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ORIGINAL ARTICLE

Artificial Intelligence Enabled Development of RP-HPLC Method for Simultaneous Determination of Metformin, Vildagliptin and Dapagliflozin in Tablet Dosage form by Application of Principles of Quality by Design

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ABSTRACT
A direct, precise, and reliable method was developed for the simultaneous determination of Vildagliptin, Dapagliflozin, and Metformin in tablet dosage form. Response surface methodology in the design of experiments was used for identifying key material attributes and critical process parameters that influence the designated critical analytical attributes. A Sun Fire C18 Column with dimensions of 3.5 µm, 4.6 mm X 250 mm was employed for the chromatogram analysis. The mobile phase, consisting of 0.01N KH2PO4 and Acetonitrile in a 60:40 v/v ratio, was passed through the column at a flow rate of 1.05 ml/min. The pH of the buffer is regulated to 3.5 by employing Orthophosphoric Acid. A constant temperature of 29.6°C was maintained. The optimal wavelength for Vildagliptin, Dapagliflozin, and Metformin was 220.0 nm. The retention time of Metformin, Dapagliflozin, and Vildagliptin were determined to be 2.382 minutes, 3.117 minutes, and 2.012 minutes, respectively. The percentage relative standard deviation (RSD) of system precision for these three drugs was determined to be 0.5, 0.9, and 0.6. The relative standard deviation (RSD) values for the method precision of Metformin, Dapagliflozin, and Vildagliptin were 0.7, 0.6, and 0.2, respectively. The rates of recovery for Metformin, Dapagliflozin, and Vildagliptin were 99.95%, 99.65%, and 99.71%, respectively. The regression equations for Metformin, Dapagliflozin, and Vildagliptin resulted in LOD (limit of detection) and LOQ (limit of quantification) values of 0.52 ppm, 1.58 ppm, 0.018 ppm, and 0.053 ppm, and 0.12 ppm, 0.36 ppm, respectively.
KEYWORDS: Metformin, Dapagliflozin, Vildagliptin, RP-HPLC, Quality by Design.

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INTRODUCTION

A QbD approach to method development gives a more durable/rugged technique since it focusses a higher attention on risk management than a usual or formal approach. Understanding dependent variables and the impact they have by using a suitable set of examinations on the responses to be investigated is a crucial component of QbD [1, 2]. With this study, a risk-based High Performance Liquid Chromatography (HPLC) technique for estimating the amounts of Vildagliptin, Dapagliflozin, and Metformin in tablet dosage form has been developed and validated. Conventional HPLC procedure optimization involves examining one component at a time while holding the other variables constant. This leads to several tests where the crucial parameters are not adequately understood. QbD has developed into an effective tool for chromatographic technique development in the present era. This approach has the advantage of providing a clear understanding of the factors influencing chromatographic separation by identifying the important ones, as well as their impact on the selected responses, both positively and negatively, and the multifaceted interactions among them. [3].

Additionally, QbD suggests the critical answers and promotes the deliberate modification of the variables to achieve the intended outcome. Early on in the development phase, the QbD method suggests assessing the quality of the analytical process [4–6].
Metformin HCl is known by its chemical nomenclature, 1,1-dimethylbiguanide hydrochloride (Figure. 1A). Dapagliflozin is [4-chloro-3- [(4- [15] ethoxy phenyl) methyl] phenyl] in chemical terms. (1S) 1, 5, anhydro-1-C-D-glucitol (Figure. 1B). 2-(3-Hydroxyadamantan-1-ylamino) acetyl] -(S)-1.
Vildagliptin's chemical name is pyrrolidine-2-carbonitrile (Figure. 1C). Type-2 diabetes mellitus is treated with biguanides, a class of anti-hyperglycemic drugs that includes metformin. Contrarily, Dapagliflozin is an inhibitor of sodium-glucose cotransporter 2 (SLGT2) that raises and decreases the quantity of carbohydrates that leave the body. Vildagliptin is a dipeptidyl protease inhibitor that lowers glucose, increases insulin and reduces the amount of glucose the liver produces [7-8]. These drugs come in tablet form, in single and double combinations. These three medications are used to treat diabetes mellitus; the FDA has just approved their fixed-dose commercial formulation. The market-available tablet dosage of 500 mg of metformin, 10 mg of Dapagliflozin, and 100 mg of Vildagliptin. Numerous distinct analytical procedures have been developed both alone and in tandem, as evidenced by a review of the literature. However, there is no HPLC method available for analyzing trio combo tablets.
Development of analytical methods and validation of this triple combination tablet is required. Thus, a sensitive HPLC technique that indicates stability for MET, DAP, and VIL is required. Studies on the drug's stability were conducted by subjecting it to a range of stressors, including thermal, oxidative, light, and hydrolysis (acid and base). The created HPLC was validated by ICH regulations. The present investigation aimed to create an HPLC technique for Metformin, Dapagliflozin, and Vildagliptin in API and formulation, and assess the stability of these medications under different stress scenarios. The QbD methodology must be applied to provide a more sensitive, economical, and dependable analytical technique for drug molecule quantification [9-10].
The analytical quality by design (AQbD) technique can be used to construct robust, dependable, and economical chromatographic procedures for the quantification of analytes from bulk pharmaceutical and biopharmaceutical samples, as recent scientific investigations have successfully proved. To ascertain the potential principal critical process parameters (CPPs) involved during the research, AQbD conducts preliminary risk assessment studies as part of a thorough understanding of the underlying interaction(s) among the various components. To give the appropriate chromatographic solution, method optimization is carried out after factor screening trials have identified the significant factors [11-12].

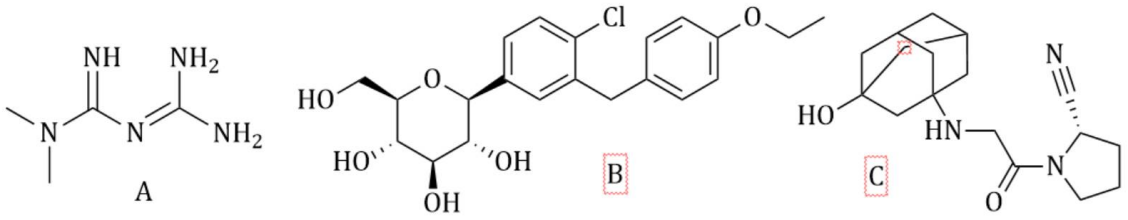

Figure 1: A) Metformin; B) Dapagliflozin; C) Vildagliptin

MATERIAL AND METHODS

Material: The commercial Daparyl-VM 1 tablets were purchased from the manufacturers, the bulk medications, Metformin, Dapagliflozin, and Vildagliptin, were graciously donated by Spectrum Laboratories PVT, LTD, Hyderabad.
Diluents: Based on the solubility of the drugs, 50:50 v/v Water: Acetonitrile were taken into consideration
Preparation of Stock Solutions: Precisely weighed, 100 mg of Metformin, 2 mg of Dapagliflozin, and 20 mg of Vildagliptin were added to individual 100 mL volumetric flasks. After adding 10 milliliters of diluent, the flasks were subjected to 20 minutes of sonication and volume was made up to 100 mL with the diluent.
Preparation of Sample Stock Solution: Five tablets were weighed to determine their average weight. The average weight of tablet was then transferred to a 250 mL volumetric flask, 25 mL of diluent was added, and the mixture was sonicated for 50 minutes. The volume was then further adjusted with diluent and filtered.
Selection of Detector Wavelength: Vildagliptin, Metformin and Dapagliflozin in a 10ppm solution were examined between 200 and 400 nm, and the wavelength maxima above 220 nm were chosen for the detection.

