

RP-HPLC Method Development and Validation for Simultaneous Quantitation of Sitagliptin Phosphate and Empagliflozin: QbD Driven

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Abstract

The recently approved combination of Sitagliptin Phosphate and Empagliflozin (September 2024) is widely used to improve glycemic control in adults with type 2 diabetes mellitus. Reported HPLC method for the quantification of Sitagliptin Phosphate and Empagliflozin lack the required robustness for regulatory approval and ignore interaction effects between method parameters. Also there is a lack of structured, risk-based methodology in the literature to optimize the separation of both drugs. Till date, no robust Quality by Design (QbD) -based RP-HPLC method has been systematically developed for their simultaneous estimation, creating a gap in reliable and optimized analytical procedures for routine quality control. This study aims to develop and validate a simple, robust, and efficient RP-HPLC method using a QbD approach for the simultaneous quantification of these drugs in a laboratory-prepared blend. Critical method parameters like percentage of acetonitrile (ACN), percentage of o-phosphoric acid (OPA), and flow rate were initially screened using a Plackett–Burman design and further optimized using a Box–Behnken design with response surface methodology. Key analytical responses, including retention time, theoretical plates, and tailing factor, were evaluated and optimized. Chromatographic separation was

achieved on an XR ODS II C18 column (150 × 3 mm, 5 µm) at 25 °C, using a mobile phase of ACN and 0.1% OPA in water (70:30, v/v), at a flow rate of 1.0 mL/min, with detection at 254 nm. The retention times were 2.626 min for Sitagliptin Phosphate and 4.041 min for Empagliflozin. In AQbD multiple factors can be varied simultaneously to optimise it as well as enables regulatory flexibility in comparison to traditional HPLC methods where one factor is varied at a time. Validation results complied with ICH Q2(R2) guidelines. Its robustness and efficiency make it particularly valuable for simultaneous estimation of this newly approved drug combination in bulk and formulation studies.

Keywords: Sitagliptin Phosphate, Empagliflozin, QbD, RP – HPLC, Validation

Introduction

Diabetes is a long-term metabolic condition driven by high blood glucose or blood sugar levels. It can lead to serious implications. Type 2 diabetes mellitus (T2DM) is the most prevalent form and is associated with long-term complications such as cardiovascular diseases, nephropathy, neuropathy, and retinopathy. Most of the people (422 million) with diabetes globally reside in low- and middle-income nations, and the disease is directly responsible for 1.5 million fatalities annually (1). The growing global

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burden of T2DM necessitates effective and patient-friendly therapeutic strategies, particularly in low- and middle-income countries where disease prevalence is high. Combination therapy targeting multiple physiological pathways has emerged as a key approach for optimal glycaemic control.

Sitagliptin Phosphate (SITA) is a selective dipeptidyl peptidase-4 (DPP-4) inhibitor that prevents the degradation of incretin hormones, primarily glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (2). This enhances glucose-dependent insulin secretion and suppresses glucagon release. In contrast, Empagliflozin (EMPA) is a sodium-glucose co-transporter-2 (SGLT2) inhibitor that lowers plasma glucose by reducing renal glucose reabsorption in the proximal convoluted tubule, thereby promoting urinary glucose excretion (3). The complementary mechanisms of these drugs provide improved glycaemic control without significantly increasing the risk of hypoglycemia. The fixed-dose combination of SITA (Figure 1A) and EMPA (Figure 1B) approved in September 2024, offers therapeutic advantages including simplified dosing and improved patient adherence (4). This has increased the need for reliable analytical methods for their simultaneous estimation in bulk and formulation studies.

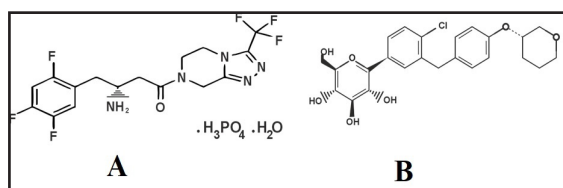


Figure 1: Structure of SITA (A) and EMPA (B)

Analytical method development using conventional One-Factor-at-a-Time (OFAT) approaches often fails to consider interactions between variables, leading to suboptimal robustness. In contrast, the Quality by Design (QbD) approach provides a systematic framework by identifying critical method parameters (CMPs) and their impact on critical quality attributes (CQAs) through Design of Experiments (DoE) (5,6). This enables the development of a robust

and reliable method within a defined design space.

