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QBD-BASED STABILITY-INDICATING RP-HPLC METHOD OF BEMPEDOIC ACID AND EZETIMIBE IN PHARMACEUTICAL DOSAGE FORMS

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Abstract:
The present study highlights a systematic Quality by Design–assisted development of an efficient analytical method for the estimation of Bempedoic acid and Ezetimibe in tablet dosage form. Daclatasvir was selected as the internal standard due to its suitable chromatographic behavior, good peak symmetry, and adequate resolution from the analytes. It showed no interference with the drug peaks and provided a consistent response, thereby improving method precision and reliability. Response surface methodology based on design of experiments was employed to identify critical method parameters influencing the critical analytical attributes. Chromatographic separation was achieved on a Sunfire C18 column (250 × 4.6 mm, 5 µm). The effects of acetonitrile content (v/v), flow rate, and column temperature on retention time, resolution, and number of theoretical plates were systematically investigated and optimized. The optimum chromatographic conditions within the design space consisted of an isocratic mobile phase of buffer and acetonitrile (60:40, v/v) at a flow rate of 1.0 mL/min with a run time of 6 min. Both drugs exhibited significant degradation under acidic, alkaline, and oxidative stress conditions, while minor degradation was observed under thermal and photolytic stress. Overall degradation ranged from approximately 6–17% under the applied stress conditions, and degradation products were well resolved from the main analyte peaks, confirming the stability-indicating nature of the method. The retention times of Bempedoic acid and Ezetimibe were 2.36 and 3.00 min, respectively. Method validation was performed in accordance with ICH and FDA guidelines. Central composite design was applied for optimization, yielding an overall desirability of 1.0 and establishing a Method Operable Design Region that ensured robust chromatographic performance.
Keywords: Critical analytical attributes, central composite design, design of experiments, Design-Expert 10.0.2 software and response surface methodology.

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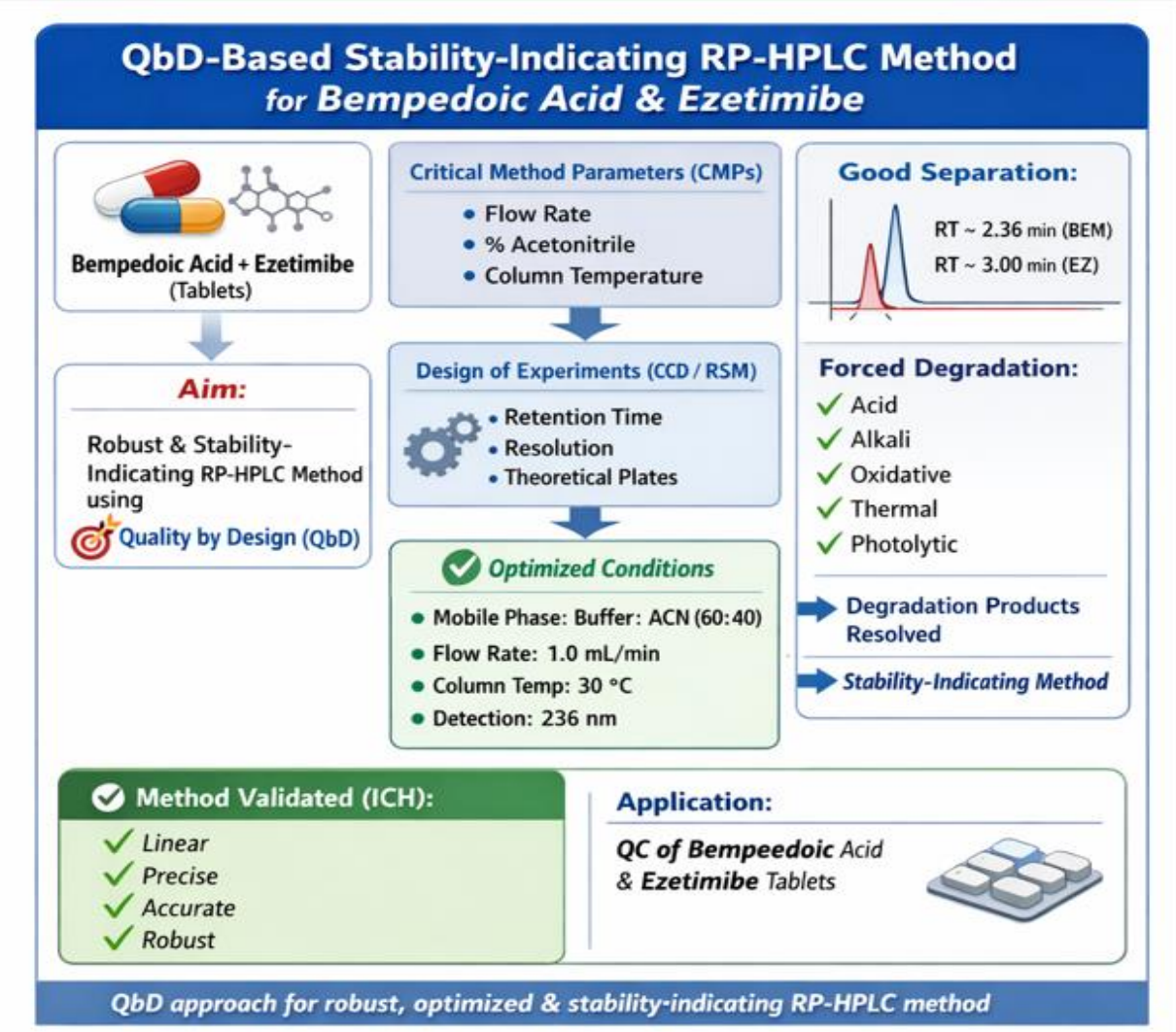
Graphical Abstract



