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Analytical Quality by Design guided RP-HPLC Method Development and Validation for estimation of Palbocilib employing Box-Behnken optimisation

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Abstract

Conventional empirical approaches (trial and error) to HPLC method design tend to yield fragile, non-consistent methods and lack regulatory flexibility, while Analytical Quality by Design offers a structured framework ensuring reliability. In this study a AQbD guided HPLC method was developed and optimised for Palbociclib, a Cyclin-dependent kinase 4/6 inhibitor (CDK4/6i) approved for the treatment of hormone receptor-positive breast cancer. Risk assessment (Ishikawa diagram) tool guided factor selection while Box-Behnken Design enabled optimisation through Design Expert (v.13) software. The optimised method employed a Shimpack C18 column (250 mm x 4.6 mm, 5 µm); mobile phase comprising Buffer (ammonium formate of pH 4.2 adjusted with glacial acetic acid) and Acetonitrile in the ratio 35:65; flow rate of 0.8 mL/min; injection volume of 10 µL; column oven temperature of 35°C and detection wavelength of 357 nm. Validation was performed in accordance with the ICH Q2 (R2) guidelines. The peak was eluted at 4.23 minutes, and the method demonstrated excellent linearity across 3–50 µg/mL with a correlation coefficient (R^2) of 0.9999, LOD of 0.75 µg/mL and LOQ of 2.27 µg/mL. Accuracy studies demonstrated recoveries between 98.91-100.88% while precision was evaluated through both intra-day and inter-day studies, consistently showing RSD deviations below 2%.

INTRODUCTION

Analytical methods are fundamental to pharmaceutical research, quality control, and regulatory compliance. Among them, High-Performance Liquid Chromatography (HPLC) is widely used for its sensitivity, reproducibility, and versatility. However, conventional trial-and-error approaches to method development are resource-intensive and often fail to ensure robustness across the method lifecycle. To address these limitations, the Analytical-Quality by Design (AQbD) provides a systematic framework that applies scientific principles and risk assessment to method development. The AQbD approach was developed in alignment with the principles outlined in ICH guidelines (Q9 and Q14), ensuring a structured and guideline-driven framework for method implementation [1,2]. Within the AQbD framework, method development is guided by defining analytical target profile (ATP), Critical Analytical attributes (CAAs), identifying Critical Method Parameters (CMPs) that influence them, and applying risk-based and statistical tools to optimise performance (Design of Experiment)[3]. This structured approach enhances method reliability under routine variations and facilitates regulatory acceptance throughout the product lifecycle. (Fig. 1) depicts the process flow of the AQbD approach in method development[4].

Palbociclib (marketed as Ibrance) is a selective CDK4/6 inhibitor that is clinically important in the treatment of hormone receptor-positive (HR+), HER2-negative advanced breast cancer in combination with an aromatase inhibitor or fulvestrant. Its pyridopyrimidinone structure (Fig. 2) underlies its kinase inhibitory activity [5,6]. Most of the available literature focuses on conventional RP-HPLC methods for its quantification in bulk drugs and formulations, including stability-indicating assays and simultaneous estimation with other anticancer agents (Table 1), but no AQbD-based RP-HPLC method has been reported currently for the estimation of Palbociclib [7–11].

Table 1
Summary of previously reported methods and the current RP-HPLC method for Palbociclib

Chromatographic conditions	Column used	Linearity (µg/mL)	Remarks	Reference
0.02 M sodium dihydrogen phosphate buffer (pH 5.5): ACN: Methanol (80:10:10 v/v/v); 1 ml/min flow rate; at 254 nm	Intersil C_8 (250 mm x 4.6 mm, 5 µm)	5–50	Simultaneous estimation of palbociclib and letrozole	8
Ammonium acetate (pH 8) adjusted with TEA: ACN (38:62 v/v); 1 ml/min flow rate; at 263 nm	Inertsil ODS-3V (250 mm x 4.6 mm, 5 µm)	5 – 1,000	Stability-indicating HPLC method for palbociclib	9
ACN: sodium acetate buffer (30:70, 0.5% TEA); 1 ml/min flow rate; at 260 nm	Kromasil C18 (250 mm x 4.6 mm, 5 µm)	4.04–20.19	Content determination of palbociclib in capsules	10
Ammonium formate buffer (pH 4.2) adjusted with glacial acetic acid: ACN (35:65 v/v), 0.8 ml/min flow rate; at 357 nm	Shimadzu Shim-pack C18 (250 mm x 4.6 mm, 5 µm)	3–50	AQbD-based RP-HPLC method for quantification of palbociclib in bulk	Proposed-present study

This study focuses on the development, optimisation and validation of a reliable RP-HPLC method for quantifying Palbociclib in bulk, employing an AQbD framework to ensure method reliability, efficiency and regulatory compliance.

MATERIALS AND METHODS

Chemicals and Reagents:

The pure API (Palbociclib) was provided by MSN Laboratories Pvt. Ltd (Telangana, India) as a gift sample, with a purity 99.60%. HPLC-grade acetonitrile and AR-grade ammonium formate were procured from Finar. Glacial acetic acid (Emplura) was procured from Merck, and ultrapure water was prepared using a Milli-Q purification system for buffer preparation.

Instrumentation:

A UV-Visible spectrophotometer (Shimadzu UV-1800) was used to determine the maximum absorbance wavelength (λ_{max}) of Palbociclib, which served as the basis for selecting the detection wavelength. Chromatographic separation was carried out using an HPLC system with a UV-Visible detector (Shimadzu LC20-series), equipped with a Shimadzu Shim-pack C18 Column consisting of 250 mm of length, 4.6 mm of internal diameter and 5 µm particle size.

Optimised Chromatographic conditions:

The mobile phase was prepared using an ammonium formate buffer (pH 4.2, adjusted with glacial acetic acid) and acetonitrile in the ratio 35:65 (v/v). It was filtered through a membrane filter (0.45 µm) and degassed by sonication. Chromatographic separation was achieved at 35°C using a flow rate of 0.8 ml/min with a 10 µL injection volume. The detection was carried out at 357 nm.

Procedure for sample preparation:

Diluent A: Acetonitrile and Water in a 50:50 ratio.

(The solubility of the drug was high in this mixture)[13]

Diluent B: Mobile phase (Buffer: Acetonitrile, 35:65 ratio)

Preparation of stock solution (200 µg/mL):

A precisely weighed quantity (5 mg) of Palbociclib was dissolved in 20 mL of diluent A. The solution was sonicated for 10 minutes to ensure complete solubilisation (clear solution), followed by dilution with Diluent A to a final volume of 25 mL.

The resulting solution was filtered through a 0.45 µm syringe filter prior to analysis.

Preparation of calibration standards:

The stock solution was diluted using diluent B (Mobile Phase) to prepare calibration standard solutions in the range of 0.05–50 µg/mL.

Selection of detection wavelength:

A 10 µg/mL solution of Palbociclib was prepared from the stock solution using diluent B and was scanned in the range of 200–400 nm on a UV-Visible Spectrophotometer. The λmax of 357 nm was obtained and was selected as a detection wavelength.

RP-HPLC Method Development by AQbD approach:

Establishment of ATP, CAAs and CMPS[1]:

The ATP, CAAs and CMPS were established systematically through risk assessment and experimental design to ensure method robustness and regulatory compliance.

Risk Assessment using Ishikawa Diagram[2]:

An Ishikawa diagram (fishbone analysis) was employed to identify potential sources of variability in HPLC performance. Critical parameters such as sample preparation, mobile phase pH and composition, injection volume, flow rate, column oven temperature, selection of column and detection wavelength were examined for their impact on CAAs like retention time, peak area, efficiency (theoretical plates) and peak asymmetry. This qualitative assessment provided a foundation for prioritising CMPs.

Preliminary Screening using the one-factor-at-a-time (OFAT) method:

Before implementing statistical optimisation, the influence of individual CMPs on CAAs was evaluated using the OFAT approach. This ensured that only the most impactful CMPs were carried forward into the DoE, thereby improving efficiency and reducing experimental burden.

Statistical optimisation using Box- Behnken Design (BBD):

Following OFAT screening, BBD was applied to systematically study the interactions among four CMPs (buffer concentration, pH of the buffer, flow rate and column oven temperature) and four CAAs (retention time, peak area, peak tailing and net theoretical plates). BBD enabled the development of predictive models and the establishment of a statistically validated method operable design region (MODR), ensuring robustness and reproducibility of the method.

Design of Experiment (DoE) Software:

Design Expert (v.13), a statistical software, was used to apply DoE approaches such as Box-Behnken Design to optimise chromatographic conditions in HPLC.

Analytical Method Validation[14]:

Validation of the developed method was performed as per ICH Q2 (R2) guideline, which includes the following parameters:

Specificity:

To establish specificity, the chromatogram of the blank was compared with the sample (10 µg/ml), which confirmed that the analyte peak was well resolved without any interference from the diluent.

System suitability studies:

The suitability of the developed chromatographic method was verified through six replicate injections of Palbociclib standard solution (10 µg/ml). The evaluation included determination of retention time, net theoretical plates and tailing factor. The acceptance criteria include retention times showing %RSD values below 2%, theoretical plates exceeding 2000, and tailing factor remaining within the limit of ≤ 2.