A review of the literature reveals that only a few spectrophotometric methods (8,10) and one conventional RP-HPLC method (9) have been reported for the simultaneous estimation of Sitagliptin and Empagliflozin. QbD approach also enables regulatory flexibility by defining a Method Operable Design Region (MODR), reducing out-of-specification (OOS) results, and strengthening regulatory submissions to agencies like the USFDA and EMA (7). Recently, ICH has announced a new guideline, ICH Q14 (11), for analytical procedure development is focusing on a proactive, science-driven approach rather than traditional reactive testing. Additionally, it focuses on applying an enhanced approach to development which can leads to more robust analytical procedures, better understanding of the impact of analytical procedure parameters and more flexibility for lifecycle management. While reported HPLC method used a minimal approach to the HPLC method development. Optimized chromatographic conditions under QbD principles can results in substantial savings in both cost and time. This improves the efficiency and dependability of results, which is particularly beneficial for routine laboratory analysis (12). However, reported HPLC method lack a systematic QbD-based optimization strategy and do not adequately address method robustness or design space establishment.

Materials and Methods

Chemicals and reagents

SITA and EMPA were procured as gift sample from LEE Pharma Ltd. Andhra Pradesh, India and Metrochem API Pvt. Ltd, Andhra Pradesh, India individually. Excipients like Cross povidone, Colloidal Silicon Dioxide, Sodium Stearyl Fumarate (SSF), Lactose, Polyethylene Glycol (PEG) and HPLC grade Methanol (MeOH), Acetonitrile (ACN) and Orthophosphoric acid (OPA) were obtained from Merck Life Science Pvt Ltd, Mumbai, India.

Instrumentation and chromatographic conditions

A Shimadzu, LC 20 AD HPLC system (Shimadzu Corporation, Japan) was linked to Empower 2 software, comprising of binary pump, UV detector and column heater. An ultra-sonicator (Toshcon), and digital pH meter (LI-120, Elico) were also employed in this research. The optimisation procedure had utilised XR ODS II C18 (150 × 3 mm, 5 µm) column, maintained at 25 °C. The FR of eluent was set at 1 mL/min and elute was analysed at 254 nm. A 20 µl injection volume was utilized. The optimised mobile phase (ACN: 0.1 % OPA in water (70:30 % v/v)) was filtered through a 0.45 µm membrane filter and utilized for further analysis.

Mobile phase preparation

To prepare the mobile phase, 0.1 % OPA was prepared by diluting 1 mL of OPA with HPLC grade water up to 1000 mL. ACN and 0.1 % OPA in water in a ratio of 70:30 % v/v were mixed and filter through 0.45 µm nylon membrane filter. The resultant solution is set away until it is needed again.

Diluent preparation

MeOH was used as diluent.

Preparation of standard solution

100 mg of SITA and 25 mg of EMPA, each precisely weighed, were transferred into a 100 mL volumetric flask (VF) and dissolved in MeOH by sonication to make a stock solution. To achieve a concentration of 1000 µg/mL SITA and 250 µg/mL EMPA, the flask was thoroughly shaken and the volume was adjusted with diluent.

Preparation of sample solution

The laboratory prepared blend was prepared by mixing 100 mg SITA, 25 mg EMPA and commonly utilised excipients like, Cross povidone, Colloidal Silicon Dioxide, SSF, Lactose, and PEG (13). To a weighed synthetic mix of 250 mg (equivalent to one tablet), 25 mL of MeOH was added, and was sonicated. Later, dilution was done to 100 mL by diluent. Filtration

was carried out utilising nylon filter. Further dilutions were done to get the concentration within linearity range. In triplicate, the experiment was carried out. The assay percentage was used to analyse the outcome.

QbD approach to RP-HPLC method development

During method development, key chromatographic parameters including mobile phase composition, flow rate (FR), detection wavelength (DL), column type, and column temperature were systematically optimized to achieve efficient, robust, and reproducible separation. A Quality by Design (QbD) approach was employed to ensure structured method development. The process began with defining the Analytical Target Profile (ATP), which specified the desired method performance in terms of adequate resolution, acceptable peak symmetry, and reasonable retention times. Based on ATP, Critical Analytical Attributes (CAAs) namely retention time (R_t), theoretical plates (N), tailing factor (T_r), and resolution (R_s) were identified, as these directly influence method performance and reliability. Potential Critical Method Variables (CMVs), including flow rate, percentage of o-phosphoric acid (%OPA), percentage and type of organic modifier (acetonitrile or methanol), column temperature, detection wavelength, injection volume, and stationary phase, were initially considered. A preliminary risk assessment using an Ishikawa diagram was conducted to qualitatively identify variables most likely to impact CAAs. For factor screening, a Plackett–Burman Design (PBD) with 16 experimental runs, including 3 center points, was employed using Design-Expert. The inclusion of center points enabled estimation of experimental error and assessment of curvature effects. Independent variables such as FR (A), column temperature (B), DL (C), injection volume (D), stationary phase (E), % organic modifier (F), type of organic modifier (G), and % OPA (H) were evaluated against responses (N , T_r , R_t , and R_s). Significant factors were identified using Pareto charts. Based on screening results, critical variables were further optimized using

