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RESEARCH

Analytical quality by design-based RP-HPLC method for dobutamine quantification: development, optimization, and validation

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Abstract

The study presents method development, optimization, and validation of RP-HPLC technique to measure dobutamine, a cardiotonic agent utilized in the treatment of acute heart failure and cardiogenic shock. An Analytical Quality by Design was used to optimize chromatographic parameters, such as composition of the mobile phase, flow rate, and temperature of the column, applied Central Composite Design. The resulting optimized procedure showed superior system suitability, consisting of tailing factor 1.0, number of plates 12036 and similarity factor of 98.9 percent, which was very high in terms of resolution and reproducibility. The six repeated injections used to measure the system precision were 2106, 310.67 in terms of mean peak area and the low %RSD was 0.3 which indicated a high reproducibility. According to forced degradation studies, dobutamine was found to be stable when exposed to acidic conditions, basic conditions and peroxide conditions and also under thermal conditions; though these findings were accompanied by a high photolytic degradation (9%) and thus need to be stored under light protection. The technique also showed linearity over the broad concentration range (50 %, 150 %) with an R^2 of 0.99996 and at three different concentrations of 50 %, 100 %, and 150 %, the recovery studies have shown accurate results with low %RSD values (0.2, 0.4). The robustness assurance ensured that there is minimum change in USP tailing, plate counts and % similarity factor with different chromatographic conditions hence robustness of this methodology. The standardized AQbD-informed RP-HPLC method exhibits great precision, accuracy, and reliability in the analysis of dobutamine during the pharmaceutical quality control with the ICH compliance, contributing to the safety of patients by the guarantee of consistent therapeutic efficacy.

Keywords Dobutamine · RP-HPLC · Analytical quality by design · Central composite design · Method validation · Pharmaceutical analysis · Forced degradation

Introduction

Dealing with acute heart failure and cardiogenic shock, the effect of dobutamine is the enhancement of cardiac output. Its short half-life and instability, however, makes some specific and accurate analytical procedure essential in the constant quantitation of this drug. RP-HPLC is also known to give high resolution, sensitivity as well as reproducibility, thus, is a good method of analyzing dobutamine [1, 2]. But the traditional procedure of trial and error of method development is costly and time-consuming. The concept of AQbD marks a paradigm shift in the development of methodologies of analytical procedures, through which there can now be a defined optimization of multiple parameters, including the make-up of a mobile phase, the flow rate and the column temperature, to produce results that are most expedient and consistent.

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The enhancing property of the myocardial contractility supplied by dobutamine, a synthetic, catecholamine, makes it widely applied in a clinical setting in the treatment of acute heart failure and cardiogenic shock. It mainly behaves as a 1-adrenergic/beta agonist, enhancing the cardiac output without significant effects on the heart rate and the peripheral resistance. Nonetheless, the high rate of metabolism and instability of dobutamine present great difficulty in stable quantification of drugs and assurance of quality and requires such analytical applications to be very effective [3–5]. Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) plays an important role in the precision and reliability of the dobutamine can be monitored in drug formulation to determine the drug efficacy and safety of patients. dobutamine activity is mostly based on the stimulation of β adrenergic receptors of the first kind and raises cardiac output with little effect on heart rate and vascular impedance. Nevertheless, due to its extensive application in therapy, the high metabolic rate and chemical instability of dobutamine pose a serious problem in regards to the quantitation and quality assurance of the drugs. Such aspects demand a creation of powerful analytical algorithms that provide accurate, reproducible and reliable measurements especially of pharmaceutical formulation and biomaterials [6–8].

Pharmacokinetics characterization of dobutamine is by its short half-life of approximately 2 min, which necessitated constant infusion of this drug through a continuous intravenous infusion in order to provide therapeutic intervention. Its clinical demand requires that effective means of ascertaining its concentration in drug products are established through constructing stable and precise methodologies to assess its concentration. Due to the inefficiency of customary quantification methods of drugs which include trial and error approaches, it is evident that more streamlined and efficient approaches are necessary. A more recent method in the development of analytical methods, AQbD focuses the optimization and control of variables that influence the performance of the method. In this study, in a bid to establish a more efficient RP-HPLC method of dobutamine analysis, the AQbD principles are adapted to develop and validate it. It is about enhancing reliability, precision, and reproducibility of the system due to the regulatory compliance of the system. The procedure will increase the quality control of pharmaceuticals and patient safety [9–11].

Monitoring of dobutamine should be based on the analytical procedures that strictly comply with the regulatory requirements toward the efficacy and safety of the drug. RP-HPLC has been established as one of the potentially effective and approved methods that could be used in measurement of dobutamine [7, 8]. RP-HPLC is highly resolving, specific, and reproducible, so this method of analysis is ideal to use on a routine basis in a pharmaceutical application. Nevertheless, due to the rigidity of creating an RP-HPLC technique that appears accurate and consistent, there is a need to adjust this chromatographic situation and will necessitate consideration of the chromatographic conditions. With this optimization, a balanced expression of variables is performed, including mobile phase composition, flow rate, column temperature, and detection wavelength.

Along with the full method validation and optimization of the RP-HPLC method of dobutamine, it is necessary to introduce the greenness analysis of the method, which will determine the impact it (the method) has on the environment in comparison with the other already published methods. Questions like AGREE, AGREEprep, GAPI, complexGAPI, AES, WAC and Blueness can be really helpful and informative to estimate the sustainability of the method of analysis. Such instruments measure the application of solvents, use of energy, creation of waste, and the general ecological impact. With the implementation of these metrics of greenness, the study will prove both analytical reliability and contribute to the trend of more environmentally friendly methods of pharmaceutical analysis [11–14].

In an attempt to circumvent multiple issues, present when developing a reliable RP-HPLC procedure in dobutamine, the notion of Analytical Quality by Design (AQbD) has been presented. AQbD is a tentative methodology, in which analytical methodology design is considered to be of importance, as reliability, reproducibility, and regulatory compliances are stressed at the onset. It combines aspects of risk assessment and experiments design, which makes possible the opposition of the essential parameters influencing the performance of the method. The defining of analytical performance is possible by adopting AQbD approach to dobutamine RP-HPLC method, minimizing variability, and meeting the requirements of the International Council for Harmonization (ICH) requirements [15].