Chromatographic conditions: A Waters HPLC 2695 System was utilized, fitted with a quaternary solvent manager, a sample manager, a TUV detector controlled by Empower 2 software, a cooling auto sampler, and a column oven facilitating temperature control of the analytical column. A Sun Fire C18 Column (3.5 µm, 4.6 mm X 150 mm) was used for this study. The temperature of 29.6 °C and flow rate of 1.05 mL/min were used for all investigations. The standard and sample injection volumes were both 20 µL. Prior to injection, every standard and sample solution were filtered using 0.2 µm filter tips. Column effluents were viewed with a photo diode array (PDA) operating at 220 nm

Selection of quality target product profile: The quality target product profile (QTPP) for the proposed HPLC method was identified as the retention time (Rt), resolution, number of theoretical plates and Asymmetry factor.

Determination of critical quality attributes: The method variables that directly influence the QTPP are the critical quality attributes (CQAs). The flow rate of the mobile phase, amount of acetonitrile (v/v) in the mobile phase, and temperature were three critical technique parameters that had to be kept under control to sustain the QTPP permissible response range.

Design for optimization: Utilizing an experimental plan based on response surface methodology (RSM), the chromatographic conditions of the proposed approach were optimized. The central composite design (CCD) was selected for optimization of the three components with a total of 20 runs. Based on prior screening and available knowledge, the independent variables and their levels were chosen. The three drugs resolution, number of theoretical plates, and retention times were the dependent variables (responses). Multiple regression analysis was carried out on design matrix and the data obtained, and the resulting second-order polynomial function was utilized to establish the connection between the independent variables and the data collected. Table 1 lists the variables, their ranges, and responses with corresponding objectives [13-14].

Table 1: Independent variables (factor) and responses investigated in CCD design

Independent variable(factor)	Level of variable		Response	Target
Flow rate of the mobile phase, mL/min	0.8318	1.17	Retention time Resolution Theoretical plates Asymmetry Factor	Minimizing the retention time of drugs while maximizing the resolution
Organic content in mobile phase, % (v/v)	21.59	38.41		
Temperature, °C	24.95	35.05		

Statistical Interpretation: Using Design-Expert10.0.2, the experimental design and statistical analysis was completed (free trial version). The importance of each model, terms, and their interactions were assessed using analysis of variance (ANOVA). A model was considered statistically significant if its *p*-value was less than 0.05. *F*-values were also checked for each model and term to determine the importance of the experiments. The null hypothesis which states that experimental part does not affect the responses is true when *F*-value is 1 or very close to 1, while high *F*-value denotes a significant effect of factors on responses. Based on the determination coefficient (R^2), adjusted determination coefficient (adj. R^2), and predicted determination coefficient, the fit of the data by regression line was evaluated (pred. R^2). Utilizing diagnostic tools like the residual vs. predicted plot and normal probability plot of residuals, suitability of the model was evaluated.

Analytical method validation: The new technique was validated in compliance with ICH, United States Pharmacopeia (USP), and FDA requirements in terms of specificity, solution stability, linearity range, accuracy, precision, system suitability, and forced degradation tests

System suitability parameters: To ensure that a method is adequate for a given analysis, system suitability is frequently used. Theoretical plate count, tailing factor, area resolution and reproducibility, limit of detection, limit of quantification were among the parameters that were confirmed in the current study

Specificity: The specificity of the method was checked to make sure that no excipients, degradation products, or impurities interfered with the drug peaks in the chromatogram. The specificity of the drugs was evaluated using peak purity indices in addition to visual inspection.

Accuracy: The data is obtained from a minimum of nine analyses at a minimum of three concentration levels that cover the recommended range. The recovery was used in the current study to evaluate accuracy at three different concentrations

Linearity: In order to construct a calibration curve, peak area was plotted against various concentrations (20–150% of nominal), and calibration curve was then used to assess linearity and operating range of the method. Calculations were made for the slope, intercept, regression coefficient (r^2), and linear equation.
Precision: The two commonly used levels of precision evaluation are intra-day (repeatability) and inter-day (intermediate precision). Three replicates at each concentration level were used in the current study evaluation of the three concentration levels of intraday precision. To assess intermediate precision, two HPLC systems were applied in two altered days. The overall variation in the relative standard deviation was determined.
Forced degradation study: These studies are conducted to purposefully degrade a sample. These investigations are carried out to assess the sensitivity of an analytical technique. The development of multiple dosage forms and the rational formulation of the treatment are both aided by the vital information on the drug breakdown pathway provided by forced degradation research. When exposed to acid, base, oxidizing, reducing agents, ultraviolet (**UV**) radiation, and water, drug compounds or drug products deteriorate by 10–30%. The degraded samples are then analyzed by means of the technique to see if there is any interference between the drug molecule and related substance(s).

RESULTS AND DISCUSSION

Preliminary screening: An initial screening and literature search were conducted to identify the significant factors as well as their effects and levels. Methanol (60%)-water (40%) mixture separates all drugs according to screening experiments, but the peaks are not symmetrical, the resolution is outside of acceptable ranges, and the retention times are also much longer than anticipated. Whereas in the second trial, 0.01 N KH_2PO_4 (45%)-methanol (55%) mixture was used as the mobile phase; by maintaining all the chromatographic conditions constant, the peaks were asymmetrical with tailing, and they were merged. In the third trial, methanol was replaced by acetonitrile, and the mobile phase consisting of 0.01 N KH_2PO_4 (60%)-acetonitrile (40%) was taken into consideration. Research was done using various pH levels and buffer systems. Phosphate buffer and buffer with 0.1% orthophosphoric acid were the two choices for the mobile phase. Since the larger molecular structure of the salt generated when the mobile phase pH is close to or over 8.0 is less damaging to the column in the current scenario, 0.1% orthophosphoric acid buffer was used [17]. Once more, it became clear that the concentration of acetonitrile significantly impacted the retention of all drugs. Therefore, we chose the content of acetonitrile, flow rate, and column temperature as the three independent variables for optimization. Separation was carried out on a Sun Fire C18 Column (3.5 µm, 4.6 mm X 250 mm) at ambient temperature. The flow rate was fixed at 1.05 mL/min with the injection volume of 20 µL. Eluents were monitored at 220 nm.

Table 2: Randomized run order, factor combinations, and corresponding responses

	Factor 1	Factor 2	Factor 3	Res 1	Res2	Res 3	Res 4	Res 5	Res 6	Res 7
Std	A:FR	B:MP	C: Temp	RT1	RT2	RT3	RS1	RS2	NTP	AF
	ml/min	%	^{0}C	%	^{0}C	min	min	min	num	num
1	0.9	25	27	2.27	2.578	3.284	2.7	6.0	7187	1.4
2	1.1	25	27	1.88	2.137	2.711	2.5	5.8	6337	1.3
3	0.9	35	27	2.23	2.683	3.832	4.4	8.5	7349	1.2
4	1.1	35	27	1.86	2.239	3.061	4.0	6.9	6253	1.1
5	0.9	25	33	2.27	2.554	3.196	2.4	5.7	7337	1.4
6	1.1	25	33	1.89	2.132	2.645	2.2	5.2	6494	1.2
7	0.9	35	33	2.23	2.634	3.582	3.9	7.7	7315	1.1
8	1.1	35	33	1.901	2.263	2.702	2.3	5.6	6223	0.9
9	0.831	30	30	2.422	2.817	3.648	3.1	6.9	7349	1.3
10	1.1681	30	30	1.77	2.062	2.691	2.9	5.8	6338	1.2
11	1	21.591	30	2.081	2.307	2.839	1.9	4.9	6483	1.0
12	1	38.409	30	2.015	2.472	3.502	4.6	7.9	6276	1.3
13	1	30	24.95	2.037	2.398	3.191	3.7	7.0	7204	1.4
14	1	30	35.04	2.063	2.362	3.014	2.7	5.9	7330	1.1
15	1	30	30	2.053	2.384	3.121	3.3	6.5	7123	1.0
16	1	30	30	2.054	2.381	3.112	3.3	6.5	7134	1.4
17	1	30	30	2.04	2.379	3.102	3.2	6.5	7146	1.2
18	1	30	30	2.03	2.379	3.103	3.3	6.5	7144	1.1
19	1	30	30	2.03	2.374	3.098	3.2	6.5	7130	1.2
20	1	30	30	2.03	2.362	3.097	3.2	6.4	7133	1.4

Experiments and statistical analysis: A full factorial design with 20 experimental conditions was carried out which included nine specifications established by the design of experiments with six repetitions at the center of the domain. A chromatogram was produced for each of these conditions. The runs and corresponding responses are depicted in Table 2. The retention of Vildagliptin differed from 1.77 to 2.81 min, while that of Metformin differed from 2.12 to 3.65 min and Dapagliflozin differed from 2.64 to 3.83 min. Therefore, these data can be utilized and examined to dispose the favorable conditions to bring about the targets of the present research. ANOVA was done to analyze the importance of 2FI experimental models, the outcomes are represented in Table 3. The importance of every model was evaluated based on Fisher's ratio (F-value). The model F-values for response 1(RT1 of 79.29), response 2 (RT2 of 29.87), response 3 (RT3 of 68.45), response 4 (RS1 of 71.5), response 5 (RS2 of 75.6), response 6 (NTP of 12.35) and response 7(AF of 14.43) imply that the model is significant.