a Box–Behnken Design (BBD). Three factors namely, % ACN, FR, and % OPA were selected for optimization due to their significant influence on chromatographic performance. The chosen range for % ACN (60–80% v/v) was based on preliminary trials, where lower percentages resulted in prolonged retention times and peak broadening, while higher percentages caused insufficient resolution between analytes. Similarly, FR (0.8–1.2 mL/min) and % OPA (0.05–0.15%) ranges were selected to balance analysis time, peak symmetry, and column backpressure. A total of 15 experimental runs (3 factors, 3 levels) were generated, and experiments were performed in randomized order to minimize systematic bias. Replication at the center point ensured reliable estimation of pure error and improved model robustness. The experimental data were fitted to multiple models, including linear, interaction (2FI), and quadratic models, with model selection based on statistical significance and goodness-of-fit parameters. Analysis of variance (ANOVA) was used to evaluate model significance, while regression analysis provided polynomial equations describing factor–response relationships. Model validation was performed using diagnostic plots (predicted vs. actual values, residual plots) and lack-of-fit tests to ensure adequacy and predictive capability. Response surface plots and contour plots were generated to visualize the effect of CMVs on CAAs. Numerical optimization using desirability functions was applied to identify optimal chromatographic conditions. The design space and Method Operable Design Region (MODR) were established using graphical optimization, ensuring robust method performance within defined parameter ranges. Future studies may extend the method to marketed formulations to further confirm its practical applicability.

Method validation

The parameters, like selectivity, system suitability test, linearity, sensitivity, precision, specificity, accuracy, and robustness were accomplished in accordance with International Conference on Harmonization (ICH) Q2(R2) (14).

System suitability

System suitability was evaluated by injecting six replicate injections of a standard solution containing SITA (100 µg/mL) and EMPA (25 µg/mL) into the HPLC system. Parameters such as retention time (R_t), theoretical plates (N), resolution (R_s), and tailing factor (T_r) were assessed to ensure adequate system performance (15).

Specificity and selectivity

To ensure the specificity of a developed method, a chromatogram of the blank, standard, placebo, & sample were recorded. The selectivity was further assessed using the peak purity tests to confirm that the chromatographic peak of the analyte is attributable to a single constituent.

Linearity

Linear response was observed for the concentration ranging from 100 - 600 µg/mL for SITA, and 25 - 150 µg/mL for EMPA. Different aliquots of standard stock solution were diluted in range 1, 2, 3, 4, 5, 6 mL in a series of 10 mL VFDiluent was utilised for dilutions. The calibration curve ($n = 3$) was obtained by graphically plotting the mean peak area against the concentration and correlation coefficient values (R^2), Slope and Y intercept were obtained.

Precision

Repeatability can be assessed by repeated scanning ($n = 6$) of binary mixture comprising of 100 µg/mL of SITA and 25 µg/mL of EMPA without changing the parameters of developed method. The Intermediate Precision; within- day can be assessed by analysis of mixture comprising of 100, 300, and 600 µg/mL SITA and 25, 75, and 150 µg/mL EMPA in triplicate ($n = 3$) on an identical day. For between day, analysis of mixture comprising of SITA (100, 300 and 600 µg/mL), and EMPA (25, 75 and 150 µg/mL), were analysed three times ($n = 3$) on different days. For each analyte at different levels of concentrations, the percentage RSD was estimated.

Accuracy

Accuracy method was executed by spiking standards at three different levels (50%, 100%, and 150%) to preanalysed blend (200 µg/mL SITA and 50 µg/mL EMPA). The parameter was assessed in a triplicate for each level. The % recovery were calculated for each.

Sensitivity

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) for SITA and EMPA were determined in accordance with ICH. The estimation was based on the standard deviation of the response (σ) and the slope of the calibration curve (S), using the following equations:
 $LOD = 3.3 \times \sigma/S$ and $LOQ = 10 \times \sigma/S$

Robustness

The Robustness was performed three times by changing various parameters like FR (± 0.2 mL/min), DL (± 2 nm), organic phase (± 2 % v/v), and % of OPA (± 0.05).