INTRODUCTION:

Compared to a traditional or formal approach, a quality by design (QbD) approach to method creation emphasizes risk management more, which results in a more robust and long-lasting method [1, 2]. Understanding how dependent variables and the effects they have on responses through a suitable analysis is a crucial component of QbD. In this work, a risk-based high performance liquid chromatography (HPLC) approach for estimating the amounts of Bempedoic acid and Ezetemibe in tablets is developed and validated. The optimization of traditional HPLC techniques involves identifying one component at a time while holding the other variables constant, which leads to several tests with inadequate comprehension of crucial parameters [3, 4]. QbD has developed into a powerful tool for creating chromatographic techniques in the present era. The advantages of this approach include a thorough understanding of the parameters influencing chromatographic separation by identifying the

most important ones, as well as their impact on the selected responses, both positive and negative, and the multifaceted interactions among them [5, 6]. Additionally, QbD suggests the key fixes and promotes the deliberate modification of the variables to achieve the intended outcome. Early on in the development phase, the QbD approach advises assessing the quality of the analytical process [7–11].

The prescription drug bempedoic acid, also known by the brand name Nexletol, lowers low-density lipoprotein cholesterol (LDL-C), or "bad cholesterol," and lowers the risk of heart attack. Patients who are unable to tolerate statin therapy because of side effects including muscle soreness are frequently treated with this oral, non-statin medication. The liver transforms the prodrug Bempedoic acid into its active form. It functions by blocking adenosine triphosphate (ATP)-citrate lyase, an enzyme involved in the liver's synthesis of cholesterol. Because of this activity, the liver produces less cholesterol, which lowers blood levels of LDL-C.

A cholesterol-lowering drug called Eezetimibe is used to treat elevated blood cholesterol levels. Ezetimibe belongs to a class of medications called inhibitors of cholesterol absorption. It functions by obstructing the Niemann-Pick C1-Like 1 (NPC1L1) protein in the small intestine's brush barrier. Ezetimibe reduces the amount of cholesterol that is delivered to the liver, which subsequently removes more cholesterol from the blood, by reducing the intestinal absorption of cholesterol. As a result, "bad" low-density lipoprotein (LDL) cholesterol levels are reduced [12-16].

Although several analytical methods have been reported for the estimation of Bempedoic acid and Ezetimibe either individually or in combination, these methods are predominantly based on trial-and-error optimization and lack a systematic Quality by Design (QbD) framework. Existing methods do not establish a defined design space or Method Operable Design Region (MODR), and they provide limited understanding of the relationship between critical method parameters and critical analytical attributes. Moreover, robustness is generally evaluated post-development rather than being predicted through statistical modeling. Importantly, no QbD-driven stability-indicating RP-HPLC method has been reported for this drug combination to date. Considering the physicochemical differences between Bempedoic acid and Ezetimibe and their susceptibility to degradation, a systematic QbD-based approach is necessary to ensure reliable separation, consistent performance, and regulatory compliance. Therefore, the present study was undertaken to develop and validate a stability-indicating RP-HPLC method using a QbD strategy with design space establishment and MODR definition, thereby addressing the limitations of existing methods and providing a robust analytical tool for routine quality control. In this study, a unique stability indicating HPLC method is developed and validated utilizing quality by design concepts for the simultaneous quantification of Bempedoic acid and Ezetemibe in pharmaceutical formulations.

MATERIALS AND METHODS

Bempedoic acid and Ezetemibe working standards were procured from Shree Icon Pharmaceutical Laboratories, Vijayawada. Commercially available fixed-dose combination tablets containing both drugs were used for analysis. HPLC-grade acetonitrile, Hydrogen peroxide, Hydrochloric Acid (Analytical Reagent), and water were purchased from Merck (India). Orthophosphoric acid (OPA, AR

grade) was used for buffer preparation. All other chemicals used were of analytical grade. Bempedoic acid and Ezetemibe combination tablet containing BEM 180 mg and EZ 10 mg was purchased from the local medical store. This study does not involve experiments on animals or human subjects.