Linearity and Calibration Curve:

The linearity was evaluated across the concentration range of 0.05–50 µg/mL with replicates (n = 3) at each concentration level. The calibration curve was obtained by plotting the mean peak area on the y-axis and the analyte concentration on the x-axis. The regression equation of the calibration curve and the correlation coefficient were determined.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The LOD and LOQ were established using two approaches: the chromatographic signal-to-noise ratio method (practical), where LOD and LOQ were defined at S/N values of approximately 3 and 10, respectively; and the calibration curve method (statistical), where values were calculated based on the standard deviation of the response and the slope of the regression line.

In the calibration curve method, the following equations were used to determine the LOD and LOQ:

LOD = 3.3 x $\frac{\sigma}{Slope}$

LOQ = 10 x $\frac{\sigma}{Slope}$

Where σ represents the standard deviation of the response.

Range:

As the LOQ represents the lowest concentration at which the analyte could be quantified with acceptable precision and accuracy, the working range of the method was defined from the LOQ (obtained from the calibration curve method) up to the highest concentration validated within the linearity study.

Precision:

Intra-day (Repeatability): Intraday precision was assessed by preparing six independent analyte solutions at a concentration of 10 µg/ml and analysing them under optimised chromatographic conditions on the same day.

Inter-day: Intermediate precision was determined by analysing six independently prepared analyte solutions at a concentration of 10 µg/ml over three consecutive days to determine the variability between days.

Accuracy:

Accuracy of the analytical procedure was evaluated through recovery experiments conducted across the validated concentration range. Three levels were selected to represent the extremes and midpoint of the linearity interval, namely 3 µg/mL, 10 µg/mL, and 50 µg/mL. Triplicate analyses were performed at each concentration, and the mean recovery values were derived to assess the method performance. The study was considered acceptable when recoveries fell within 98–102%, and the relative standard deviation did not exceed 2%, in accordance with established validation criteria. The following equation was used to calculate the % Recovery for each level.

% Recovery = $\frac{Measured\ value}{true\ value}$ X 100

Robustness:

It was evaluated by introducing minor changes in the analytical method parameters, such as composition of mobile phase (± 2% of the buffer), column oven temperature (± 2 °C) and detection wavelength (±2 nm). The method is considered robust if these changes do not significantly influence the system suitability parameters (retention time, peak tailing and net theoretical plate) and all factors remain within the predefined acceptance limits (%RSD ≤ 2, tailing factor ≤ 2 and theoretical plates > 2000).

Benchtop solution stability:

Freshly prepared stock solution and working solutions were kept at an ambient laboratory condition (25 ^{o}C ±2), exposed to normal light and were analysed at different time intervals (0, 4, 16, 24, 48 hours). At each timepoint, replicate injections (n=3) were performed, and chromatographic parameters, including peak area, retention time, tailing factor and theoretical plates, were compared with the reference solution.

RESULTS AND DISCUSSION

Initial method trials using acetonitrile-water mixtures in varying ratios resulted in peaks with retention times exceeding 25 minutes and poor peak shape. Subsequent experiments employing different buffer systems across a pH range of 2.5–6.5 were evaluated. Among these, ammonium formate provided optimal performance, yielding a well-defined peak that eluted within 8 minutes. Hence, ammonium formate was selected as the buffer for the final method development.

Wavelength selection:

A working standard solution of Palbociclib (10 µg/ml) showed a λmax of 357 nm. Additional peaks were observed at 220nm, 264nm, and 302 nm, as shown in Fig. 3.

Method Development by an AQbD approach:

Risk assessment:

The possible factors, as described in Fig. 4, were studied for their influence on the method's performance using an Ishikawa diagram. Each factor was grouped into one of the following categories: Method Parameters, Sample preparation, Materials, Instrumentation, Human Factors, and Environment. After mapping the potential cause, the most impactful parameters were selected based on the risk assessment score. The Key parameters identified were - mobile phase composition and its pH, flow rate,

column oven temperature, type of column (C18), diluent for sample preparation and wavelength for detection.

Optimisation of the method using BBD:

The BBD experimental design comprised 27 runs, incorporating two factor levels (-1,+1) along with a central point (0) to assess the curvature effects. The coded values of the independent variables and their corresponding experimental runs are summarised in Table 2 and Table 3, respectively.

Table 2
Coded values for CMPs in Box-Behnken Design

Factors	Levels		
	-1	0	1
A: pH of Buffer	2.8	3.5	4.2
B: Buffer concentration (%)	30	35	40
C: Flow Rate (mL/min)	0.8	0.9	1
D: Column temperature (^{o}C)	30	35	40

BBD optimisation yielded the following equations explaining how each CMP influences the CAAs:

Retention time = +12.57450 - 1.00369A + 0.031433B - 11.38583C - 0.080967D - 0.003357AB + 0.185714AC – 0.002357AD - 0.072500BC + 0.000050BD + 0.198500CD + 0.188350 A^2 + 0.000807B^2 + 1.32917C^2 -0.001413D^2

Interpretation: The coded equation represents a quadratic polynomial model for retention time and suggests that there is a strong negative effect of pH (-1.00369A) and flow rate (11.3858C) on Retention time. Which implies that increasing Factor A and C reduces the retention time as shown in Figure 5.

Peak Area = + 738994.666 – 3152.75A -12876.25B – 72343.58C-362.08D + 3043.50AB + 5068.75AC + 336.00AD – 365.00BC – 2390.75BD + 2979.50CD + 47348.87A^2 + 195.87B^2 + 7446.63C^2 – 2655.37D^2

Interpretation: The coded equation represents a quadratic polynomial model for peak area, while suggesting that there is a very strong negative effect of flow rate (-72343.58C) on peak area, implying that faster flow significantly reduces the area, as shown in Figure 6.

Table 3: Experimental Run of Box-Behnken Design (Sample concentration: 20 µg/mL)

Run	Factor A	Factor B	Factor C	Factor D	Retention time	Peak Area	Theoretical Plates	Tailing Factor
1	3.5	35	0.8	30	4.173	821691	2770	1.441
2	3.5	35	0.9	35	3.638	737803	2721	1.458
3	4.2	35	1	35	3.563	716183	3710	1.331
4	3.5	40	0.8	35	4.204	807909	4002	1.34
5	2.8	30	0.9	35	3.454	806391	10422	1.469
6	3.5	30	1	35	3.205	686924	4297	2.268
7	3.5	35	1	40	3.236	674981	2617	1.471
8	3.5	30	0.9	40	3.563	750896	5496	2.383
9	3.5	35	0.8	40	3.752	806951	2734	1.533
10	3.5	40	0.9	40	3.663	723208	3782	1.321
11	2.8	35	1	35	3.134	718375	6303	1.671
12	4.2	35	0.9	40	3.874	783057	3921	1.353
13	2.8	35	0.9	40	3.539	786753	7256	1.655
14	4.2	30	0.9	35	3.907	798085	3616	1.318
15	3.5	30	0.8	35	3.968	833274	5401	2.493
16	2.8	35	0.9	30	3.493	785820	6608	1.747
17	2.8	35	0.8	35	3.97	877565	7911	1.764
18	3.5	30	0.9	30	3.605	741111	3843	2.287
19	3.5	35	0.9	35	3.639	739428	2694	1.461
20	4.2	35	0.9	30	3.861	780780	3797	1.355
21	3.5	35	0.9	35	3.633	739753	2718	1.453
22	3.5	40	0.9	30	3.7	722986	3652	1.319
23	4.2	35	0.8	35	4.347	855098	4082	1.357
24	3.5	35	1	30	3.26	677803	2522	1.383
25	3.5	40	1	35	3.296	660099	3484	1.312
26	4.2	40	0.9	35	4.002	775916	4172	1.362
27	2.8	40	0.9	35	3.596	772048	4631	1.47

Tailing Factor = + 4.94243 – 0.202381A – 0.068233B – 0.41C + 0.003067D

Interpretation: The coded equation represents a linear model for the tailing factor. It suggests that there is a negative effect of pH (-0.202381A) on the tailing factor, implying that as the pH increases, the tailing is reduced and the peak symmetry is improved, as shown in Figure 7.

Theoretical plates = + 215254.5 – 54061.66A – 4603.783B – 75838.333C + 248.15D + 453.35714AB + 4414.28AC – 37.42857AD + 293.00BC – 15.23BD + 65.50CD + $4738.60544A^2$ +$44.72167B^2$ +$24741.667C^2$ + $5.72167D^2$

Interpretation: The coded equation represents a quadratic second-order polynomial model for theoretical plates. It suggests that there is a strong negative effect of flow rate (-75838.333C), implying that higher flow reduces efficiency, as shown in Figure 8.

Method Operable Design Region (MODR):

The MODR was established by overlaying contour plots of all critical responses, identifying the 3D design space where method performance consistently met the acceptance criteria. The two critical parameters that were optimised are pH of the buffer (A) and the buffer concentration (B), while the other two parameters, flow rate (C) and column oven temperature (D), were kept constant during the overlay analysis. The yellow area shown in Figure 9 represents the optimised operable design space for the developed method. This region was derived by overlaying contour plots of multiple responses (retention time, peak area, net theoretical plates and tailing factor). The red dots represent the experimental runs.