Results and Discussion

RP-HPLC method development by QbD approach

The present study aimed to apply QbD approach to RP-HPLC method development and validation of SITA and EMPA in synthetic blend. Initially, preliminary experiments were carried out wherein different stationary phases and different eluents were attempted so as to get well separated and sharp peak. Different solvent combinations, such as MeOH, Water, ACN, and OPA, with varying FR were used to vary the mobile phase. Different solvent combinations, such as MeOH, Water, ACN, and OPA, with varying FR were used to vary the mobile phase. The sharp and resolved peak was obtained by employing XR ODS II C18 column (150 x 3 mm x 5 µm), ACN: 0.1 % OPA in water (70: 30 % v/v) as eluent flowing at rate of 1 mL/min in isocratic mode. Further screening and optimisation were done by the application of experimental design. The ATP of present study is to separate SITA and EMPA. The screening study was accomplished using PBD design wherein total 16 runs were carried out by incorporating 8 factors at two levels. Further analysis

of factors were done using Pareto chart (Figure 2). The variables were ranked from 1 to 8 based on their prioritised rank towards CAAs. Few factors were discovered to be positive on the outcome of the technique attributes, while others were antagonistic. The variable that crossed the t-value limit was deemed significant (t-value and Bonferroni limits as shown in Figure 2). In traditional Pareto charts, the most important factors influencing the independent variables are FR, % ACN, and % OPA as shown in Figure 2 (A) – Figure 2 (C). The length of each bar in the Pareto chart corresponds to the value of the regression coefficient of that factor. The optimization was carried out by using BBD wherein 15 runs were carried out in which 3 factors (% ACN, FR, % OPA) at 3 levels were taken (Table 1 and Table 2). It makes it easier to find the best chromatographic separation for SITA and EMPA. The variables that formed the central point were ACN: 0.1% OPA in water (70:30%v/v) and flow rate (1 mL/min), which were determined via design space selection. To determine the design space, the impact of these factors on the replies was assessed. Several polynomial models, including linear, second-order, and quadratic, were fitted to the response data. The high regression coefficient ($R^2 = 0.9981$), which greatly outperformed other models ($p < 0.0001$), indicated that the quadratic model was the best fit. After doing ANOVA tests on each model and determining metrics like sum of squares, F value, mean square, and p value for every response, it was discovered that the models N of SITA, T_f of SITA, and R_t of EMPA were significant. Response surface analysis was used to investigate the factor–response connection and likely interaction effects. Significant variables were used to create contour (2D) and response surface (3D) plots, while the third variable was kept constant at a predetermined level. The enormous curvatures created by the majority of the response surfaces on the method CAAs demonstrate the high degree of interaction among the examined CMVs. For a particular set of input variables, these charts are used to determine the response. Figure 3(A) depicts the effect of % ACN and FR on N of SITA, It can be understood that with the increase in FR and %

ACN, the N of SITA also increases. Figure 3(B) represents the effect of % ACN and FR on T_f of SITA, The 3d surface chart interprets as FR increases and % ACN decreases, the T_f of SITA decreases. Figure 3(C) depicts the effect of % ACN and FR on R_f of EMPA and it can be concluded, with the decrease in FR and increase in % ACN; the R_f of EMPA increases. Figure 3(D) displays Desirability plot and it illustrates the simultaneous optimisation of multiple chromato-

graphic responses. The contour plots in Figure 3 (D) represents the combined effect of % ACN and FR while keeping % OPA constant. The central red region in the graph represents the optimum zone where all the responses meet the desired criteria. The overlay plot illustrating the correlation between independent factors and responses for graphical optimization is presented in Figure 3 (E).

