RESEARCH PAPER

Rp-Hplc Method Development And Validation Of By Using Qbd Approach

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Abstract

Objective: Using an analytical quality by design approach, the current work aimed to establish a robust, precise, accurate, and specific hplc method for the quantification of dronedarone hcl in tablet dosage form and in bulk.

Materials and Methods: By using both observation and head part examination, the fundamental boundaries were accurately determined. To create mathematical models, two independent factors—flow rate and wavelength—were used. Using a c18 segment with a portable stage that contained a mobile phase composition of 0.05 m potassium dihydrogen orthophosphate buffer ph 3 and acetonitrile in a ratio of (60:40 v/v) at a flow rate of 1.1 ml/min and column temperature of 40 °c, the optimised and expected data was achieved.

Results: These ideal circumstances allowed for the baseline drug separation with good resolution and a run time of less than 3.0 minutes. A pda indicator at 290 nm was used to finish the discovery process. Ich q2 (r1) guidelines were followed in the validation of the optimised assay settings.

Conclusion: Because the results were found to be robust and specific, it was evident that the quality by design approach could be used to successfully optimise the rp-hplc method for routine quantification of dronedarone hcl in bulk and tablet format.

Keywords: Rp-Hplc, Method Development, Dronedarone.

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INTRODUCTION

A novel antiarrhythmic medication, Dronedarone, is primarily prescribed for the management of atrial fibrillation and atrial flutter. It exhibits multichannel blocking activity and demonstrates properties of all four classes of the Vaughan Williams classification, including inhibition of potassium, sodium, and calcium channels along with antiadrenergic effects[1-4]. Dronedarone helps maintain sinus rhythm and reduces cardiovascular hospitalization in patients with paroxysmal or persistent atrial fibrillation. Compared to its structural analogue Amiodarone, it has reduced lipophilicity and lacks iodine moieties, resulting in fewer adverse effects and improved safety profile[5,6].

A survey of the literature indicates that various analytical methods have been developed for the estimation of dronedarone in bulk drug, pharmaceutical dosage forms, and biological matrices. These include high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC-MS), and spectrophotometric methods, offering high sensitivity, precision, and reproducibility[7–10].

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use has introduced guidelines such as Q8, Q9, and Q10 focusing on pharmaceutical development, quality risk management, and pharmaceutical quality systems[11–13]. Quality by Design (QbD) is a systematic and science-based approach that begins with predefined objectives and emphasizes process understanding and control. It has become an essential component in modern pharmaceutical development to ensure consistent product quality. However, implementation at an industrial level remains challenging due to the complexity in identifying critical quality attributes and process parameters. The establishment of a design space and control strategy is a key aspect of QbD, enabling optimization of process conditions to achieve reproducibility, robustness, and accuracy. Various statistical and mathematical tools are employed to construct the design space and evaluate experimental data[14–15].

Therefore, the aim of the present study was to develop a simple, economical, and time-efficient analytical method

for the estimation of dronedarone using a QbD approach. Among the available techniques, High-Performance Liquid Chromatography (HPLC) is widely used due to its specificity, sensitivity, and reliability. Application of QbD principles to analytical method development enhances robustness and ensures consistent performance throughout the analytical lifecycle. This approach minimizes variability, reduces method failures in routine analysis, and ultimately saves time and cost by decreasing the need for repeated investigations[16].

Materials and methods:

Materials: Dronedarone received as gift sample from Emcure Pharmaceutical Ltd, Pune, M.S. and other chemicals were used of analytical grade (Merck).

Instrumentation: An Agilent 1260 Infinity instrument including an implicit degasser and an autosampler (AS-4050). Additionally, a PDA (Shimadzu, SPD-20A) detector with a wavelength of 228 nm was part of the system. Software for programming Open Lab EZ Chrome was used to gather and handle data. With a C18 column, chromatographic separation was carried out.

Chromatographic conditions: Following various preliminary steps, a C18 column with a portable stage was selected. The mobile phase used in the experiment consisted of a combination of 0.05 M potassium dihydrogen orthophosphate buffer pH 3 and acetonitrile in a 60:40 v/v ratio. The mobile phase was pre-mixed, degassed, and filtered using a 0.4µm nylon filter. A finder was set to 290 nm in frequency, and the flow rate was maintained at 1.0 ml/min. Using an autosampler with a variable circle volume of 10-150 µl, 20 µl was infused in this method. Because of the framework's segment stove, section temperatures could be programmed throughout the run. It was decided to keep the segment temperature at 40 °C throughout the procedure following an initial run at various temperatures.

