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Development and validation of a robust RP-HPLC method for Landiolol using analytical quality by design

S. Harshini[1], Panneerselvam Theivendren[1] and Saravanan Govindaraj[2*]

*Correspondence:
Saravanan Govindaraj
sarachem1981@gmail.com
[1]Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai, Tamilnadu 600117, India
[2]Department of Pharmaceutical Chemistry, Vel Pharmacy College, Vels Institute of Science, Technology and Advanced Studies, Manjankaranai Village, Uthukkottai Taluk, Tiruvallur, Tamilnadu 601102, India

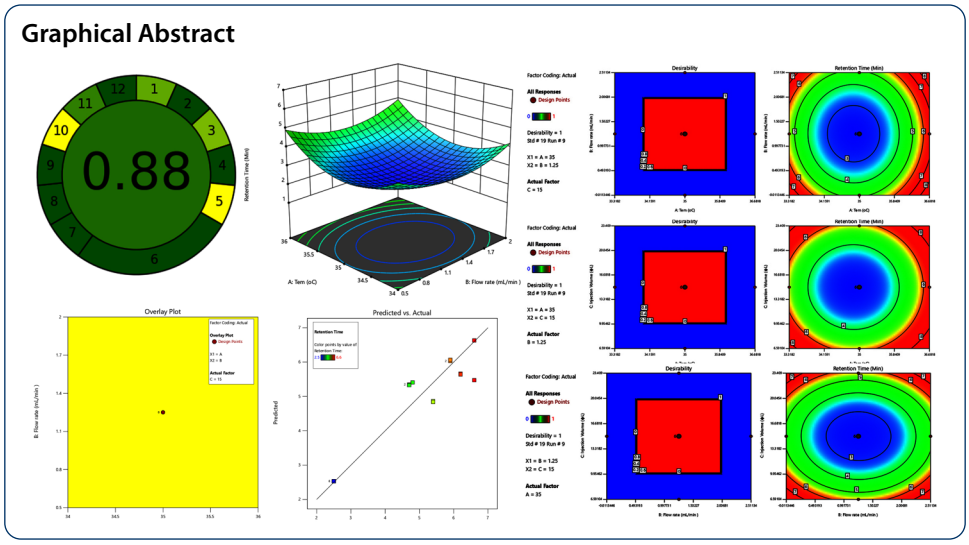
Abstract

A systematic Analytical Quality by Design based reverse-phase high-performance liquid chromatography method was successfully developed and validated for the quantification of Landiolol in pharmaceutical formulations. The method optimization employed Central Composite Design with Analysis of Variance revealing a statistically significant model (F-value = 11.60, $p = 0.0003$). The optimized chromatographic conditions included an Inertsil ODS column (150 × 4.6 mm, 5 µm), mobile phase comprising 55% sodium dihydrogen phosphate and 0.2% orthophosphoric acid, flow rate of 1.25 mL/min, injection volume of 15 µL, and detection wavelength of 240 nm, yielding a retention time of 2.70 min. The validated method demonstrated excellent system suitability with USP tailing factor of 1.0, plate count of 10,925, and similarity factor of 98.4%. System precision showed exceptional reproducibility with %RSD of 0.3%, while linearity was established over the concentration range with $R^2 = 0.9999$. Accuracy studies revealed recoveries between 100.8 and 101.1% across 50%, 100%, and 150% levels. Forced degradation studies indicated Landiolol stability under thermal (3% degradation) and oxidative conditions (4% degradation) but significant photolytic degradation (9%). The greenness assessment yielded a score of 0.88, confirming environmental sustainability. This robust, precise, and environmentally conscious analytical method complies with regulatory guidelines for routine quality control and pharmaceutical analysis of Landiolol formulations.

Keywords Analytical quality by design, RP-HPLC, Landiolol, Method validation, Central composite design

1 Introduction

Medicine analysis is an essential step in assuring quality, efficacy and safety of drug products. RP-HPLC stands out as one of the most potent analytical techniques for the quantitation and characterization of pharmaceutical compounds owing to several advantages including enhanced resolution, sensitivity, and reproducibility [1]. The challenge with traditional RP-HPLC method development is that it is often a trial-and-error process leading to inefficient method optimization and method validation. In order to address these challenges, Analytical Quality by Design (AQbD) has been introduced as



a systematic approach to ensure an understanding of the method, risk assessment and ensuring continuous improvement, leading to establishing robust and reliable analytical procedures [2, 3]. This research work aims to develop and validate the AQbD approach for the RP-HPLC method to quantify the mostly used non-selective beta-blocker named Landiolol drug used for hypertension, heart failure and left ventricular dysfunction [4]. Landiolol, 1-(9 H-carbazol-4-yloxy)-3-{[2-(2-methoxyphenoxy) ethyl]amino}spropan-2-ol, which are competitive antagonists of β and α adrenergic receptors widely used for the treatment of cardiovascular diseases [5]. Landiolol demonstrates poor aqueous solubility and a significant first-pass effect in vivo, necessitating accurate and precise quantification of the drug in pharmaceutical dosage forms in order to ensure reproducible therapeutic effects. RP-HPLC-based methods to quantify Landiolol often suffer from challenges associated with peak tailing, poor resolution, and inconsistent retention times, making an AQbD approach imperative to improve the method performance and robustness [6, 7].

Analytical quality by design (AQbD) builds upon the Quality by Design (QbD) principles and advocates for a science- and risk-based approach to analytical method development. For example, process analytical technology (PAT) follows a systematic approach to establishing the analytical target profile (ATP), identifying CQAs, risk assessment and identification of the critical method parameters (CMPs) which lead to meaningful method performance [8]. By implementing the AQbD principles in RP-HPLC method development, systematic optimization of the method is possible through the use of statistical tools, such as DoE, RSM, and multivariate analysis, to make the method robust and compliant with regulatory requirements [9]. In addition, regulatory bodies like the International Council for Harmonisation (ICH) promote the use of AQbD in the development of analytical methods for improved lifecycle management and more seamless implementation of post-approval changes with minimal regulatory impact [10–13]. The objective of this research work was to develop and optimize an AQbD-enabled RP-HPLC method for quantification of Landiolol, which can be achieved with high specificity, precision and robustness. This is done by first screening all the significant chromatographic parameters such as mobile phase composition, column type, flow rate, and detection wavelength, followed by optimisation with DoE [14]. This work is anticipated to enhance

both our understanding of analytical variability and the performance of methods in general, thus establishing a new standard for quality control during the method development process, especially for routine quality control and regulatory submissions. However, no such study currently exists highlighting the key benefits of AQbD applications in pharmaceutical analysis and certainly will add to the existing literature towards the supporting evidence of AQbD applications in pharmaceutical analysis [15–17].

This study is a full systematic AQbD framework combining risk assessment, DoE and green analytical chemistry principles in quantifying Landiolol, unlike the traditional trial and error methodologies [18–20]. It is novel because it uses the Central Composite Design to simultaneously optimize critical parameters of the method with AGREE metric-based greenness assessments, as it is rarely documented in the case of ultra-short-acting beta-blockers. Although the existing approaches of Landiolol are only analytical performance oriented, our approach is the only one that balances the strength of the method with the sustainability of the environment, by using low consumption of organic solvents and low waste production. Besides guaranteeing regulatory compliance, it has also created a paradigm of eco-friendly pharmaceutical analysis, responding to the increasing need to have sustainable analytical practice in quality control laboratories.

2 Materials and materials

Landiolol was obtained as a gift sample from Dr. Reddy's Laboratories (Hyderabad, India). All chemicals used in the experiments were of HPLC grade and purchased from reputable suppliers. HPLC-grade water was prepared in-house using an in-house laboratory water purification system.

The chromatographic method was developed and validated using a Shimadzu HPLC system equipped with an LC-20AD pump, SPD-M20A photodiode array (PDA) detector, SIL-20 A autosampler, CTO-20AC column oven, and DGU-20A5R degasser. The system was controlled and data were acquired using LabSolutions software (Version 5.119, Shimadzu Corporation, Kyoto, Japan). Complete instrument specifications including manufacturer details, model numbers, software versions, and accessories are comprehensively detailed in the Methods section.