Optimised Chromatographic conditions:

The selection of optimised chromatographic conditions was guided by desirability solutions derived from BBD, ensuring robustness and compliance with ICH guidelines. The conditions are shown in Figure 10 with a desirability of 1.0000. The final chromatographic conditions, as shown in Table 4, were further used for method validation.

Control strategy:

A control strategy is planned for the developed method to deliver reliable and robust results within the obtained MODR. The elements identified as controls are as follows:

Mobile phase pH: To obtain reproducible retention time and analyte stability, the pH should be controlled within 2.8-4.2.

Buffer concentration in the mobile phase (%): The buffer concentration can be varied in the range 33 - 40%.

Flow rate: The flow is set to 0.8 mL/min, with a variation of ±0.05mL.

Column Oven Temperature: The temperature is maintained at 35 ^{o}C± 2.

Table 4: Optimised Chromatographic conditions for Palbociclib estimation:

Parameters	Chromatographic **Conditions**
Stationary Phase	Shimadzu Shim-pack C18 Column (250 mm of length; 4.6 mm of internal diameter and 5 µm particle size)
Mobile Phase	Ammonium formate buffer (pH 4.2, adjusted with glacial acetic acid) and Acetonitrile ratio 35:65 (v/v)
Flow rate	0.8 mL/min
Injection Volume	10 µL
Column oven temperature	35 ^{o}C (±2 ^{o}C)
Elution	Isocratic mode
Run time	8 minutes
Detector and Wavelength	UV-Visible, 357 nm
Retention time	4.236 minutes

Analytical Method Validation:

Specificity:

Specificity was assessed by analysing a blank solution and a sample prepared at a concentration of 10 µg/mL, where the chromatogram of the blank showed no interfering peaks Figure 11A, and the analyte peak in the sample was clearly resolved with a retention time of 4.236 minutes Figure B, confirming the absence of solvent interference.

System suitability studies:

System suitability testing demonstrated consistent retention time, acceptable peak symmetry (≤ 2), and adequate theoretical plates, confirming the reliability of the chromatographic method. The results are summarised in Table 5.

Table 5
Summary of system suitability parameters for Palbociclib

Replicate No.	Retention Time (minutes)	Peak Area (mV)	Tailing Factor	Theoretical Plate
1	4.29	403617	1.374	3870
2	4.28	417189	1.354	3766
3	4.28	409646	1.359	3760
4	4.24	419608	1.361	3843
5	4.28	410679	1.368	3865
6	4.30	413617	1.367	3846
Mean	4.279	412392.7	1.364	3825
SD	0.020	5728.141	0.007	44.900
%RSD	0.4773	1.3890	0.5276	1.1739

Linearity:

Linearity study confirmed direct proportionality between concentration and peak area over the range of 0.05–50 µg/ml, with a correlation coefficient (R^2) of 0.9999. The results are summarised in Table 6:

Table 6
Calibration curve and Linearity Results for Palbociclib method validation

Concentration (µg/mL)	Mean Peak area (mV)	% RSD	Intercept	Slope	Corelation coefficient (R2)
0.05	2428	0.7946	2709.7	40496	0.9999
0.1	5773	1.0415			
0.5	25963	1.5387			
1	44789	1.1917			
5	207718	1.3682			
10	412793	1.0761			
30	1198567	0.8434			
50	2037597	1.5311			

LOD and LOQ:

The LOD and LOQ derived from the S/N approach were found to be 0.05 µg/ml and 0.5 µg/ml, respectively. By applying the calibration curve method, it yielded higher LOD and LOQ of 0.7 µg/ml and 2.2 µg/ml, respectively. Results of both methods are given in Table 7, with the significance of each method.

Range:

Based on the method's LOQ value (2.2 µg/mL), the working range for Palbociclib quantification was set between 3–50 µg/mL, ensuring accuracy and precision throughout.

Table 7
LOD and LOQ results obtained by two approaches with their analytical significance

Method	LOD (µg/ml)	LOQ (µg/ml)	Significance
Signal-to-noise	0.05	0.5	Provides the lowest possible values, showing the method's sensitivity (Qualitative)
Calibration curve	0.7	2.2	Defines the lowest concentration that can be quantified with acceptable accuracy and precision (Quantitative)

Intra-day and Inter-day precision:

The intra-day precision, assessed by analysing replicates within a single day, demonstrated a % RSD of less than 2% across all levels, as shown in Table 8, confirming excellent repeatability of the developed method.

Similarly, analysis repeated over three days (inter-day) showed minimal %RSD, supporting method's reproducibility as shown in Table 9.

Table 8
Intra-day precision results (repeatability)

Intra-day Precision				
Concentration (µg/mL)	**Peak Area (mV)**	**Mean (n = 3)**	**SD**	**% RSD**
3	121589	122917.33	1664.8617	1.3544564
3	122378			
3	124785			
10	416591	420735.67	5983.232	1.4220881
10	418021			
10	427595			
50	2033798	2034263.3	9211.8191	0.4528332
50	2025293			
50	2043699			

Table 9
Inter-day (Intermediate precision) results:

Inter-day Precision						
Concentration (µg/mL)	**Mean Peak Area (mV) (n = 3)**			**Mean**	**SD**	**%RSD**
	Day 1	**Day 2**	**Day 3**			
3	121255	123157	124768	123060	1758.50761	1.4289839
10	418542	419608	413028	417059.333	3531.68874	0.8468073
50	2063659	2100199	2083877	2082578.33	18304.5842	0.8789386

Accuracy (% Recovery study):

Accuracy testing demonstrated satisfactory recovery of Palbociclib, with mean percentage values between 98.91% to 100.88% at concentrations of 3 µg/ml, 10 µg/mL and 50 µg/mL, as summarised in Table 10.

Robustness:

The robustness testing was performed by changing mobile phase composition, column temperature and detection wavelength. The system suitability results consistently met the acceptance criteria, indicating

the method’s consistency under slight variations in the chromatographic conditions. The result of robustness is summarised in Table 11.

Benchtop solution stability:

Solution stability was monitored under ambient laboratory conditions for 24 hours, with assay values remaining consistent and replicate injections showing an RSD below 2%. The data confirms that the solutions are stable for a minimum of 24 hours under benchtop storage.

Table 10
Accuracy study showing recovery results for Palbociclib

Concentration (µg/mL)	Peak Area (mV)	Mean (%RSD)	(% Recovery)
3	121389	122880.667	98.91
3	122478		
3	124775		
10	416000	412793.667	100.88
10	414658		
10	407723		
50	2003798	2037596.67	100.49
50	2065293		
50	2043699		

Table 11
Robustness data for a sample concentration of 10 µg/mL

Parameters	Variation (± 2)	%RSD (n = 3)		System Suitability Parameters (n = 3)	
		Retention Time	Area	Tailing factor (range)	Theoretical Plates (range)
Buffer concentration (%)	33	1.26	0.59	1.481–1.498	5257–5277
	37	1.45	1.14	1.465–1.484	4899–4966
Column Temperature (^{o}C)	33	0.604	1.14	1.491–1.503	5059–5121
	37	0.987	0.605	1.483–1.491	5227– 5263
Detection wavelength (nm)	355	0.856	0.588	1.478–1.486	5079–5106
	359	0.70	0.590	1.465–1.484	5080–5099

Life-cycle management and continuous improvement:

Managing the life cycle of an AQbD-based HPLC method ensures its reliability and reproducibility while maintaining regulatory standards. Post-validation, the method will be continually monitored, documented and controlled modifications within the design space, ensuring its continued suitability for routine analysis of Palbociclib.

Table 12
ANOVA data for all the CMPs and CAAs using BBD

Statistical Terms	Retention Time (R1)	Peak Area (R2)	Tailing Factor (R3)	Theoretical Plates (R4)
F-value	43.66	228.55	5.70	15.19
p-value	< 0.0001	< 0.0001	0.0026	< 0.0001
Model	Quadratic	Quadratic	Linear	Quadratic
A-Buffer conc.	0.4673	$1.19x10^{8}$	0.2408	$3.27x10^{7}$
B-Buffer pH	0.0480	$1.99x10^{9}$	1.40	$7.28x10^{6}$
C- Flow rate	1.86	$6.28x10^{10}$	0.0202	$1.31x10^{6}$
D-Column temp	0.0180	$1.57x10^{6}$	0.0028	$5.69x10^{5}$
AB	0.0006	$3.705x10^{7}$	-	$1.00x10^{7}$
AC	0.007	$1.02x10^{8}$	-	$3.81x10^{5}$
AD	0.003	$4.51x10^{5}$	-	68644
BC	0.0053	$5.32x10^{5}$	-	85849
BD	6.250×10^{-6}	$2.28x10^{7}$	-	$5.79x10^{5}$
CD	0.0394	$3.55x10^{7}$	-	4290.25
A^2	0.0454	$1.19x10^{10}$	-	$2.87x10^{7}$
B^2	0.0022	$2.04x10^{5}$	-	$6.66x10^{6}$
C^2	0.0009	$2.95x10^{8}$	-	$3.26x10^{5}$
D^2	0.0067	$3.76x10^{7}$	-	$1.09x10^{5}$
Residual Analysis	Significant	Significant	Significant	Significant
Lack of fit F-value	476.31	27.25	4906.01	2250.69
p-value	0.0021	0.0359	0.0002	0.0004
R^2	0.9807	0.9963	0.5089	0.9466
Adjusted R^2	0.9583	0.9919	0.4196	0.8843

Conclusion

The integration of Ishikawa-based risk assessment, OFAT screening, and BBD optimisation highlights the strength of the AQbD framework in delivering a reliable and reproducible RP-HPLC method. This structured workflow ensures scientific rigour, regulatory flexibility, and lifecycle management, representing a significant advancement over conventional trial-and-error strategies for Palbociclib quantification.