Preparation of stock solution:

A 10 ml volumetric flask containing a little amount of 0.05 M potassium dihydrogen orthophosphate buffer pH 3 and acetonitrile in the ratio of (60:40 v/v) was filled with precisely weighed 10 mg of Dronedarone. To achieve a 1000 ppm concentration, the volume was increased to 10 ml using the same mobile phase composition.

Preparation of working solution:

Take out 1 millilitre (ml) of the stock solution, transfer it to a volumetric flask, and dilute it with the mobile phase to make 10 millilitres (100 ppm). After that, the mixture is sonicated for thirty minutes.

Method development:

Selection and Preparation of Mobile Phase:

Various amounts and flow rates of mobile phases comprising methanol, water, acetonitrile, and cradles at different pH values were tried. With a flexible stage consisting of 60 sections of potassium dihydrogen orthophosphate buffer pH 3 and 40 pieces of acetonitrile, good peaks were obtained at a stream pace of 1.0 ml/min. Before being inserted into the framework, the two components of the portable stage were vacuum-separated through 0.45µm film channels and sonicated for 30 minutes.

Preparation of Standard Stock Solutions:

The drug's standard solutions were made with acetonitrile in a 60:40 v/v ratio and 0.05 M potassium dihydrogen orthophosphate buffer pH 3. To create standard stock solutions with a concentration of 1000 µg/mL, 10 mg of the medication was weighed and then dissolved in diluents in 10-milliliter volumetric flasks. To get the necessary medication concentrations, diluent was added to the normal stock solutions. Every day, all solutions—including the stock solution—were made from scratch.

Preparation of Calibration Curve:

The drug's standard stock arrangements were transferred to a 10 mL volumetric flask and appropriately diluted using a 60:40 v/v ratio of acetonitrile to 0.05 M potassium dihydrogen orthophosphate buffer pH 3. Aliquots were obtained so as to obtain final fixations within the range of 10–150 µg/mL. Plotting the peak area of the drug chromatograph on the x-axis and the top areas recorded for each concentration on the y-axis allowed for the creation of the drug calibration curve. Calculations were made for the calibration curve's slope, Y-intercept, and correlation coefficient.

Experimental Design

Factorial Design

A 2-factor, 3-level design used is suitable for exploring quadratic response surfaces and constructing second order polynomial models with Design Expert®

Table 1: Coded Values for Independent Variables

Name of Factor	Coded Values	Levels		
		Small	Medium	High
Detection wavelength (nm)	A	55:45	60:40	65:35
Flow rate (ml/min)	B	0.8	1.0	1.2

Table 2: Different Batches with their Respective Composition

Batch Code	Detection wavelength (nm)	Flow rate (ml/min)
B1	55:45	0.8
B2	55:45	1.0
B3	55:45	1.2
B4	60:40	0.8
B5	60:40	1.0
B6	60:40	1.2
B7	65:35	0.8
B8	65:35	1.0
B9	65:35	1.2

Validation of analytical method: According to ICH Q2 (R1) recommendations [30], the suggested RP-HPLC method of analysis was verified for criteria such system appropriateness, specificity, linearity, precision, accuracy, and robustness, as well as limit of detection (LOD) and limit of quantitation (LOQ).

System suitability: Before sample analysis can start, the chromatographic systems that will be employed for the analysis must pass system suitability limits. After setting up the chromatographic system, give the RP-HPLC system forty minutes to stabilise. To assess the system suitability parameters, such as resolution (NLT 2.0), tailing factor (NMT 1.5), theoretical plate count (NLT 3000), and percentage RSD for peak area of six replicate injections of the Dronedarone standard (% RSD NMT 2.0), inject the blank preparation (single injection) and the standard preparation (six replicates). Then, record the chromatograms. After analysis, the tailing factor, percent RSD, and theoretical plates were found to be satisfactory. Table 3 presents the ideal chromatographic conditions as well as the system suitability data.