All models were found to have p-values less than 0.0001, demonstrating their significance. According to the corresponding F- and p-values for each term, all other terms were also significant. On the basis of the determination coefficient (R^2), adjusted determination coefficient (adj. R^2), and predicted determination coefficient, the quality of the obtained polynomial regressions was evaluated (pred. R^2). In every instance, the R^2 values were discovered to be very close to 1, indicating data fit by the regression curve with an accuracy of greater than 99%. The adjusted R^2 values and predicted R^2 values had a good degree of agreement (i.e., difference less than 0.2). Based on the signal-to-noise ratio which should be greater than 4 adequate precisions can be determined. High precision values were discovered in the current study, indicating a sufficient signal. Table 5 summarizes the model.

Table 3: ANOVA for response surface 2FI and Quadratic models (Degrees of freedom, F value)

Model	Predicted Mean	Predicted Median	Observed	F-value	p-value	Std Dev
RT1	2.04769	1.94769	2.012	1003.15	< 0.0001	0.00734843
RT2	2.30458	2.30458	2.382	29.87	< 0.0001	0.0113273
RT3	3.0799	3.0799	3.117	68.45	< 0.0001	0.0519826
RS1	3.5545	3.5545	3.0	71.5	< 0.0001	0.238602
RS2	6.72129	6.72129	6.4	75.6	< 0.0001	0.147959
NTP1	6742.3	6742.3	6742	12.35	< 0.0001	108.347
AF	9107.22	9107.22	9140	14.43	< 0.0001	96.6259

Table 4: Regression model summary

Model	R^2	Adj. R^2	Pred. R^2	Adeq. Precision	Std. dev	CV (%)
Model for RT1	0.9734	0.9611	0.8739	33.3650	0.0279	1.48
Model for RT2	0.9324	0.9012	0.6175	21.5468	0.1284	2.65
Model for RT3	0.9876	0.9076	0.8654	25.8765	0.1247	2.98
Model for RS1	0.9547	0.9338	0.8077	27.4801	0.1395	3.23
Model for RS2	0.9704	0.9568	0.8314	29.0253	0.2939	5.79
Model for NTP	0.9468	0.8642	0.4111	10.3576	0.1567	5.29
Model for AF	0.9876	0.8234	0.5643	22.8976	0.1678	4.98

The polynomial equations in coded form for each model were as follows (Eqs. (1)– (7)):

RT1 of Vildagliptin = +0.4875– 0.4782× A + 0.0034× B +0.0007× C + 0.0005× AB + 0.0002× AC + 0.0001× BC+0.0042 A^2 (1)

RT2 of Metformin = +0.6878– 0.6423× A + 0.0364× B +0.0012× C + 0.0005× AB + 0.0008× AC + 0.0000× BC+0.0064A^2 (2)

RT3 of Dapagliflozin= +2.03+ 1.44× A + 0.4413× B +0.0822× C + 0.0348× AB + 0.0009× AC + 0.0258× BC (3)

RS1 = +9.23+ 0.5483× A + 6.39× B +1.47× C + 0.3200× AB + 0.1800× AC + 0.3200×BC (4)

RS2 = +14.90+2.86× A + 8.93× B +1.72× C + 1.12× AB + 0.0800× AC + 0.1800× BC (5)

NTP = +0.6878– 0.6423× A + 0.0364× B +0.0012× C + 0.0005× AB + 0.0008× AC + 0.0000× BC+0.0064A^2 (6)

Asymmetry factor = +0.453– 0.4253× A + 0.0254× B +0.0023× C + 0.0015× AB + 0.0048× AC + 0.0004× BC+0.0043A^2 (7)

where A is the flow rate, B is acetonitrile content (%), and C is column temperature. A, B, and C illustrate the main effect terms, while AB, AC, and BC exhibit their interaction effect. Visual inspection of the residual vs. predicted plot and normal probability plot was done to determine whether the model was adequate. The residuals were normally dispersed along the

straight line with little scatter, indicating a good fit to the data. It is possible to predict the response for specific levels of each factor using the equation expressed in terms of coded factors. The absence of any systematic bias or outliers was indicated by the residual plots which displayed a random distribution of residuals between +4 and 4 without any trend.

Derringer desirability function: The specification for optimizing every single response is displayed in Figure. 2 using the Derringer desirability function. Desirability value for each response serves as the foundation for this methodology. A value close to one is required for a fully desired response on the scale of desirability function which runs from zero to one; zero is thought to be the most undesirable response. The most desirable trials were chosen based on their value. Thus, for method optimization, the first trial with desirability one (i = 1) was chosen. Table 6 displays the outcome.

Table 5: Optimized trials suggested by software based on desirability value

	Flow rate ml/min	Organic phase content	Temp °C	RT1	RT2	RT3	RS1	RS2	NTP1	NTP2	NTP3	Desirability
Predicated Value	1.05	33.08	29.00	1.947	2.304	3.0799	3.5545	6.721	6742.36	9107.2	10487.5	1.0

Design space and optimal separation conditions: Utilizing three-dimensional (3D) response surface graphs and two-dimensional (2D) overlay contour plots, the standardized effects of independent factors on the responses as well as their interactions with one another are visualized in Figure.3–9. To figure out the design space and the ideal composition of the mobile phase, an overlay contour plot was built using the data for the experimental conditions and corresponding responses. In this instance, the goal was to increase resolution while simultaneously reducing the retention time of both drugs. More exactly, the target was to attain minimum and maximum responses of 1.77 ≥ Rt of Vildagliptin ≤ 2.42, 2.06 ≥ Rt of Metformin ≤ 2.68, 2.64 ≥ Rt of Dapagliflozin ≤ 3.83, RS1 ≥ 4.6, RS2 ≥ 8.5, 2845 ≥ NTP ≤ 4498 and AF≥ 5.6 and. Various specifications within the design space were analyzed for desirability before the most advantageous ones were selected. The entire target was achieved with a desirability value of 1.00 using a mobile phase containing roughly 40% (v/v) acetonitrile and a buffer concentration of about 10 mM. Finally, at a column temperature of 29.6°C and a flow rate of 0.298 mL/min, acetonitrile blend of 29.06% (v/v) and 0.1% orthophosphoric acid buffer was determined to be the best isocratic mobile phase. Furthermore, the method operable design region (MODR) and ideal chromatographic conditions were delineated by the graphical optimization as shown in Figure10.