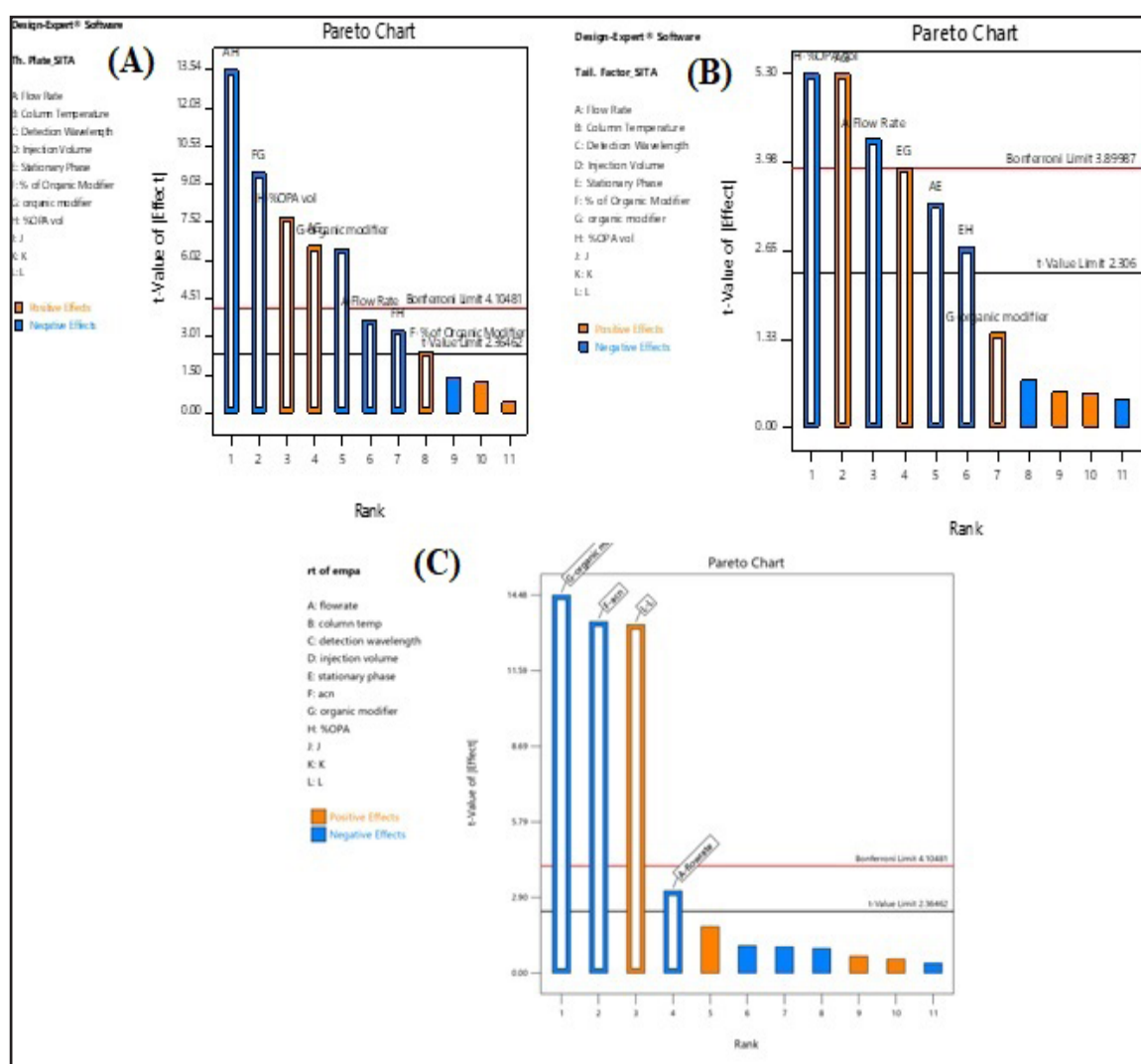


Figure 2: Pareto chart indicating factors influence on response variables of RP-HPLC method (A) N of SITA, (B) T_f of SITA, (C) R_f of EMPA

Table 1: Experimental factors and levels used in BBD

Sr. No.	Factors	Low	Medium	High
Independent variables				
1	% ACN	60	70	80
2	Flow rate	0.80	1	1.2
3	% OPA	0.05	0.1	0.15
Dependent variables				
4	N of SITA	Y1		
5	T _f of SITA	Y2		
6	R _t of EMPA	Y3		

Table 2: Optimization of responses using BBD

Run	Std	Factor 1 A: % ACN	Factor 2 B: FR	Factor 3 C: % OPA	Response 1N (SITA)	Response 2 T _f (SITA)	Response 3 R _t (EMPA; min)
1	8	80	1	0.15	1781.28	1.95	7.188
2	10	70	1.2	0.05	1648.56	1.57	3.22
3	1	60	0.8	0.1	1620.14	1.54	4.813
4	6	80	1	0.05	1827.72	1.42	7.212
5	5	60	1	0.05	1390.35	1.61	3.916
6	11	70	0.8	0.15	1830.37	2.11	4.12
7	7	60	1	0.15	1351.27	1.93	3.867
8	2	80	0.8	0.1	1945.37	1.26	7.421
9	13	70	1	0.1	2467.23	1.21	4.037
10	4	80	1.2	0.1	1883.84	1.26	7.171
11	15	70	1	0.1	2450.6	1.28	4.044
12	3	60	1.2	0.1	1281.69	1.21	3.17
13	12	70	1.2	0.15	1775.82	1.82	3.129
14	9	70	0.8	0.05	1987.37	1.68	4.12
15	14	70	1	0.1	2484.75	1.17	4.051