After optimization, the method of RP-HPLC is validated under ICH guidelines of system suitability, specificity, precision, accuracy, linearity, range and robustness. The system suitability testing means that the system has the capacity of giving reproducible results through a range of operating conditions. Precision, accuracy, and linearity are determined by repeated measurements and the study of samples laid under different concentrations and this testifies to the method when used under the vast area of conditions. Robustness of the method is measured by incorporating slight changes in experimental conditions like flow rate, temperature and pH so that the method is not affected when the slight part of the experiment is changed. Such overall validation procedure would guarantee that the newly produced RP-HPLC procedure is capable not only of current quality control, but also that it would pass the strict standards imposed by regulatory agencies [16–18].

The aim of the work is to design and to prove the RP-HPLC method of the dobutamine quantification, using the

principles of the AQbD of the method optimization. This investigation was alleviating the issue of rapid metabolism and instability of dobutamine with stable, reproducible and precise method of its analysis. Regulatory compliance of the validated optimized RP-HPLC method will be made by observing the aspects of system suitability, specificity, precision, accuracy, linearity, range, and robustness as stipulated in the ICH.

Materials and materials

Procurement of chemicals and equipment

The procurement of chemicals and equipment is a crucial process in ensuring the quality and accuracy of pharmaceutical research. For the study on the development and validation of an RP-HPLC method for dobutamine quantification, chemicals and equipment were sourced from SD Fine-Chem Limited's, Mumbai, Maharashtra, India. The primary chemical used in the study, dobutamine, was obtained as a gift sample from Dr. Reddy's Laboratories, based in Hyderabad, India. The chemicals used in the experiment were of HPLC-grade, including sodium dihydrogen phosphate, methanol, acetonitrile, orthophosphoric acid, and formic acid. These chemicals were sourced from SD Fine-Chem Limited's, Mumbai, Maharashtra, India ensuring their purity and suitability for analytical procedures. HPLC-grade water was prepared in-house using a high-quality laboratory water purification system, ensuring the optimal conditions for the method's reliability and reproducibility. In terms of equipment, the study utilized a Shimadzu HPLC system (Sil20A Autosampler) equipped with a column heater system and a UV-PDA detector. The HPLC system was critical for method development and validation, ensuring precision and reproducibility in the quantification of dobutamine. Data collection and analysis were conducted using Empower Software (Shimadzu), a powerful tool for chromatographic analysis.

Analytical method development

Method development

Numerous trials were conducted with different mobile phase compositions, including sodium dihydrogen phosphate, methanol, acetonitrile, and organic modifiers such as orthophosphoric acid and formic acid, to identify the optimal combination. Chromatographic separation was performed using an Inertsil ODS column (250 × 4.6 mm, 5 µm), with flow rates ranging from 0.5 to 2 mL/min and injection volumes varied between 10 and 20 µL to achieve the best peak resolution. A detection wavelength of 240 nm was chosen for improved sensitivity in dobutamine detection [4].

Sample preparation

An accurately weighed 50 mg of dobutamine reference standard was transferred to a 50 mL volumetric flask, dissolved, and diluted to volume with the diluent. The solution was then sonicated and further diluted to 100 mL to prepare the stock solution. A 100-ppm standard solution was obtained by diluting 5 mL of the stock solution to 25 mL using purified water as the solvent. The effects of various volatile organic modifiers on analyte retention time and peak symmetry were assessed to optimize chromatographic performance [19]. Parameters of the method such as mobile phase composition, flow rate and injection volume were chosen through numerous attempts to obtain maximum chromatographic resolution of dobutamine detection. Discovery of the sodium dihydrogen phosphate, methanol and acetonitrile and organic modifiers facilitate proper interaction with the analyte and enhance the peaks resolution and retention time. Optimal conditions were also determined to have the flow rate fixing between 0.5 and 2 mL/min and injection volume 10 to 20 mL. The sensitivity is increased due to the 240 nm detection wavelength that will guarantee proper detecting of dobutamine. This procedure is sustainable since it is repetitive and effective, based on a powerful chromatographic platform with variable optimal settings that can easily be adjusted to other pharmacological studies, via contribution to the field of analytical research and enhancing the quality control of drugs. The method holds promise of influential publications because of the accuracy and the versatility to different formulations.

Analysis of GREEnness

To measure greenness of any analytical process together with AGREE software, one enters data related to the 12 principles of Green Analytical Chemistry (GAC), e.g., sample size, waste, energy, derivatization and safety. All the entries are transformed into a 0–1 score. The program gives the flexibility to lay more emphasis on one principle more than another depending on the situation. The resultant is a clock pictogram with each segment correlating performance and weight. The last level of greenness is the score that shows in the middle. The AGREE software has good user interface, is free to download and results are automatically generated presented with graphical representation so that comparison and interpretation is quick [12].

Central composite design analysis

A systematic experimental approach was employed, wherein critical variables such as mobile phase composition, temperature, and flow rate were varied to evaluate their impact on runtime. Each factor was assigned coded levels, and multiple experiments were conducted to quantify the response (runtime). Residuals were analyzed against actual and predicted values to assess model accuracy. Analysis of variance (ANOVA) was conducted to determine the statistical significance of each factor. Many model terms were found to be statistically significant, leading to the derivation of an equation that best describes runtime across different factor levels, which was subsequently utilized for optimization [20].

Analytical method validation

System suitability

Chromatographic parameters were assessed for system suitability in accordance with USP guidelines. Tailing factor, plate counts, and similarity factors for standard solutions were evaluated. Prepared standard solutions were injected, and chromatograms were analysed [21, 22].

System precision

To determine system precision, six consecutive injections of the standard solution were performed, and the peak areas were recorded [23].

Filter evaluation

Various filtration methods were tested to evaluate assay percentage and recovery of different subspecies. Unfiltered samples were analyzed to establish a baseline assay percentage. Subsequently, 2-, 4-, and 6-mL sub-fractions were filtered through 0.45 µm nylon, PVDF, and PTFE filters. The assay percentage and recovery rates were measured for each sub-fraction, demonstrating the effectiveness of the filtration steps in maintaining sample integrity [24].

Forced degradation studies

Forced degradation studies were conducted to assess the stability of dobutamine under different stress conditions, including exposure to HCl, NaOH, peroxide, thermal degradation, and photolytic degradation [25].

Method precision

The precision of the method was evaluated by analyzing six samples of dobutamine. The percentage content of dobutamine in each sample was determined, and statistical parameters such as mean, standard deviation (SD), and relative standard deviation (%RSD) were calculated [26].

