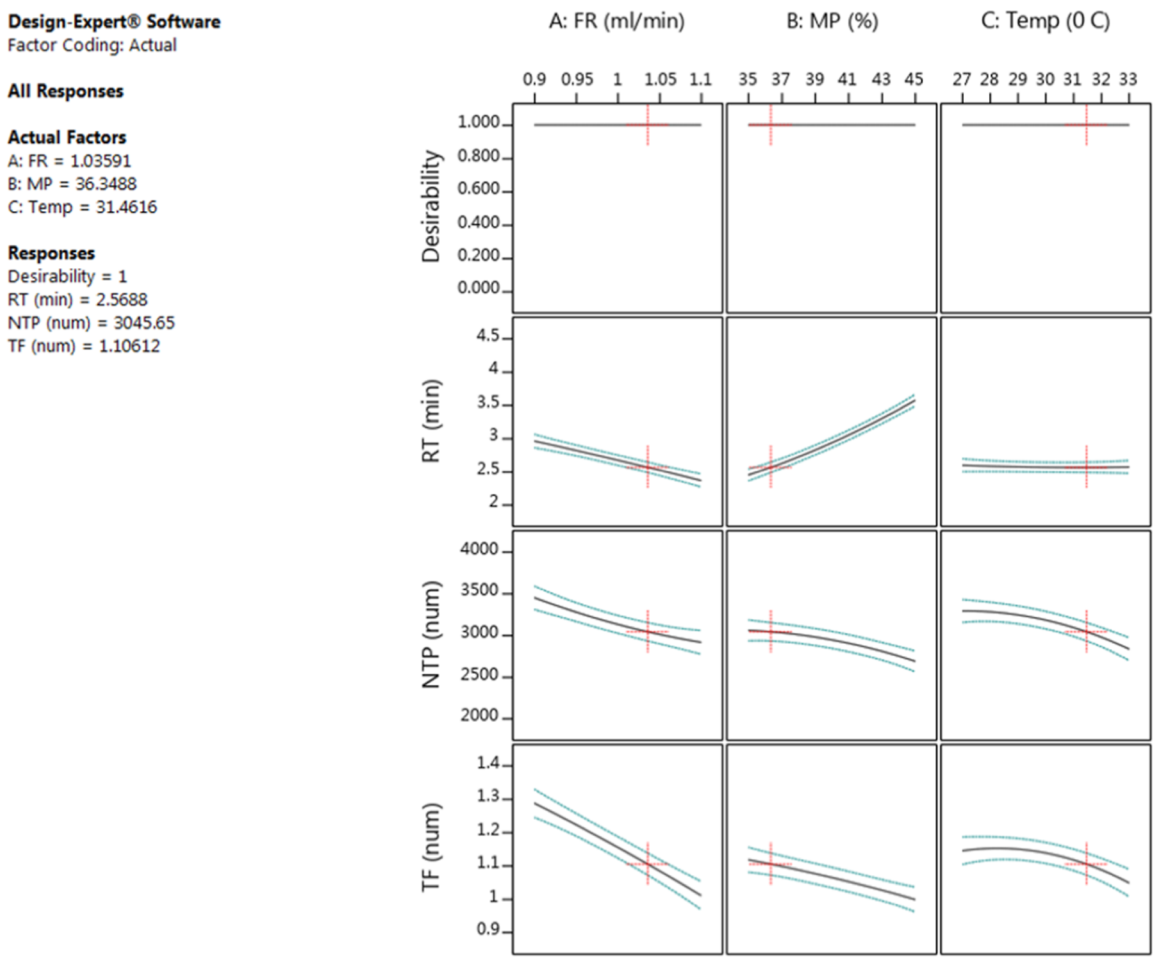

Figure 5. Final optimized solution for treprostinil injection.

Table 3. Optimized Method Parameters

flow rate	1.04 mL/min
column	Agilent Express C18 column (5 μm particle size, $L \times$ I.D. 15 cm $\times$ 4.6 mm)
detector	photo diode array
detector wavelength	276.0 nm
column temperature	31.4 °C
injection volume	10.0 μL
run time	6.0 min

methods. The mobile phase used is cost-effective with a lower consumption of organic solvents compared to other methods.

The results of method validation, as per ICH guidelines, show that all of the parameters fall within the acceptance criteria.

Degradation Kinetic Studies. The degradation of treprostinil injection under varied stress conditions was further studied by following ICH Q1A and Q1B guidelines. Different stress conditions, as outlined in the protocol, were applied by using standard solutions. The drug showed 6.6% degradation under acidic conditions (Figure S2), 2.01% degradation under alkali conditions (Figure S3), and 1.09% degradation under oxidation conditions (Figure S4). In thermal and photolytic conditions, the drug showed 0.24% and 6.49% degradation (Figures S5 and S6). Under neutral conditions, the drug showed a 2.11% degradation (Figure S7).

Degradation of drug substances between 5% and 20% is acceptable for the validation of chromatographic assays.[24,25] The results indicate that treprostinil injection was notably below the acceptable limit.