Method Validation

Specificity: No interference from any other peak of impurities or excipients was discovered after visual examination of the standard chromatogram, assay sample chromatogram, and forced degradation sample chromatogram. Additionally, peak purity indices in each case were reviewed and were found to be greater than 0.9998, demonstrating the method specificity for the drug molecules. The chromatograms of the standard mixture and sample mixture are shown in Figure 11b, c. The chromatogram of a blank obtained by a PDA detector is shown in Figure 11a.[15]

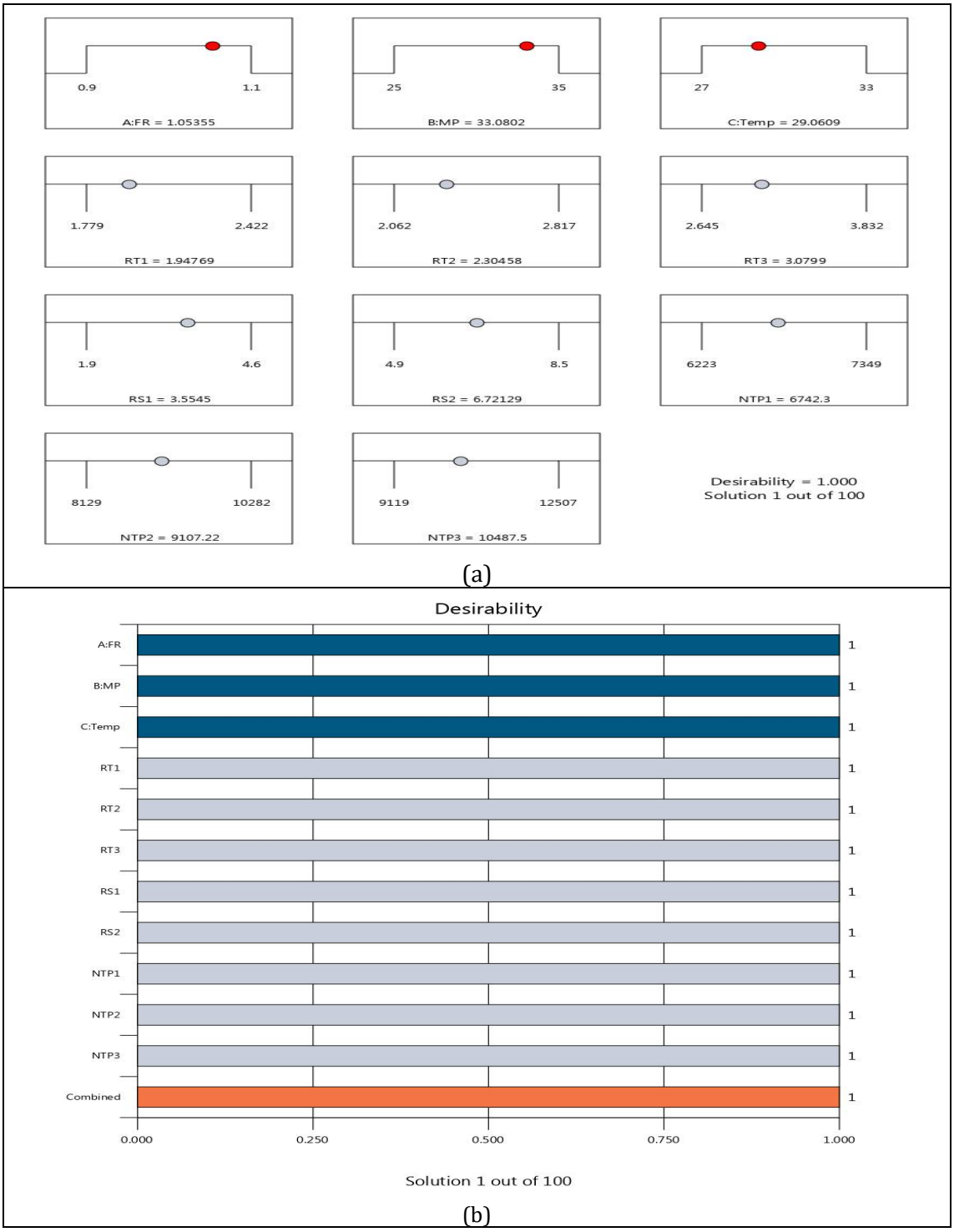

Figure 2: Ramps (a) and bar graph (b) of Derringer's desirability function representing the optimized experimental conditions, the individual and combine desirability values.

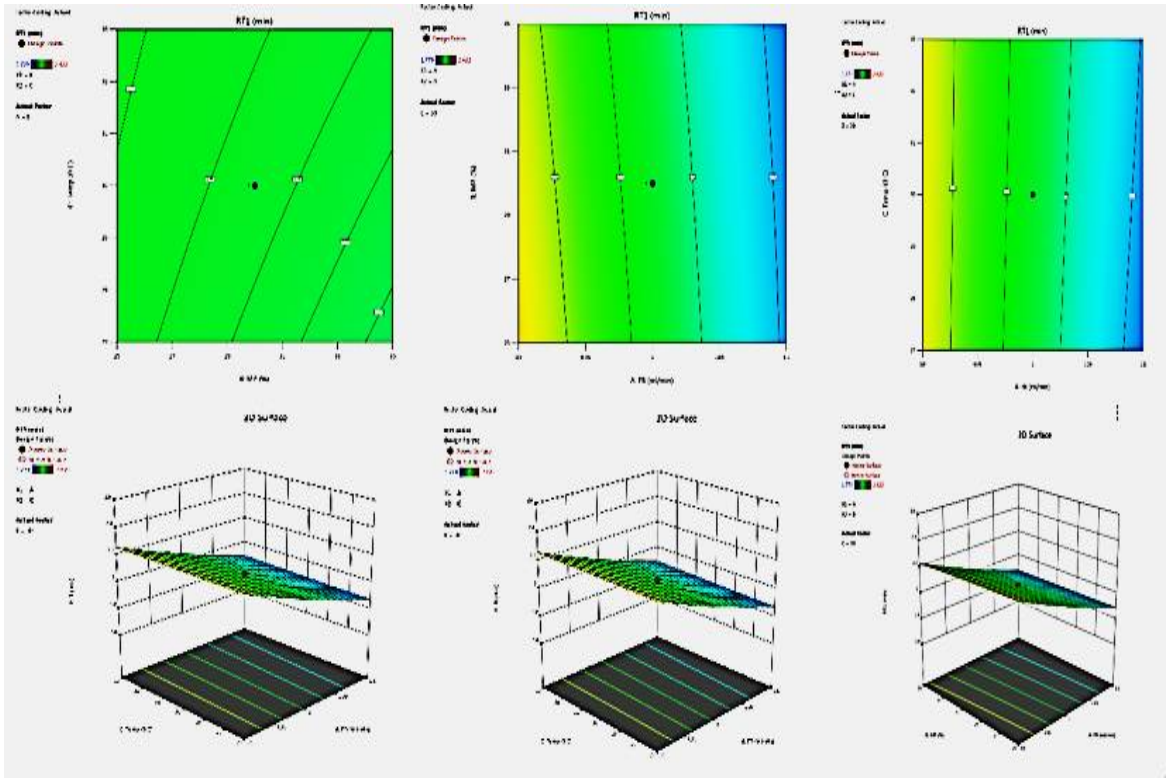

Figure 3: 2D-contours and 3D-response surface plots showing the influence of CMPs a) Flow rate (A) and % Organic phase (B), b) % Organic phase (B) and Oven temperature (C) and c) Flow rate (A) and Oven temperature (C) on Retention time (RT1) as the CAA