Linearity: Dronedarone was produced as a stock solution of 1000 µg/mL in 0.05 M Potassium-Di-Hydrogen Phosphate Buffer pH 3: Acetonitrile (60:40) v/v. Working standard solutions for the drug, ranging from 10 to 150 µg/mL, were created from this stock and introduced into the HPLC apparatus. The medication has been shown to exhibit linearity in the 10-150 µg/mL range. Plotting the peak regions of the medication under study against its concentration produced the calibration graph, which was produced by repeat analysis at all concentration levels. The Microsoft Excel® tool was then used to determine the linearity of the relationship.

Precision: For each of the drugs, the precision of the developed method was confirmed. The peak regions identified by a real study of six simulated infusions of a typical centralisation of every drug. By calculating the RSD, the accuracy of the method was also verified with regard to the intra- and inter-day variation in the peak zones.

Accuracy: To evaluate the method's accuracy for the substance, a known concentration of the drug was spiked at three distinct concentration levels: 50%, 100%, and 150%. The difference between the theoretical and predicted values was then compared to the concentration determined by the method.

LOD and LOQ: The lowest quantity in a sample that can be identified under the specified experimental conditions, but not necessarily measured, is known as the limit of detection. The lowest analyte concentration in a sample that can be found with reasonable accuracy and precision is known as the limit of quantitation. The limit of quantitation and the limit of detection were determined using the following formula. LOD is equal to 3.3 σ/S and LOQ is equal to 10 σ/S, where σ is the response standard deviation and S is the calibration curve's slope. Table 4 displays the LOD and LOQ values. The new method's sensitivity was validated by the LOD and LOQ values.

RESULTS AND DISCUSSION:

Chromatographic Separation: Following several experiments, the ideal chromatographic conditions were determined by taking into account the system suitability factors. The results of this process are listed in Table 3, and the representative HPLC chromatograms of the drug and blank are displayed in Figs. 2 and 3, respectively. According to ICH Q2 (R1) recommendations, all system suitability parameter findings correlate within acceptance criteria.

Table 3. The system suitability data and the optimum chromatographic conditions.

Parameter	Chromatographic conditions

Instrument	Agilent 1260 Infinity instrument with Autosampler (AS-4050)
Column	Discovery C18 (250mm x 4.6ID, Particle size: 5 micron)
Detector	PDA (Shimadzu, SPD-20A) detector
Diluents	0.05 M Potassium - Di-Hydrogen Phosphate Buffer pH 3: Acetonitrile (60:40)
Mobile phase	0.05 M Potassium - Di-Hydrogen Phosphate Buffer pH 3: Acetonitrile (60:40)
Flow rate	1.0 ml/min
Detection wave length	290 nm.
Run time	5 minutes
Column Temperature	40 °C
Volume of injection loop	20 µL
Retention time (tR)	4.17 Min
Theoretical plates [th.pl] (Efficiency)	94699
% RSD Tailing factor (asymmetry)	0.2154
% RSD of minimum 5 replicate of calibration standard.	1.4