GAPI Assessment. The current study uses the GAPI tool, which consists of pictograms representing 15 different characteristics, to evaluate the greenness of the developed method. These characteristics cover sample preparation, collection, preservation, transportation, storage, reagent compounds used, cleanup, and final quantification by instrumentation. The GAPI pictogram for the analytical method developed for treprostinil injection is shown in Figure 8. The method has a total score of 83, indicating that it is overall green. Based on the GAPI parameters, the method was considered greener. The research indicates that the developed method is safer and more environmentally friendly in terms of sample preparation, reagents and solvents, instruments, and analytical procedures.

BAGI. All parameters were incorporated into the web application, considering all relevant attributes. In the BAGI tool, dark blue corresponds to 10 points, blue corresponds to 7.5 points, light blue corresponds to five points, and white corresponds to 2.5 points. The parameters used to establish the green metric are shown in Figure S8. The BAGI pictogram for the analytical method developed for treprostinil injection is depicted in Figure 9. Consequently, our findings reveal that the

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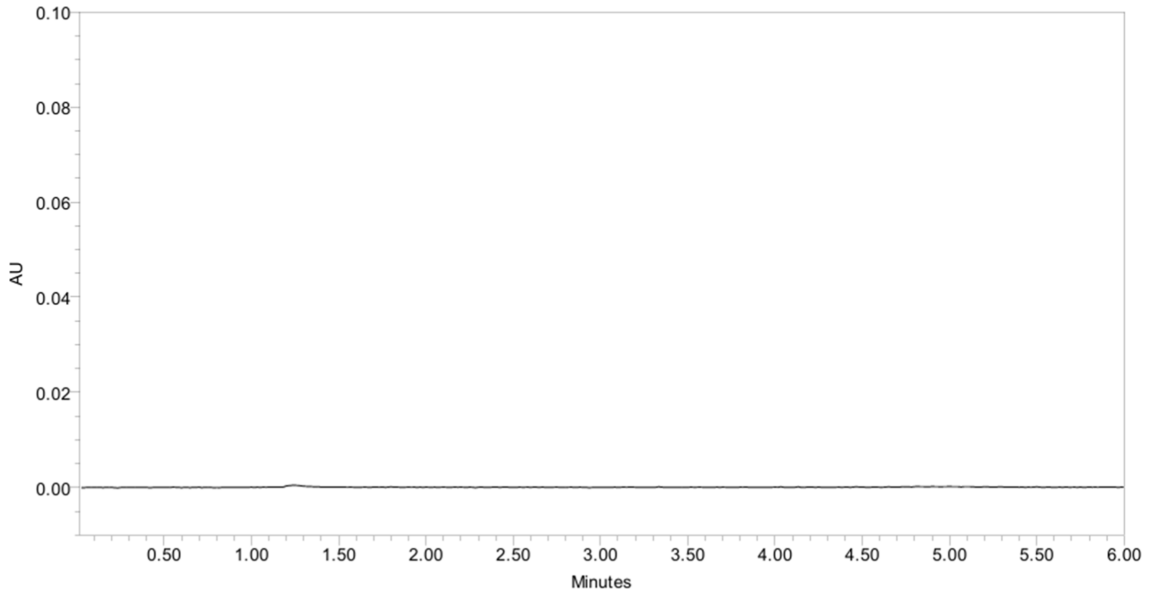

Figure 6. Blank chromatogram.

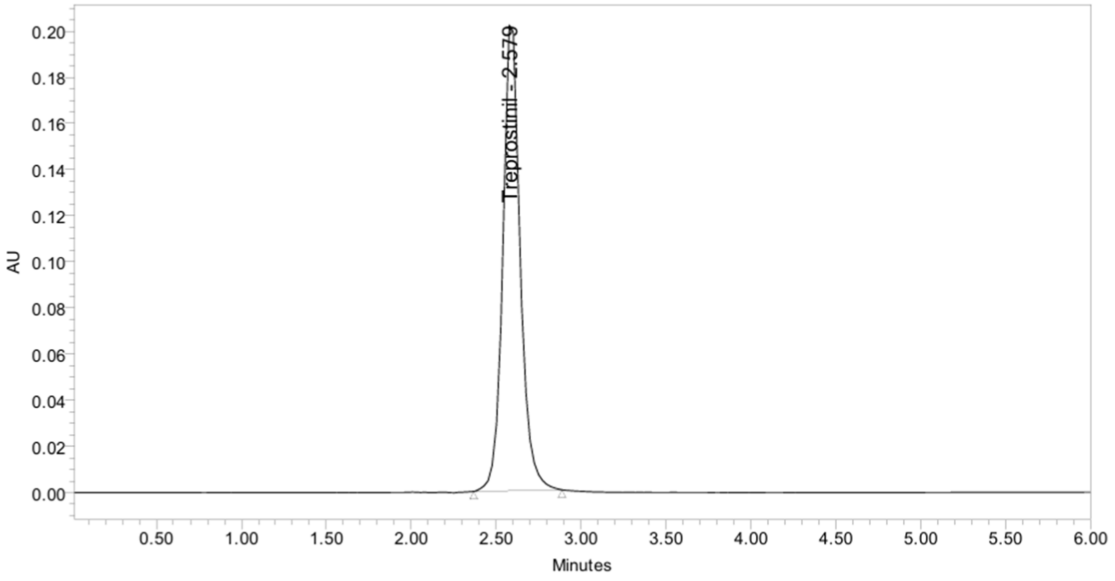

Figure 7. Optimized chromatogram of treprostinil injection.