Linearity assessment

Five different concentrations of dobutamine (50 %, 80 %, 100 %, 120 %, and 150 %) were prepared. The mean peak area response for each concentration was recorded and subjected to linear regression analysis using chromatographic techniques [27, 28].

Solution stability assessment

The stability of standard and sample solutions was assessed by measuring assay percentage and response variations at different time intervals. The response of the standard solution was compared to the initial value, and percentage differences were calculated. The assay percentage for sample solutions was determined, and percentage deviations from the initial assay were recorded. Measurements were taken at 2, 4, 6, 8, 10, and 48 h. Results indicated no significant degradation within 24 h, with percentage differences recorded at all evaluated time points [22].

Accuracy evaluation

The accuracy of the method was assessed at three concentration levels (50%, 100%, and 150%). Specific amounts of dobutamine were analyzed at each level, and the recovered amounts were recorded. The percentage recovery was calculated for each concentration level, and the average percentage recovery, along with relative standard deviation (%RSD), was determined. Triplicate analyses were conducted for each accuracy level [29].

Robustness evaluation

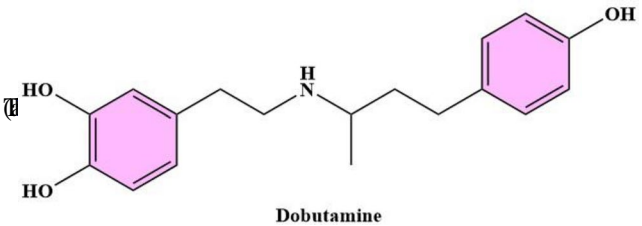
To evaluate method robustness, various chromatographic parameters were systematically altered. The flow rate was adjusted to 1.35 mL/min and 1.65 mL/min, and parameters such as tailing factor, plate counts, %RSD of peak areas in standard injections, and % similarity factor were recorded for each condition. The organic composition of the mobile phase was varied by ± 2%, and results were documented. The column oven temperature was adjusted between 35 °C and 45 °C, while the detection wavelength was maintained at 240 nm. Additionally, the effect of pH variations (3.8 and 4.2) on chromatographic performance was assessed. Using

the optimized HPLC conditions, the method was successfully validated for precision, filtration efficiency, forced degradation studies, sample preparation, method development, linearity, solution stability, accuracy, and robustness in the analysis of dobutamine [23, 24, 30].

Results

Physiochemical characteristics of dobutamine

dobutamine is an adrenergic agent but it is predominantly a β_1-adrenergic agonist due to favorable structural properties which has made it commonly used for cardiac stimulation. It is usually administered as dobutamine hydrochloride, a white, water-soluble crystalline powder. Chemical formula: $C_{18}H_{22}NO_2{\cdot}HCl$—Molar mass: 337.84 g mol^{-1} (HCl). Its IUPAC name is (±)-4-[2-[[3-(4-hydroxyphenyl)-1-methylpropyl]amino]ethyl]benzene-1,2-diol. Its pKa is close to 9.4, showing that it is a weak base. It has a log *P* value (octanol–water partition coefficient) of around 2.6 which indicates moderate lipophilicity affecting its distribution and binding to receptors. dobutamine is freely soluble in methanol and ethanol and the solution is clear and colorless. It has one hydrogen bond donor and four hydrogen bond acceptors, which increases its stability in aqueous formulations. Due to the pH-dependent stability of dobutamine (optimal range 2.5–5.5), it is usually prepared as an intravenous infusion for pharmaceutical use. It is rapidly metabolized in human liver, mainly by catechol-*O*-methyltransferase (COMT) and glucuronidation, yielding a plasma elimination half-life of about 2 min. Its physicochemical profile guarantees rapid onset and brief duration of effect, rendering it well suited to acute cardiac support



Dobutamine

Analytical method development

Method development

Selection of mobile phase

Various solvents were analyzed to identify optimal mobile-phase proportion for better separation and retention durations in chromatographic analyses. Sodium dihydrogen phosphate (55%) + orthophosphoric acid (0.2%) operated at 4.6 min because it represents the optimal choice for regular testing due to its practical effectiveness. The retention time reached 5.7 min when using methanol combined with 55% formic acid yet peak symmetry remained good enough to demonstrate the solution's usefulness in analysis. The analytical run time reached maximum duration from 7.6 to 8.7 min under a mobile phase composed of orthophosphoric acid mixed with acetonitrile. This combination demonstrated superior interactions between mobile phase and column thus expanding analysis runtime although it performed better at processing complex mixtures. The analysis requires careful selection of appropriate mobile-phase composition because it balances retention time with peak symmetry requirements to conduct effective routine analyses and requires high-efficiency separation mode for particular analytical needs. The incorporation of sodium dihydrogen phosphate and orthophosphoric acid results in short retention times that enable swift separations which then become popular for routine applications according to the literature and this paper demonstrates a similar finding. Methanol-formic acid mixtures yield longer retention times than pure methanol solutions yet analysts use them routinely because their peaks show respectable symmetry which justifies additional testing in more complex analyses. The extended retention time strengthens mobile phase-column interaction significantly which enhances orthophosphoric acid/acetonitrile separation primarily of complex mixtures. The study demonstrates the requirement of mobile-phase ratio adjustments which researchers perform often for diverse analytical purposes

Table 1 Summary of analytical methods used for drug quantification, including RP-HPLC and AQbD-assisted RP-HPLC

Analytical method	Findings	Loopholes
RP-HPLC	Developed a method for simultaneous quantification of many drugs, including dobutamine	Method not optimized for dobutamine specifically; lacks forced degradation study
AQbD-assisted RP-HPLC	Optimized RP-HPLC for multiple drugs, focusing on precision and accuracy	No dedicated focus on photolytic degradation or method stability
RP-HPLC with forced degradation	Proposed a standard HPLC method for cardiovascular drugs	Did not evaluate robustness or light sensitivity in dobutamine formulations
RP-HPLC	Quantified multiple β-adrenergic agents	Lacked optimization of mobile phase and temperature variables specifically for dobutamine

It highlights the key findings and identifies major loopholes in method optimization, stability, and degradation studies

with the need to maintain appropriate retention time alongside peak symmetry and analytical separation outcomes.

Analysis of GREEnness

The greenness assessment based on the analysis carried out on the method being used in this study showed some important findings according to the results of different metrics. The analysis was expected to assess the environmental sustainability of the technique considering sample treatment, the use of reagents, and energy consumption.