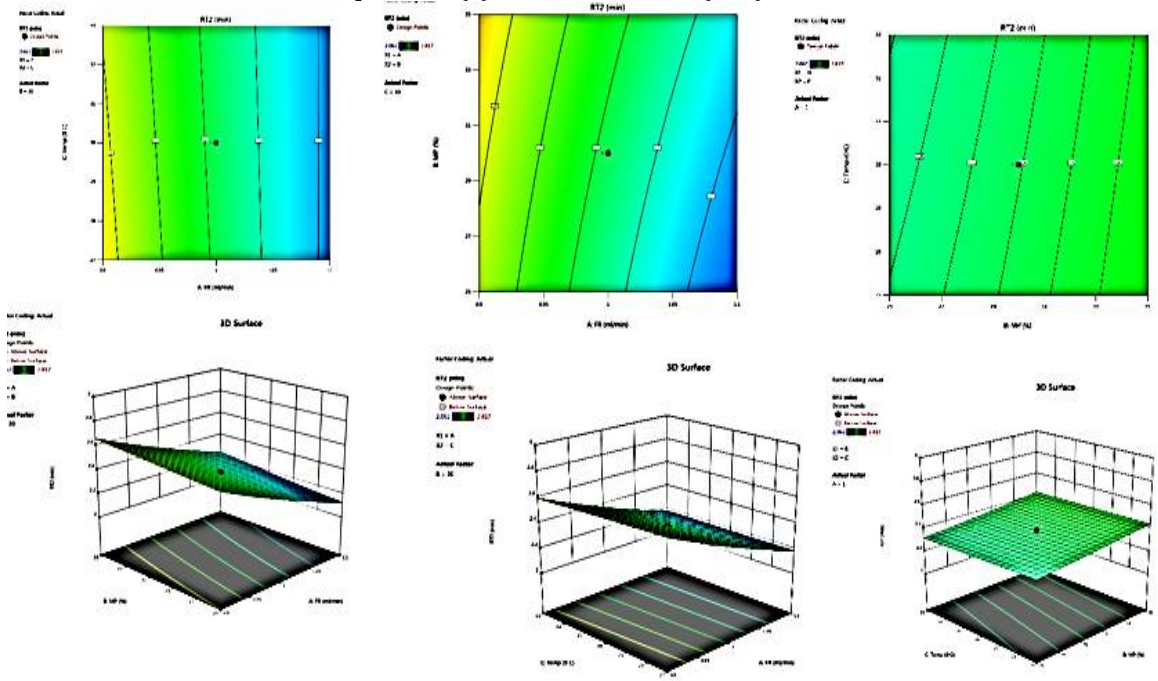

Figure 4: 2D-contours and 3D-response surface plots showing the influence of CMPs a) Flow rate (A) and % Organic phase (B), b) % Organic phase (B) and Oven temperature (C) and c) Flow rate (A) and Oven temperature (C) on Retention time (RT2) as the CAA.

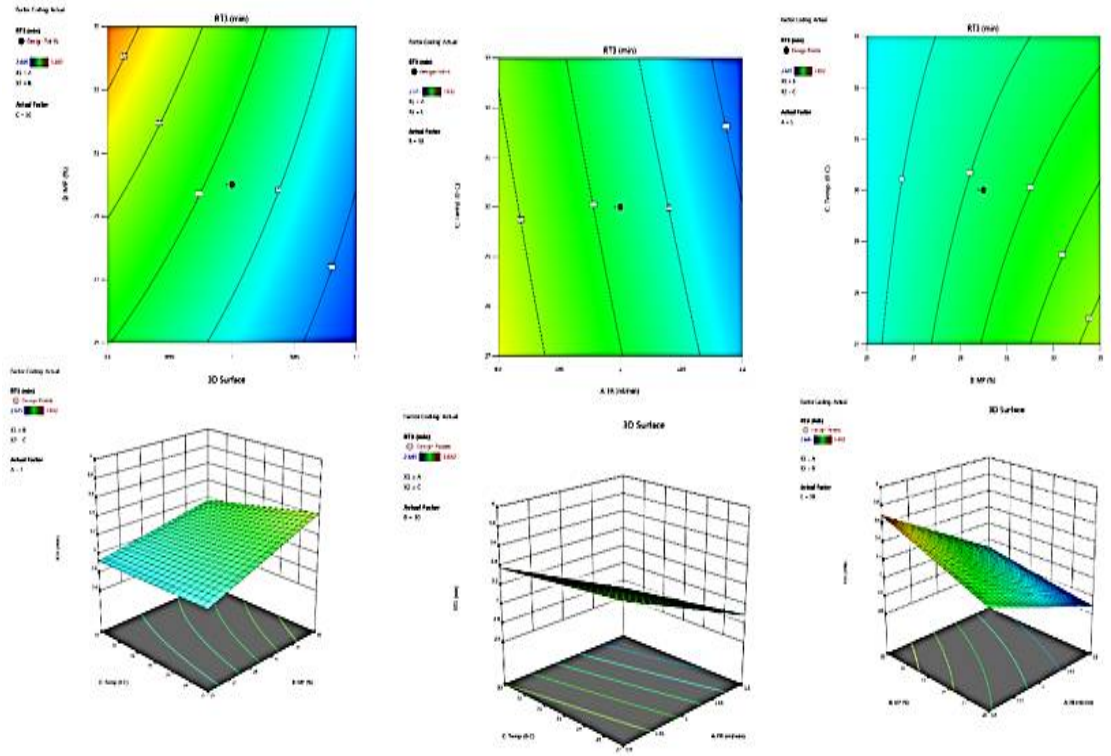

Figure 5: 2D-contours and 3D-response surface plots showing the influence of CMPs a) Flow rate (A) and % Organic phase (B), b) % Organic phase (B) and Oven temperature (C) and c) Flow rate (A) and Oven temperature (C) on Resolution (RT3) as the CAA.

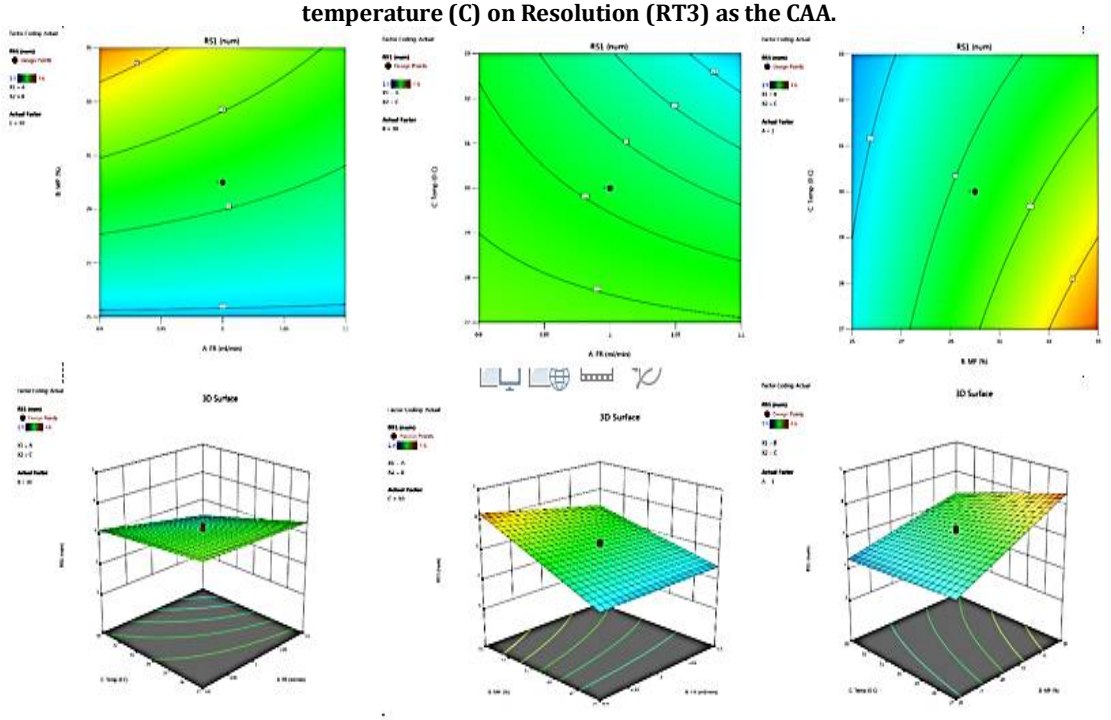

Figure 6: 2D-contours and 3D-response surface plots showing the influence of CMPs a) Flow rate (A) and % Organic phase (B), b) % Organic phase (B) and Oven temperature (C) and c) Flow rate (A) and Oven temperature (C) on Resolution (RS1) as the CAA.

