RESEARCH PAPER

Analytical Quality By Design Approach For Development Of Validated RP-HPLC Method For Estimation Of Acalabrutinib And Capecitabine In Their Respective Formulations

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ABSTRACT

Analytical control of anticancer drugs requires highly reliable quantification methods due to their therapeutic sensitivity and clinical significance. This study applies an Analytical Quality by Design (AQbD) framework to develop and validate RP-HPLC methods for acalabrutinib and capecitabine. The Analytical Target Profile was defined, and potential chromatographic factors were screened using risk assessment tools to identify critical method parameters for each drug. These parameters were optimized using a Box–Behnken response surface design to establish a statistically supported Method Operable Design Region (MODR), ensuring robust performance under variable analytical conditions.

Acalabrutinib was separated on a Zorbax XDB-C18 column (250 × 4.6 mm, 5 µm) using phosphate buffer (pH 6.4):methanol (80:20, v/v) at 1.0 mL·min⁻¹ with a 15-min run time. Capecitabine was determined on a Hypersil BDS C8 column (250 × 4.6 mm, 5 µm) using phosphate buffer (pH 4.0):acetonitrile (80:20, v/v) at 1.2 mL·min⁻¹ in 15 min.

The obtained MODR demonstrated a wide operational flexibility and enhanced method reliability for routine quality control. Validation according to ICH Q2 confirmed linearity ($r^2 = 0.999$), accuracy within 98–102%, precision with %RSD < 2%, robustness to deliberate variations, and 48-h solution stability. The developed AQbD-based methods are therefore suitable for routine pharmaceutical analysis of the selected anticancer drugs.

Keywords: Analytical Quality by Design, RP-HPLC, Anticancer, Method Development

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INTRODUCTION

Cancer remains one of the most serious global health challenges, responsible for millions of deaths each year, and the development of effective anticancer drugs is essential for controlling disease progression and improving patient outcomes. Among the various therapeutic agents available, small-molecule chemotherapy and targeted drugs play a critical role in inhibiting malignant cell growth through diverse molecular mechanisms.Acalabrutinib ($C_{25}H_{24}N_6$) is a selective Bruton's tyrosine kinase inhibitor used in chronic lymphocytic leukemia and mantle cell lymphoma, acting through suppression of B-cell receptor signaling [1]. Capecitabine ($C_{15}H_{22}FN_3O_6$) is an orally administered prodrug of 5-fluorouracil used in breast and colorectal cancers, where it interferes with DNA synthesis and induces apoptosis [2]. Given the clinical relevance of these drugs and increasing expectations for reliable analytical control, a structured and science-based approach to method development becomes essential. Analytical Quality by Design (AQbD) enables systematic understanding of method variables, ensuring robustness, regulatory confidence, and consistent assessment of the drug's quality throughout its lifecycle.There have been several HPLC methods developed for these individual anticancer drugs and combination with other drugs [3] [4] [5] [6] [7]. However, the analytical quality by design approach is not yet explored for all these drugs. In this study, risk-based approach and analytical quality by design approach have been used for the HPLC method development for the acalabrutinib, capecitabine. The developed HPLC method was validated according to ICH Q2 guidelines.

MATERIALS AND METHODS

Instrumentation and Software

Chromatographic analysis was performed on a Jasco LC-4600 RP-HPLC system equipped with a UV detector and a Fine-pack Jasco C18 column (250 mm × 4.6 mm, 5 µm). A Jasco V-630 UV spectrophotometer was used for wavelength selection. Supporting instruments included a Mettler Toledo analytical balance, Mettler Toledo pH meter, and Lab India ultrasonic sonicator.

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MATERIALS AND REAGENTS

The study utilized Acalabrutinib (Batch 205550007, 99.3% purity, 0.2% LOD) and Capecitabine (WS-005, 99.96% purity, 0.10% LOD. Dosage forms including Calquence 100 mg capsules, Caplive 500 mg tablets, along with analytical-grade reagents such as HPLC-grade water, methanol, acetonitrile, phosphoric acid, orthophosphoric acid, buffers, and sodium hydroxide were procured from Merck Life Science and RANKEM (India). A placebo excipient blend (≈100 g) was also used.

Chromatographic conditions

For analytical method validation, following chromatographic conditions were used.

Acalabrutinib

The chromatographic conditions for Acalabrutinib employed a Zorbax XDB-C18 column (250 × 4.6 mm, 5 µm) with an isocratic mobile phase consisting of phosphate buffer (pH 6.4) and methanol in the ratio 80:20 v/v. The flow rate was maintained at 1.0 mL/min, the detection wavelength was 238 nm, the injection volume was 20 µL, and the total run time was 15 minutes. The mobile phase served as both blank and diluent.

Capecitabine

The chromatographic conditions for Capecitabine utilized a Hypersil BDS C8 column (250 × 4.6 mm, 5 µm), with a mobile phase comprising phosphate buffer (pH 4.0) and acetonitrile (80:20 v/v) in isocratic mode. The analysis was performed at a flow rate of 1.2 mL/min, using a detection wavelength of 240 nm, injection volume 20 µL, and a run time of 15 minutes. The mobile phase was used as the blank.

Preparation of standard solutions

Acalabrutinib

Weigh accurately about 50 mg of Acalabrutinib Working standard and transfer to a 50 ml volumetric flask. Add 25 ml of diluent and sonicate to dissolve. Dilute to volume with diluent and mix. Transfer 1.0 ml of solution into a 10 ml of volumetric flask and dilute to volume with the diluent and mix.