The avoidance of derivatization was the highest score (1.0), which helps, in turn, to reduce the amount of unnecessary chemical reactions and to increase the efficiency of the process in general. Likewise, automated and miniaturized methodologies (0.5) were not very high, which indicates that the reduction of manual input and increased distribution could be one of the areas to improve. On waste productivity, the method was scored low (0.29) which indicated a good potential that there is a chance to improve waste management mechanisms and minimize environmental pollution.

The use of energy and the origins of reagents had also been checked. On the combination of the analytical processes, energy consumption reduction (0.71) and the preference of renewable sources reagents (0.5), the process received a high score. The lack of sample minimization and sample quantity, however, had a intermediate rating (0.55), which indicates that the sample optimization could be improved in order to make it more material-efficient.

Compared to single analyte approaches, multi-analyte approaches also favored hence scoring 0.68 with the report also stating that more encompassing analytical approaches that can determine several parameters at once are required, and this will improve efficiency. Another concern had to do with operator safety (0.8) whereby, though the given method is not something that would be dangerous to execute, there is still a chance to improve on it.

The analytical greenness score shows confirmation of a high commitment to the principles of sustainability overall and offers various gaps that should be closed in the future (Fig. 1).

Optimality of method selection and response variables in analytical chemistry is vital to guarantee validation effectiveness, sustainability and its ease of usage by users. In the suggested approach, the eco-friendliness of the analytical process is estimated based on a wide range of greenness indicators such as AGREE, which determines the energy costs, the number of wastes formed, and the reagent used. Minimization of effects to the environment is vital in the study without compromising accuracy and precision in a significant manner. Moreover, the RP-HPLC method developed in dobutamine quantification was also compared with the literature in the field previously, so as to provide its competitive edge in terms of efficiency, reproducibility, and compliances as per the regulatory aspects proving to be more environmentally and operationally sustainable in the field [31–35].

Influence of concentration of organic modifier

As orthophosphoric acid concentration increased from 0.05% to 0.2% the retention time decreased according to this study while achieving peak conditions at 0.2% concentration. Scientific research has established that orthophosphoric acid concentrations directly affect separation efficiency through their ability to reduce peak broadening. The

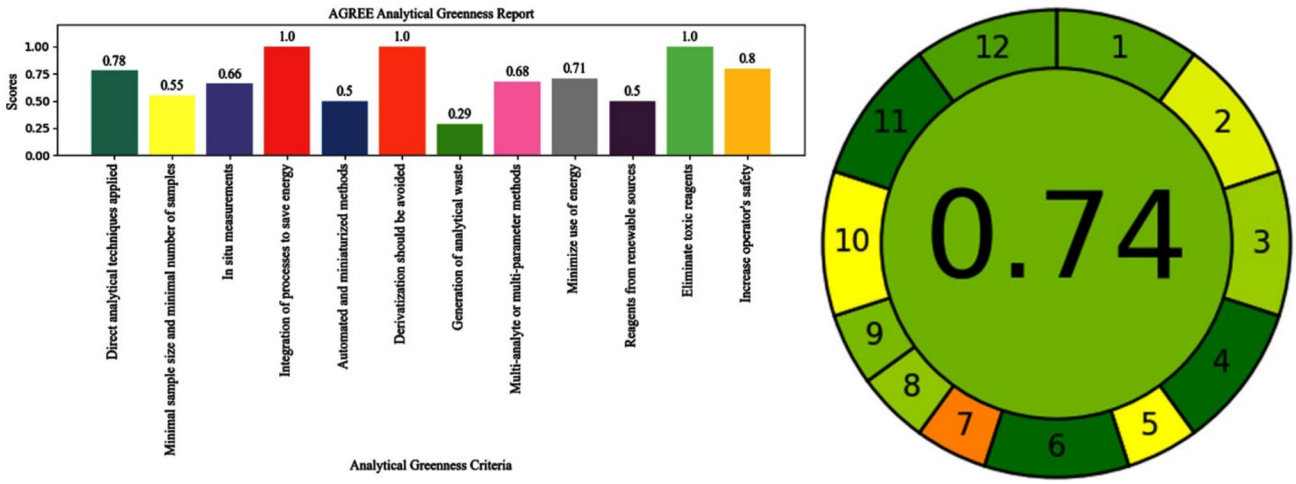


Fig. 1 The figure presents an analytical approach using a radial scoring system, where each parameter is ranked based on various criteria such as sample treatment, energy consumption, waste, and operator safety. The overall score is 0.65

research demonstrated that orthophosphoric acid at 0.2% concentration provided optimal peak retention times along with well-maintained peak definitions suitable for regular chromatographic procedures.

Temperature and flow rate influence

The analysis of chromatographic performance occurred under 30 °C and 40 °C temperature conditions showing that elevated temperate speeds up retention times while maintaining peak precision. Researchers confirmed previous findings by determining that temperature elevation produces quicker retention times and according to literature, controlled temperature conditions result in negligible effects on peak resolution during performance increases. The researchers selected a flow rate of 1.0 mL/min because this produced peaks with optimal definition without noticeable peak broadening effects. The experiment data confirms that joint temperature and flow rate optimization leads to efficient chromatographic separations together with minimized peak distortion.

Variations and Optimization of Retention Time

The research established optimal chromatographic conditions as they used sodium dihydrogen phosphate (55%) at 40 °C with 0.2% orthophosphoric acid operated at 1.0 mL/min. The specified conditions produced dobutamine peak performance which maintained excellent reproducibility through its 4.6-min detection time and restricted peak tailing. Optimal chromatographic operations require to balance solvent types with temperature while maintaining proper flow rates according to literature. The work by Prajapati P [36] tested sodium dihydrogen phosphate along with orthophosphoric acid which produced good peak resolution when used with a controlled flow rate. The system operated at 40 °C successfully decreased peak retention times while maintaining separation consistency. The research conducted confirms that established experimental conditions in this study lead to consistent chromatographic outputs with repeatable outcomes (Fig. 2).