2.1 Analytical method development

2.1.1 Analysis of GREEnness

To quantify greenness of any analytical process along with AGREE software one puts on record the data associates with the 12 principles of Green Analytical Chemistry (GAC) e.g. sample size, waste, energy, derivatization and safety. All the entries are converted into a 0–1 score. The program leaves a choice to focus more on one principle than another relating to the situation. The outcome is a pictogram of a clock where every segment relates performance and weight. The final score of greenness is the one that appears in the middle. The AGREE software is well user interface, free to download and the results automatically pop out with graphical representation that one can easily compare and interpret.

2.1.2 Analysis of central composite design

The experimental approach consists in varying important variables mobile phase, temperature and flow rate to assess their impact on runtime [21–24]. Each of the factors is

assigned coded levels and several experiments are performed to quantify the response, in this case, the runtime. One collection where residuals are calculated against actual values and predicted values to model accuracy on formation. An analysis of variance (ANOVA) to measure the significance of each factor. Several model terms are statistically significant, and an equation is obtained that best fits a given runtime at different observed factor levels which can then be used for optimization [25].

2.1.3 Method development

Multiple attempts were optimized with a variety of compositions of the mobile phase including sodium dihydrogen phosphate, methanol, acetonitrile and organic modifiers like orthophosphoric acid and formic acid to settle on the best one. Column Inertsil ODS column (150 × 4.6 mm, 5 µm) of flow rate was varied from 0.5 to 2 mL/min and injection volume was varied from 10 to 20 µL for best resolution of the peaks. To obtain better sensitivity in detecting Landiolol detection wavelength of 240 nm was selected [26].

2.1.4 Sample preparation

Take accurately about 50 mg of Landiolol reference standard, transfer it to 50-mL volumetric flask, dissolve and dilute to 50 mL with diluent. This solution was processed by sonication and diluted to 100mL to initiate the stock solution. A 100-ppm standard solution by dilution of 5 mL of this into 25 mL purified water is used as solvent and tested for influence of different volatile organic modifiers on analyte retention time and peak symmetry to reach improved chromatographic performance.

2.2 Analytical method validation

2.2.1 System suitability

All chromatographic parameters were evaluated for system suitability according to USP tailing, plate counts, similarity factor of standard solutions. Sample of prepared standard solutions injected and analyzed chromatograms [27].

2.2.2 System precision

To assess the accuracy of the system, six successive injections of the standard solution were performed, and the corresponding peak areas were obtained from the injections [28].

2.2.3 Evaluation of filters

Different filtration processes were used to assess the degree of assay percentage and recovery of different subspecies. Next unfiltered samples were assayed to form a baseline percentage. Then 2, 4, and 6 mL sub-fractions were passed through 0.45 µm nylon, PVDF, and PTFE filters. Assay % and recovery were therefore measured across subfractions, showing the effectiveness of these filtration steps at each stage in maintaining sample integrity [29].

2.2.4 Assessment of forced degradation studies

In order to evaluate the drug's stability under various stress conditions, forced degradation studies were performed; HCl, NaOH, peroxide, thermal degradation and photolytic degradation were employed [30].

2.2.5 Method precision

This is a test percentage analysis of Landiolol, within this procedure. Landiolol, across a total of six samples. Each sample is tested to obtain the percentage of Landiolol contained in it. The mean, standard deviation (SD) and relative standard deviation (%RSD) can be calculated for the data in question [30].

2.2.6 Evaluation of linearity

Five different concentrations of Landiolol should be prepared (50%, 80%, 100%, 120%, and 150%). Each solution's mean peak area response can be documented and subjected to linear regression analysis utilizing chromatographic analysis [31, 32].

2.2.7 Assessment of solution stability

In order to avail stability of standard and sample solutions it was testing at various time intervals for assay percentage and response variations. The standard solution's response was determined, and a percentage difference from the original value was calculated. The assay % for the solution of sample was calculated and the percentage deviation was recorded from the first assay. Measurements were taken at regular intervals of 2, 4, 6, 8, 10, and 48 h. These data point show no degradation of assay stability after 24 h, with % differences between the two solutions recorded at all evaluative time points [33].

2.2.8 Evaluation of accuracy

The accuracy of Landiolol was evaluated at three concentrations (50%, 100%, and 150%). Specific dosages of Landiolol were given at each level, and the amounts yielded were further recorded. For each concentration level, the percent recovery was calculated for both solutions and the average percent recovery and the relative standard deviation (%RSD) was determined. And triplicate was assessed for each accuracy level [34].

2.2.9 Evaluation of robustness

Certain parameters were systematically varied to evaluate the effect of conditions on chromatographic performance. Flow rate was changed to 1.35 mL/min & 1.65 mL/min, tailing, plate counts, %RSD of areas in standard injections, & % similarity factor was calculated for every condition. The organic phase of mobile was altered by ± 2%, results were recorded. A column oven temperature was set between 35 °C and 45 °C, and the detection wavelength varied from 218 nm, and 222 nm. All detection was reported for the analysis of the effect of change in Ph of mobile phase, at 3.8, and 4.2 [35]. Using the optimum setting of HPLC, the applicate, precision, filtering efficacy, forced degradation, sample preparation, method development, precision, linearity, solution stability, accuracy and robustness for Landiolol has been determined.

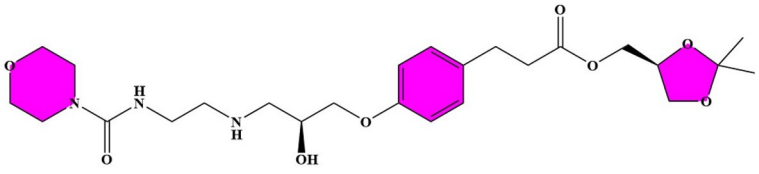
3 Results and discussion

Based on different physiochemical properties and various analytical parameters, a HPLC method was developed and validated for the analysis of Landiolol, as focused on the study. The received results of the experimental procedures were analyzed according to the standard system suitability, precision, filtration efficiency, degradation studies, assays performance, linearity, stability, accuracy and robustness. Optimizing these parameters is crucial for providing the developed method to be reliable, reproducible, and cost-effective.

3.1 Physiochemical characteristics of landiolol

The most relevant physiology-chemical properties of Landiolol (Table 1) show crucial characteristics affecting solubility, stability, bioavailability. The compound is leading to a white to off-white crystalline powder with a melting point in the range of 114–118 °C, which proves to be the crystalline compound [36]. The solubility profile represented indicates that Landiolol is freely soluble in methanol, ethanol and DMSO but sparingly soluble in water, indicating a need to choose a suitable solvent system in chromatographic method development [37, 38]. logP value of 3.97 gives us an idea about the lipophilicity of the compound and thus influences its bioavailability and its interaction with the stationary phase in HPLC analysis [39]. Moreover, Landiolol exists as a racemic mixture of R(+) and S(-) enantiomers, factor in pharmacodynamic and pharmacokinetic aspects [40].