Capecitabine

Weigh accurately about 25 mg of Capecitabine working standard and transfer to a 25 ml volumetric flask. Add 10 ml of diluent and sonicate to dissolve. Dilute to volume with diluent and mix. Transfer 1.0 ml of solution into a 10 ml of volumetric flask and dilute to volume with the diluent and mix.

Preparation of sample solutions

Acalabrutinib

Determine the average weight of 10 Capsules. Powdered it Weigh accurately about 88mg of Calquence 100mg Capsules sample powder (equivalent to 50mg of Acalabrutinib) and transfer to a 50 ml volumetric flask. Add 25 ml of diluent and sonicate to dissolve. Dilute to volume with diluent. Transfer 2ml of the solution to 10ml volumetric flask, dilute and mix. Filter the solution through 0.2µm nylon membrane filter.

Capecitabine

Weigh and transfer 40mg of sample powder into a 25 ml volumetric flask. Add about 10 ml of diluent and shake for 20 minutes by mechanical means or manually and further sonicate for 30 minutes. Dilute up to mark with diluent. Centrifuge this solution at 8000 rpm for 10 minutes. Decant the supernatant solution into another test tube and transfer 1.0 ml of supernatant solution into another 10 ml volumetric flask and make up the volume with diluent. Further transfer 1.0ml of solution into another 10 ml volumetric flask and make up the volume with diluent. Filter the solution through 0.2µm nylon membrane filter.

Analytical target profile (ATP), critical method parameters (CMPs), critical method attributes (CMAs) and risk assessment

Analytical Target profile is important element of analytical QBD. It helps to establish goals of the method developments and gives a strict criteria for method development. ATP was prepared for analytical method development. The ATP is shown in Table 1.

Table 1 Analytical Target Profile for Acalabrutinib, Capecitabine

Parameters	Details and Acceptance Limits
Objective	Estimation of selected anticancer agents
Selection of Target Analyte	Acalabrutinib, Capecitabine (Separate dosage forms)
Selection of Analytical Method	RP-HPLC

Accuracy	98-102%
Precision	% RSD <2%
Resolution	>2
Number of Theoretical Plates	>2000
Tailing Factor	<2

To identify Critical Method Parameters the Ishikawa Fishbone diagram (***Figure 2***)

Figures:

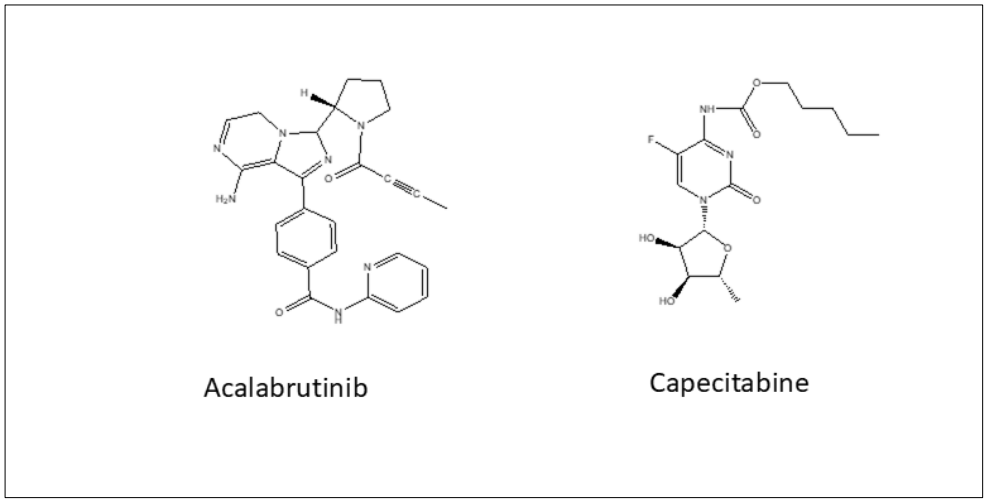

Figure 1 Chemical Structure of Acalabrutinib and Capecitabine.