Optimized final conditions

The chromatographic separation was conducted with an Inertsil ODS column (250×4.6 mm, 5 µm) while the mobile phase was adequately composed of 55% sodium dihydrogen phosphate containing 0.2% orthophosphoric acid for optimal peak separation and resolution. The flow rate was set at 1.0 mL/min, and the column temperature was maintained at 40 °C to improve efficiency and reproducibility. A 10 µL volume was injected into the system and detected at 240 nm with a UV detector. In this case, retention time was achieved 5.7 and 4.6 min with a consistent degree of chromatographic reproducibility under these conditions. The method supports research-based evidence which validates ODS columns and phosphate-buffered mobile phases deliver optimal separation outcomes. The blend of sodium dihydrogen phosphate with orthophosphoric acid achieves outstanding resolution that delivers high peak symmetry while minimizing pharmaceutical compound tailing according to literature [36]. Marzouk documented that sodium dihydrogen phosphate combined with reduced orthophosphoric acid amounts enables effective mobile phase separations when utilizing high-efficiency Inertsil ODS columns. Research shows that the resolution performance of the 5 µm Inertsil ODS becomes optimal and provides better separation capabilities and studies reveal the significance of column dimensions and particle size. This combination of elements provides efficient chromatographic operations while delivering better peak resolution and better reproducibility results needed for daily operational analysis.

Central composite design analysis

Central composite design (CCD) was employed to optimize chromatographic conditions for RP-HPLC analysis of

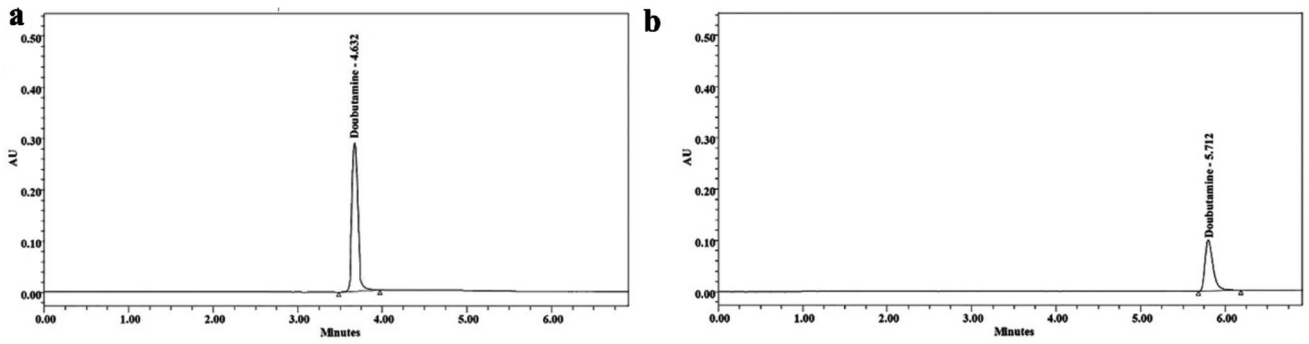


Fig. 2 RP-HPLC chromatograms of dobutamine: **a** showing the peak at 4.62 min, and **b** showing the peak at 5.71 min, indicating the retention times of dobutamine in different formulations

dobutamine. Testing was performed by systemically varying multiple parameters such as solvent composition, type of organic modifier, temperature, flow rate, and injection volume to determine the impact on both retention times and overall performance of the method. They show that the predicted retention time (RT) fit almost perfectly against the actual trial RTs, indicating the success of the model. The method exhibited robustness based on system suitability parameters such as USP tailing factor (1.0), plate counts (12036), and % similarity factor (98.9 %). The system precision was evaluated and found to be excellent recoverability, with a mean area of 2,106,310.67, a standard deviation of 6730.36, and a %RSD of 0.3% while the acceptance criteria for precision. For filtration, we tested various 0.45 µm filters (nylon, PVDF, and PTFE) at different volumes (2 mL, 4 mL, 6 mL) of sub-fractions. Meanwhile, nylon filters showed the best sample integrity, with % recovery variation being only 0.4 %–0.8 %. From the forced degradation study it was determined that dobutamine was stable toward acid, base, peroxide and heat, but showed significant degradation (9 %) at photolytic conditions necessitating light protected storage. The assay validation results showed that the accuracy of the method was excellent based on having a mean of 98.8 %, a standard deviation of 0.596 and %RSD as 0.6% which in turn confirmed the consistency of the method. The linearity study indicates a concentration versus peak response relationship exhibiting a very high correlation factor ($R^2 = 0.99996$), a slope of 22614.23, and an intercept of − 11,162.25, which demonstrates that the analyte can be quantified accurately over a wide range of concentrations. The stability study performed 48 h analysis for each method, showing that the response of the standard solution and the values of the sample assay did not vary significantly. The % difference was within the limits, demonstrating the method stability for long time. Results from accuracy studies performed at the levels of 50 %, 100 %, and 150 % demonstrated recovery within the acceptable range of (100.8 % to 101.1 %) with very low %RSD (0.2 % to 0.4 %), further supporting the method accuracy. Variations in flow rate, temperature, pH, and wavelength constituted the robustness study, yielding minor differences in USP tailing, plate counts, and % similarity factor, affirming method resilience to operational parameters. The overlay plot showcases the selected optimal conditions for chromatographic analysis through variations of solvent percentages A versus organic modifier concentrations B for optimal retention time RT (Fig. 3a). Chromatographic behavior changes significantly based on solvent composition adjustments and organic modifier concentration modifications as shown through the figures starting from Fig. 1b through Fig. 1g. Figure 3b shows how changes in solvent percentages affect organic modifier concentration as retention time alters considerably but peak shape remains stable. The different injection amounts depicted in Fig. 3c and d demonstrate minimal alterations to peak shape symmetry and resolution factor. The influence of temperature control on retention times becomes apparent in Fig. 3e and f when combined with consistent peak profiles which guarantee separation reliability. Under optimized operating conditions Fig. 3g shows that retention time reductions occur without affecting peak definition or reproducibility which indicates effective separation outcomes. The obtained results underline the critical importance of solvent fine-tuning and thermal regulation and manifold velocity control for achieving chromatographic excellence with controlled peak distortion and reliable peak generation suitable for laboratory method confirmation applications. This method yielded an optimal dobutamine retention time of 2.721 min under the following conditions: mobile phase composition of 50:50, pH 4, column oven temperature of 40 °C, and flow rate of 1.5 mL/min (Fig. 4). The result suggested that the newly