Standard Preparation:

Accurately weighed and transferred 45mg of Bempedoic acid and 2.5 mg of Ezetemibe working Standards into a 50ml clean dry volumetric flask, added 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Sample Preparation:

1tablet was weighed, powdered and then was transferred into a 100 mL volumetric flask, 50mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.5 ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Selection of appropriate detection wavelength

Bempedoic acid and Ezetemibe concentrations were scanned in the 200–400 nm range with the wavelength maxima of 236 nm choosen as the detecting wavelength.

Chromatographic specifications

A Waters Acquity HPLC system was utilized, fitted with a quaternary solvent manager, a sample manager, a TUV detector controlled by Empower 2 software, a cooling autosampler, and a column oven facilitating temperature control of the analytical column. A Sunfire C18 (250x 4.6 mm x 5µm) was used for this study. The temperature of 30°C and flow rate of 1 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 was filtered using 0.2 µm filter tips. Column effluents were viewed with a photo diode array (PDA) operating at 236 nm.

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

Independent variable(factor)	**Level of variable**			**Response**	**Target**
	low (–1)	**medium (0)**	**high (+1)**		
Flow rate of the mobile phase, (mL/min)	0.8318	1.0000	1.17	Retention of Bempedoic acid RT_1; retention of Ezetemibe RT_2; Resolution RS; Resolution NTP1; number of Theoretical plates NTP2; number of Theoretical plates	Minimizing the retention time of both drugs while maximizing the resolution and no of theoretical plates
Acetonitrile content in mobile phase, % (v/v)	51.59	60.00	68.41		
Temperature, °C	24.95	30.00	35.05		

Choosing a High-Quality Target Product Profile.

The number of theoretical plates, resolution, and retention time (Rt) of Bempedoic acid and Ezetimibe were determined to be the quality target product profile (QTPP) for the suggested HPLC method.

Identification of the Critical Quality Attributes:

The QTPP is directly impacted by the critical quality attributes (CQAs), which are method variables. To maintain the QTPP allowable response range, three crucial procedure parameters had to be maintained: temperature, the mobile phase flow rate, and the amount of acetonitrile (v/v) in the mobile phase.

Ooptimization Design: The chromatographic conditions of the suggested method were refined through the use of an experimental design based on response surface methodology (RSM). After 20 tests, the central composite design (CCD) was chosen to optimize the three components. The independent variables and their levels were selected based on previous screening and existing knowledge. The dependent variables (responses) were retention times, the number of theoretical plates, and the resolution of the two medicines. The design matrix and the acquired data were subjected to multiple regression analysis, and the relationship between the independent variables and the data was established using the second-order polynomial function that resulted [24]. The variables, their ranges, and responses with matching objectives are listed in Table 1.

Statistical interpretation. Using Design-Expert 10.0.2, the experimental design and statistical analysis were 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 diagnostic tools like the residual vs. predicted plot and normal probability plot of residuals, suitability of the model was evaluated.

Validation of analytical methods. In terms of specificity, solution stability, linearity range, accuracy, precision, system applicability, and forced degradation tests, the novel method was verified in accordance with ICH, USP, and FDA criteria.

Specificity. To ensure that no contaminants, degradation products, or excipients affected the drug peaks in the chromatogram, the method's specificity was examined. In addition to ocular assessment, peak purity indices were used to assess the drugs' specificity.

Stability of the solution. To find out if the drug combination would stay stable in the diluting solvent, test solutions were kept in tightly sealed vials and refrigerated at 5°C for 24 hours at room temperature. The solutions at 0 and 24 hours were analyzed using the established approach. The area does not appear to have changed. The relative standard deviation (RSD) of the drug's stability in the diluting solvent was found to be less than 2.0%.

Range of linearity. Peak area was plotted against several concentrations (20–150% of nominal) to create a calibration curve, which was then used to evaluate the method's linearity and operating range. The linear equation, regression coefficient (r^2), slope, and intercept were all calculated.

Precision. The information comes from at least nine analyses at three concentrations. The intra-day (repeatability) and inter-day (intermediate precision) levels of precision evaluation are the two most

often utilized. In the current study, the three concentration levels of intraday precision were evaluated using three replicates at each concentration level. Two HPLC systems were used in two different days to evaluate intermediate precision. The relative standard deviation's overall fluctuation was calculated.

System Suitability. It is common practice to employ system appropriateness to make sure that a method is suitable for a certain analysis. Among the parameters that were validated in the current investigation were theoretical plate count, tailing factor, area resolution and repeatability, limit of detection, etc.