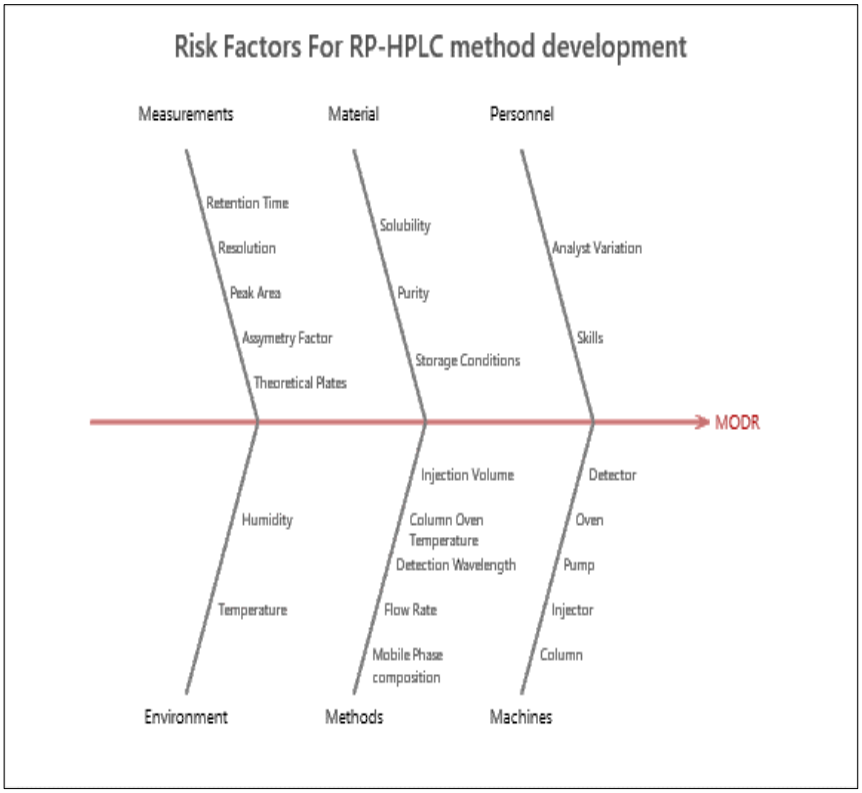

Figure 2 Cause effect analysis of the risk factors for HPLC method development using the Ishikawa Fishbone Diagram