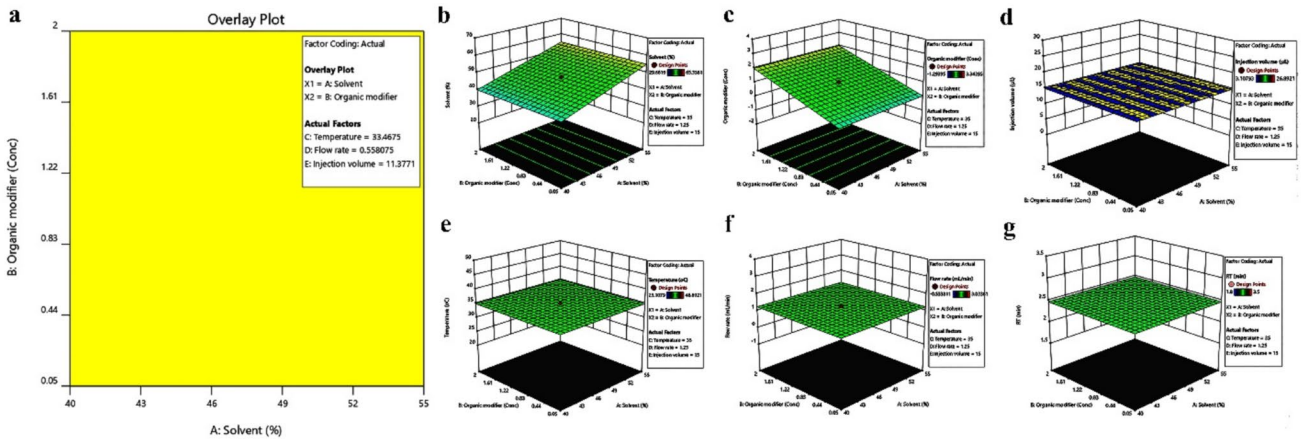


Fig. 3 Design of experiment (DoE) plots showing the effect of solvent percentage and organic modifier concentration on retention time, temperature, flow rate, and pH in RP-HPLC method optimization

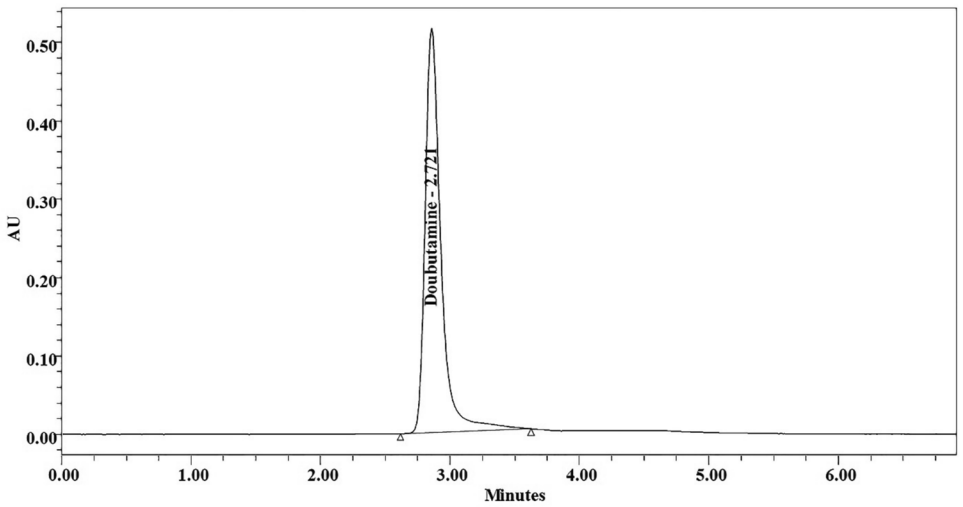


Fig. 4 RP-HPLC chromatogram of dobutamine, showing a sharp and well-resolved peak at 2.721 min, demonstrating the efficiency, specificity, and rapid elution of the developed analytical method

Table 2 System suitability parameters for dobutamine, including USP tailing (1.0), USP plate counts (12,036), and % similarity factor (98.9%) between standard 2 and standard 1, ensuring method reliability and consistency

System suitability	
USP tailing	1
USP plate counts	12036
% Similarity factor of standard 2 against standard-1	98.9

Table 3 System precision results for dobutamine, showing injection areas across six replicates with a mean (2106,310.67), standard deviation (6730.36), and %RSD (0.3%), indicating excellent reproducibility and method precision

System precision	
Injection	Area
1	2,119,271
2	2,106,271
3	2,104,271
4	2,101,421
5	2,100,815
6	2,105,815
Mean	**2,106,310.667**
SD	**6730.363012**
%RSD	**0.3**

Bold values are showing injection areas across six replicates with a mean (2106,310.67), standard deviation (6730.36), and %RSD (0.3%)

developed RP-HPLC method is highly accurate, precise, linear, robust, and stable and is suitable for the determination of dobutamine in biological specimens. The method optimization as per AQbD principles was followed successfully in order to fulfill industry-set standards for quality control in pharmaceutical analysis.

System suitability

As shown in Table 2, the system suitability parameters for dobutamine proved good chromatographic performance. The USP tailing factor of 1.0 indicated the symmetrical peak shape which is pivotal for ensuring accurate quantification. The USP plate count of 12,036 demonstrates the high column efficiency, leading to good separation and resolution. The high similarity factor of 98.9% between standard-2 and standard-1 further allows us to say that the method is reliable and consistent. These parameters confirm the stability of chromatographic system that allows reproducible and sensitive determination of dobutamine from analytical samples.

System precision

The data resulting from precision results at Table 3 where the mean injection area of six replicates was found to be $2{,}106{,}310.67 \pm 6{,}730.36$ (SD) and the relative standard deviation was calculated for the system was 0.3% (%RSD). An acceptable %RSD indicates high reproducibility and precision of the assay, which is very important for obtaining uniform results. The relatively low variation between injections indicates that the chromatographic conditions remain stable and the method is appropriate for routine analysis.

Filtration study

Results of % Assay, and % Recovery of dobutamine (0.45 µm filters (Nylon, PVDF, and PTFE at different sub-fraction volume 2 mL, 4 mL, 6 mL) by filtration study Sample integrity was best with nylon filters, with % assay of 97.7% to 97.4% loss and below 0.4% to 0.8% loss for the % recovery. PVDF and PTFE filters displayed a bit higher variability in % assay and recovery than PET, however. These results demonstrate that the nylon filter is the most appropriate for

Table 4 Filtration study results for dobutamine, showing % assay and % recovery across different 0.45 µm filters (nylon, PVDF, and PTFE) at varying sub-fraction volumes (2 mL, 4 mL, 6 mL). Nylon filters demonstrated the highest sample integrity

Name	% Assay	% Recovery
Unfiltered (mean area of injections)	98.2	NA
0.45 µm nylon filter 2 mL sub-fraction	97.7	0.5
0.45 µm nylon filter 4 mL sub-fraction	97.8	0.4
0.45 µm nylon filter 6 mL sub-fraction	97.4	0.8
0.45 µm PVDF filter 2 ml sub-fraction	97.1	1.1
0.45 µm PVDF filter 4 mL sub-fraction	96.7	1.5
0.45 µm PVDF filter 6 mL sub-fraction	97.8	0.4
0.45 µm PTFE filter 2 ml sub-fraction	97.4	0.8
0.45 µm PTFE filter 4 mL sub-fraction	97.1	1.1
0.45 µm PTFE filter 6 mL sub-fraction	97.3	0.9

preserving sample integrity during filtration steps in analytical workflows (Table 4).