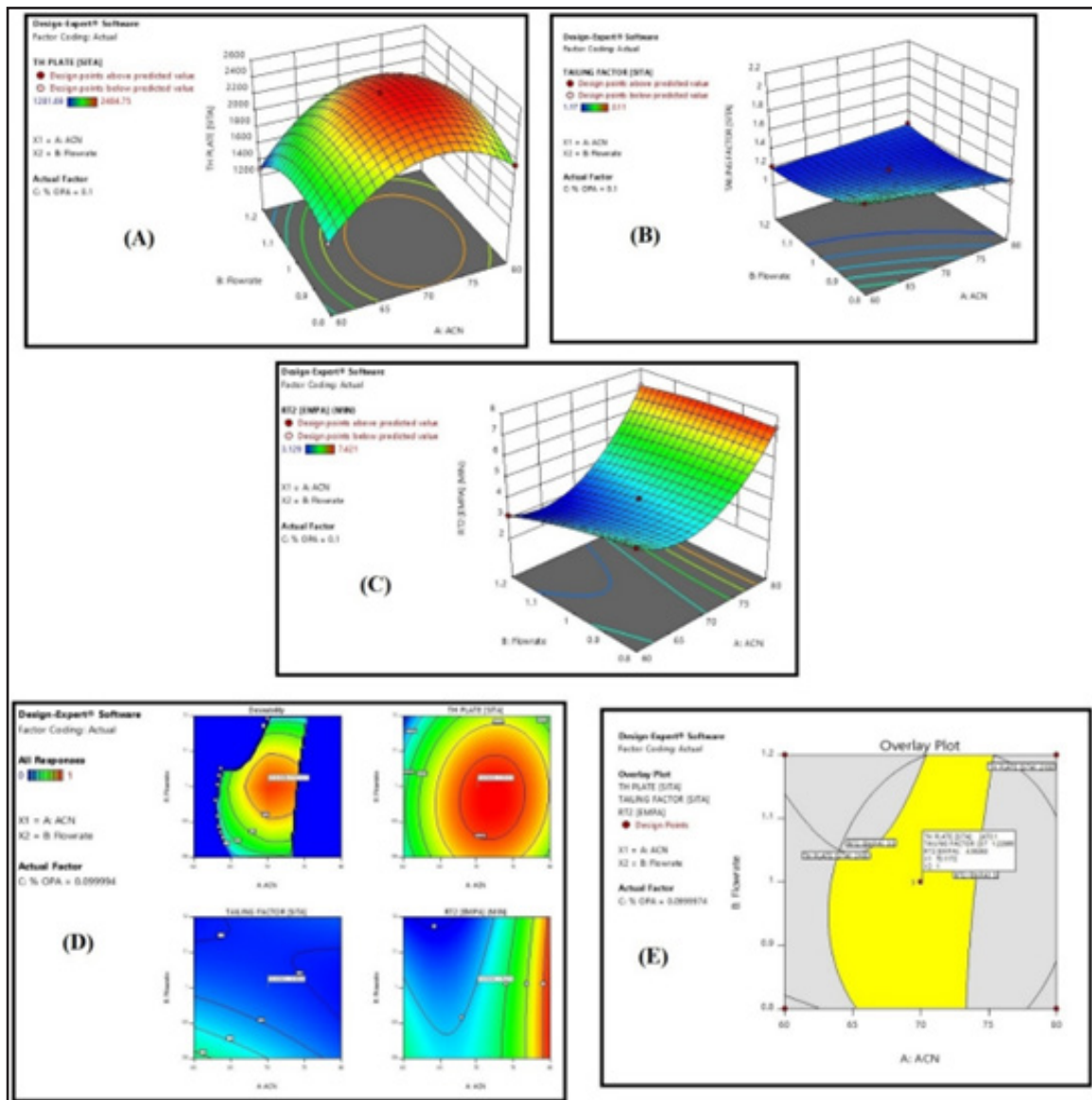


Figure 3: 3D-response surface plot showing the relationship among the factors on (A) N of SITA (B) T_f of SITA, (C) R_t of EMPA, (D) Desirability Plot for obtaining optimised method parameters, (E) Overlay Plot

Effect of independent variables on N (SITA) (Y1)

The reduced quadratic model for theoretical plates was significant ($F = 1071.89$, $p < 0.0001$) with significant terms A, B, C, AB, BC, A², B², and C², while AC was insignificant, the R² (0.9993), adjusted R² (0.9984) and predicted R²

(0.9952) indicates excellent model accuracy and predictive ability, and the non-significant lack of fit confirms good model adequacy.