Forced degradation. The objective of this research is to intentionally deteriorate a sample. The purpose of these studies is to evaluate an analytical technique's sensitivity. The crucial information on the drug breakdown pathway that forced degradation research provides helps with both the rational formulation of the treatment and the development of multiple dose forms. Drug compounds or products deteriorate by 10–30% when exposed to water, oxidizing and reducing chemicals, bases, acids, and ultraviolet (UV) light. The procedure is then used to the degrade samples to determine whether the drug molecule and associated substance(s) interfere in any way.

RESULTS AND DISCUSSION

Method Development. *Initial Screening.* To determine the important elements and their levels and effects, a preliminary screening and literature search were carried out. According to screening experiments, Bempedoic acid and Ezetimibe are separated by a 40% methanol and 60% water mixture and the column used was Agilent C18 (4.6 x 250mm, 5µm); nevertheless, the resolution is outside of acceptable ranges, and the peaks are not symmetrical and tailing is observed. In contrast, the mobile phase in the second trial was a 0.01 N KH_2PO_4 (50%) acetonitrile (50%) mixture; all chromatographic conditions were kept constant, resulting in peaks that were asymmetrical with tailing and merged. In the third trial, mobile phase, which was composed of 0.01N Ammonium acetate (60%): Acetonitrile (40%) was examined. In this trail also Bempedoic acid peak shape is not symmetrical, so further trail is carried out. In the fourth trial, mobile phase, which was composed of 0.01 N KH_2PO_4 (60%)− acetonitrile (40%) was examined by changing the column to Sunfire C18 (4.6 x 250mm, 5µm).In this trail also Bempedoic acid peak shape is not symmetrical and base line noise was observed, so further trail is carried out. In the fifth and final trial, mobile phase, which was composed of 0.1% OPA (60%)− acetonitrile (40%) was examined by the mobile phase in this final trail bempedoic acid and Ezetimibe peak shapes are symmetrical with good resolution and no base line noise is observed.

Statistical analysis and experiments. Nine specifications derived from the design of trials with six repetitions at the domain's center were incorporated in the entire factorial design, which encompassed 20 experimental situations. Each of these situations resulted in a chromatogram. Table 2 shows the

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

		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4	Response 5
Std	**Run**	**A:FR**	**B:MP**	**C:Temp**	**RT1**	**RT2**	**RS**	**NTP1**	**NTP2**
		ml/min	%	0 C	min	num	num	num	num
1	17	0.9	55	27	2.736	3.395	3.7	4108	5448

2	13	1.1	55	27	2.215	2.749	3.4	3505	4845
3	5	0.9	65	27	2.842	3.715	4.7	4263	5603
4	9	1.1	65	27	2.313	3.021	4.4	3549	4889
5	6	0.9	55	33	2.438	3.023	3.4	3795	5135
6	1	1.1	55	33	2.036	2.522	3.2	3289	4629
7	3	0.9	65	33	2.547	3.342	4.6	3892	5232
8	2	1.1	65	33	2.131	2.791	4.3	3434	4774
9	7	0.831821	60	30	2.823	3.594	4.2	4445	5785
10	15	1.16818	60	30	2.04	2.593	3.6	3422	4762
11	19	1	51.591	30	2.356	2.903	3.2	3527	4867
12	14	1	68.409	30	2.479	3.321	5	3713	5053
13	16	1	60	24.9546	2.614	3.321	4.2	3835	5175
14	11	1	60	35.0454	2.153	2.735	3.9	3390	4730
15	18	1	60	30	2.351	2.993	4.1	3812	5152
16	12	1	60	30	2.352	2.993	4	3818	5158
17	4	1	60	30	2.357	2.997	4.1	3900	5240
18	10	1	60	30	2.358	2.999	4.1	3835	5175
19	20	1	60	30	2.366	3.016	4.1	3868	5208
20	8	1	60	30	2.386	3.026	4.2	3899	5239

runs and the responses that go with them. Bempedoic acid's retention time varied from 2.03 to 2.84 minutes, whereas Ezetimibe retention time varied from 2.52 to 3.71 minutes. As a result, these data can be used and analyzed to create the ideal environment for achieving the current study's goals. The significance of 2FI experimental models was examined using ANOVA; the results are shown in Tables 3 and 4. Fisher's ratio was used to assess each model's significance. The model appears to be significant based on the model F-values for responses 1 (RT1 of 408.99), 2 (RT2 of 402.73), 3 (RS of 144.12), 4 (NTP1 of 142.39), and 5 (NTP2 of 142.39). It was discovered that all three models had p-values below 0.0001, indicating their relevance. Every other term was also significant based on the associated F- and p-values. The quality of the resulting polynomial regressions was assessed using the determination coefficient (R^2), adjusted determination coefficient (adj. R^2), and anticipated determination coefficient (pred. R^2). The R^2 values were found to be extremely near to 1 in each case, suggesting that the regression curve fit the data with an accuracy of more than 99%. There was a good degree of agreement (difference less than 0.2) between the modified R^2 values and the projected R^2 values. It is possible to estimate sufficient precision based on the signal-to-noise ratio, which should be higher than 4 [25]. The current investigation found high accuracy values, which suggests a sufficient signal. The model is summarized in Table 5. Each model's coded polynomial equations were as follows (Eqs. (1)– (5)):