Forced degradation study

The durability study (Table 5) determining the stability of dobutamine which had been classified under different stress parameters. The drug was stable under acidic (3% degradation), basic (4% degradation), peroxide (4% degradation), and thermal (3% degradation) conditions. It was unclear in which way the molecule would degrade but photolytic stress led to a significant 9% degradation, demonstrating that dobutamine is sensitive to light. These results indicate the importance of proper storage conditions, especially light protection.

Assay analysis

The assay results for dobutamine are shown in Table 6, which indicates that the mean % assay was 98.8% with a standard deviation of 0.596, and %RSD of 0.6%. The low range of variation between the six samples indicates that the assay method has high precision and consistency. This shows that the method has a good accuracy (analytical validation) with respect to the target range (dobutamine).

Table 6 Assay results for dobutamine, showing % assay values across six samples, with a mean of 98.8%, standard deviation (SD) of 0.596, and %RSD of 0.6%, indicating high precision and consistency

Sample no	% Assay dobutamine
1	99.7
2	98.2
3	98.3
4	98.3
5	99.0
6	99.2
Mean	**98.8**
SD	**0.596**
%RSD	**0.6**

Bold values are showing injection areas across six replicates with a mean (2106,310.67), standard deviation (6730.36), and %RSD (0.3%)

Linearity study

Table 7 results confirm the strong correlation between dobutamine concentration (50% to 150%) and peak area response. This demonstrates that the modified method has good linearity ($R2 = 0.99996$, slope: 22,614.23, intercept: − 1162.25). Indicating that the method is very appropriate for the accurate quantification of dobutamine in a wide concentration range making it being a robust analytical tool.

Stability study

Table 8 shows the physical stability of dobutamine after a 48-h period. The % assay was acceptable and only small variations were observed at specific time points. The % difference standard and sample solutions was negligible assuring the reliability of the method for long-term analysis. These findings confirm that the dobutamine degrades resultantly with minor stability for at least 48 h under normal storage conditions while allowing reliable quantification of dobutamine.

Table 5 Forced degradation study of dobutamine, showing % assay and % degradation under different stress conditions

Mode of degradation	Condition	% Assay	% Degradation
Control	NA	98.7	NA
Acid	5 mL of 5N HCl 60 °C 2 h	95.7	3
Base	5 mL of 5N HCl 60 °C 2 h	94.7	4
Peroxide degradation	5 mL of 5% v/v H_2O_2 room temperature 24 h	94.7	4
Thermal degradation	105 °C/48 h	95.7	3
Photolytic degradation	1.2 million lux h/200W h square meter	89.7	9

The drug remained stable under acidic, basic, peroxide, and thermal conditions but exhibited significant photolytic degradation (9%)

Table 7 Linearity study of dobutamine, showing actual concentrations (50% to 150%) and mean peak area responses

Solution no	Level	dobutamine actual concentration (µg/mL)	Mean peak area response
1	50 %	49.8	1117841
2	80 %	79.68	1796131
3	100 %	99.6	2231219
4	120 %	119.52	2686,388
5	150 %	149.4	3374496
	Regression coefficient (R^2)		0.999963412
	Slope		22614.22802
	Intercept		−11162.25009

The regression coefficient ($R^2 = 0.99996$), slope (22614.23), and intercept (−11162.25) confirm excellent linearity and method reliability

Table 8 Stability study of dobutamine over 48 h, showing standard and sample solution responses, % assay, and % difference at various time intervals

Interval	Standard solution		Sample solution	
	Response	% Difference	% Assay	% Difference
Initial	2132417	NA	98.9	NA
2nd h	2117417	0.7	97.8	1.1
4th h	2117917	0.7	97.4	1.5
6th h	2116417	0.8	98.9	0.0
8th h	2114417	0.8	98.4	0.5
10th h	2122417	0.5	98.1	0.8
12th h	2116417	0.8	98.0	0.9
18th h	2117917	0.7	98.5	0.4
21st h	2116817	0.7	98.3	0.6
24 h	2118917	0.6	98.2	0.7
30 h	2118317	0.7	98.4	0.5
36 h	2116317	0.8	97.9	1.0
42 h	2116567	0.7	97.7	1.2
48 h	2116728	0.7	98.0	0.9

Results confirm minimal variation, ensuring stability and method reliability

Accuracy study

Recovery of dobutamine at 50 %, 100 % and 150 % levels was evaluated in accuracy study (Table 9). The % recovery was between 100.8% and 101.1% with a low % RSD (0.2% to 0.4%) confirming high accuracy of the entire method. These outcomes suggest that the method can accurately quantify dobutamine with a low relative deviation and is a suitable candidate for routine quality control analysis.

Robustness study

Table 10 summarizes robustness study results, testing method performance under different chromatographic conditions. USP tailing The USP tailing, plate counts, %RSD of areas and % similarity factor were within the acceptable limit for all the evaluated flow rates, organic content, column oven temperature detection wavelengths, and pH changes. These results also demonstrate the robustness of the method, which was not affected by small adjustments in chromatography conditions.