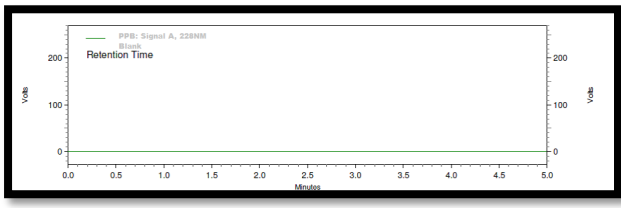


Figure 2: Chromatogram of blank solution

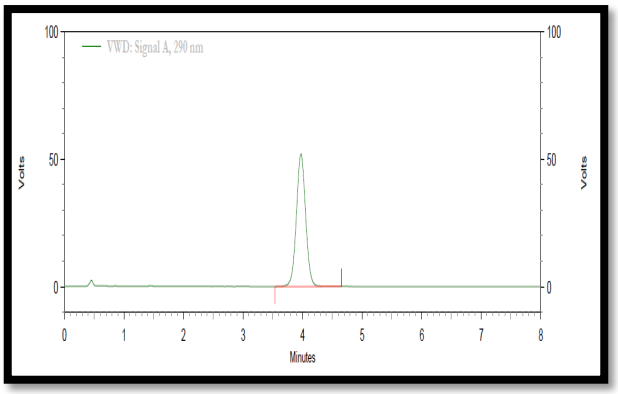


Figure 3: Chromatogram of drug Dronedarone

Linearity

Under proposed experimental conditions, the relationship between the area and concentration of Dronedarone was studied. Linearity was checked by preparing standard solutions at 5 different concentration levels of each of Dronedarone. Standard solutions (40, 40,80,120,160 µg/mL) of Dronedarone were injected into the RP-HPLC system to get the chromatograms. The average peak area and retention time were recorded. The calibration curve was constructed between concentrations versus peak area by the prepared concentration of 20-160 µg/mL of stock solution. The linearity range was found to be 20-160 µg/mL and the calibration graph of Dronedarone shown in Figure 4. Results show that a phenomenal correlation exists between peak area and concentration of drug within the linearity range. The Summary of validation parameters are shown in Table 4.

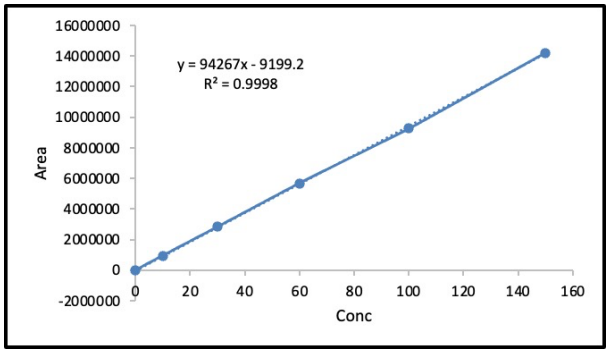
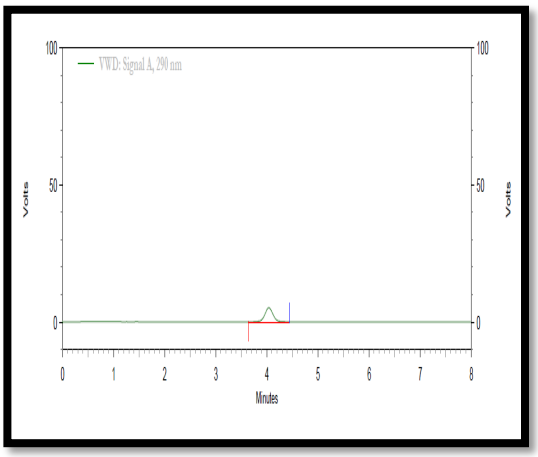
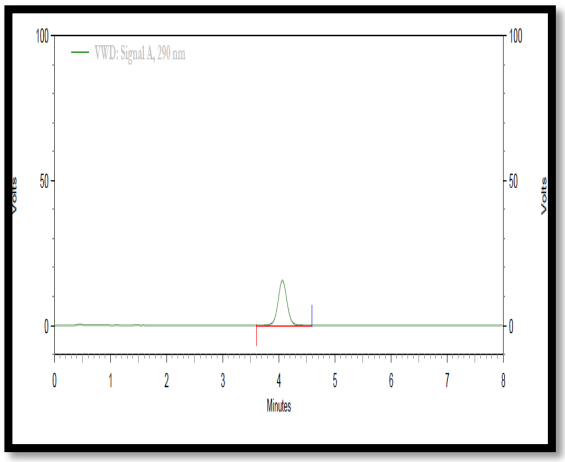
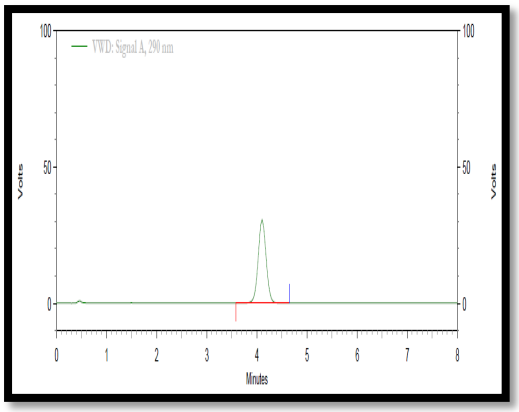


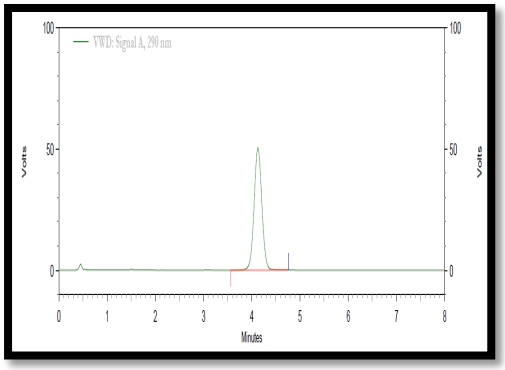
Figure 4. Calibration curve of Dronedarone