Theoretical plates (Y1) = + 2467.53 + 224.34 A - 99.17 B - 14.41 C + 69.23 AB + 71.06 BC - 503.82 A² - 280.95 B² - 376.05 C²

Effect of independent variables on T_f (SITA) (Y2)

The reduced quadratic model for T_f was found to be significant ($F = 56.70$, $p < 0.0001$) with significant terms B, C, AB, B^2 , and C^2 , while A was insignificant. The high predicted R^2 (0.9770), and adjusted R^2 (0.9598) indicates good model accuracy and predictive ability, and non-significant lack of fit confirm good model adequacy.

Tailing factor (Y2) = $+1.23 - 0.0500 A - 0.0912 B + 0.1913 C + 0.0825 AB - 0.045 BC + 0.052 AC + 0.015 A^2 + 0.0813 B^2 + 0.4913 C^2$

Effect of independent variables on R_t (EMPA) (Y3)

The reduced quadratic model for R_t was highly significant ($F = 89605.11$, $p < 0.0001$) with all model terms (A, B, C, AB, BC, AC, A^2 , B^2 ,

C^2) ... - 0.2474 C^2 were found significant. The predicted R^2 (0.9892) and adjusted R^2 (0.9917) indicate perfect model fitting and predictive ability and the non-significant lack of fit confirms excellent model adequacy.

Retention time (Y3) = $+ 4.04 + 1.65 A - 0.4730 B - 0.0205 C + 0.3483 AB - 0.0228 BC + 0.006 AC + 1.75 A^2 - 0.1494 B^2 - 2474 C^2$

Model validation and confirmatory experiments

To validate the predictive ability of the model, confirmatory (checkpoint) experiments were conducted at checkpoint conditions within the design space (Table 3) The experimentally obtained values for R_t , N, and T_f were compared with predicted values, and % prediction error was found to be less than 5%, confirming the reliability of the model and validating the design space (Table 3).

Table 3: Comparison between predicted and actual of dependent variables

% ACN	Flow rate	% OPA	N of SITA			T_f of SITA			R_t of EMPA		
			Exp.	Pred.	% error	Exp.	Pred.	% error	Exp.	Pred.	% error
65	0.9	0.1	2263.060	2232.880	1.35	1.317	1.337	-1.50	4.064	3.930	3.41
65	1	0.1	2207.730	2233.780	-1.17	1.256	1.253	0.24	3.661	3.650	0.30
70	1.1	0.1	2331.700	2343.53	-0.50	1.211	1.203	0.67	3.652	3.770	-3.13

Optimization and design Space

Numerical optimization (Figure 3 (D)) using Derringer's desirability function yielded an optimal desirability value of 0.944, indicating a balanced compromise between all responses. The overlay plot defined a Method Operable Design Region (MODR), where all CAAs met acceptance criteria. This region ensures robustness against small variations in method parameters as shown in Figure 3 (E).

Method validation

Before performing the validation, the system suitability was performed (15) and the parameters were within the acceptance range ($N > 2000$, $T_f < 2$ and $R_s > 2$ and % RSD of peak areas of six injections were not more than 2%). The outcomes of system suitability was tabulat-

ed in Table 5. The chromatogram of blank, placebo, standard and sample was compared to assess the specificity of the method (Figure 5). The method was found specific as no interference of excipients and eluent was found at R_t of drugs. The selectivity was confirmed from the peak purity value (SITA: 0.99999 and EMPA: 0.99998) that no excipients are co eluted with the cited drugs. The proposed approach shows linear response for SITA from 100 – 600 $\mu\text{g/mL}$ and 25 -150 $\mu\text{g/mL}$ for EMPA. The regression equation and coefficient were tabulated in Table 4. The % RSD of SITA and EMPA for repeatability were 1.86 and 1.64 correspondingly. The outcomes for intermediate precision (within-day and between-day) study for SITA and EMPA indicates that the % RSD results were within limits (Table 4). The method was accurate because

% recovery for SITA and EMPA fell within desired range. The outcomes of sensitivity were tabulated in Table 5. The % RSD in robustness study (Table 5) for various conditions was found less than 2 %, indicates that the method is robust. The proposed approach was applied for the analysis of blend and outcomes (Table 5) showed that the % assay findings were in good agreement.