RT1 of Bempedoic Acid = +2.36-0.2332 A +0.0450 B -0.1266 C -0.0028 AB +0.0290 AC +0.0000 BC +0.0233 A^2+0.0184 B^2 +0.0063 C^2 (1)

RT2 of Ezetimibe = +3.00 -0.2984 A+0.1379 B -0.1602 C -0.0123 AB +0.0360 AC+0.0005BC+ $0.0290A^2$+$0.0355 B^2$ +$0.0058 C^2$(2)

RS = +4.10-0.1544 A +0.5365 B -0.0882C -0.0125 AB +0.0125 AC +0.0375 BC -0.0811 A^2-$0.0104 B^2$ -$0.0280 C^2$(3)

NTP1 = +3854.83-293.00 A +55.20 B -129.12 C -7.88 AB +44.12AC +5.37 BC +30.90 A^2 -79.94 B^2 -82.59 C^2(4)

NTP2 = +5194.83 -293.00 A +55.20B -129.12 C -7.88 AB +44.12AC +5.37 BC +30.90 A^2 -79.94 B^2 -82.59 C^2(5)

Where A represents the flow rate, B the percentage of acetonitrile, and C the temperature of the column. While AB, AC, and BC show their interaction impact, A, B, and C show the main effect terms. To ascertain whether the model was sufficient, a visual examination of the residual vs. predicted plot and the normal probability plot was conducted. A satisfactory fit to the data was shown by the residuals' normal distribution along the straight line with minimal scatter. The equation in terms of coded factors can be used to anticipate the response for particular levels of each factor. The residual plots showed a random distribution of residuals between +4 and 4 with no trend, indicating the lack of any systematic bias or outliers.

Function of Derringer desirability. Fig. 1 shows the specification for utilizing the Derringer desirability function to optimize each and every response. This methodology is based on the desirability value for each response. On the desirability function scale, which goes from zero to one, a completely wanted response requires a value around one; zero is considered the most undesired response. Based on their worth, the most attractive trials were selected. Therefore, the first trial with desirability one (i = 1) was selected for method optimization. The results are shown in Table 6.

Table 6. Optimized trials suggested by software based on desirability value

Trail No.	Flow rate, mL/min	Organic phase content, %	Temperature, °C	RT_1, min	RT_2, min	RS_1	NTP1	NTP2	Desirability
4	1.000	60.000	30.000	2.362	3.004	4.102	3854.834	5194.834	1.000

Selected Design area and ideal conditions for separation. Figs. 2–6 illustrate the standardized impacts of independent factors on the responses and their interplay with each other using two-dimensional (2D) overlay contour plots and three-dimensional (3D) response surface graphs. Using the data for the experimental settings and accompanying responses, an overlay contour plot was constructed in order to determine the design space and the optimal mobile phase composition. In this case, the objective was to decrease the retention time of both medications while also increasing resolution. To be more precise, the goal was to achieve minimum and maximum responses of 2.03 ≥ Rt of Bempedoic acid ≤ 2.84, 2.15 ≥ Rt of Ezetimibe ≤ 3.71,3.2 ≤ RS ≥ 5.6, 3289 ≥ NTP1 ≤ 4445, and 4730 ≥ NTP 2≤ 5785.. 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 30°C and a flow rate of 1.0mL/min, acetonitrile blend of 60.0% (v/v) and 0.1% ortho-phosphoric 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 Fig. 7.