Table 1 Chemical and physicochemical properties of Landiolol

Chemical Name	 **Landiolol**
IUPAC Name	[(4 S)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl 3-[4-[(2 S)-2-hydroxy-3-[2-(morpholine-4-carbonylamino)ethylamino]propoxy]phenyl]propanoate
Molecular Formula	C25H39N3O8
Molecular Weight	509.6 g/mol
Physical Appearance	white to almost white powder or crystalline powder
Melting Point	122–127 °C
Boiling Point	± 60.0 °C
Solubility	Freely soluble in methanol, ethanol and DMSO Sparingly soluble in water
pKa Values	-3.4 (strongest acidic) 15.05 (strongest basic)
LogP (Partition Coefficient)	0.95 (lipophilic)
Topological Polar Surface Area (TPSA)	127.82 $Å^2$
Hydrogen Bond Donors, Acceptors	Hydrogen Bond Donors: 3 Hydrogen Bond Acceptors: 9 or 10
Rotatable Bonds	14
XLogP3	2.37760
Refractive Index	1.7400
Density	1.201 g/cm3.
Vapour Pressure	Negligible
Half-life ($t_1/_2$)	4.5 min

3.2 Analytical method development

3.2.1 Analysis of GREEnness

The greenness score of 0.88 is high and implies the high sustainability of the analytical method in consideration of the 12 criteria that have been used to assess it. This figure shows that the approach works outstandingly in most items, which indicates the high regards to the aspects of environmental safety and operator safety. The green color sections which follow criteria of sample treatment, device positioning, stages of sample preparation, derivatization, waste, throughput of analysis, energy consumed, toxicity as well as to safety of operator demonstrates that the method is able to reduce the number of negative impacts in these areas. As an example, the effective sample pre-treatment and placement may decrease the consumption of resources and mistakes related to handling items, whereas the improved preparation phases and derivatization minimize the usage of the chemicals and the generation of the waste products. It should be pointed out that an appropriately designed method in the aspects of automation and miniaturization raises throughput and decreases human contact and energy requirements accordingly, which adds to the green score.

The yellow areas in sample amount, automation and miniaturization, source of reagents however indicate an area of improvement. The amount of sample needed to be more than optimal can correspond to the use of more material, which can lead to more wastes or reagents consumption. On the same note, low automation and miniaturization may wilt into an aspect of being dependent on manual work or large-scale operations which decrease the efficiency and on the other side, human labour or energy consumption. The origin of the reagents identified as yellow point out that the technique may involve the usage of reagents of less sustainable or safer source, which requires the investigation of the greener sources. Overall, the 0.88 greenness index indicates that the analytical process is well rounded, environmentally friendly with a few limitations that are best linked to the size of the sample, level of automation, and availability of reagents. Further streamlining the sustainability of the method through further emphasis on these particular parameters including the implementation of more automated or miniaturized workflow and the choice of greener reagents sources would be possible. These results stress the importance of a whole picture assessment table in attaining greener analytical sciences which reveals advantages and areas of enhancements (Fig. 1).

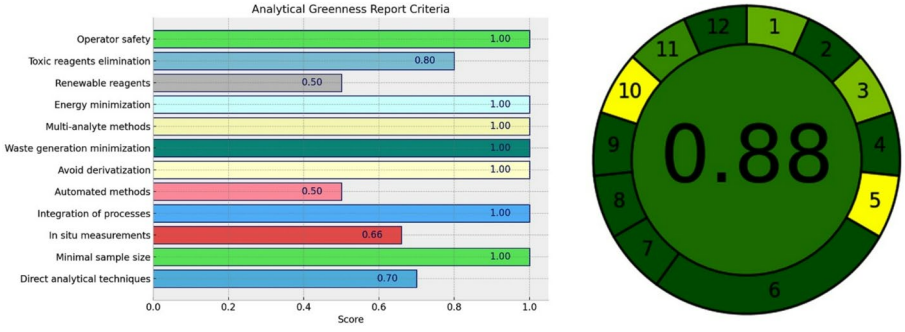


Fig. 1 The greenness assessment for the analytical method, scoring twelve criteria to generate an aggregate score. Color codes indicate performance: green (excellent), yellow (needs improvement). Criteria are listed numerically

3.2.2 Central composite design analysis

3.2.2.1 Analysis of variance (ANOVA) The analysis of variance (ANOVA) presented in the table provides significant insights into the influence of various factors on the response variable. The model was found to be statistically significant with a p-value of 0.0003 and an F-value of 11.60, indicating that the model adequately fits the experimental data and reliably explains variations in the response. Among the linear terms, temperature (A) showed a notable influence on the response, with an F-value of 3.89 and a p-value of 0.0767, suggesting a marginal significance at the 10% confidence level. In contrast, flow rate (B) and injection volume (C) yielded extremely low F-values of 0.0018 and corresponding p-values of 0.9673, indicating that these individual linear effects are not significant within the considered range of experimental conditions. The interaction terms (AB, AC, and BC) were all found to be statistically insignificant, with identical low F-values (0.0030) and high p-values (0.9573), demonstrating that there are no substantial synergistic effects between temperature, flow rate, and injection volume in this model. This implies that the response variable is not significantly influenced by the combinations of these factors, simplifying the interpretation of the results. However, the quadratic terms revealed a contrasting and highly significant impact on the response. The square of temperature (A^2) produced an exceptionally high F-value of 53.75 with a p-value < 0.0001, highlighting a strong curvature in the response surface due to temperature changes. Similarly, the quadratic terms for flow rate (B^2) and injection volume (C^2) were also statistically significant with F-values of 23.35 and 42.23, and p-values of 0.0007 and < 0.0001, respectively. These results underscore the importance of non-linear effects, indicating that optimal conditions for the response cannot be identified by examining linear effects alone. The residual error, representing unexplained variations, was relatively low with a sum of squares of 4.15 and a mean square of 0.4154, supporting the model's accuracy. Moreover, the lack of fit was not significant, as it equally matched the pure error (sum of squares = 4.15, df = 5, MS = 0.8308), suggesting that the model does not suffer from systematic bias and fits the data well. The total variation captured by the model (SS = 43.36) was high compared to the corrected total (SS = 47.51), signifying that a substantial portion of variability in the response is effectively explained by the selected model factors (Table 2).

3.2.2.2 Statistical analysis The statistical summary of the model further confirms its adequate performance and reliability in explaining the experimental data. The standard deviation (0.6445) reflects the average deviation of the data points from the predicted values and indicates acceptable prediction precision, given the scale of the response variable. The mean response value of 4.70 serves as a reference point for evaluating variability, and the coefficient of variation (C.V.%), calculated at 13.73%, suggests moderate variability in the data. Since a C.V. value below 20% is generally acceptable for experimental models, this result affirms the model's consistency.

The coefficient of determination (R^2) value of 0.9126 suggests that approximately 91.26% of the variability in the response is explained by the model, indicating very good model fitness. However, it is important to consider the adjusted R^2, which accounts for the number of predictors in the model. The adjusted R^2 value of 0.8339 remains high and confirms that the model remains significant even after adjusting for the number of terms included. Notably, the predicted R^2 value of 0.3389, which measures the model's ability to predict new observations, is quite low in comparison to R^2 and adjusted R^2. This discrepancy implies that although the model fits the current data well, its predictive capability for unseen data is limited and may require refinement, such as removing insignificant terms or improving experimental design.

The Adeq Precision value of 8.9950, which measures the signal-to-noise ratio, exceeds the threshold of 4, indicating adequate model discrimination and that the model signal is

Table 2 ANOVA table showing source of variation, sum of squares, degrees of freedom (df), mean square, F-values, and p-values

Source	Sum of squares	df	Mean square	F-value	*p*-value	
Model	43.36	9	4.82	11.60	0.0003	Significant
A-Tem	1.62	1	1.62	3.89	0.0767	
B-Flow rate	0.0007	1	0.0007	0.0018	0.9673	
C-Injection Volume	0.0007	1	0.0007	0.0018	0.9673	
AB	0.0012	1	0.0012	0.0030	0.9573	
AC	0.0012	1	0.0012	0.0030	0.9573	
BC	0.0012	1	0.0012	0.0030	0.9573	
A^2	22.33	1	22.33	53.75	< 0.0001	
B^2	9.70	1	9.70	23.35	0.0007	
C^2	17.54	1	17.54	42.23	< 0.0001	
Residual	4.15	10	0.4154			
Lack of Fit	4.15	5	0.8308			
Pure Error	0.0000	5	0.0000			
Cor Total	47.51	19				

Significance of model terms assessed for experimental factors and interactions. Factor coding is Coded. Sum of squares is Type III - Partial. The Model F-value of 11.60 implies the model is significant. There is only a 0.03% chance that an F-value this large could occur due to noise

P-values less than 0.0500 indicate model terms are significant. In this case A^2, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model

Table 3 Model summary statistics including standard deviation, R^2, adjusted and predicted R^2, coefficient of variation (C.V. %), and Adeq Precision, which evaluate model fit, reliability, and prediction accuracy

Std. Dev.	0.6445	R^2	0.9126
Mean	4.70	Adjusted R^2	0.8339
C.V. %	13.73	Predicted R^2	0.3389
		Adeq Precision	8.9950

The Predicted R^2 of 0.3389 is not as close to the Adjusted R^2 of 0.8339 as one might normally expect; i.e. the difference is more than 0.2. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc. All empirical models should be tested by doing confirmation runs. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 8.995 indicates an adequate signal. This model can be used to navigate the design space

much stronger than the background noise. Overall, these diagnostic statistics suggest a well-fitted model with high explanatory power, though with some limitations in predictive performance that should be addressed for enhanced future application (Table 3).