Declarations

Author's Contributions

All authors contributed to the study conception and design. Tanvi Painginkar carried out the experimental work of HPLC method optimization, DoE analysis, data interpretation/analysis, and drafting the paper. Dr Gundawar Ravi was involved in conception of research work, supervising the experimental work, data analysis, resources, and drafting and reviewing the paper. All authors have made a significant contribution to the research in the manuscript, approved its claims, and agreed to be an author.

Competing interests:

The author(s) declares no competing interests

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Data Availability

All data generated during this study are included in this published article.

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Figures

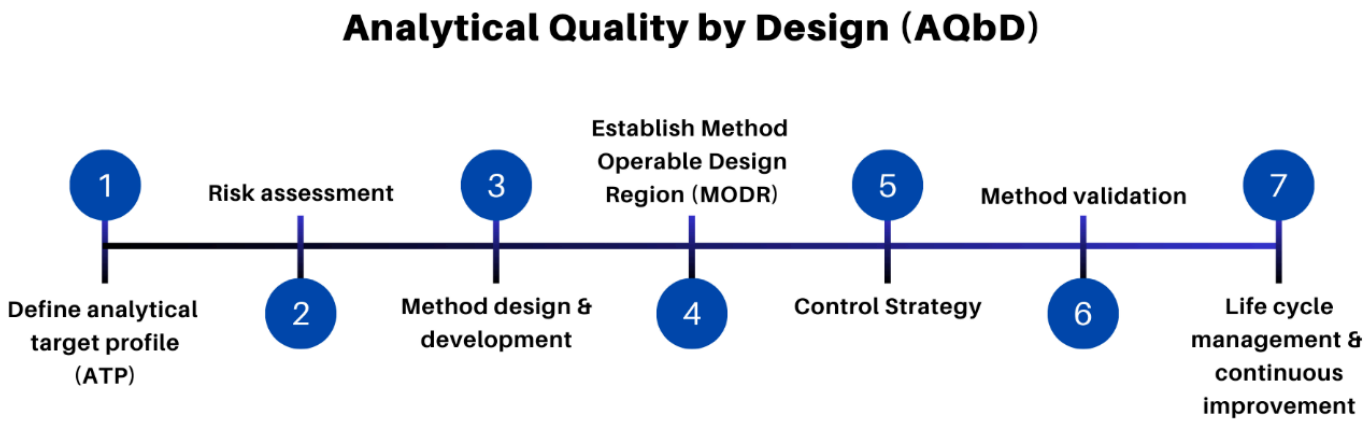


Figure 1

Analytical QbD process flow in HPLC method development and validation

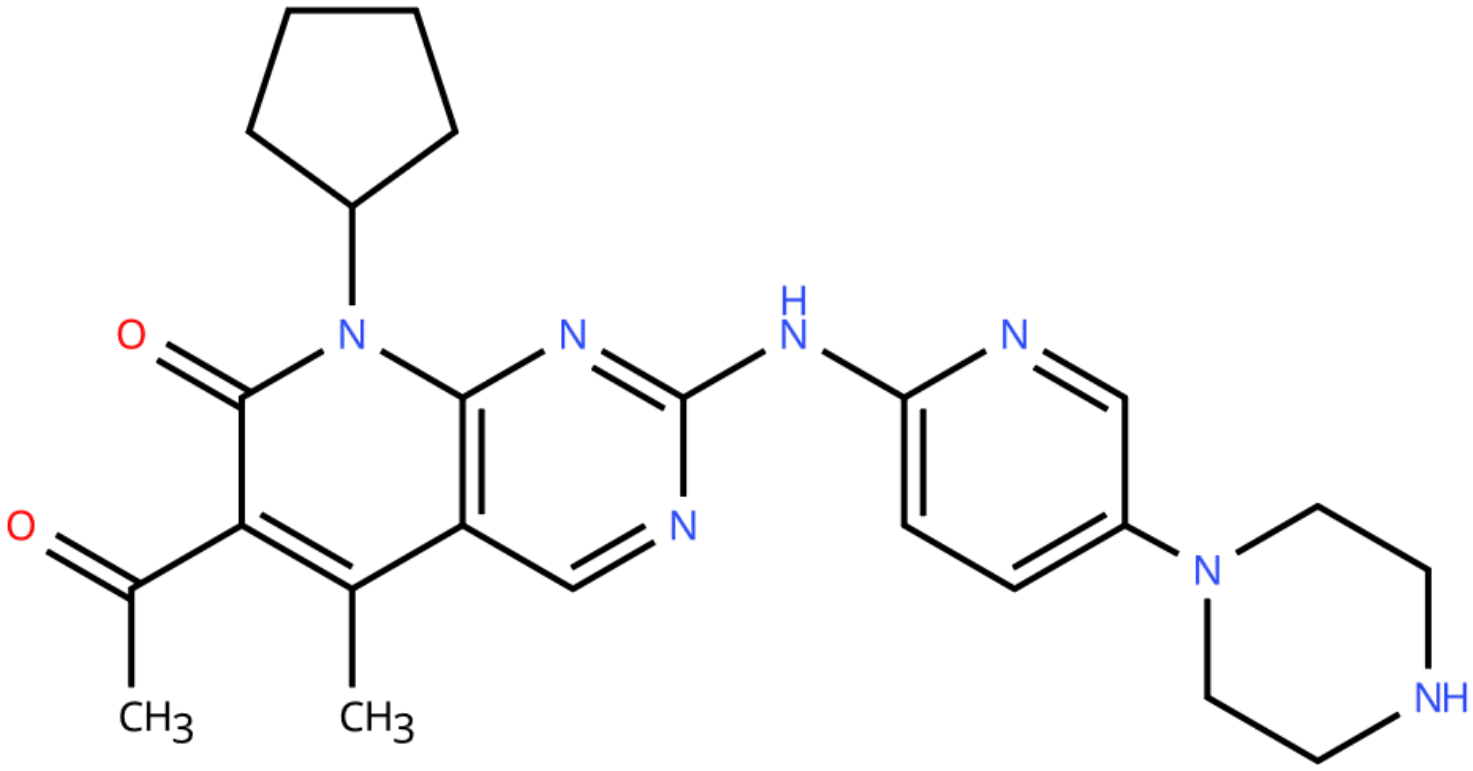


Figure 2

Molecular structure of Palbociclib[12]