Table 3. ANOVA for response surface 2FI and quadratic models (degrees of freedom, *F*-value)

Source	Degrees of freedom					*F*-value				
	RT of BA	RT of EZ	RS	NTP1	NTP2	RT of BA	RT of EZ	RS	NTP1	NTP2
Model	9	9	9	9	9	408.99	402.73	144.12	142.39	142.39
A	1	1	1	1	1	2712.63	2363.12	94.36	904.18	904.18
B	1	1	1	1	1	101.10	504.46	1138.86	32.09	32.09
C	1	1	1	1	1	799.76	680.83	30.78	175.60	175.60
AB	1	1	1	1	1	0.2210	2.33	0.3621	0.3826	0.3826
AC	1	1	1	1	1	24.57	20.15	0.3621	12.01	12.01
BC	1	1	1	1	1	0.0000	0.0039	3.26	0.1782	0.1782
A2	1	1	1	1	1	28.59	23.54	27.44	10.61	10.61
B2	1	1	1	1	1	17.73	35.35	0.4484	71.02	71.02
C2	1	1	1	1	1	2.11	0.9532	3.28	75.81	75.81

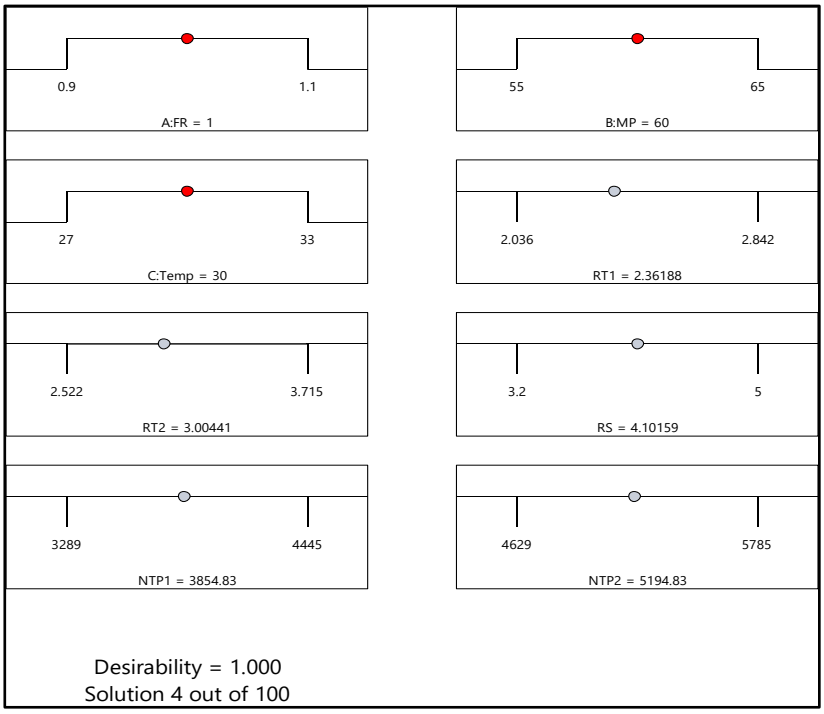
Table 4. ANOVA for response surface 2FI and quadratic models (*p*-value)

Source	*p*-value, prob > *F*				
	RT of BA	RT of EZ	RS	NTP1	NTP2
Model	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
A	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
B	< 0.0001	< 0.0001	< 0.0001	0.0002	0.0002
C	< 0.0001	< 0.0001	0.0002	< 0.0001	< 0.0001
AB	0.6484	0.1577	0.5607	0.5500	0.5500
AC	0.0006	0.0012	0.5607	0.0061	0.0061
BC	1.0000	0.9515	0.1012	0.6818	0.6818
A^2	0.0003	0.0007	0.0004	0.0086	0.0086
B^2	0.0018	0.0001	0.5183	< 0.0001	< 0.0001

C^2	0.1768	0.3519	0.1001	< 0.0001	< 0.0001

Table 5. Regression model summary

Model	R^2	Adj. R^2	Pred. R^2	Adeq. precision	Std. dev.	CV%
Model for RT1	0.9973	0.9949	0.9846	69.3035	0.0165	0.6910
Model for RT2	0.9972	0.9948	0.9822	74.3667	0.0227	0.7432
Model for RS	0.9923	0.9855	0.9688	43.4387	0.0588	1.46
Model for NTP1	0.9923	0.9853	0.9667	44.8695	36.01	0.9564
Model for NTP2	0.9923	0.9853	0.9667	44.8695	36.01	0.9564