3.2.2.3 Analysis of regression coefficients The regression equation for retention time illustrates the influence of linear, interaction, and quadratic terms on the response variable. The intercept of the model is + 2.53, representing the baseline retention time when all input variables are at their coded zero levels. Among the linear terms, temperature (A) exhibits the most pronounced positive effect on retention time, with a coefficient of + 0.3441, suggesting that an increase in temperature leads to a noticeable increase in retention time. On the other hand, the coefficients for both flow rate (B) and injection volume (C) are − 0.0073, indicating a negligible and negative impact on retention time. These small values are consistent with the earlier ANOVA findings, where B and C were statistically insignificant.

The interaction terms AB, AC, and BC each have coefficients of + 0.0125, showing a minimal combined effect between the variables. Such small coefficients imply that the

interaction between factors has little to no practical impact on retention time and might be excluded from future model refinements without compromising accuracy.

In contrast, the quadratic terms demonstrate a much more substantial influence. The squared term of temperature (A^2) has the highest coefficient at + 1.24, reinforcing that temperature has a strong curvilinear effect on the retention time. Similarly, the square of flow rate (B^2) and injection volume (C^2) exhibit positive coefficients of + 0.8205 and + 1.10, respectively. These large coefficients confirm the significance of non-linear relationships and highlight the importance of optimizing each variable rather than relying on linear trends.

Overall, the regression coefficients reveal that while linear and interaction effects are relatively minor (except for temperature), the curvature (quadratic) effects play a dominant role in determining the retention time, suggesting a need for careful process optimization, especially around extreme values of each factor (Table 4).

3.2.2.4 Analysis of regression model The regression model describing the relationship between process variables and retention time provides valuable insight into how each factor influences the system. The model intercept is + 1529.67152, representing the estimated retention time when all independent variables (temperature, flow rate, injection volume) are at their baseline values. Among the linear terms, temperature has the most significant negative effect, with a coefficient of – 86.84777, indicating that an increase in temperature leads to a substantial decrease in retention time. This is consistent with chromatographic principles, where higher temperatures often reduce retention due to increased analyte volatility or faster solute movement through the column (Table 5).

The flow rate and injection volume also negatively impact retention time, with coefficients of – 4.28977 and – 1.41714 respectively, though their influence is much less dramatic compared to that of temperature. These negative coefficients suggest that increasing the flow rate and injection volume marginally reduces the retention time, possibly by accelerating the elution process or reducing mass transfer resistance.

Interaction effects between variables are minimal but not entirely negligible. The coefficients for temperature × flow rate (0.016667), temperature × injection volume (0.002500), and flow rate × injection volume (0.003333) are all small positive values,

Table 4 Regression coefficients table displaying factor effects, coefficient estimates, degrees of freedom (df), standard errors, 95% confidence intervals, and variance inflation factors (VIF) to assess multicollinearity and model significance

Factor	Coefficient estimate	df	Standard error	95% CI Low	95% CI high	VIF
Intercept	2.53	1	0.2629	1.95	3.12	
A-Tem	0.3441	1	0.1744	-0.0445	0.7328	1.0000
B-Flow rate	-0.0073	1	0.1744	-0.3959	0.3813	1.0000
C-Injection Volume	-0.0073	1	0.1744	-0.3959	0.3813	1.0000
AB	0.0125	1	0.2279	-0.4952	0.5202	1.0000
AC	0.0125	1	0.2279	-0.4952	0.5202	1.0000
BC	0.0125	1	0.2279	-0.4952	0.5202	1.0000
A^2	1.24	1	0.1698	0.8665	1.62	1.02
B^2	0.8205	1	0.1698	0.4422	1.20	1.02
C^2	1.10	1	0.1698	0.7250	1.48	1.02

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-colinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable

Table 5 Regression equation coefficients for retention time, showing intercept and effects of factors A (temperature), B (flow rate), C (injection volume), their interactions, and quadratic terms in the model

Retention time	**=**
+ 2.53	
+ 0.3441	A
-0.0073	B
-0.0073	C
+ 0.0125	AB
+ 0.0125	AC
+ 0.0125	BC
+ 1.24	A^2
+ 0.8205	B^2
+ 1.10	C^2

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as + 1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients

Table 6 Regression coefficients for retention time model showing intercept, linear terms (temperature, flow rate, injection volume), interaction effects, and quadratic terms with their estimated values, reflecting contribution magnitude and direction

Retention time	**=**
+ 1529.67152	
-86.84777	Tem
-4.28977	Flow rate
-1.41714	Injection Volume
+ 0.016667	Tem * Flow rate
+ 0.002500	Tem * Injection Volume
+ 0.003333	Flow rate * Injection Volume
+ 1.24477	Tem^2
+ 1.45867	$Flow\ rate^2$
+ 0.044134	$Injection\ Volume^2$

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space

implying minor synergistic interactions which slightly increase retention time when these parameters are adjusted simultaneously.

More notably, the quadratic terms indicate strong curvature in the system response. The squared effect of temperature (Tem^2) is particularly large at + 1.24477, confirming significant non-linearity and reinforcing that the relationship between temperature and retention time is not purely linear. Additionally, the squared terms for flow rate (+ 1.45867) and injection volume (+ 0.044134) also suggest some degree of curvature, though less pronounced.

In summary, temperature dominates the system both linearly and quadratically, while flow rate and injection volume exert milder effects. Optimization should therefore focus primarily on precise temperature control to achieve desired retention times (Table 6).

3.2.2.5 Analysis of regression diagnostic results The regression diagnostic results reveal important insights into the model's fit and the influence of individual runs on the response variable. The actual retention times ranged from 2.50 to 6.60, while the predicted values varied similarly but with some deviations, as seen in the residuals. Notably, runs 1, 2, 8,

17, and 18 showed relatively large negative residuals (e.g., run 1: -0.6433), indicating that the model overpredicted the retention time in these cases. Conversely, run 7 had the highest positive residual of 1.13, suggesting underprediction by the model. The magnitude of residuals and their internal studentized values highlight certain outlying observations, with run 7 displaying an internally studentized residual of 2.79 and an externally studentized residual of 5.62, marking it a highly influential point.

Leverage values provide a measure of each observation's influence on the fitted model. The highest leverages (~0.670) were associated with runs 1, 2, 8, 12, 13, 14, 17, and 18, implying these points are located farther from the centre of the predictor space and have a stronger effect on the regression estimates. However, runs with both high leverage and large residuals, such as run 7, indicate potential outliers that may disproportionately affect the model.

Cook's Distance, which evaluates the influence of each point on the regression coefficients, showed a particularly high value for run 7 (1.203), corroborating its role as an influential observation. Similarly, DFFITS values greater than ±2 suggest considerable impact on the fitted values; run 7's DFFITS of 6.988 further confirms its influence.

The majority of other runs exhibited low residuals, small Cook's Distances, and DFFITS values within acceptable limits, suggesting that these data points conform well to the model assumptions. Overall, the diagnostics indicate that while the model fits most observations satisfactorily, certain data points, especially run 7, should be further investigated for their outlier behavior or measurement error. Addressing these influences may improve model robustness and predictive accuracy (Table 7).