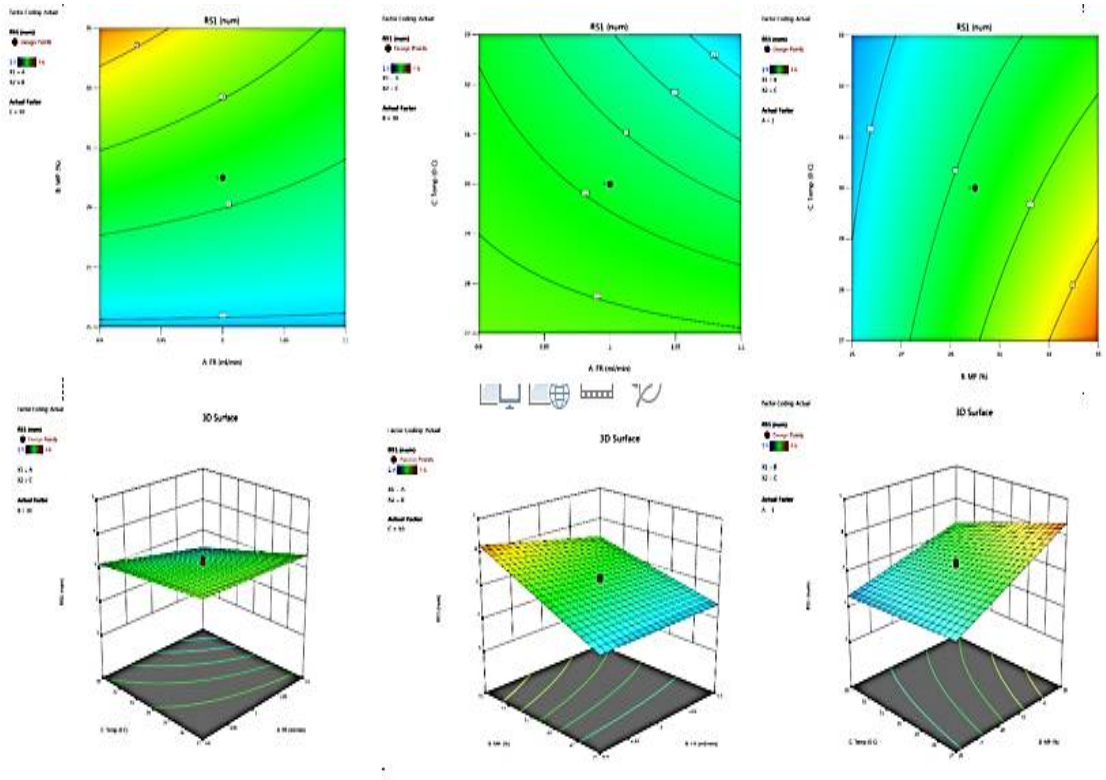

Figure 7: 2D-contours and 3D-response surface plots showing the influence of CMPs a) Flow rate (A) and % Organic phase (B), b) % Organic phase (B) and Oven temperature (C) and c) Flow rate (A) and Oven temperature (C) on Resolution (RS2) as the CAA.

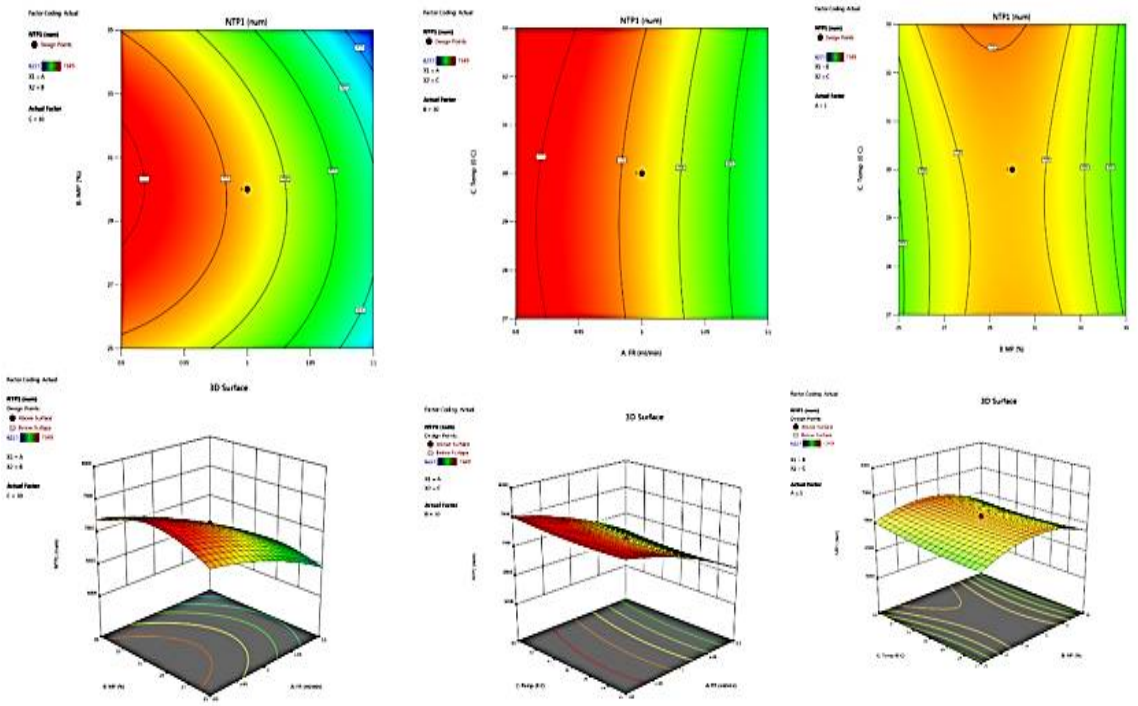

Figure 8: 2D-contours and 3D-response surface plots showing the influence of CMPs a) Flow rate (A) and % Organic phase (B), b) % Organic phase (B) and Oven temperature (C) and c) Flow rate (A) and Oven temperature (C) on Number of Theoretical Plates as the CAA.

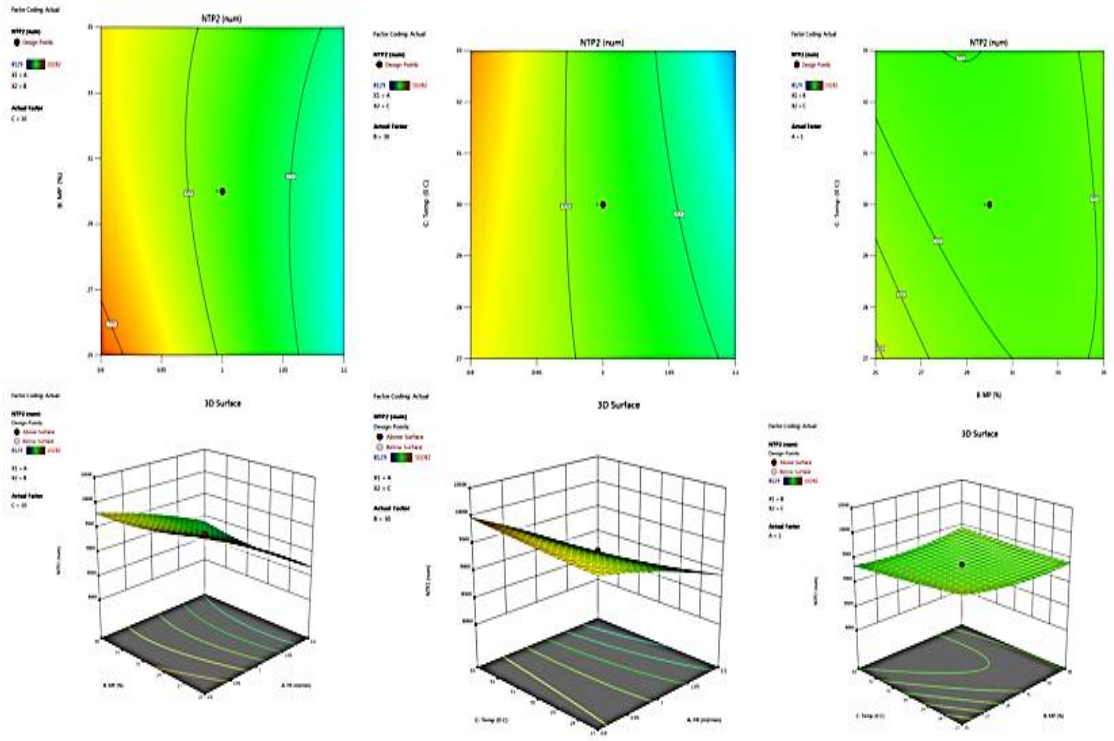

Figure 9: 2D-contours and 3D-response surface plots showing the influence of CMPs a) Flow rate (A) and % Organic phase (B), b) % Organic phase (B) and Oven temperature (C) and c) Flow rate (A) and Oven temperature (C) on Asymmetry Factor (AF) as the CAA.