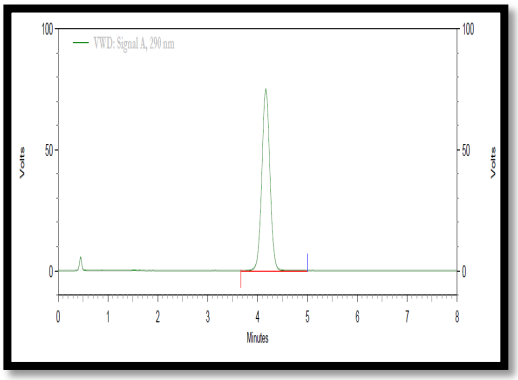


a) 10
b) 30





c)60 **d)100**



e) 150

Figure 5. Chromatogram of different ppm samples to determine linearity

Table 4. Summary of validation parameters.

Parameter	**Result**
Linearity range (µg/mL)	10-150 µg/mL
Liner Regression equation	y = 94267x
R^2	0.9998
Intraday precision (% RSD)	1.40 %
Interday precision (% RSD)	1.38 %
Recovery	99.80 %

LOD (µg/mL)	0.548
LOQ (µg/mL)	0.754
Robustness	Robust

Accuracy

The accuracy of the method was found to be good with the overall % RSD for recovery at 50 %, 100 % and 150 % levels were all within the limits which indicate that the proposed method was found to be accurate. The results are tabulated in table 5.

Table 5: Results of Accuracy (%Recovery)