The regression analysis revealed several important insights into the significance and reliability of the model predictors. As shown in the parameter metrics, all linear terms (A, B, and C) exhibited a standard error of 0.2706, indicating relatively consistent variability across the predictors. The interaction terms (AB, AC, and BC) showed slightly higher standard errors at 0.3536, suggesting increased variability when considering joint effects, however still within acceptable limits for interpretability.

Table 7 Regression terms with their standard errors, variance inflation factors (VIF), coefficient of determination (R^2), and statistical power, assessing variable influence and multicollinearity in the regression model

Term	Standard error*	VIF	R_i^2	Power
A	0.2706	1	0.0000	91.4%
B	0.2706	1	0.0000	91.4%
C	0.2706	1	0.0000	91.4%
AB	0.3536	1	0.0000	72.2%
AC	0.3536	1	0.0000	72.2%
BC	0.3536	1	0.0000	72.2%
A^2	0.2634	1.01827	0.0179	99.9%
B^2	0.2634	1.01827	0.0179	99.9%
C^2	0.2634	1.01827	0.0179	99.9%

For a standard deviation of 1. Power calculations are performed using response type "Continuous" and parameters: Delta = 2, Sigma = 1. Power is evaluated over the −1 to +1 coded factor space. Standard errors should be similar to each other in a balanced design. Lower standard errors are better. The ideal VIF value is 1.0. VIFs above 10 are cause for concern. VIFs above 100 are cause for alarm, indicating coefficients are poorly estimated due to multicollinearity. Ideal R_i^2 is 0.0. High R_i^2 means terms are correlated with each other, possibly leading to poor models. If the design has multilinear constraints, then multicollinearity will exist to a greater degree. This inflates the VIFs and the R_i^2, rendering these statistics useless. Use FDS instead. Power is an inappropriate tool to evaluate response surface designs. Use prediction-based metrics provided in this program via Fraction of Design Space (FDS) statistics. Click on the Graphs tab to find the FDS graph. More information about FDS is available in the Help. Be sure that the model you selected contains only terms you expect to be significant

Variance Inflation Factors (VIFs) for all terms remained close to 1, with the highest being 1.01827 for the quadratic terms (A^2, B^2, and C^2), signifying minimal multicollinearity issues. The near-unity VIFs validate the independence of predictors and reinforce the stability of estimated coefficients. Additionally, the R_i^2 values for all terms were either zero or close to zero (maximum of 0.0179), which further supports the absence of problematic collinearity. This implies that no single term is significantly explained by other predictors in the model.

From a power analysis perspective, the regression model showed considerable strength. The power associated with linear terms A, B, and C was 91.4%, while the interaction terms demonstrated slightly lower power values at 72.2%. Most notably, the quadratic terms registered power values as high as 99.9%, strongly affirming the ability to detect true effects if they exist. Such high statistical power is indicative of a robust model capable of identifying meaningful relationships in the data set.

Overall, the low standard errors, VIFs near 1, negligible R_i^2 values, and high-power estimates collectively demonstrate that the fitted regression model is statistically reliable, free from significant bias due to multicollinearity, and highly sensitive in detecting contributing effects (Table 8).

3.2.2.6 Analysis of correlation matrix The correlation matrix provides valuable insights into the degree of multicollinearity and interdependencies among the regression terms used in the model. As expected, the diagonal elements all hold a value of 1.000, affirming perfect self-correlation for each term. The off-diagonal elements, however, are of primary interest for discerning the extent of correlation between different model terms.

The main effects -A (Temperature), B (Flow Rate), and C (Injection Volume) - each show zero or near-zero correlation with one another. This independence is favorable and indicates that the experimental design successfully isolated the effects of individual variables. Interaction terms such as AB, AC, and BC also show negligible correlation with the main factors, supporting the orthogonality built into the regression design. Notably, all three interaction terms exhibit identities with a zero correlation to each other and with quadratic terms, suggesting they do not share variance, which strengthens the model's interpretability.

Table 8 The correlation matrix displays relationships among model terms, including main effects, interactions, and quadratic terms

	Intercept	**A-tem**	**B-flow rate**	**C-injection volume**	**AB**	**AC**	**BC**	**A^2**	**B^2**	**C^2**
Intercept	1.000	0.000	0.000	-0.000	-0.000	0.000	0.000	*-0.529*	*-0.529*	*-0.529*
A-Tem	0.000	**1.000**	0.000	0.000	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000
B-Flow rate	0.000	0.000	**1.000**	-0.000	-0.000	-0.000	-0.000	0.000	-0.000	-0.000
C-Injection Volume	-0.000	0.000	-0.000	**1.000**	-0.000	-0.000	-0.000	0.000	0.000	-0.000
AB	-0.000	-0.000	-0.000	-0.000	**1.000**	-0.000	-0.000	0.000	0.000	0.000
AC	0.000	-0.000	-0.000	-0.000	-0.000	**1.000**	0.000	-0.000	-0.000	-0.000
BC	0.000	-0.000	-0.000	-0.000	-0.000	0.000	**1.000**	-0.000	-0.000	-0.000
A^2	*-0.529*	-0.000	0.000	0.000	0.000	-0.000	-0.000	**1.000**	***0.099***	***0.099***
B^2	*-0.529*	-0.000	-0.000	0.000	0.000	-0.000	-0.000	***0.099***	**1.000**	***0.099***
C^2	*-0.529*	-0.000	-0.000	-0.000	0.000	-0.000	-0.000	***0.099***	***0.099***	**1.000**

Bold values represent perfect self-correlation (1.000), while italic values indicate inter-term correlations

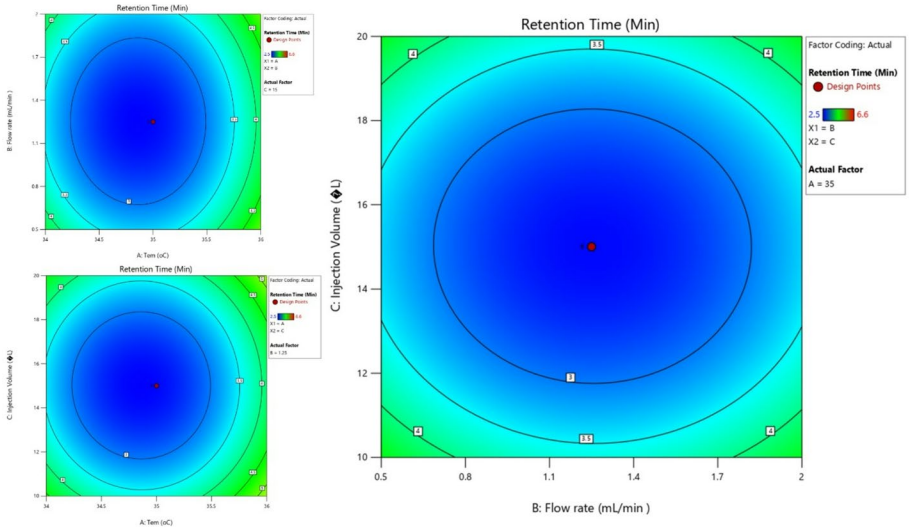


Fig. 2 Contour plots depicting the effect of temperature, flow rate, and injection volume on retention time. The color gradient represents retention time (min), with design points highlighted in red

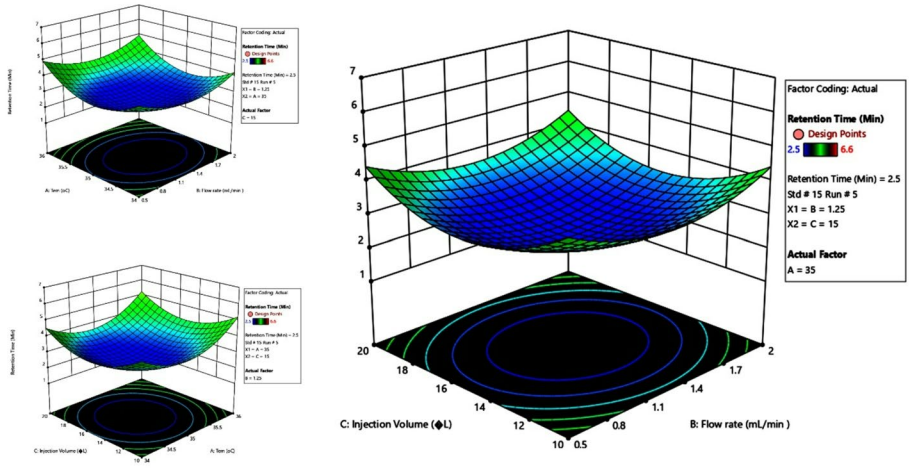


Fig. 3 Three-dimensional response surface and contour plots display the influence of flow rate, injection volume, and temperature on retention time. Color scale indicates retention time; red circles show experimental design points