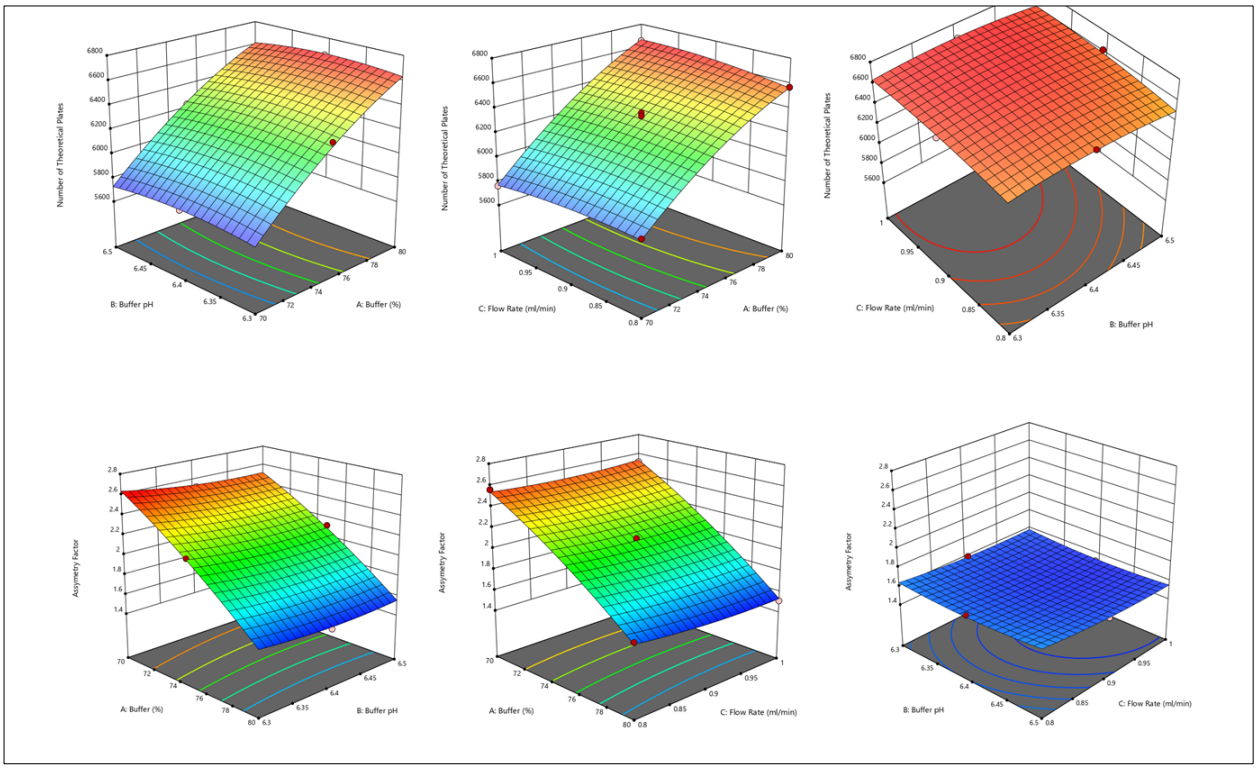

Figure 3 3D plots for effect of CMPs on CMAs for the Acalabrutinib

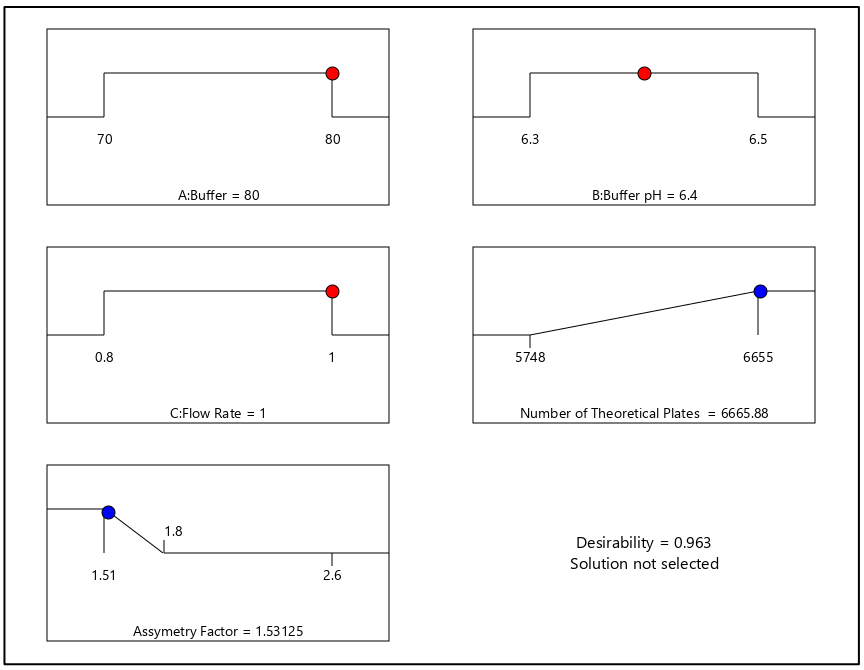

Figure 4 Numerical optimization desirability ramps for the Acalabrutinib

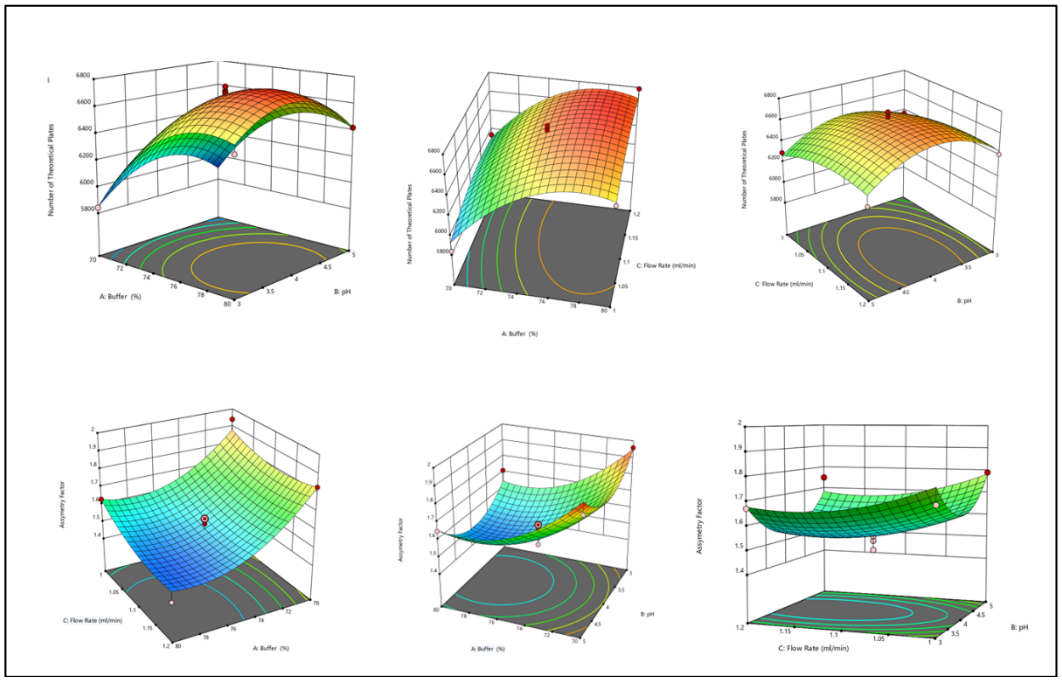

Figure 5 3D plots for effect of CMPs on CMAs for the Capecitabine

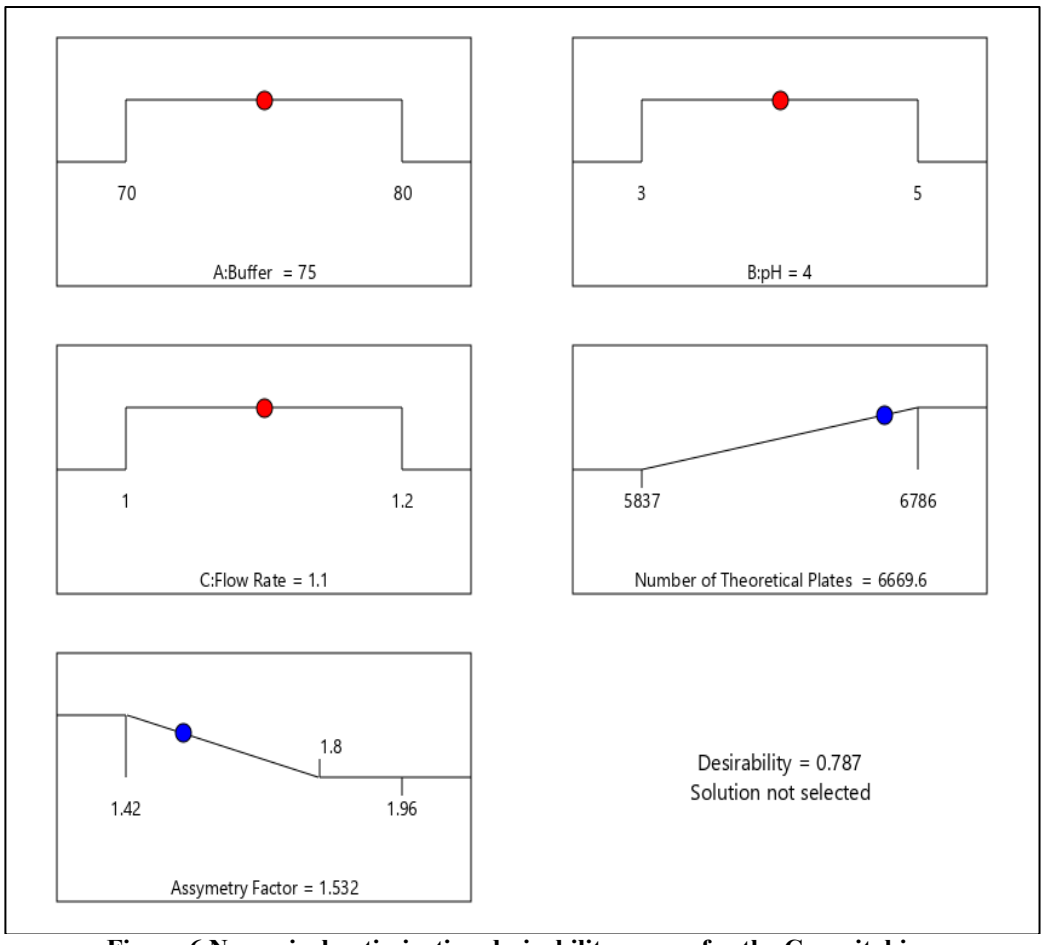

Figure 6 Numerical optimization desirability ramps for the Capecitabine

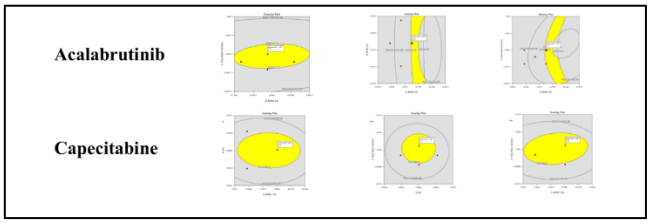

Figure 7 Method Operable Design Region for Acalabrutinib, Capecitabine

was used and cause effect relationship between various risk factors was established.

RESULTS AND DISCUSSION

Screening and verification of the critical method parameters (CMPs)

Acalabrutinib

During the initial phase of method development, a comprehensive risk assessment was carried out using an Ishikawa cause-and-effect evaluation followed by Failure Mode and Effect Analysis (FMEA) to determine which chromatographic factors had the highest likelihood of influencing the performance of the method. All potential chromatographic variables—including mobile phase components, pH, organic solvent composition, column dimensions, flow rate, temperature, injection volume, gradient strength, diluent composition and detection wavelength—were initially listed and qualitatively evaluated for their expected impact on method performance.

The predefined critical method attributes (CMAs) for this method were Number of Theoretical Plates and Asymmetry Factor, as these directly reflect column efficiency and peak acceptability during quantitative analysis. Each potential parameter was assessed based on its anticipated effect on these CMAs, and relative risk was quantified using RPN, where severity of impact on CMAs, likelihood of routine variation, and detectability through system suitability were scored. Parameters with the highest overall Risk Priority Number (RPN) were prioritized for further experimental evaluation, while low-risk parameters were fixed at a controlled level for subsequent work.