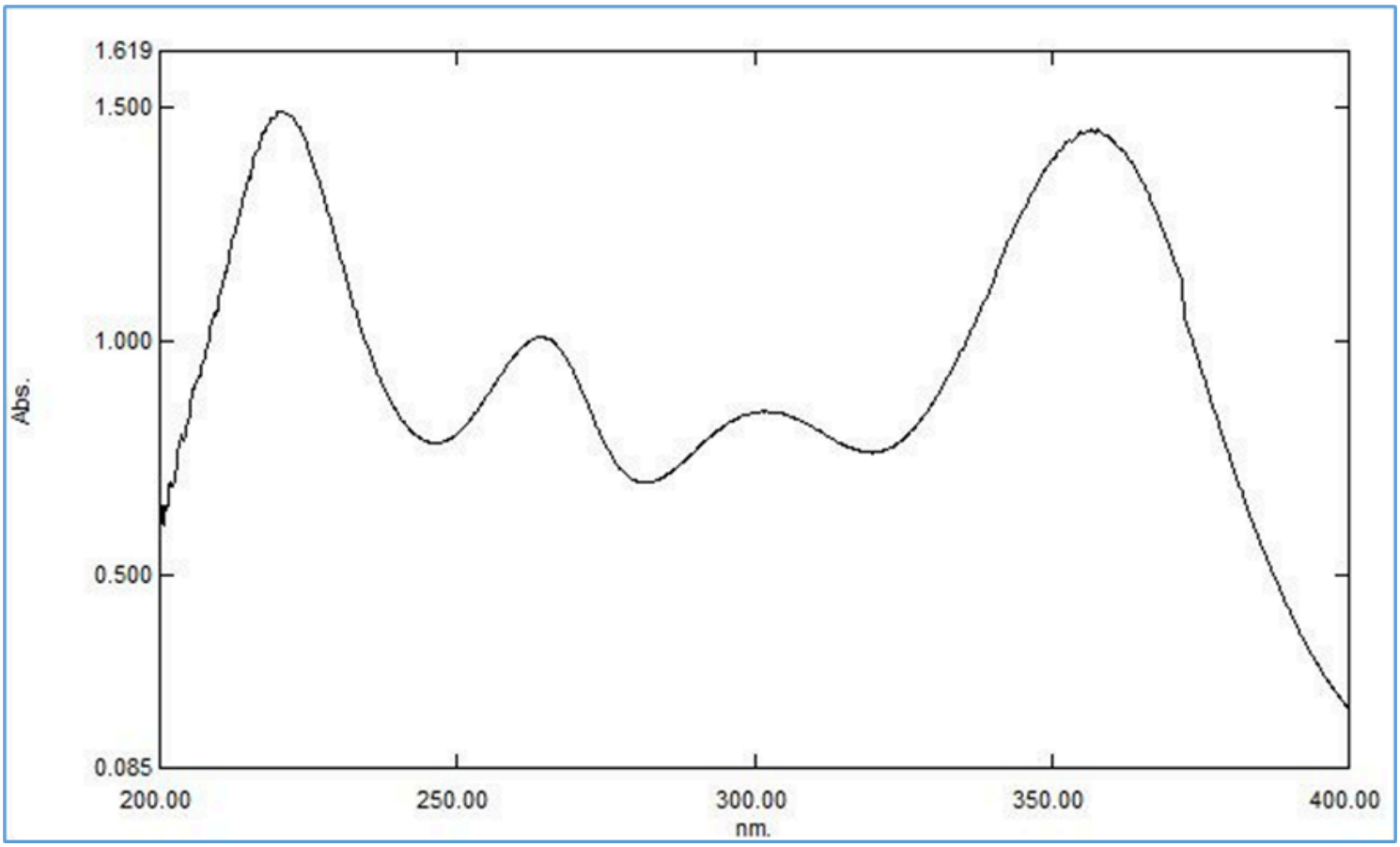


Figure 3

Represents the UV absorption spectrum of Palbociclib (10µg/ml)

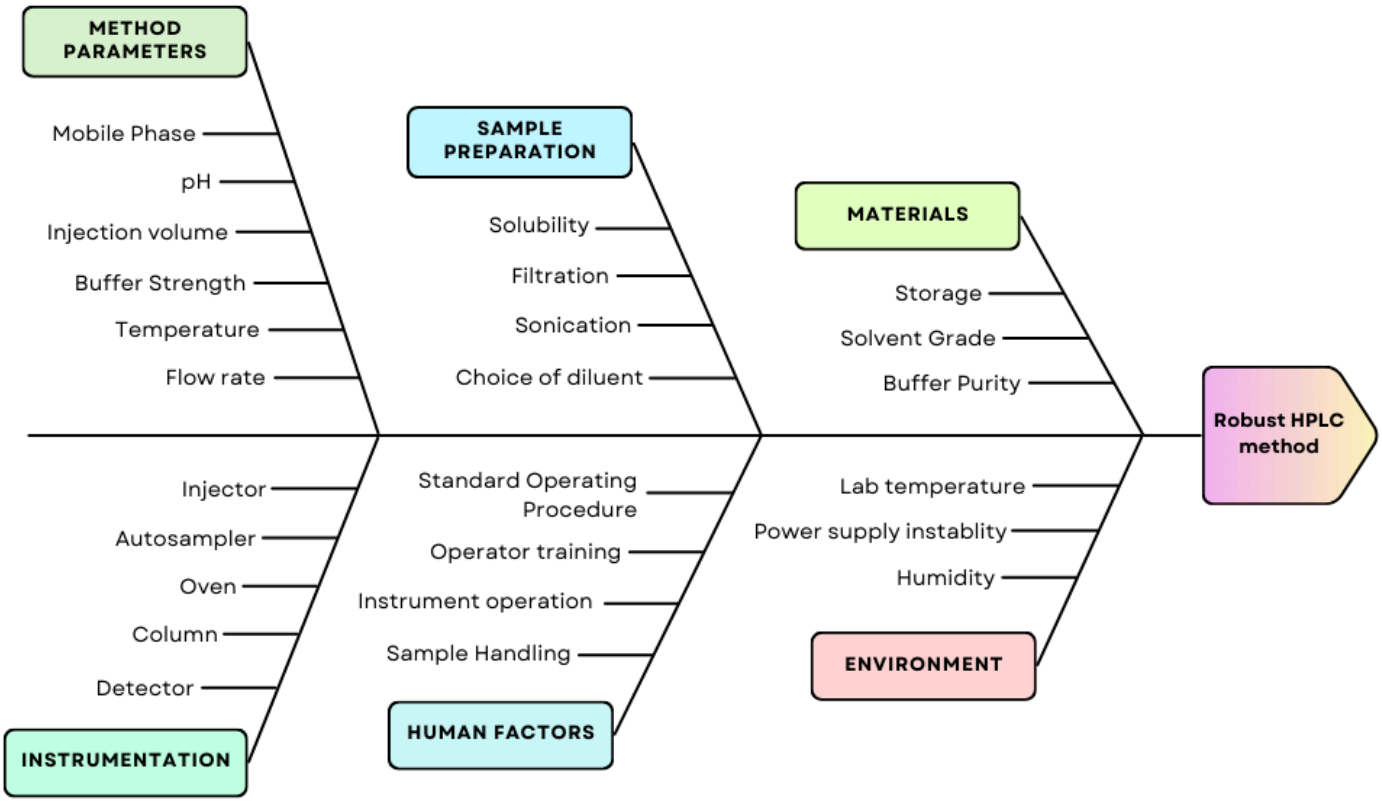


Figure 4

Systematic cause-and-effect mapping for development of a robust HPLC method using Ishikawa (fishbone) diagram.

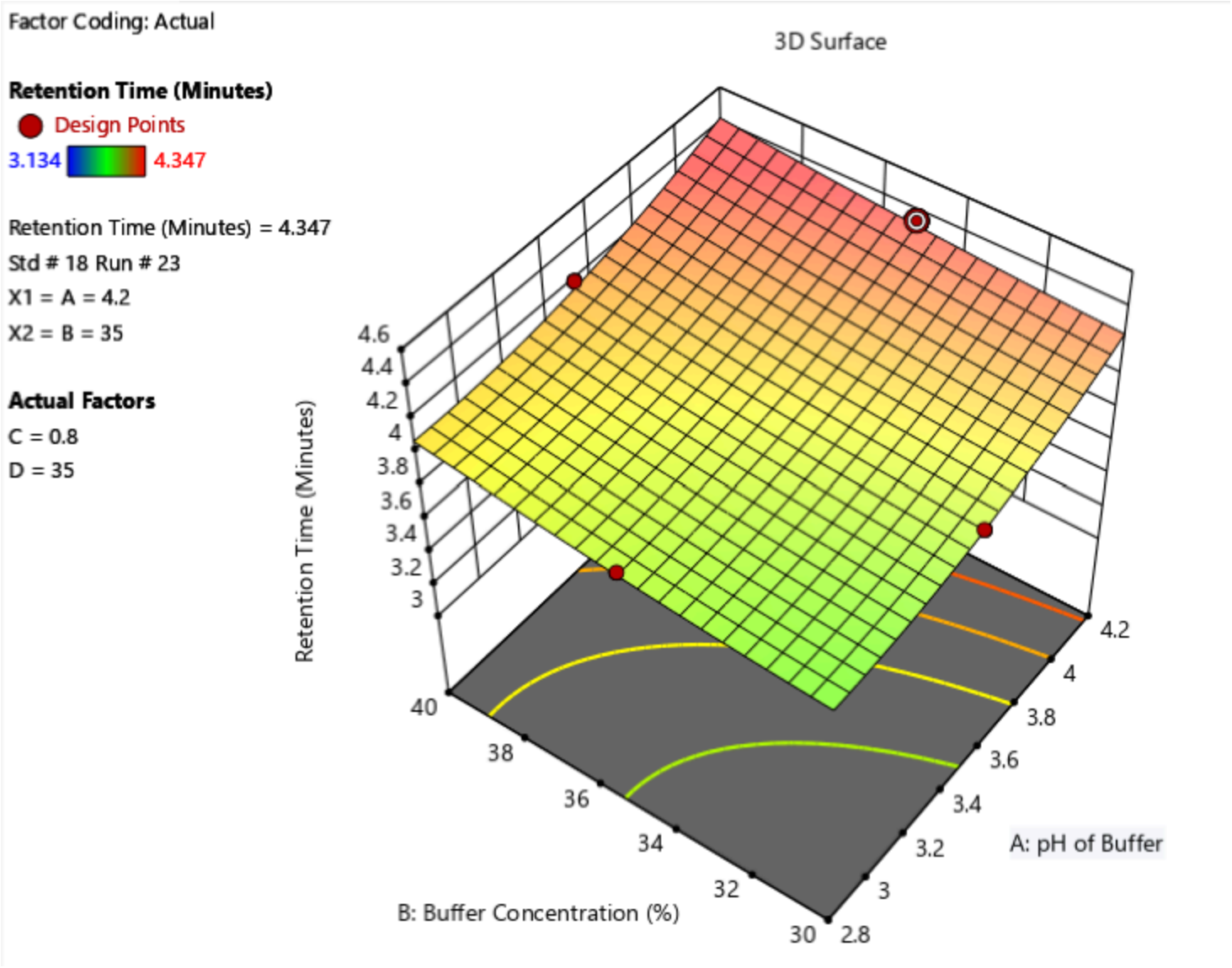


Figure 5

3D-surface plot depicting the effect of other factors on Retention time using BBD.