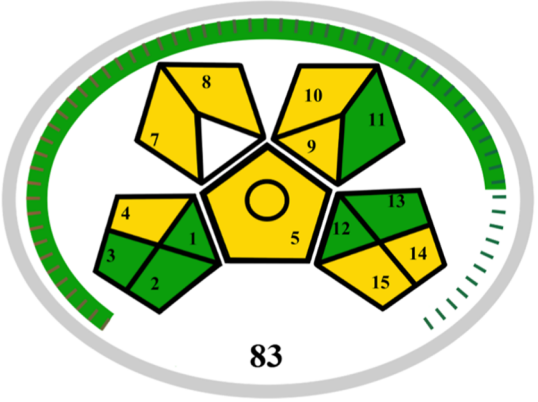

Figure 8. GAPI index pictogram for the reported method.

current analytical method has a BAGI score of 82.5, indicating good applicability potential for both the method and the study.

The GAPI method achieved a total score of 83, indicating an overall green rating. A similar score was obtained when the method was assessed by using BAGI.

The current method is novel, precise, sensitive, stable, and cost-effective compared with reported methods. The mobile phase is cost-effective, using fewer organic solvents than in other methods. The summarized results of method validation per the ICH guidelines show that all parameters meet the acceptance criteria.

CONCLUSIONS

The AQbD approach was applied to the RP-HPLC method development based on the analytical target product profile. This QbD approach provided a deep understanding of the method variables at different levels. The CCD experimental design, using the Design-Expert 11.0 version, evaluated the

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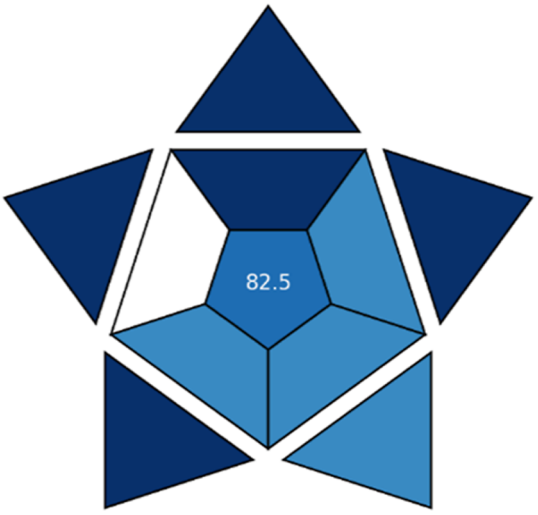

Figure 9. BAGI pictogram for treprostinil injection.

interrelationships between flow rate, buffer, and column temperature, with responses such as retention time, TPs, and TF. This approach provides valuable insights for future chromatography optimization. The AQbD approach enhanced the creation of the treprostinil injection HPLC technique, identifying the optimal system and ultimate design space for several crucial process parameters. In the AQbD-based RP-HPLC method, the peak purity of treprostinil was studied using a PDA detector for all degradation products, the control sample, and the reference solution, and the developed technique was validated according to the USP and ICH guidelines. The automated QbD method development approach, using Design-Expert software, provided a more robust, higher-performing method in less time compared with manual method development. Moreover, degradation of treprostinil injection under various conditions, such as acidic, alkaline, oxidation, thermal, and photolytic conditions, was observed and quantitatively analyzed using this HPLC method. The method demonstrated linearity, precision, accuracy, specificity, and robustness in determining treprostinil injection.

The greenness and practicality of the developed method were assessed by using the GAPI and BAGI tools. According to GAPI parameters, the developed method is considered highly green, with a total score of 83. BAGI facilitates the evaluation of various analytical techniques, identifying strengths and weaknesses in terms of application and practicality. A similar score was obtained when the method was assessed using BAGI. We conclude that the chemical community will increasingly trust and adopt GAPI and BAGI metrics for evaluating methods.

The method demonstrates superiority in terms of practicality and applicability. The treprostinil injection QbD-developed method is efficient, cost-effective, fast, and stability-indicating, making it suitable for use in quality control laboratories. It is designed to meet the demands of daily testing and stability monitoring. The developed method not only reduces environmental impact but also offers long-term economic and social benefits. Furthermore, it aligns with both regulatory requirements and sustainability goals, establishing it as a leader in environmentally responsible practices.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.5c00373.

Additional information on design of the experiment (critical test parameter/attributes), results of forced degradation study, method validation, and Green technology (PDF)

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Notes

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