The quadratic terms (A^2, B^2, and C^2), however, show modestly correlated values among themselves, particularly a correlation of 0.099 between each pair. Additionally, these quadratic terms share a small negative correlation of -0.529 with the intercept, which may reflect model centering or design scaling techniques. Importantly, the lack of substantial correlation (absolute values remain below 0.6) among all predictors indicates minimal multicollinearity problems, ensuring more stable and reliable estimation of coefficients in the regression model.

In sum, the structure of the correlation matrix confirms that the regression predictors are well-suited for model fitting. The low inter-term correlation supports parameter precision and reduces the risk of inflated variances. This enhances the robustness of statistical inferences drawn from the model and underscores soundness in experimental design and variable selection (Table S9 and Figs. 2, 3, 4 and 5).

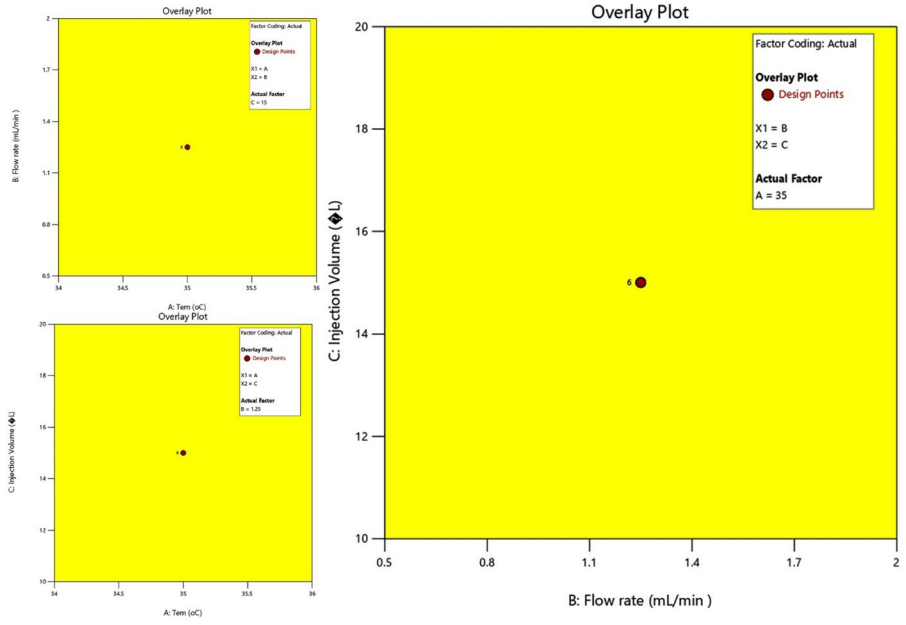


Fig. 4 Overlay plots show the relationship between temperature, flow rate, and injection volume. Yellow regions indicate response outcomes. Red dots represent design points. Designated actual factor levels are marked in each plot

3.2.2.7 Method development The developed method for the Landiolol quantification by reverse-phase high-performance liquid chromatography (RP-HPLC) in isocratic mode over a Inertsil ODS column (150 × 4.6 mm, 5 µm). Several trials were performed with varying compositions of the mobile phase such as sodium dihydrogen phosphate, methanol, acetonitrile, and organic modifiers such as orthophosphoric acid and formic acid were optimized to arrive at the best one. for the flow rate, it was set between 0.5 and 2 mL/min, and the injection volume was set between 10 and 20 µL in order to achieve the best resolution of the peaks. A detection wavelength of 240 nm was chosen to obtain the best possible sensitivity for Landiolol detection. Weigh accurately about 50 mg Landiolol reference standard, and transfer to 50-mL volumetric flask, dissolve and dilute to 50 mL with diluent. This solution was then sonicated and diluted to 100 mL to give the stock solution. 100 ppm standard solution: From this, 5 mL was diluted to 25 mL The effect of various solvents and organic modifiers on analyte retention time and peak symmetry was analysed to optimise the chromatographic performance. For the best option, orthophosphoric acid (55%) provided sodium dihydrogen phosphate with 4.90 min (Figure S6), and with methanol and formic acid, it provided 5.70 min (Figure S7), which marginally increased the retention. Retention times were greatest with acetonitrile with orthophosphoric acid, between 7.30 min (Figure S8) and 8.70 min (Figure S9). The effect of organic modifier concentration was also studied, and as the orthophosphoric acid concentration increased from 0.05% to 0.2%, a gradual decreased in retention time was observed. A 0.2% orthophosphoric acid concentration ensured well-resolved peaks while optimizing retention time. Effect of temperature at 30 °C and 40 °C showed that increase in temperature has a minor effect on retention time and there was not a large impact on peak efficiency. Likewise, when the flow rate was 1 mL/min a symmetrical peak with no tailing was observed making the method more robust. As a result of these investigator trials, the pre-final chromatographic conditions consisted of an Inertsil ODS column using sodium

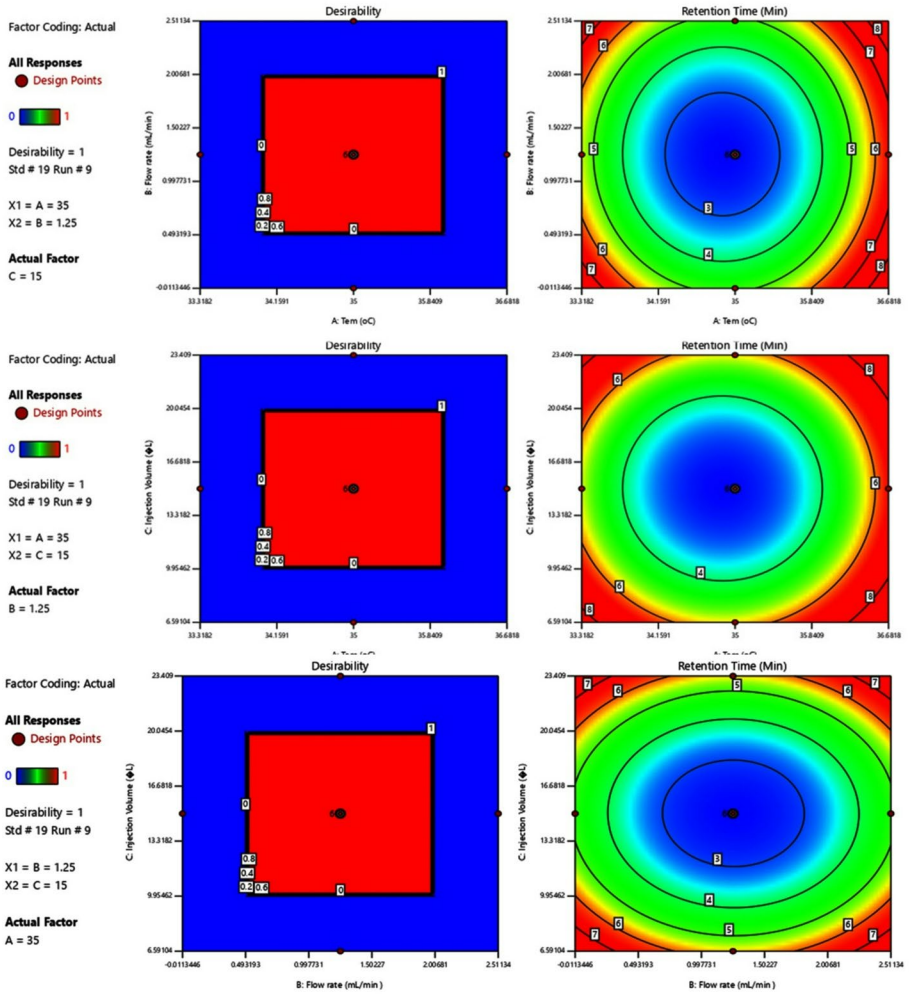


Fig. 5 Contour and overlay plots illustrate effects of flow rate and injection volume on retention time and desirability. Color gradients indicate responses; red dots mark design points. Actual factor levels are specified

dihydrogen phosphate (55%) and 0.2% orthophosphoric acid as a mobile phase, at 35 °C, a flow rate of 1.25 mL/min. 15 µL was used as the fixed injection volume, while 240 nm was used as the detection wavelength to ensure reproducibility and accuracy. Landiolol eluted at 2.7 min with sharp peak symmetry and negligible interference under these conditions (Fig. 6).