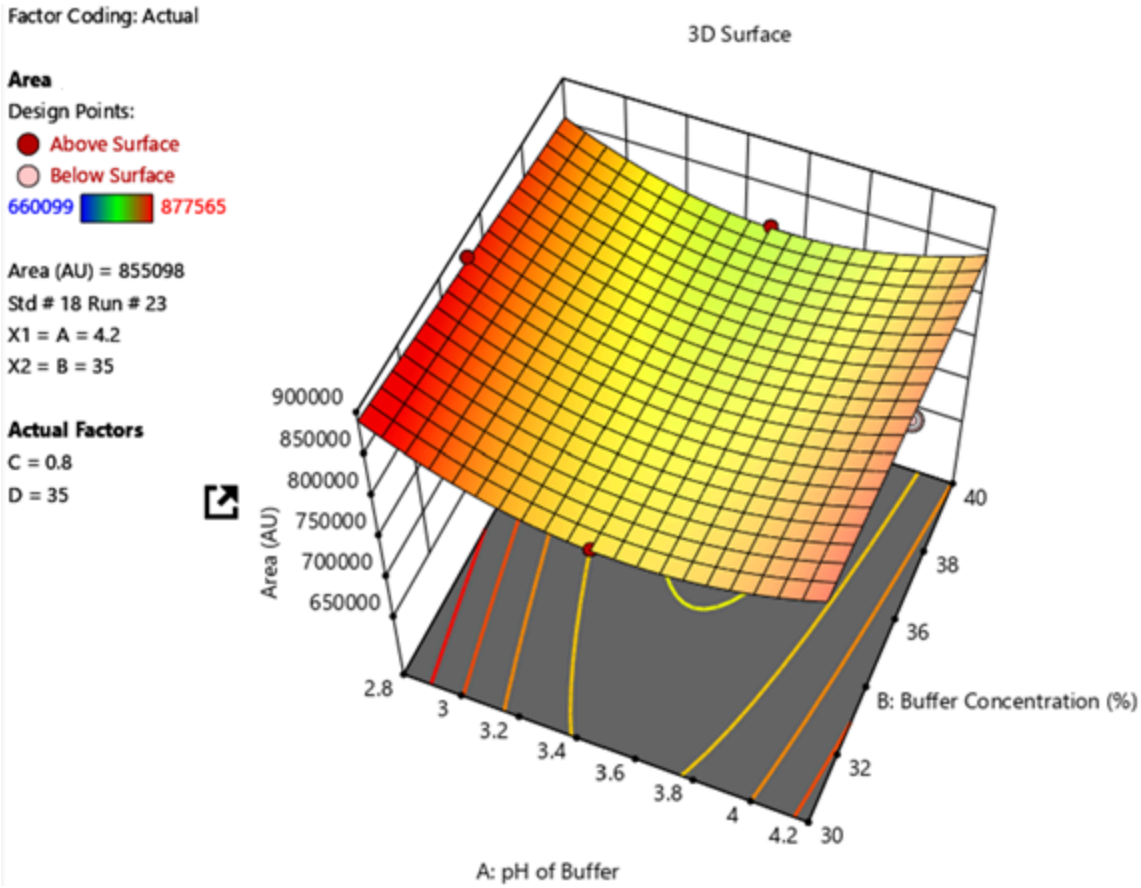


Figure 6

3D-surface plot depicting the effect of other factors on Area using BBD.

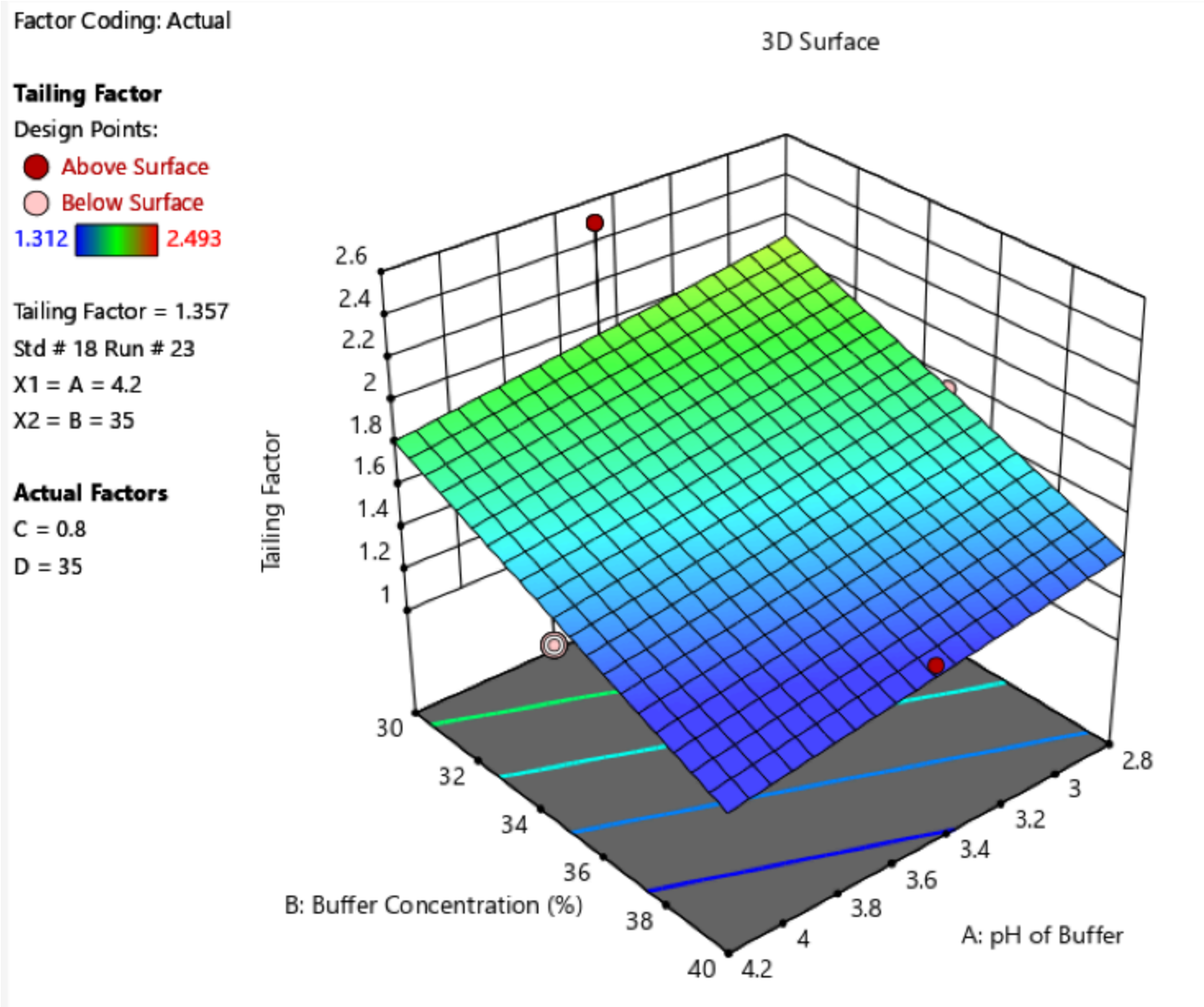


Figure 7

3D-surface plot depicting the effect of other factors on the tailing factor using BBD