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Table 2 Risk assessment for CMAs and CMPs for HPLC method development for Acalabrutinib

Parameter	S	O	D	RPN	Risk Ranking	Outcome
% Buffer in Mobile Phase	5	4	3	60	High	**Selected CMP**
Buffer pH	5	3	3	45	High	**Selected CMP**
Flow Rate	4	3	3	36	High	**Selected CMP**
Column Temperature	3	2	3	18	Medium	Fixed
Buffer Concentration	3	2	3	18	Medium	Fixed
Gradient Profile	3	2	2	12	Low–Medium	Fixed
Injection Volume	2	3	3	18	Low–Medium	Fixed
Detection Wavelength	2	2	2	8	Low	Fixed
Diluent	2	2	3	12	Low	Fixed

presents the summarized Risk assessment outcomes. % buffer in the mobile phase, buffer pH, and flow rate demonstrated the highest cumulative risk scores, indicating that variability in these parameters could significantly impair chromatographic efficiency or peak symmetry. In contrast, parameters such as column temperature, injection volume, wavelength and buffer concentration showed low RPN values and were therefore controlled at constant values in subsequent experiments.

Based on the results of this risk assessment, three parameters were classified as Critical Method Parameters (CMPs) and carried forward for structured statistical optimization through Box–Behnken experimental design: A –% Buffer in Mobile Phase, B – Buffer pH, C – Flow Rate. For Acalabrutinib, preliminary chromatographic trials were carried out using phosphate buffer with methanol and acetonitrile as organic modifiers on a C18 stationary phase to evaluate baseline retention, peak symmetry, and system pressure behavior. Initial isocratic compositions in the range of 70–90% aqueous buffer and pH between 6.3–6.5 showed stable retention and acceptable peak shape, whereas lower buffer proportions caused peak distortion and co-elution. Flow rates from 0.8–1.2 mL/min were evaluated; pressures remained within instrument limits and theoretical plates showed sensitivity to flow variation. Based on these empirical observations, % buffer, pH and flow were recognized as key influencing factors and their workable ranges were defined as 70–90%, pH 6.3–6.5 and flow 0.8–1.2 mL/min.