Sr. No.	Assay Level	% Recovery
1	50	99.5
2	100	99.7
3	150	100.1

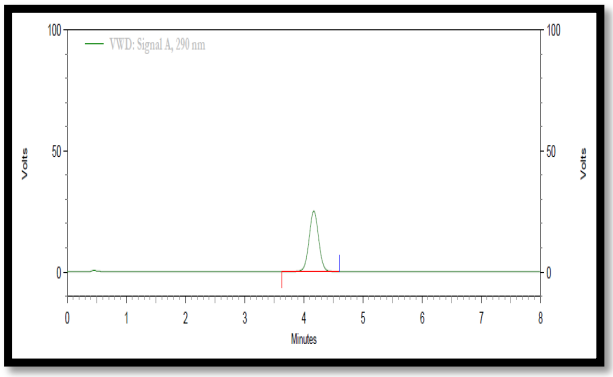


Figure 6. Accuracy study chromatogram of 50% Dronedarone

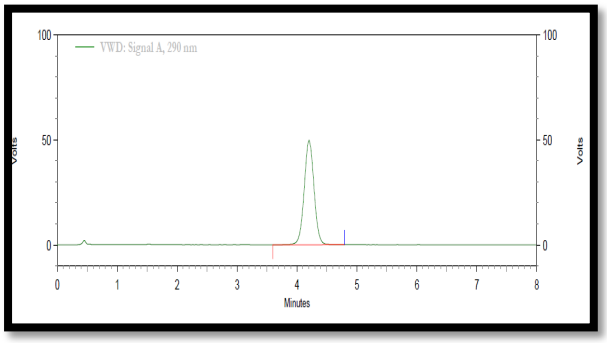


Figure 7. Accuracy study chromatogram of 100% Dronedarone

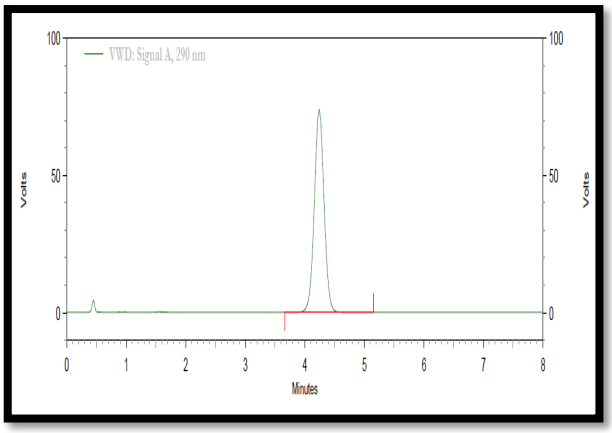


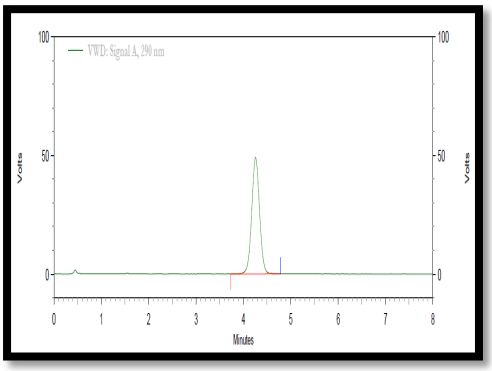
Figure 8. Accuracy study chromatogram of 150% Dronedarone

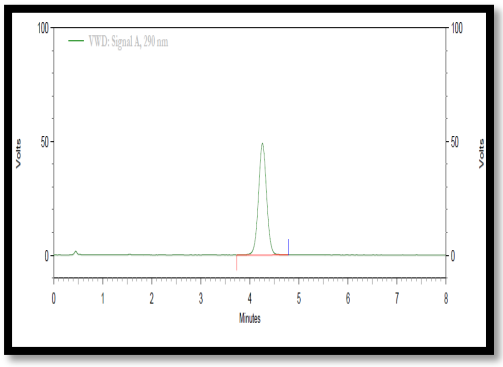
Precision

The value of Dronedarone were found within limit, which indicates that the developed method is precise.

Table 6: Result of Precision (% Recovery)

Sr. No.	Evaluation Parameter	Results	Acceptance Criteria
1	% Assay values obtained by six test solutions (Average)	99.7	NLT 98% and NMT 102 %
2	% RSD for Assay values obtained by six test solutions	1.4	NMT 2.0 %





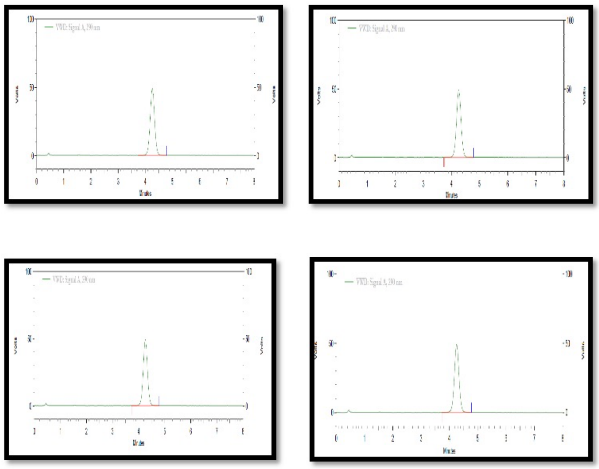


Figure 7. Precision study chromatogram of Dronedarone

Specificity:

The value of Dronedarone were found within Limit, which indicates that the developed method is specific.

Table 7: Result of Specificity

Sr. No.	Results	Acceptance Criteria
1	Retention time of Dronedarone peak in test solution is comparable to that in standard solution.	Retention time of Dronedaronepeak in test solution should be comparable to that in standard solution.
2	Peak purity of standard and test solution is within acceptance criteria	NLT 99.9

LOD and LOQ

Method validation following ICH guidelines indicated that the developed method had high sensitivity with LOD of 0.266 µg/mL and LOQ of 0.712 µg/mL.

5. Conclusion:

The current study focusses on systematic QbD and the creation of an easy-to-use, quick, accurate, and affordable RP-HPLC method for the simultaneous measurement of Dronedarone. The experimental design delineates the process of scouting essential components, such as flow velocity and detecting wavelength. The 0.05 M Potassium-Di- Hydrogen Phosphate Buffer pH 3: Acetonitrile in the ratio of 60:40 in the optimised model confirms the eligibility for drug estimation. The mobile phase's flow rate and column temperature were optimised to 1 ml/min and 40 °C, respectively. The validation study verified that the approach was resilient, linear, exact, accurate, selective, and specific, which helped to justify the choice of the ideal conditions. As a result, applying the response surface technique offers improved insight for developing methods and conducting robustness tests. Under regulatory flexibility, this created technique is suitable for regulatory submission and satisfies the design space concept.

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