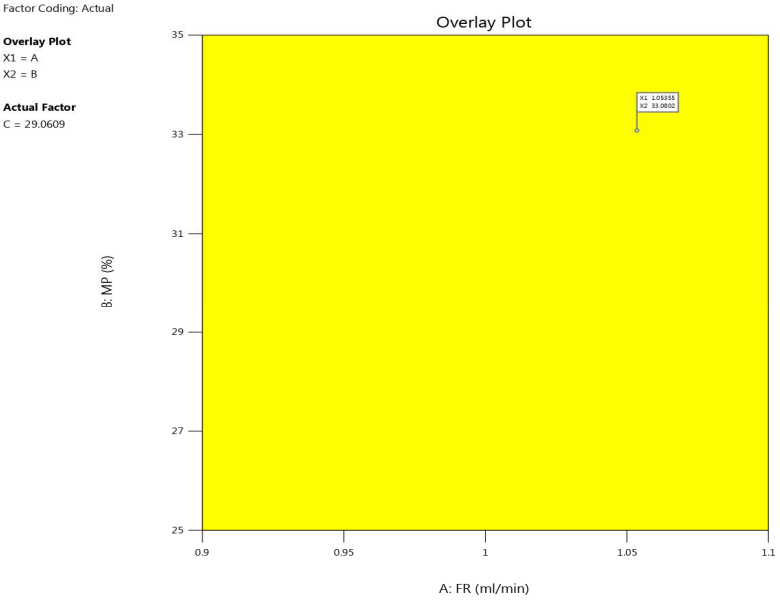

Figure 10: An illustration of the overlay plot in graphical form displaying the ideal design space or technique operational design region (MODR).

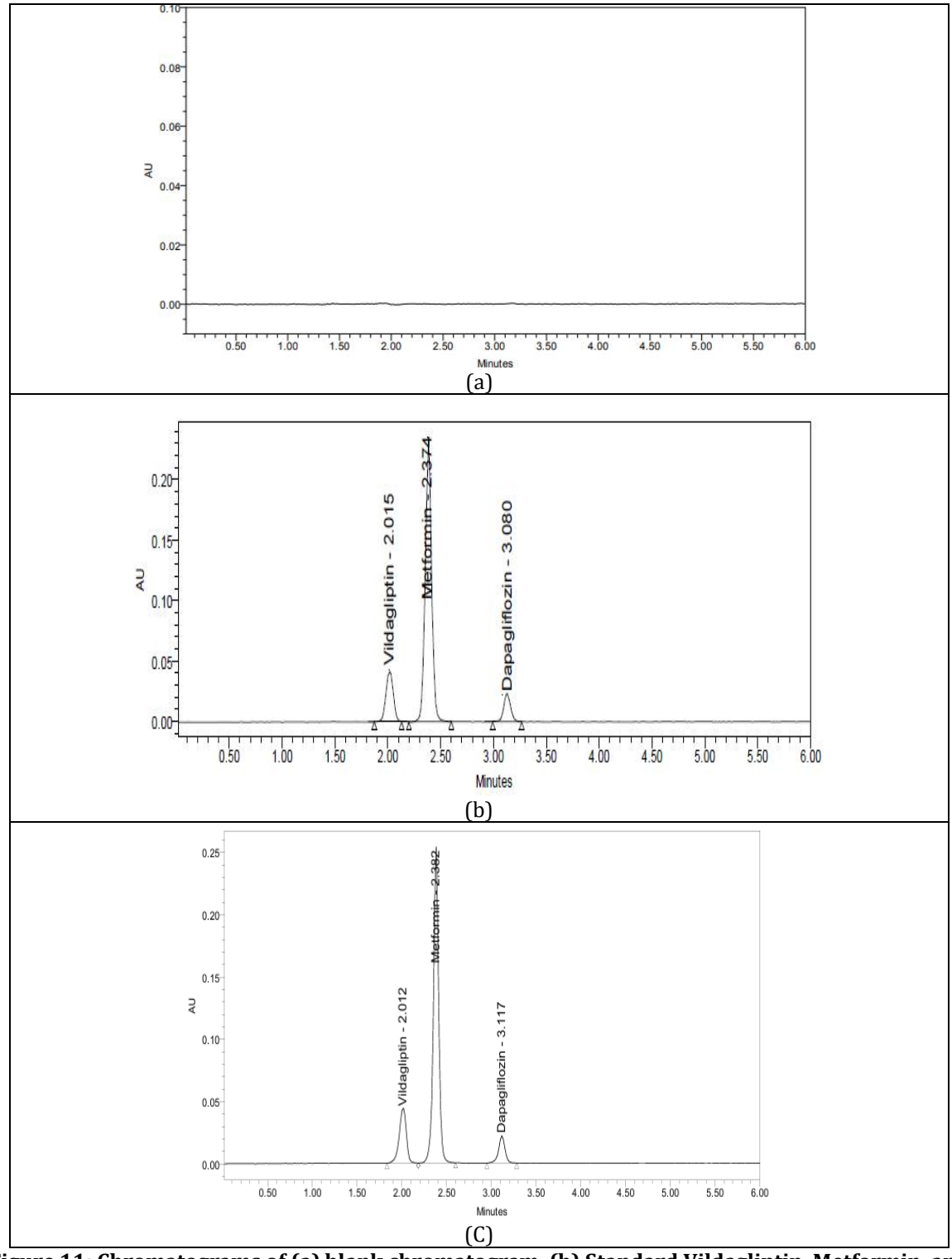

Figure 11: Chromatograms of (a) blank chromatogram, (b) Standard Vildagliptin, Metformin, and Dapagliflozin chromatogram, and (c) Sample chromatogram

Solution stability: Vials with working standards at the nominal concentration were placed at room temperature and in the refrigerator, and tests were performed at 0 and 24 h to determine stability in this solvent. The peak areas were then contrasted with those at 0 h. No appreciable alterations in the area were seen. The drugs appeared to be stable in the diluting solvent as the RSD was discovered to be less than 2.0% [16].

Linearity, working range, and accuracy*:* The nominal standard concentration in the current validation procedure was 1 mg/mL of Metformin, 0.02 mg/mL of Dapagliflozin and 0.2 mg/mL of Vildagliptin.

Plotting the peak areas against the corresponding concentration, we prepared standard solutions at 20, 50, 80, 100, 120, and 150% of the nominal concentration. All drugs had determination coefficients (r^2) above 0.999, indicating a satisfactory fit of the data by the regression line. The linearity range was found to be 25–150 µg/mL for Metformin,0.5-3 µg/mL for Dapagliflozin and 5- 30 µg/mL Vildagliptin. The regression equations were as follows (Eqs. (6) (7) and (8)):

Metformin: $y = 10407x + 1862.5$ ($r^2 = 0.9998$) ----------(6)

Dapagliflozin: $y = 51736x + 319.29$ ($r^2 = 0.9998$) ----------(7)

Vildagliptin: $y = 11818x + 417.71$ ($r^2 = 0.9998$) ---------- (8)

The accuracy of the method for the simultaneous estimation of the drugs was demonstrated by the percent recoveries which were well within the limit (100 ±2%). Tables 6, 7 present the findings.