Capecitabine

A preliminary risk assessment using Ishikawa analysis followed by FMEA was performed to identify chromatographic variables with the highest probability of affecting method performance. Potential parameters were screened against the predefined CMAs—Number of Theoretical Plates and Asymmetry Factor and ranked by Risk Priority Number (RPN).

The results are summarized in **Table *3***.

Table 3 Risk Assessment for CMAs and CMPs for HPLC method development for Capecitabine

Parameter	S	O	D	RPN	Risk Ranking	Outcome
% Buffer in Mobile Phase	5	4	3	60	High	Selected CMP
Buffer pH	5	3	3	45	High	Selected CMP

Flow Rate	4	3	3	36	High	Selected CMP
Column Temperature	3	2	3	18	Medium	Fixed
Injection Volume	2	3	3	18	Medium	Fixed
Detection Wavelength	2	2	2	8	Low	Fixed
Diluent	2	2	3	12	Low	Fixed

Based on the RPN outcomes, % buffer in mobile phase (A), buffer pH (B), and flow rate (C) were identified as Critical Method Parameters and taken forward for DOE optimization.

For Capecitabine, initial experimental runs were performed using phosphate buffer and acetonitrile mixtures on a C8 column to assess peak shape and resolution. Screening of mobile phase compositions revealed that an aqueous buffer proportion below 70% resulted in inadequate retention, while buffer above 90% led to excessive run times without chromatographic improvement. pH conditions within the range of 3–5 were explored, with notable changes in peak symmetry observed across the evaluated pH range. Flow rate adjustments between 1.0–1.2 mL/min demonstrated measurable variation in theoretical plates and asymmetry. These screening outcomes supported identifying % buffer, pH and flow rate as CMPs, and establishing the working ranges of 70–90%, pH 3–5 and flow 1.0–1.2 mL/min.

Optimization of the critical method parameters (CMPs) using response surface methodology (RSM)

The obtained parameters from the screening experiments were extensively studied using response surface methodology. Response surface methodology is one of the best statistical tools for opti mization, which helps in determining the individual and combination effect of the experimental variables. Box Behenken Design (BBD), an RSM-based design, was employed to optimize and finalize the CMPs. BBD was useful for identifying the method operable design space region (MODR) by employing numerical and graphical optimization.

Acalabrutinib

The parameters obtained from the screening experiments were further evaluated using response surface methodology (RSM), a statistical tool that enables assessment of both individual and interactive effects of variables during method optimization. A Box–Behnken Design (BBD) was employed to optimize and finalize the CMPs, generating 17 experimental runs, which were performed and the resulting responses were incorporated into the design. The data were analyzed through ANOVA using a quadratic model, which was found to be statistically significant, while the lack of fit was non-significant, confirming model adequacy.

The coefficient estimates for linear, squared, and interaction terms are presented in equation XX and p-values are given in ***Table 5***.

Table 4 Box Behenken Design for the Acalabrutinib

Factor 1	Factor 2	Factor 3	Response 1	Response 2
A:Buffer (%)	B:Buffer pH	C:Flow Rate (ml/min)	Number of Theoretical Plates	Assymetry Factor
70	6.5	0.9	5794	2.49
75	6.5	1	6237	2.12
75	6.4	0.9	6312	2.08
75	6.5	0.8	6178	2.14
75	6.4	0.9	6341	2.1
75	6.3	0.8	6263	2.16
70	6.3	0.9	5748	2.6
75	6.3	1	6299	2.18
80	6.5	0.9	6618	1.57
80	6.3	0.9	6592	1.59
70	6.4	1	5763	2.52
70	6.4	0.8	5817	2.56
75	6.4	0.9	6368	2.06
75	6.4	0.9	6245	2.08
80	6.4	1	6655	1.51
80	6.4	0.8	6571	1.63
75	6.4	0.9	6310	2.11

Table 5 P-Values for the Individual, Squared and combined effects of CMPs on CMAs for Acalabrutinib

	Intercept	A	B	C	AB	AC	BC	A^2	B^2	C^2
TP	6315.2	**414.25**	-9.375	15.625	-5	34.5	5.75	**-84.975**	-42.225	-28.725
p-values		**< 0.0001**	0.5847	0.37166	0.83511	0.17977	0.81099	**0.00070**	0.1034	0.2436
AF	2.086	**-0.487375**	**-0.02625**	-0.002	0.00225	-0.002	-0.001	**-0.059225**	**0.035775**	0.0028225
p-values		**< 0.0001**	**0.0279**	0.07311	0.13766	0.17988	0.48055	**0.00027**	**0.0292**	0.0677

Table 6 Box Behenken Design for the Capecitabine

	Factor 1	Factor 2	Factor 3	Response 1	Response 2
Run	**A:Buffer**	**B:pH**	**C:Flow Rate**	**Number of**	**Assymetry Factor**

				Theoretical Plates	
	%		ml/min		
1	70	4	1	5837	1.94
2	80	5	1.1	6421	1.65
3	75	4	1.1	6593	1.59
4	70	5	1.1	5958	1.88
5	75	4	1.1	6736	1.47
6	80	3	1.1	6452	1.71
7	75	4	1.1	6689	1.53
8	80	4	1.2	6786	1.42
9	75	4	1.1	6627	1.56
10	75	5	1.2	6319	1.75
11	75	3	1.2	6412	1.67
12	80	4	1	6514	1.63
13	75	5	1	6293	1.79
14	70	3	1.1	5842	1.96
15	70	4	1.2	6148	1.83
16	75	3	1	6371	1.71
17	75	4	1.1	6703	1.51