3.3 Analytical method validation

3.3.1 System suitability

The method was validated as per system suitability test in Table S9. Peak symmetry: USP tailing factor was 1. The plate count was 10,925, indicating good efficiency and peak resolution for the column. The similarity factor between standard 1 and standard 2 was 98.4%, confirming the consistency of standard preparation and instrument performance. These results indicate that the chromatographic system is properly validated and fulfills standard acceptance criteria.

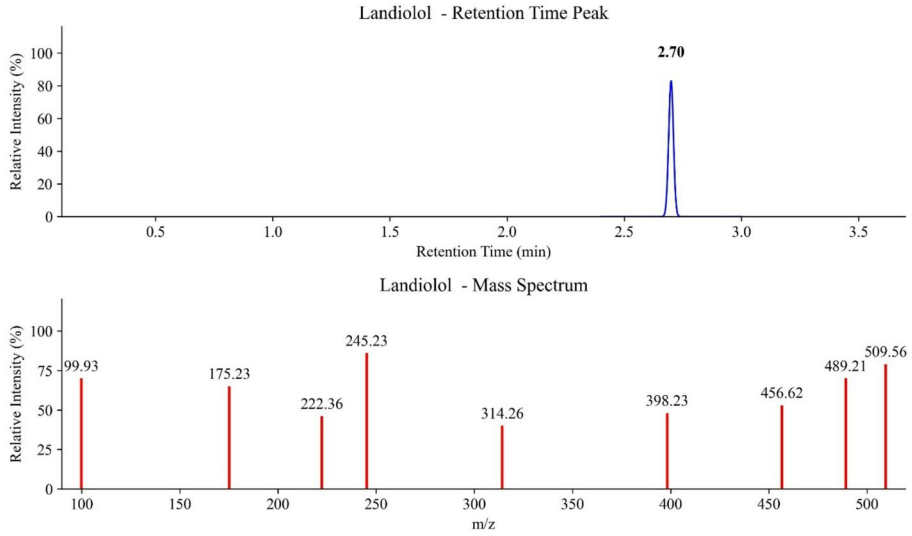


Fig. 6 Chromatographic and mass spectrometric analysis of Landiolol showing retention time at 2.70 min and fragmentation pattern with molecular ion peak at m/z 509.55 and major fragments

3.3.2 System precision

System precision (Table S10) was measured by multiple injections, evaluating peak area variation. =2061273.667 and the standard deviation (SD) 6,730.36 and relative standard deviation.

(%RSD 0.3). With a low %RSD (< 2%) on the retention time, the method can be considered highly reproducible and showed very little variation between injections, making it suitable for determination of CrS in routine analysis.

3.3.2.1 Particulate filtration efficiency Filtration study (Table S11) was performed with different membrane filters (nylon, PVDF, and PTFE) at different sub-fraction volumes (2 mL, 4 mL, and 6 mL). The recovery results show slight loss in the assay values, with recovery percentages varying between 96.6% and 97.7% across filters. The 0.45 µm PVDF filter at sub-fraction 4 mL demonstrated the lowest assay recovery (96.6%) and with a very marginally increased % recovery deviation (1.5%) suggesting there may be some adsorption to the filter. However, in all results, this was still in an acceptable manner and suggested that there is no significant loss of analytes from filtration.

3.3.3 Studies of forced degradation

Forced degradation tests (Table S12) investigated the stability of Landiolol in various stress factors such as acidic, basic, oxidative, thermal and photolytic degradation. The control sample indicated that the retention time of 2.70 min produced a sharp, symmetric peak with 98.2% assay and formed the background of degradation evaluation. In the acidic environment (5 N HCl at 60 °C during 2 h), the chromatogram indicated the appearance of degradation products with the major one with 95.2% assay, which corresponds to 3% degradation. The acidic stress chromatogram showed good separation between the parent drug peak and the degradation products, which proves the specificity of the methods. Simple hydrolysis under similar conditions to 5 N NaOH caused a slightly better degradation of 4% and the assay reduced to 94.2. The simplified degradation chromatogram was found to separate degradation products with the main peak of

the analyte, as well, which implies that the method is capable of separating effectively closely related materials.

Landiolol is moderately susceptible to oxidative conditions, as the degradation of 4% by oxidative stress induced by 5% hydrogen peroxide was similar. The chromatogram of oxidative degradation obtained clearly indicated that the method was stable because its peaks had a baseline separation. 48 h of thermal degradation at 105 °C had a little degradation of 3, which means that the compound has good thermal stability. The thermal stress chromatogram retained the integrity of the peaks and did not have any serious interference of thermal degradation products.

The greatest percentage degradation of 9 was obtained at the photolytic degradation after exposure to 1.2 million lux hours where the assay decreased to 89.2. The photolytic stress chromatogram showed several degradation products with different retention times, indicating that the drug was very sensitive to light. The chromatographic picture was clear to demonstrate the resolution of photodegradation products of the main peak, which underlines the importance of light protection of a formulation, storage, and manipulation. The homogeneity of the major peak under all stress conditions was verified by peak purity analysis by photodiode array detector, which is the specificity of the method. The findings indicate that, Landiolol is relatively stable at thermal and oxidative conditions but needs very serious protection against light exposure during its pharmaceutical lifetime.

3.3.4 Assay performance

Results (Table S13) obtained from the assay held similar performance across samples. Results for the percentage assay values were 98.2% to 99.2%, mean value of 98.6%, standard deviation 0.41, %RSD 0.4%, showing the accuracy and precision of the method. The low variation ascertains the appropriateness of the method for routine quality control analysis.

3.3.5 Linearity study

Linearity: The linearity was calculated in terms of peak area response (50%, 80%, 100%, 120% and 150%) (Table S14) for Landiolol. The obtained results showed a good linearity between the concentration and response, with R^2 = 0.9999, slope = 22591.54, and intercept = -11162.25. This achievement reveals the method to be one that provides a strong correlation between analyte concentration and detector response, making it ideal for accurate quantification for a wide range.

3.3.6 Stability studies

The stability testing (Table S15) was carried out over 48 h for the analysis of standard and sample solutions. The quantification of response as a percentage difference also indicated that Landiolol solutions are stable up to the study period as demonstrated by the percentage response which was still between only 0.4% and 0.8%. These results demonstrate the robustness and suitability of the method for longer analysis times in QC laboratories.

3.3.7 Correctness and reaggregation

Recovery of Landiolol at levels of 50%, 100% and 150% were determined in accuracy study (Table S16). The overall percentage recoveries were 100.8% (50%), 101.1% (100%), and 101% (150%), with %RSD values between 0.2 and 0.4. Hence, the results show that the method was quite sensitive and could recover nearly 100% of the analyte in different concentration levels.