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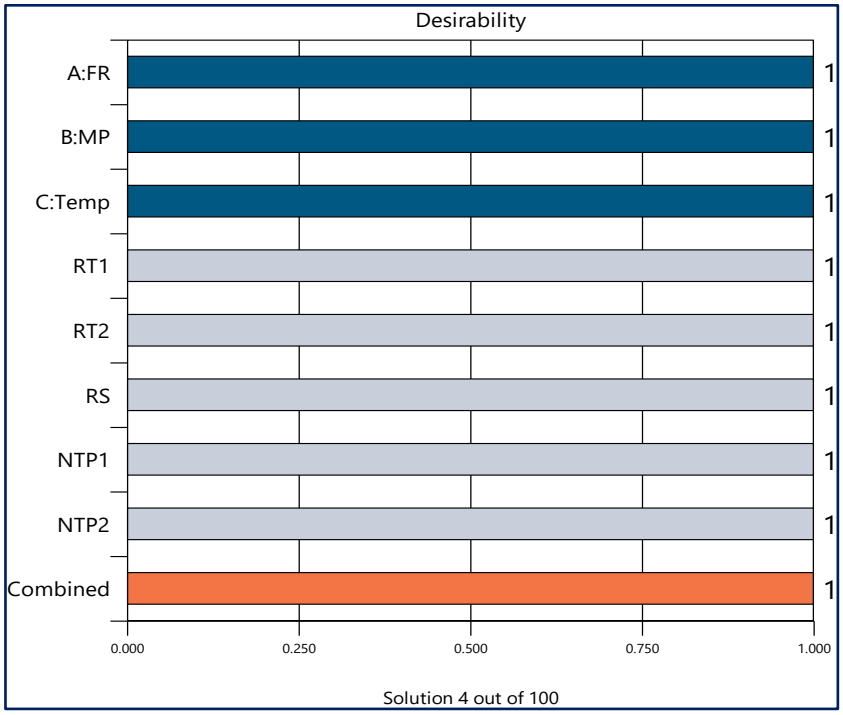
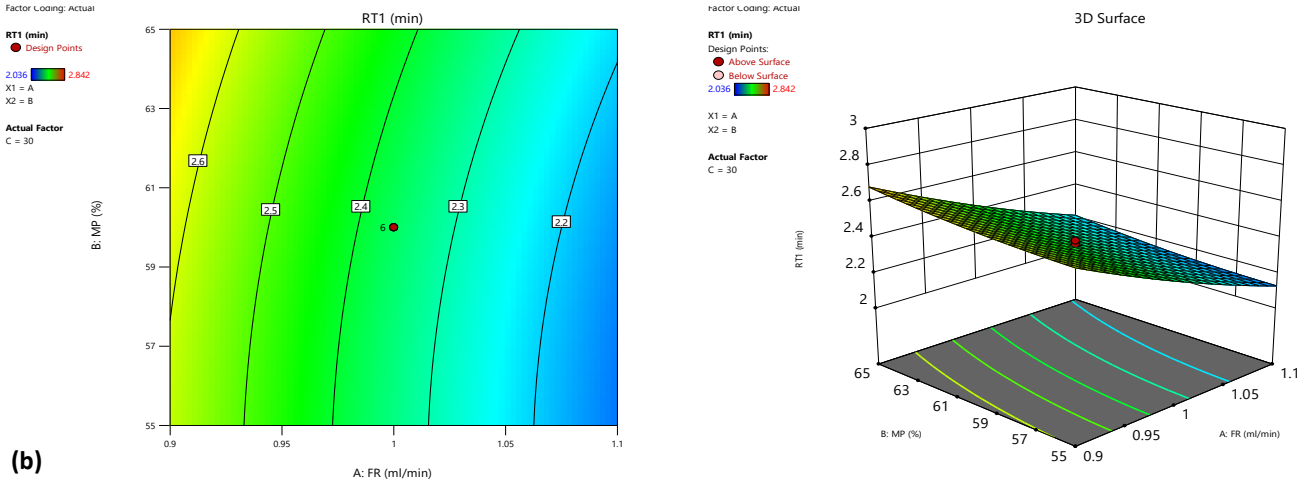


(a) g. 1. Ramps (a) and bar graph (b) of Derringer's desirability function representing the optimized perimental conditions, the individual and combined desirability values.



(b)