Table 7 P-Values for the Individual, Squared and combined effects of CMPs on CMAs for Capecitabine

	Intercept	A	B	C	AB	AC	BC	A^2	B^2	C^2
TP	6669.6	**298.5**	-10.75	**81.25**	-36.75	-9.75	-3.75	**-264.425**	**-236.925**	-83.925
p-values		**<0.00001**	0.75688	**0.04511**	0.46117	0.84222	0.9389	**0.00007**	**0.00013**	0.11099
AF	1.532	**-0.15**	0.0025	-0.05	0.005	-0.025	2.89591E-18	**0.1215**	**0.1465**	0.05155
p-values		**0.0005**	0.92222	0.08225	0.89902	0.49711	1.0000	**0.00091**	**0.0035**	0.17399

For R1, CMPs-A, A^2 and For R2, CMPs-A, A^2, B, B^2 have the significant effect.

Number of Theoretical Plates= 6315.2 + 414.25A-9.375B + 15.625C-5AB + 34.5AC + 5.75BC-84.975A^2-42.225B^2 - 28.725C^2

Assymetry Factor= 2.086 -0.48375A -0.02625B -0.02C + 0.0225AB + -0.02AC -0.01BC -0.05925A^2 + 0.03575B^2 + 0.02825C^2

The 3D plots was analyzed for the relation between CMPs and Responses. The R1 (theoretical plates) was maximum (6632.28) at higher % buffer (80%), lower pH (6.3) and higher flow rate (1.0 mL/min); and minimum (5724.78) at lower % buffer (70%), lower pH (6.3) and moderate flow rate (1.0 mL/min). The R2 (asymmetry factor) was maximum (2.63325) at lower % buffer (70%), higher pH (6.5) and higher flow rate (1.0 mL/min); and minimum (1.55325) at higher % buffer (80%), higher pH (6.5) and higher flow rate (1.0 mL/min).

The numerical optimization was carried out to determine the most favorable combination of CMPs for a robust chromatographic method. The variables buffer percentage (CMP-A), buffer pH (CMP-B), and flow rate (CMP-C) were optimized within the defined experimental ranges with the objective of maximizing theoretical plates and minimizing asymmetry. The solution proposed buffer at 80%, pH 6.4156, and a flow rate of 0.9738 mL min^{-1}. At these conditions, the predicted responses were 6663.21 for theoretical plates (R1) and 1.528 for asymmetry (R2).

The global desirability value obtained was 0.968, indicating approximately 97% achievement of the optimization criteria and confirming the suitability of the selected conditions.

Capecitabine

Response surface methodology (RSM) using a Box–Behnken design was employed to evaluate and optimize the chromatographic performance for capecitabine. A total of 17 experimental runs were generated based on three control method parameters: percentage of buffer in the mobile phase (A), buffer pH (B) and flow rate (C), and the obtained responses for number of theoretical plates (TP) and asymmetry factor (AF) were incorporated into the model. The experimental data were analyzed using a quadratic model through ANOVA, which was found to be statistically significant for both responses, while the lack of fit remained non-significant, confirming the suitability of the model.

For TP, buffer percentage (A), flow rate (C), and the squared terms A^2 and B^2 exhibited significant effects, whereas for AF, the linear term A and the quadratic terms A^2 and B^2 were identified as significant contributors.**Number of Theoretical Plates=** 6669.6 + 298.5A -10.75B + 81.25C - 36.75AB - 9.75AC - 3.75BC -264.425A^2-236.925B^2-83.925C^2

Assymetry Factor= 1.532 - 0.15A + 0.0025B - 0.05C + 0.005AB - 0.025AC + 2.89591e^{-18}BC + 0.1215A^2 + 0.1465B^2 + 0.0515C^2

The 3D plots were analyzed to evaluate relationships between the CMPs and responses for capecitabine. R1 (number of theoretical plates) reached a maximum of 6505.58 at higher buffer (% A = 80%), higher pH (B = 5) and higher flow rate (C = 1.2 mL·min^{-1}), and a minimum of 5665.07 at lower buffer (% A = 70%), lower pH (B = 3) and lower flow rate (C = 1.0 mL·min^{-1}). R2 (asymmetry factor) showed a maximum of 2.029 at lower buffer (% A = 70%), lower pH (B = 3) and lower flow rate (C = 1.0 mL·min^{-1}), and a minimum of 1.619 at higher buffer (% A = 80%), lower pH (B = 3) and lower flow rate (C = 1.0 mL·min^{-1}).

For capecitabine, the numerical optimization was carried out to determine the most favorable combination of CMPs for a robust chromatographic method. The variables buffer percentage (CMP-A), buffer pH (CMP-B), and flow rate (CMP-C) were optimized within the defined experimental ranges with the objective of maximizing theoretical plates and minimizing asymmetry. The solution proposed buffer at 78.1189%, pH 3.96059, and a flow rate of 1.15682 $mL \cdot min^{-1}$. At these conditions, the predicted responses were 6769.57 for theoretical plates (R1) and 1.46507 for asymmetry (R2).
The global desirability value obtained was 0.931, indicating approximately 93% achievement of the optimization criteria and confirming the suitability of the selected conditions for capecitabine.