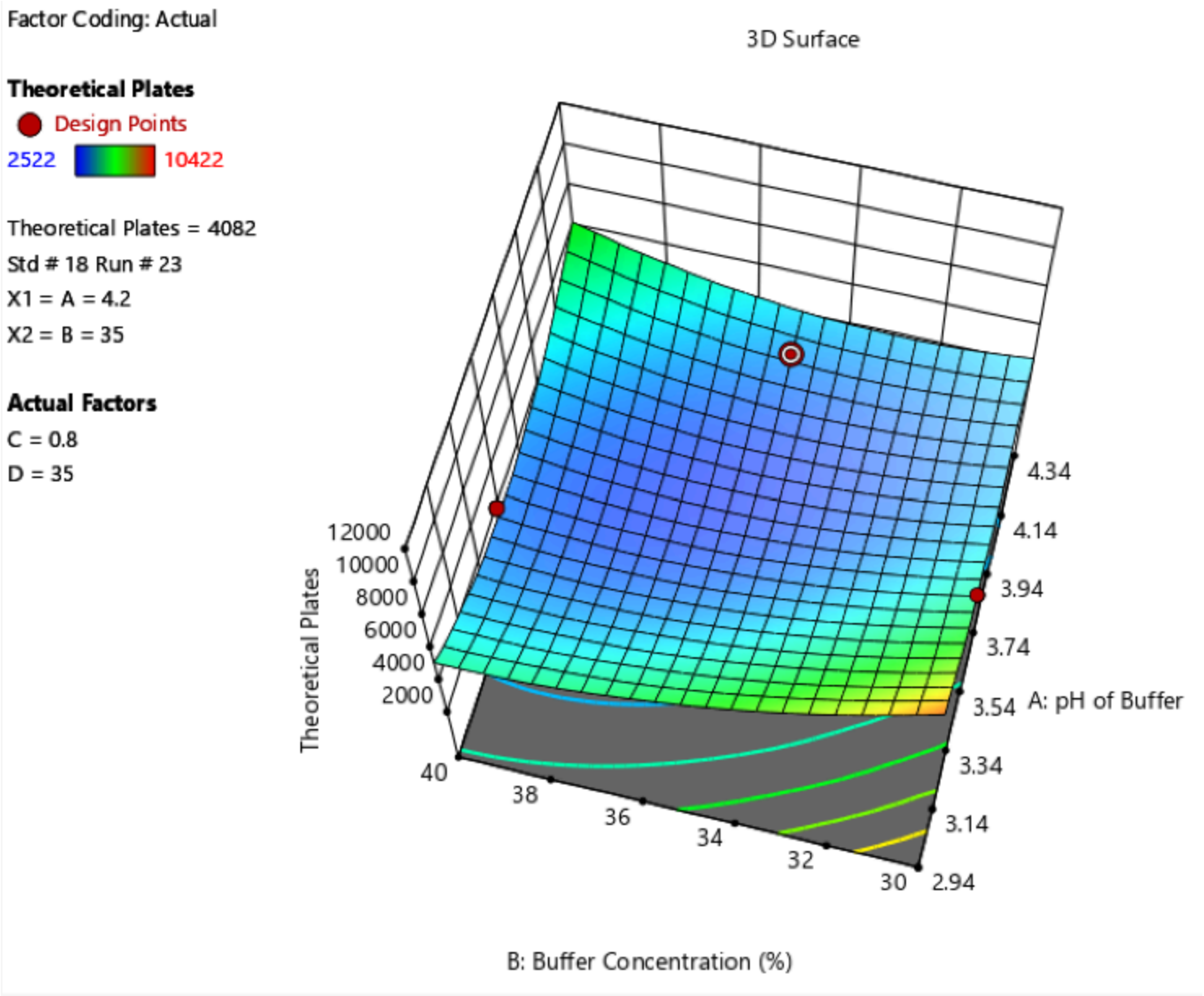


Figure 8

3D-surface plot depicting the effect of other factors on theoretical plates using BBD

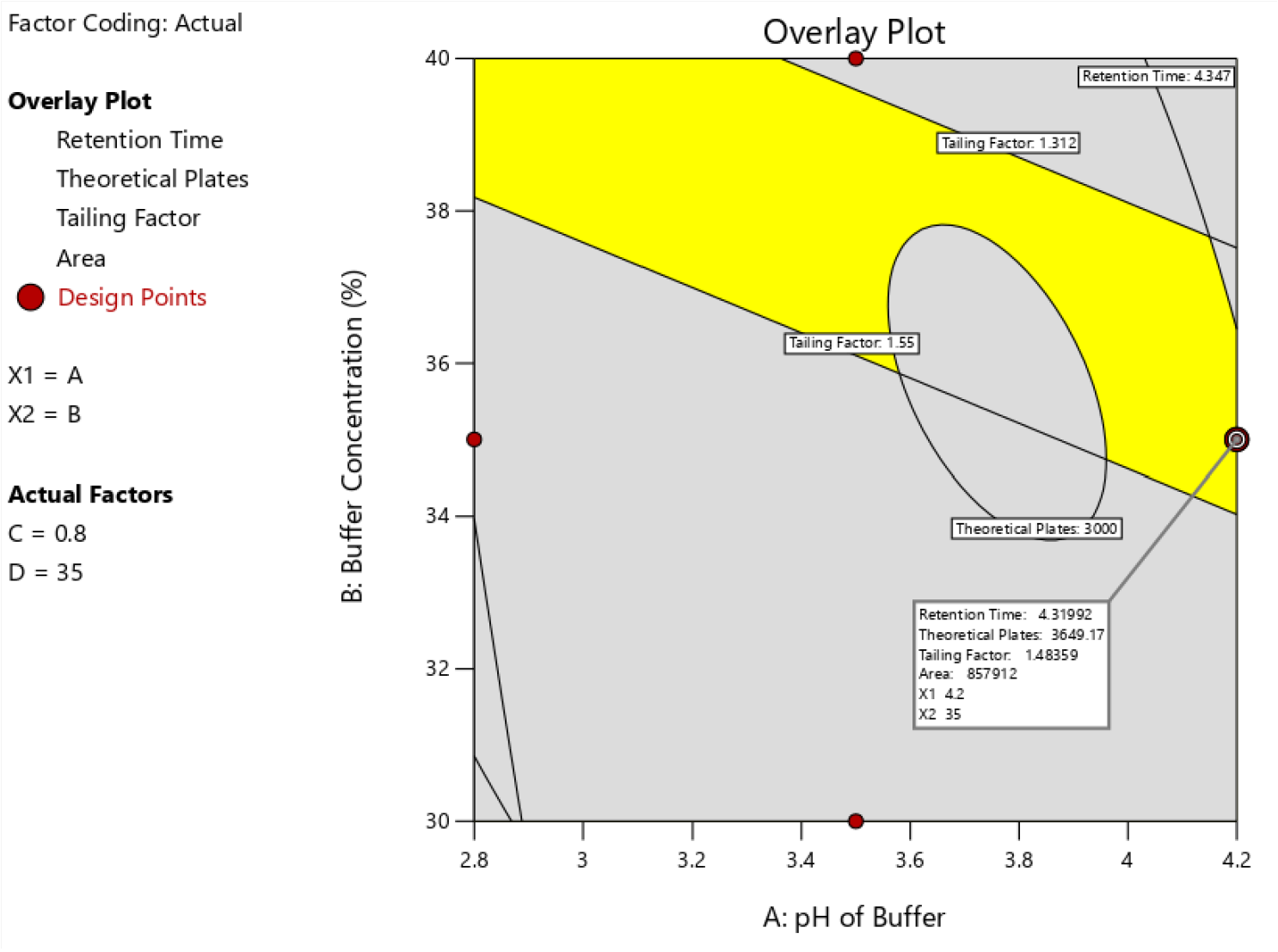


Figure 9

Graphical representation of MODR derived from BBD

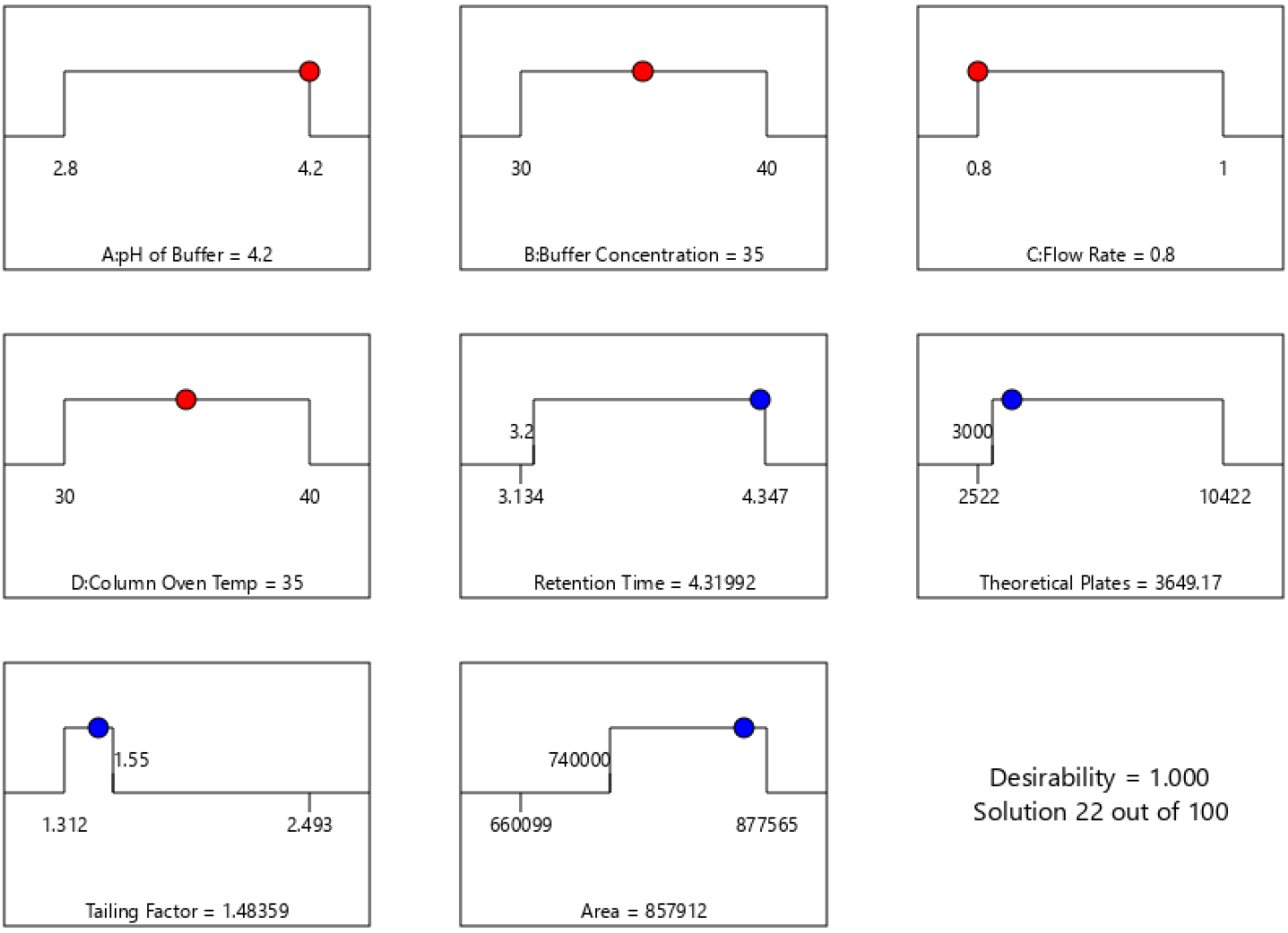


Figure 10

Numerically optimised plots showing ideal chromatographic conditions and responses with maximum desirability (1.000)