3.3.8 Evaluation of robustness

Robustness (Table S17) was evaluated by modifying periodic significant chromatographic parameters including flow rate (1.35 and 1.65 mL/min), organic content (± 2%), column oven temperature (35 °C and 45 °C), detection wavelength (218 and 222 nm), and mobile phase pH (3.8 and 4.2). The results showed little change in USP tailing (1.0–1.4), plate counts (~ 11,000), and % similarity factor (99.1–100.2) for all conditions. The %RSD was still less than 1%, suggesting that small changes in the chromatographic conditions have no considerable effect on the behaviour of the method. The HPLC method developed for Landiolol analysis was found precise, accurate, rugged and linear over a wide range of concentration. The system suitability tests were indicative of the optimal performance of the chromatography with high plate numbers, low tailing and excellent reproducibility of the peaks. Forced degradation studies showed that Landiolol is thermally and oxidatively stable but prone to photolytic degradation; and therefore, should be stored under proper conditions. The filtration study validated that assay recovery is not significantly affected by different filter membranes, enabling their use as sample preparation tools. System suitability, assay performance, and robustness studies illustrate the precision of the method, %RSD was very well in all the studies and < 1% The method proved to reliably quantify Landiolol in pharmaceutical formulations as confirmed by linearity and accuracy results. In conclusion, the validated HPLC method is compliant with the regulatory guideline and can be applied for other purposes like routine quality control, stability studies and pharmaceutical analysis of Landiolol formulations. The results offer a scientifically rigorous and reproducible analytical solution that guarantees batch-to-batch uniformity and regulatory adherence during drug manufacturing. The method adaptation for enantiomeric separation, bioanalytical applications, and dissolution profiling will be possible for future studies.

3.3.9 Method comparison using MUP approach

AQbD has proven to be much more beneficial than traditional approaches as assessed in Method Uncertainty Profile (MUP) framework. The overall comparison entails the critical analytical performance attributes such as precision, trueness, and linearity to determine strong tolerance levels which are indicative of actual method ability in the real world. MUP methodology offers a comprehensive analysis, by looking at all the sources of uncertainty in the analysis process, such as variability caused by sample preparation procedures, instrument variability, and calibration procedures, which provides a more realistic estimate of method performance when compared to conventional validation methods, which typically analyze these variables one at a time.

Quantitative analysis indicates that the AQbD-optimized approach attains the total uncertainty of +/-1.8% which is significantly lower than the conventional methods that have uncertainties of +-3.5 to +/-4.2 on average. This improved performance is also

witnessed by high levels of precision, the relative standard deviation (RSD) values are always less than 0.5% and excellent linearity is witnessed in the form of a correlation coefficient (r^2) values greater than 0.9998 throughout the analytical span. Such features of performance suggest the high robustness and reproducibility of the methods that are directly reflected in the high rate of the confidence in the analytical results and the low level of erroneous decision-making in the quality control processes.

The combination of the data of precision, trueness, and linearity with the help of the MUP framework allows building the tolerance intervals that consider the collective impact of the random and systematic errors which additionally is a more precise prediction on the future measurement outcome. This uncertainty analysis as a whole is what proves that the AQbD methodology can not only lead to the minimization of the overall variability of measurements, but also contributes to a better control over all the critical analytical parameters. As such the AQbD method is more reliable when used in routine quality control and makes more accurate decisions based on batch disposition, better process understanding and enhanced regulatory compliance and at the same time the possibility of out-of-specification results is minimized as a result of analytical variability and not the quality of the product.

4 Discussion

The results obtained in the developed reverse-phase high-performance liquid chromatography (RP-HPLC) design to measure Landiolol concentration denote the high degree of enhancement of the methods of analysis optimization and validation. A methodical modification of the chromatographic conditions makes the approach be effectively optimized on mobile-phase composition, flow rate, injection volume, detection wavelength and temperature. The ultimate optimum conditions were Inertsil ODS column, mobile phase with sodium dihydrogen phosphate 55% and orthophosphoric acid 1.025%, flow rates, injection volume of 15 and 240 nm of detector wavelength. These conditions resulted in the high and repeatable retention of Landiolol at a retention time of 2.52 min; this is an indication of a great resolution and peak symmetry. System suitability tests revealed that the method met the criterion of standard method acceptance because their tailing factor was 1.0 and plate count was 10,925 and that demonstrated the efficiency of the method and its capability in the resolution of Landiolol.

On the point of accuracy, the system demonstrated good relative standard deviation (RSD) = 0.3%, which indicated good reproducibility of the following injections. Additionally, it was established that the procedure was precise with recovery rates of up to almost 100% of 100.8 to 101.1% of the measurements which revealed the accuracy of the procedure in determining Landiolol in various levels. The outcome of the linearity test was that the value of R^2 was 0.9999, therefore, the applicability of the method in the quantification of the sample originating a wide fixation was tested. The two solutions, the standard and the sample, were found to be stable after a duration of 48 h and the response did not differ significantly and this demonstrates that the technique is strong and sound in the long term of the analysis.

The forced degradation tests revealed that Landiolol was resistant in thermal and oxidative conditions but highly degraded in photolytic conditions which justifies the practice of storing the drug under lights. Combined, the validated RP-HPLC method offers a satisfactory, precise and consistent method of analysis in the quality control procedure

and pharmaceutical examination with regulatory compliance as well as the batch-to-batch reproducibility and repeatability.

5 Conclusion

The study successfully developed and validated reverse-phase high-performance liquid chromatography (RP-HPLC) method for the quantification of Landiolol in pharmaceutical formulations. The method was optimized by systematically varying chromatographic conditions like mobile phase composition, flow rate, injection volume, detection wavelength, temperature to obtain the best resolution, peak symmetry, reproducibility. The conditions finally optimized were (1) Inertsil ODS column (150 × 4.6 mm, 5 µm), (2) mobile phase (55% sodium dihydrogen phosphate and 1.025% orthophosphoric acid) (3) flow rate (1.25 mL/min.), (4) injection volume (15 µL) (5) detection wavelength (240 nm) which yielded higher peak response eliminating significant interference. The methods were validated for accuracy, precision, linearity, stability and robustness and hence can be used for routine quality control analysis. System suitability parameters are within standard acceptance criteria with a tailing factor of 1 and plate count of better than 10,000 and a similarity factor between standard solutions of 98.4%. System precision tests showed a %RSD of 0.30% and the assay validation showed a %RSD of 0.4% both of which demonstrates the high precision of the method. Linearity was observed over a wide concentration range with a correlation coefficient (R^2) of 0.9999 between the analyte concentration and peak response indicating excellent agreement. All the Accuracy studies conducted at 50%, 100% and 150% levels yielded recoveries in the range of 100%, indicating that the method is able to quantify Landiolol accurately in various sample concentrations. Landiolol's stability in thermal and oxidative conditions but high photolytic degradation susceptibility was identified in forced degradation studies that highlighted the importance of light-protected storage. However, filtration studies showed that there is only minimal analyte loss, indicating that the method is not dependent on a specific filtration technique and any filtration procedure should not significantly impact assay results. The results of the stability study showed that standard and sample solutions were stable for out to 48 h, suggesting that the method is applicable to prolonged analysis times. Robustness testing proved that changes in flow rate, mobile phase composition, temperature and detection wavelength had no significant effect on method performance; thus, this method can be used in different laboratories. The newly developed, validated RP-HPLC method for Landiolol analysis is straightforward, exact, correct and strong making it worthy of use in regular quality control, hostility studies, and pharmaceutical examination. It complies with the regulatory requirements for pharmaceutical manufacturing, where batch to batch consistency is essential. Future directions can explore in the areas of enantiomer separation, bioanalytical application, and dissolution profiling giving it a scope in drug development and clinical research.

Supplementary Information

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Supplementary Material 1.

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Author contributions
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Data availability
All data generated or analysed during this study are included in this published article [and its supplementary information files].

Declarations

Ethics approval and consent to participate
Ethics approval is not applicable, as this study did not involve human participants or animals. Not applicable. This study did not involve human participants.

Consent for publication
Not applicable. This manuscript does not contain any individual person's data in any form (including images or identifiable information).

Competing interests
The authors declare no competing interests.

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