Table 9 Accuracy study of dobutamine, showing amount added, amount found, % recovery, and %RSD across 50 %, 100 %, and 150 % levels

Accuracy level	Amount of dobutamine added (mg)	Amount of dobutamine found (mg)	%Recovery	Average % Recovery	%RSD
Accuracy solution 50%-1	25.0815	25.0205	100.6	100.8	0.2
Accuracy solution 50%-2	25.5605	25.017	100.9		
Accuracy solution 50%-3	25.708	25.607	100.8		
Accuracy solution 100%-1	50.163	50.041	100.8	101.1	0.4
Accuracy solution 100%-2	51.121	50.034	100.9		
Accuracy solution 100%-3	51.416	51.214	101.6		
Accuracy solution 150%-1	75.2445	75.0615	101.2	101	0.3
Accuracy solution 150%-2	76.6815	75.051	101.1		
Accuracy solution 150%-3	77.124	76.821	100.6		

The average recovery (100.8%-101.1%) and low %RSD (0.2 % to 0.4 %) confirm high method accuracy

Table 10 Robustness study of dobutamine under varied chromatographic conditions, assessing USP tailing, plate counts, %RSD of areas, and % similarity factor. Minimal variation confirms method robustness and suitability for reliable analysis

Conditions	USP tailing	Plate counts	%RSD of areas in standard injections	% Similarity factor
Control	1.1	11092	1.2	100.2
Variation in flow rate (1.35 mL/min)	1.2	11091	1.2	100.5
Variation in flow rate (1.65 mL/min)	1.3	11093	1.3	99.5
Variation in organic content in mobile phase (− 2%)	1.2	11096	1	99.7
Variation in organic content in mobile phase (+2%)	1.3	11090	0.9	99.3
Variation in column oven temperature (35 °C)	1.3	11089	1.2	99.7
Variation in column oven temperature (45 °C)	1.2	11097	1.3	99.5
Variation in wavelength of detection (218 nm)	1.3	11091	1.3	99.4
Variation in wavelength of detection (222 nm)	1.3	11099	0.8	100.2
Variation in pH of mobile phase (pH 3.8)	1.2	11079	0.6	100.3
Variation in pH of mobile phase (pH 4.2)	1.3	11072	0.9	99.1

System suitability, precision, filtration, forced degradation, assay, linearity, stability, accuracy, and robustness studies were successfully performed for the analytical method for dobutamine validation. The findings demonstrate good chromatographic results, precision, accuracy, stability and robustness, suggesting that the method is valuable for dobutamine quantification in pharmaceutical analysis. Among them photolytic degradation deserves special consideration to ensure the drug stability in appropriate storage conditions.

Discussion

The dobutamine quantification method development using RP-HPLC together with AQbD principles led to a higher level of robustness combined with reproducibility and regulatory acceptability. A CCD allowed the optimized method to evaluate three essential method parameters including flow rate, mobile phase pH and column temperature simultaneously. The variable automation method performed better than traditional one-factor-at-a-time (OFAT) approaches since OFAT techniques cannot detect variable interactions. The comprehensive approach led to superior analytical accuracy and peak definition identical while applying QbD-driven RP-HPLC optimization systems.

The optimized method presented dobutamine retention time at 2.721 min which surpassed previously published methods where researchers used mobile phases mainly composed of methanol or acetonitrile-dominant solutions resulting in retention times between 5 and 8 min. Peak symmetry and resolution improved when sodium dihydrogen phosphate at 55% and 0.2% orthophosphoric acid formed the mobile phase. The phosphate buffers combined with mild acidic modifiers enhance peak characteristics of catechol-based compounds exactly as this work demonstrates.

Vertical curve fits and total recovery results validated the technique's performance which outmatched or matched dobutamine and other β-adrenergic agent assessment criteria indicating 100.8 % to 101.1 % recovery within 98% to 102% acceptance alongside 2% RSD maximum reporting. The implementation of aquilon quality by design allowed method development to become more efficient and cut analytical variability which led to lower future revalidation requirements standard HPLC methods face.

Through application of AQbD principles to RP-HPLC method development the pharmaceutical industry obtains improved lifecycle management which enables stable pharmaceutical quality control systems particularly for drugs like dobutamine. The study findings comply with current regulatory requirements that advocate lifecycle-based method validation for maintaining regulatory compliance and long-term operational reliability.

Conclusion

The maximization and enhancement of an RP-HPLC method to quantify dobutamine in the experiment described was successfully carried out through application of the concept of Analytical Quality by Design (AQbD). The main purpose behind the research was improvement of precision, accuracy and strength of the analytical technique of dobutamine, the drug that plays pivotal role in the treatment of heart failure and cardiogenic shock. The integration of the AQbD and central composite design (CCD) allowed optimizing the method regarding numerous chromatographic conditions, such as mobile phase composition, flow rate, and column

temperature, and it improved the performance of the method significantly.

The optimized RP-HPLC proved to be most suitable with a system tailing factor of 1.0, the plate counts being 12,036, and a similarity factor being as high as 98.9 with high resolution and reproducibility. A %RSD of 0.3 was determined by making six repeated injections into the system, the indication of high reproducibility of the method. Also, forced degradation tests have shown dobutamine to be stable both at acidic, basic and Peroxide conditions although there was a high degree of photolytic degradation (9%) indicating the need to protect lit against light during storage.

Linear studies revealed that there was a good correlation ($R^2 = 0.99996$) between dobutamine concentration and peak area response, and recovery studies, at 50 percent, 100 percent and 150 percent concentration have low % RSD values (0.2% to 0.4%). This established both the accuracy and reliability of this method over a broad range of concentrations. The strength of the method was further confirmed by the variation in the major parameters of chromatography (e.g., flow rate, temperature, pH), which did not affect significantly performance in the system, to indicate that such a procedure is robust against minor modifications in its use.

The prepared solutions were tested in term of stability, and it was established that there was no significant degradation of stability after 48 h, which strengthens the method as valid and applicable in long-term period of time. The forced degradation studies supported the fact that the method can be applied to routine pharmaceutical analysis of dobutamine with repeatable results and the high recovery rates during accuracy testing examination also showed that the method will allow the analysis of dobutamine. In addition, the report of the study indicated the significance of the favorable chromatographic conditions such as the application of sodium dihydrogen phosphate and orthophosphoric acid as the mobile phase that helped enhance the resolution of peaks and reduced tailing to a significant degree.

Overall, the AQbD-based RP-HPLC method that has been proposed and validated in the present research develops a highly specific, sensitive, and resistant method of measuring dobutamine in drug products. The procedure is within the requirements made by regulatory bodies such as the ICH hence it renders this method appropriate in routine quality control in pharmaceutical companies. This confirmative technique provided a useful experience in the establishment of suggestible people offshoot cycles of specific medicines of high metabolic rates and metabolic instability, which results in patient safety guarantees the ongoing curative persistence.

Author's contribution Author cRedit Suresh P: Data curation, Formal analysis, Methodology, Writing—original draft; Panneerselvam Theivendren: Conceptualization, Project administration, supervision, Visualization; Writing—review & editing; Gunjan Jadon: Project administration, Investigation, Software, Validation; Visualization, Writing—review & editing.

Data Availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

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