Precision: In the current study, triplicate injections of three different solutions with known quantities of added drugs (50, 100, and 200% of the nominal concentration) were conducted. Regression equations were used to calculate percent recoveries (concentrations). Table 8 displays the findings of the precision study. The method is precise within the required recovery range, as shown by the low RSD of the total variation [17].

System suitability: All the system suitability parameters like peak area, USP plate count, tailing, and resolution met the desired level [18]. Results are presented in Table 9.

Robustness: The robustness was studied by the slight but deliberate change in intrinsic method parameters. The flow rate, buffer concentration, and column temperature were examined in our robustness investigation [19]. The RSDs for peak areas were found to be less than 2%. Table 10 displays the outcomes.

Table 6: Linearity data for Metformin, Dapagliflozin, and Vildagliptin

Metformin		Dapagliflozin		Vildagliptin	
Conc (µg/mL)	Average area ratio	Conc (µg/mL)	Average area Ratio	Conc (µg/mL)	Average area ratio
25	260999	0.5	25693	5	59673
50	522868	1	52507	10	119156
75	784587	1.5	78745	15	179591
100	1038218	2	103268	20	234968
125	1318201	2.5	130817	25	293268
150	1551615	3	154430	30	357183

Table 7: Accuracy data for Metformin, Dapagliflozin, and Vildagliptin

% of nominal	Amount Spiked (µg/mL)			Amount recovered (µg/mL)			% Recovery (µg/mL)		
	Metformin	Dapagliflozin	Vildagliptin	Metformin	Dapagliflozin	Vildagliptin	Metformin	Dapagliflozin	Vildagliptin
50%	50	1	10	49.75	1.01	9.90	99.50	100.62	98.98
	50	1	10	50.22	1.00	9.97	100.44	100.20	99.69
	50	1	10	49.84	0.99	10.16	99.67	99.41	101.62
100%	100	2	20	100.49	2.00	19.87	100.49	99.88	99.34
	100	2	20	99.78	1.99	19.80	99.78	99.53	99.01
	100	2	20	100.00	1.98	19.86	100.00	99.08	99.31
150%	150	3	30	149.68	2.99	29.90	99.78	99.73	99.67
	150	3	30	149.67	2.94	30.03	99.78	98.01	100.09
	150	3	30	150.17	3.04	29.90	100.11	100.41	99.66
Mean % recovery							99.95%	99.65%	99.71%

Table 8: Summary of Precision

	Metformin	Dapagliflozin			Vildagliptin	
Injection	Method precision	Intermediate precision	Method Precision	Intermediate precision	Method Precision	Intermediate Precision
	Peak area	Peak area	Peak area	Peak area	Peak area	Peak area
1	1038705	1007359	103131	102874	239644	238186
2	1029248	1025659	104196	102401	238783	236719
3	1048668	1013152	103883	104630	238795	238328
4	1048833	1012068	104790	103069	239534	238584
5	1034448	1021742	104874	103850	238661	237528
6	1040259	1016564	104205	103274	238598	237613
Mean	1040027	1016091	104180	103350	239003	237826
SD	7763.1	6704.8	639.3	0.6	461.6	680.6
%RSD	0.7	0.7	787.4	0.8	0.2	0.3

Table 9: Analysis of System Suitability

Parameter	Metformin	Vildagliptin	Dapagliflozin	Limit
Tailing factor	1.04	1.06	1.07	USP $Tf \leq 2.0$
LOD	0.52	0.12	0.018	S/N =3:1
LOQ	1.58	0.35	0.053	S/N =10:1

RSD is Relative Standard Deviation. LOD is Limit of Detection.
LOQ is Limit of Quantification. [a] N = 6. [b] Based on visual detection.

Table 10: Robustness study

Variation of Parameter	Peak area			%RSD		
	Metformin	Vildagliptin	Dapagliflozin	Metformin	Vildagliptin	Dapagliflozin
Flow rate minus (-10%)	1129736	247033	112771	1.0	0.2	0.7
Flow rate plus (+10%)	1015163	230712	101070	0.3	0.2	0.3
Mobile phase minus (-10%)	1095849	240519	102945	0.3	0.5	0.8
Mobile phase plus (+10%)	1056329	240868	1086448	0.5	0.5	0.1
Temperature minus (-5^{0}C)	1084650	246450	106981	0.8	0.9	1.2
Temperature plus (+5^{0}C)	1027296	233332	101073	0.2	0.9	0.6

Table 11: Study of Forced Degradation

S. No	Degradation parameter	Metformin		Vildagliptin		Dapagliflozin	
		% Drug Undegraded	% Drug Degraded	% Drug Undegraded	% Drug Degraded	% Drug Undegraded	% Drug Degraded
1.	Acid degradation (0.1N HCl/60^{o}c/30min)	94.67	5.33	94.04	5.91	94.09	5.91
2.	Alkaline degradation (0.1N NaOH/60^{o}c/30min)	94.23	5.77	93.58	6.42	94.11	5.89
3.	Oxidative degradation (20%w/v H_2O_2/60^{o}c/30min)	95.90	4.10	94.73	5.27	94.54	5.46
4.	Photo degradation (UV radiation at 200-Wh/m^2)	96.56	3.44	96.69	3.31	96.38	3.62
5.	Temperature degradation (105^{o}c/1day)	98.64	1.36	97.15	2.85	97.66	2.34
6.	Neutral degradation (water/60^{o}c/6hrs)	99.10	0.90	99.01	0.99	99.78	0.22

***Forced degradation study**:* These experiments were conducted in 0.1 M HCl, 0.1 M NaOH, 10% H_2O_2 solution, and a UV chamber for seven days or 200 W·h/m2. After being kept in the dark for 24 h, samples were examined using the recently created technique. Their peak areas were compared with those of a freshly made standard solution [20]. Metformin was found to be significantly stable to all environmental

factors, degrading the most in alkaline media. In contrast, Dapagliflozin displayed sensible stability under all circumstances, degrading the most in acidic media and Vildagliptin displayed sensible stability under all circumstances, degrading the most in alkali media. Marked degradation took place, and it was found that 6.42% of Vildagliptin and 5.89% of Dapagliflozin degraded at low base concentrations (0.1 M NaOH) and relatively high percent of drug, i.e., 5.91% of Vildagliptin and 5.91% of Dapagliflozin, degraded at low concentrations of the acid (0.1 M HCl), when compared to basic conditions. As the percentage of degradation is within the limits (5–20%).it has been proven that all drugs are stable at pH 4. These data can be used for the analytical method development for the determination of Vildagliptin, Dapagliflozin, and Metformin in tablet preparations. The results are given in Table 11.

CONCLUSION

The development of the HPLC analytical method has been investigated using a QbD methodology. The approach's goals are explained in light of the analytical target product profile. The experimental design identifies and specifies the main elements of the HPLC method, such as the flow rate, column temperature, and percentage of organic phase composition. In order to create the HPLC technique for metformin, vildagliptin, and dapagliflozin, the analytical QbD ideas were extended. Three elements were combined in a multivariant evaluation of many crucial process parameters: the temperature of the column at three distinct levels, the flow rate, and the mobile phase composition. The goal of this study was to determine the ultimate design space and the system with the highest performance. Central Composite Design was used to analyze and optimize their interactions with one another. Here, the factors affecting the effectiveness of the procedures and chromatographic separation are made clear. This method provides useful information that makes it easier to create chromatographic optimization that will be relevant in the future. It was found that every verified parameter complied with the approval specifications. It was discovered that the validated method for determining dapagliflozin, metformin, and vildagliptin was robust, linear, precise, and accurate. The QbD approach to method creation has improved our understanding of the method variables and allowed for the verification and transfer of methods with fewer failures. The automated QbD method creation process that makes use of the Design Expert software has produced a more reliable and effective approach.

COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies with human participants performed by any of the authors.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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AUTHORSHIP CONTRIBUTION STATEMENT

Ramya Kuber Banoth: Supervision, Conceptualization, Writing - review & editing, Validation. Jyothi Pusapati and Theja Indireddy: Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Visualization, Resources, Validation.

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