Table 4: System Suitability Data

Limit (n = 6)	SITA (Average $\pm$ SD), % CV	EMPA (Average $\pm$ SD), % CV
R_t	2.626 $\pm$ 0.0036, 0.18	4.043 $\pm$ 0.0026, 0.06
N	2314.82 $\pm$ 41.576, 1.80	7895.33 $\pm$ 2.581, 0.03
T_f	1.245 $\pm$ 0.0187, 1.50	1.406 $\pm$ 0.0027, 0.18
R_s	--	7.165 $\pm$ 0.0022, 0.03

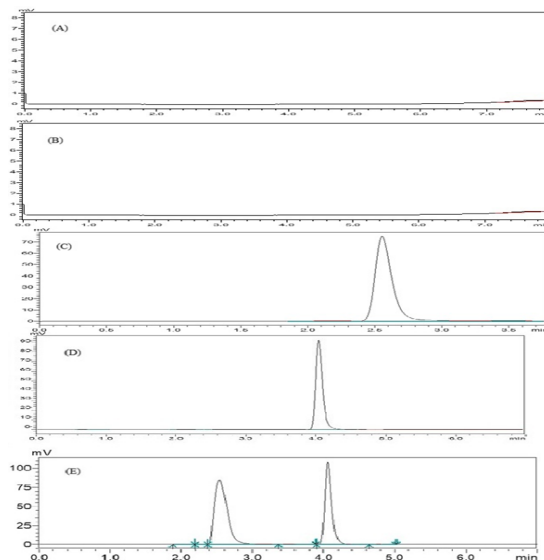


Figure 4: Chromatogram of (A) Placebo, (B) Blank, (C) 300 µg/mL SITA, (D) 75 µg/mL EMPA, (E) Mixture (300 µg/mL SITA and 75 µg/mL EMPA)

Table 5: Summary of Validation Outcomes

Sr. No.	Parameter	SITA	EMPA
1	Specificity	No interference	No interference
2	Range for linearity (µg/mL)*	100 - 600	25 - 150
3	Equation of Regression	$y = 1471.8x + 470780$	$y = 9484x - 135253$
4	Coefficient of determination (R^2)	0.9999	0.9998
5.	Precision		
a.	Repeatability#	1.86	1.64
b.	Within - day*	1.12-1.22	1.24-1.51
c.	Between day*	0.56-1.65	1.52-1.72
6	Accuracy (% recovery) *	99.92-100.98	98.59-100.18
7	LOD (µg/mL)	25.25	1.45
8	LOQ (µg/mL)	76.51	4.44
9.	Robustness (% RSD)		
a.	Change in DL (± 2 nm)*	(+) 0.025, (-) 0.006	(+) 0.184, (-) 0.16
b.	Change in FR (± 0.2 mL/min)*	(+) 0.037, (-) 0.011	(+) 0.087, (-) 0.176
c.	Change in % OPA (± 0.05 %)*	(+) 0.015, (-) 0.012	(+) 0.175, (-) 0.279
d.	Change in composition of organic phase (± 2 %)*	(+) 0.011, (-) 0.006	(+) 0.200, (-) 0.134
10	Assay* (% label claim) Mean $\pm$ SD	101.2 $\pm$ 0.08	99.15 $\pm$ 0.13

* Mean of three determinations; # Mean of six determinations

Conclusion

The proposed work describes QbD approach to HPLC method for the simultaneous quantitation of SITA and EMPA in blend. The goal of the approach was clearly defined by ATP.

The study utilizes PBD for the screening of variables and on the basis of Pareto analysis, three factors (% of ACN, % of OPA and FR) have a significant effect on CAAs. BBD was utilised as an optimization tool for the identification of significant response variables. Based on ANOVA results ($p < 0.001$), three CAAs (R_t of EMPA, N of SITA and T_f of SITA) were found significant. Design Space (MODR) was illustrated using 3D response graph as well as contour plot. Numerical Optimisation yields optimised method having a high desirability of 0.944. The optimised chromatographic conditions were % ACN (70), % OPA (0.1) and FR (1 mL/min). The % of recovery of drugs in the synthetic mixture implies that the excipients included in the blend had no impact on the outcome of the analysis. The validation study confirmed the method's linearity, robustness, specificity, precision, and accuracy for the concurrent quantitation of SITA and EMPA. By better understanding the method variables, the QbD approach to method development has reduced the likelihood of failure during method validation and transfer. The use of a QbD framework reduced method validation and transfer failures by using a structured, science-based approach defining the analytical target profile (ATP), performing risk assessments (FMEA, Ishikawa diagram, etc), and establishing a design space (DoE). To ensure the robustness, the confirmatory runs were conducted and experimental value as well as predicted value for the responses were compared. This approach could be utilised for routine analysis in QC laboratories for the concurrent quantitation of SITA and EMPA.

Declaration of competing interest

According to the authors, no known conflicting financial interests or close relationships could have influenced any of the work reported in this study.

Data availability

On request, data will be provided.

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