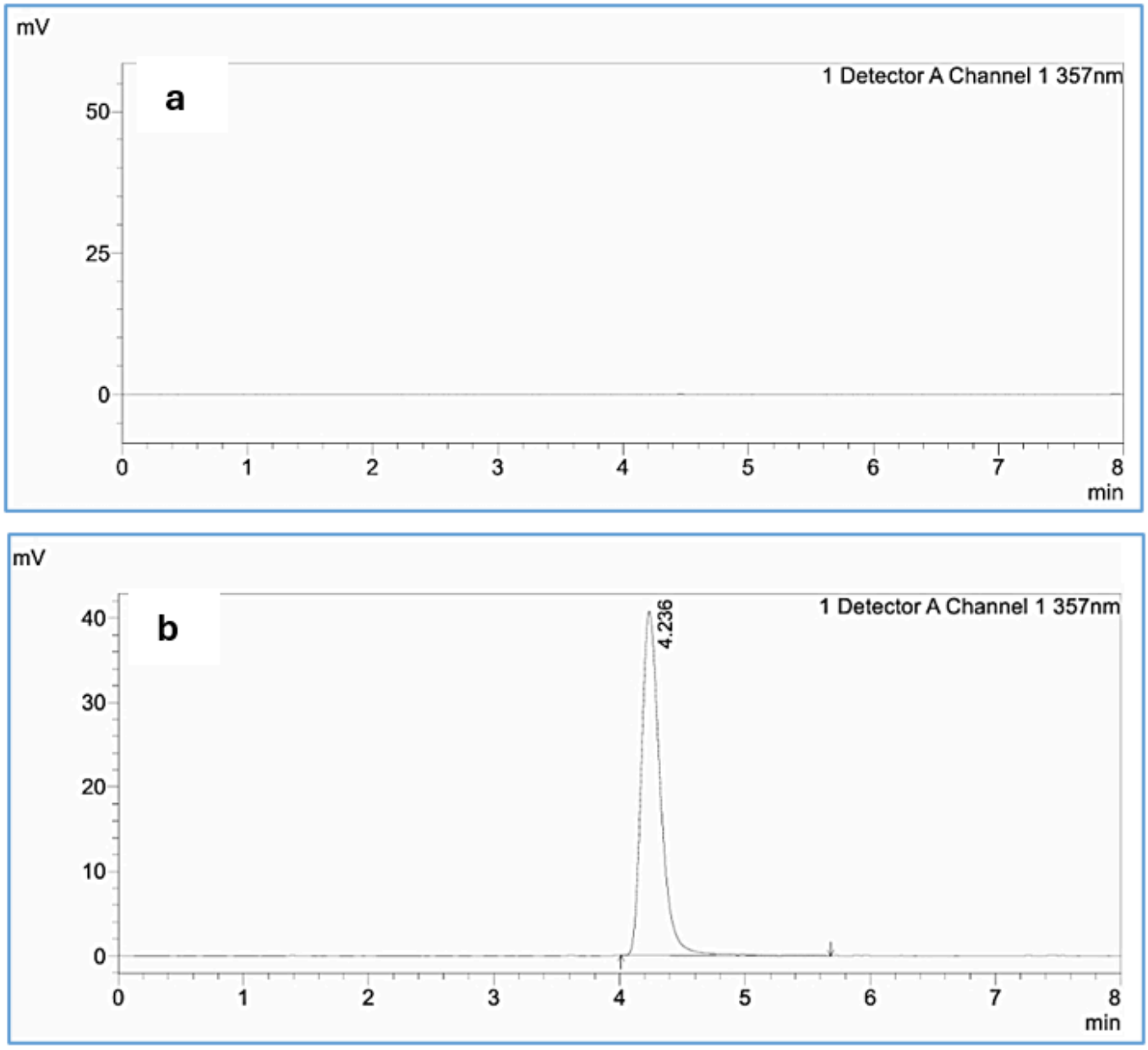


Figure 11

Chromatogram of (a) Blank and (b) Palbociclib (10 µg/ml)

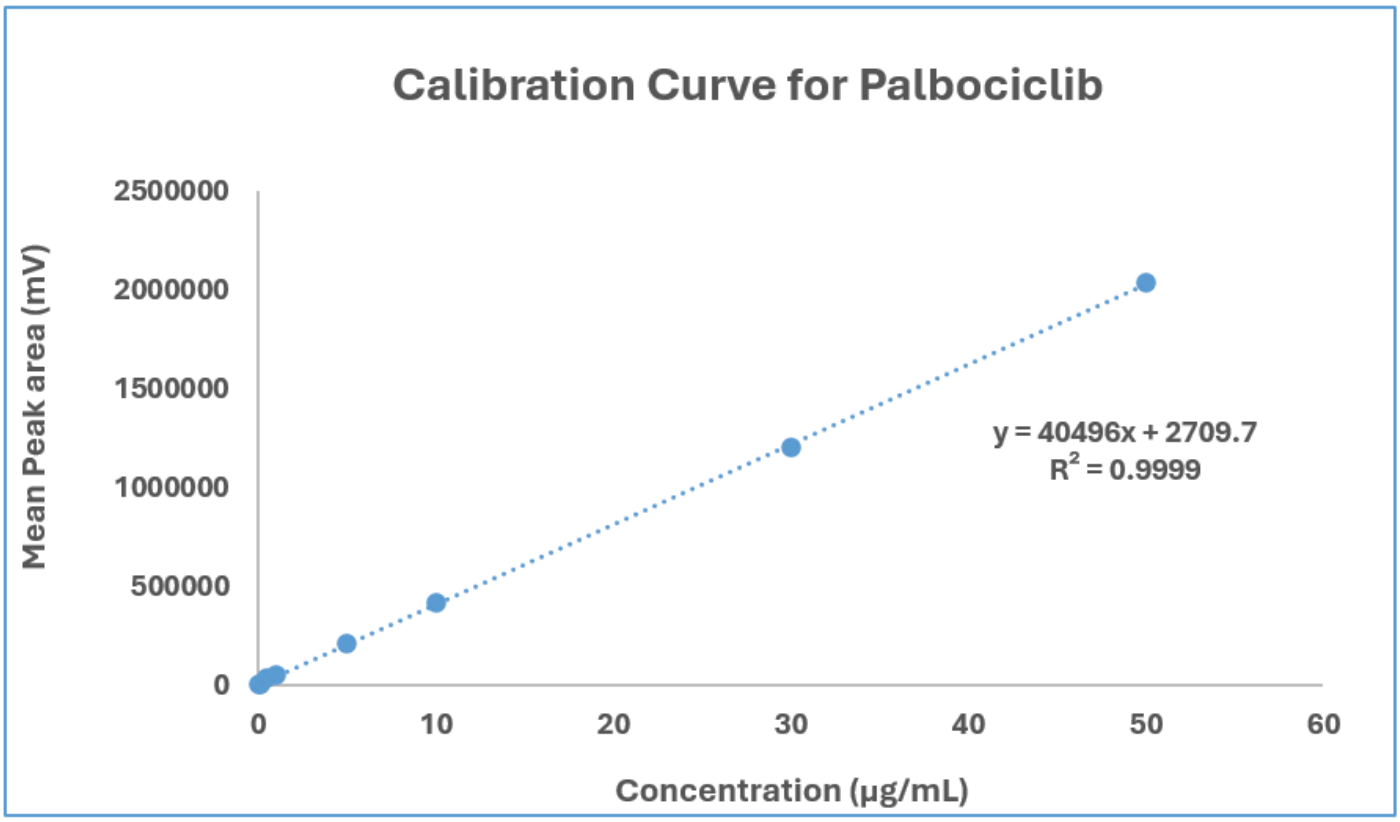


Figure 12

Calibration Curve for Palbociclib (0.05 – 50 µg/mL)