Identification of method operable design region (MODR)
The method operable design region was found out by graphical optimization. The MODR for Acalabrutinib, Capecitabine are shown in
Figure 7. The method operable design is highlighted with yellow shade. The optimum method conditions, is well within method operable design region.

Method validation

The method validation was carried out according to ICH Q2 guidelines.

System Suitability

System suitability was evaluated by analyzing five replicate injections of standard solutions of Acalabrutinib, Capecitabine under the optimized chromatographic conditions. The parameter assessed was peak area repeatability, and the %RSD was calculated to determine system precision. All analytes exhibited %RSD values below 2%, with Acalabrutinib (0.49%), Capecitabine (1.13%) indicating excellent repeatability and confirming that the system met the predefined suitability criteria.

LOD LOQ
The Quantitation and Detection Limits were calculated by use of standard deviation of the response and slope. The LOD and LOQ for all four drugs are shown in Table 8.

Table 8 Detection and Quanitation Limits for the HPLC method development of Acalabrutinib, Capectiabine

Drug	UV LOD	UV LOQ	HPLC LOD	HPLC LOQ
Acalabrutinib	0.3822	1.1583	1.0719	3.2480
Capecitabine	0.3699	1.1210	5.4042	16.3765

Linearity
The linearity was verified by injecting the five different concentrations of the drug between 50% to 150% concentration of the target concentration. The results for linearity are given in Table 9.

Table 9 Results for analytical method validation of Acalabrutinib, Capecitabine

Test	Details	Acceptance Criteria	Acalabrutinib	Capecitabine
Linearity	Range	-	50-150 ug/ml	50-150 ug/ml
	Correlation Coefficient (r^2)	>0.99	0.9997	0.9993
Accuracy (% Recovery)	n=9 (triplicates of std addition 50%, 75% and 150%)	98-102%	99.66±0.61	99.77±0.25
Method Precision	N=6	RSD <2%	0.97	0.56
Intermediate Precision	N=12	RSD <2%	1.65	0.54
Robustness (RSD)	Change in Column lot (n-2)	RSD <2%	0.44	0.21
	Change in flow rate (± 0.2 ml/min)	RSD <2%	0.82	0.79
	Change in wavelength (± 0.2 nm)	RSD <2%	0.73	1.61
	Change in mobile phase pH (± 2%)	RSD <2%	0.76	1.91
Stability of analytical solution	48 Hrs	RSD <2%	0.64	0.37

Specificity/Selectivity
Selectivity was performed by injecting the diluent blank solution, excipient blend, system suitability solution, test solution. The diluent blank solution, excipient blend solution did not show any peak at the retention time of the Acalabrutinib, Capecitabine.

Precision

Method precision was evaluated by performing six replicate injections of the test solution at the same concentration under identical operating conditions, and the precision was expressed in terms of %RSD of peak areas.

Intermediate precision (ruggedness) was assessed by analyzing a total of twelve injections, where six replicates were performed by Analyst 1 using Column 1 on System 1 on Day 1, and the remaining six replicates were carried out by a different analyst on a different day using an independent system and column. This design enabled evaluation of the method's reproducibility across analysts, instruments, columns, and days. Results are shown in Table 9.

Accuracy

Accuracy of the method was established through recovery studies conducted using the standard addition technique. Known quantities of the analyte were spiked into the pre-analyzed sample matrix at five concentration levels corresponding to 50%, 75%, 100%, 125% and 150% of the target concentration, with each level analyzed in triplicate. The percentage recovery and %RSD at each level were calculated to determine the closeness of the measured values to the true added concentrations, thereby demonstrating the accuracy and reliability of the analytical method across a wide concentration range. Results for the Accuracy shown in Table 9.

Robustness

Robustness of the method was evaluated by intentionally introducing deliberate variations in key chromatographic parameters and assessing their impact on system suitability results. The parameters subjected to variation included change in column lot, alteration of flow rate (±0.2 mL/min), modification of detection wavelength (±2 nm), and adjustment of mobile phase composition (±2%). The results were compared with those obtained under optimized conditions to determine the method's capacity to remain unaffected by small but purposeful changes, thereby confirming its reliability during routine analysis (Table 9).

Solution stability

Solution stability was assessed to determine the integrity of the sample and standard solutions over a defined period under normal laboratory storage conditions. Both sample and standard solutions were stored at room temperature and analyzed at predetermined time intervals (initial, 12 h, 24 h, and 48 h) using the optimized chromatographic conditions. The results were evaluated by comparing the measured peak areas and system suitability parameters against the initial values. Percentage assay difference and %RSD were calculated to ensure that no significant degradation or variability occurred, thereby confirming the stability of the solutions for the specified duration, results are shown in Table 9.

CONCLUSION

The Analytical QbD approach enabled systematic development and optimization of RP-HPLC methods for acalabrutinib, capecitabine. Individual risk assessments identified distinct critical method parameters for each drug, which were optimized using Box–Behnken RSM to establish robust method conditions and a well-defined MODR. The finalized methods complied with the predefined ATP and fulfilled ICH Q2 validation criteria, showing excellent linearity, precision, accuracy, robustness, and solution stability. These results confirm suitability of the optimized methods for routine quality control of the respective dosage forms.

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