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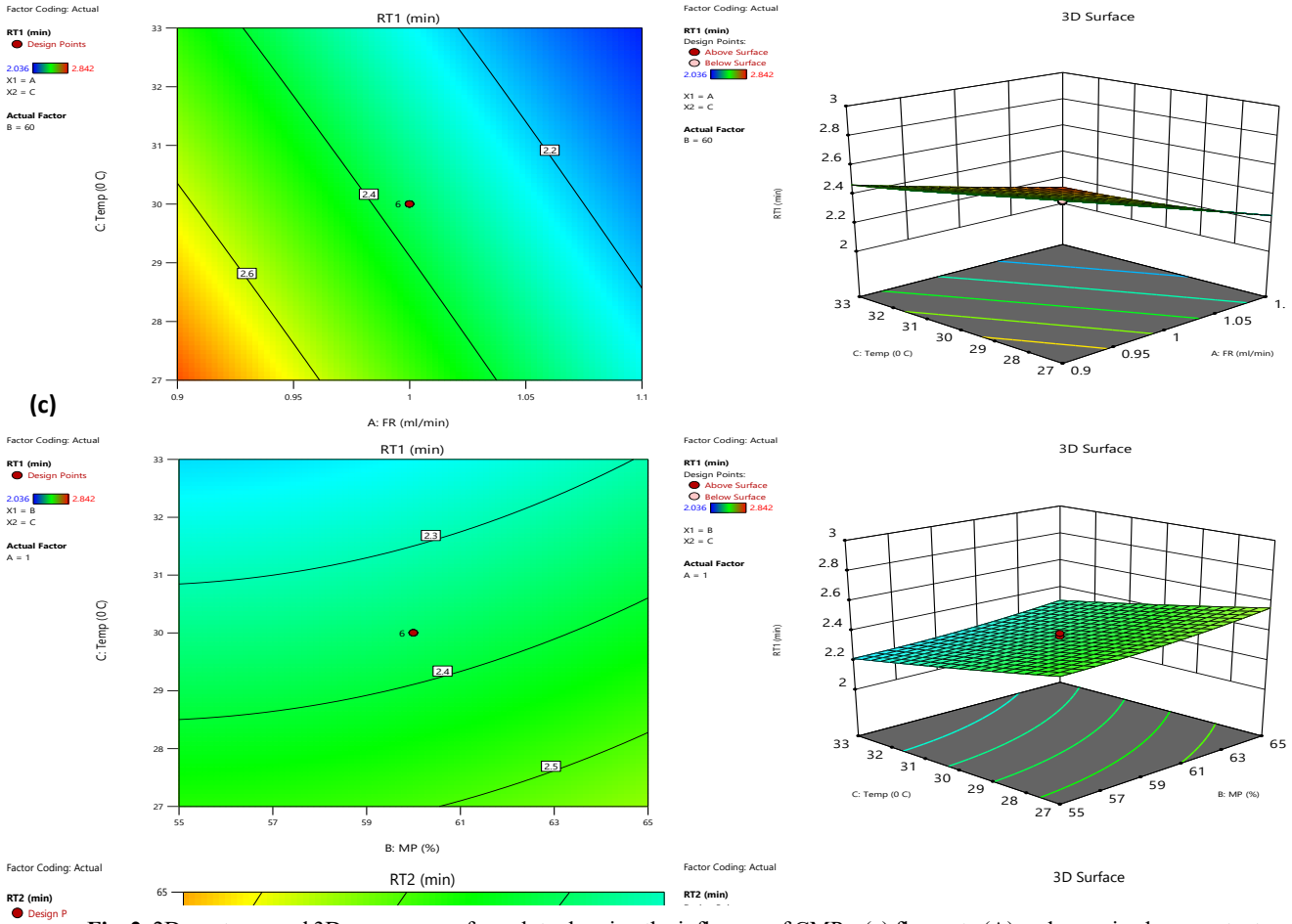
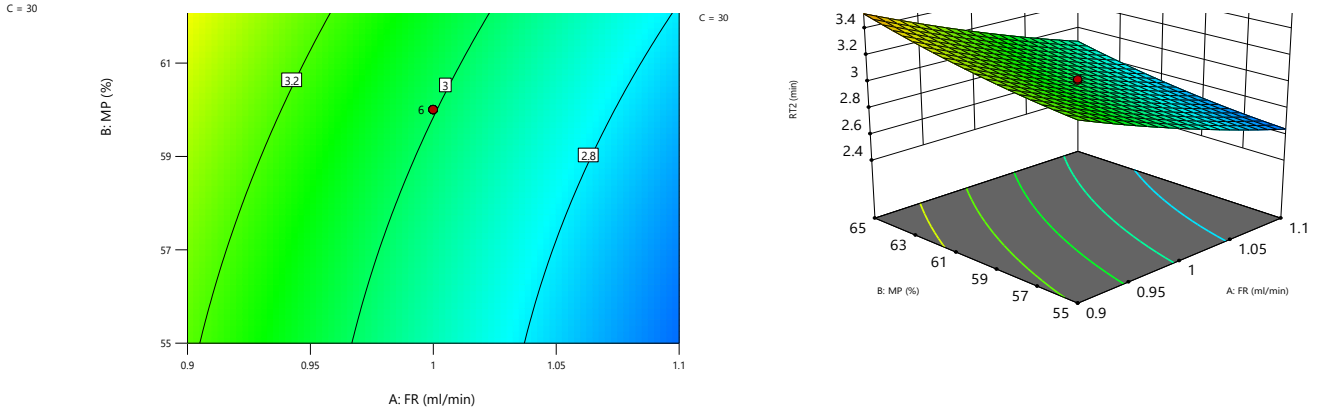


Fig. 2. 2D-contours and 3D-response surface plots showing the influence of CMPs: (a) flow rate (A) and organic phase content (%) (B); b) organic phase content (%) (B) and oven temperature (C); (c) flow rate (A) and oven temperature (C) on retention time (RT1) as the Critical Analytical Attribute(CAA).



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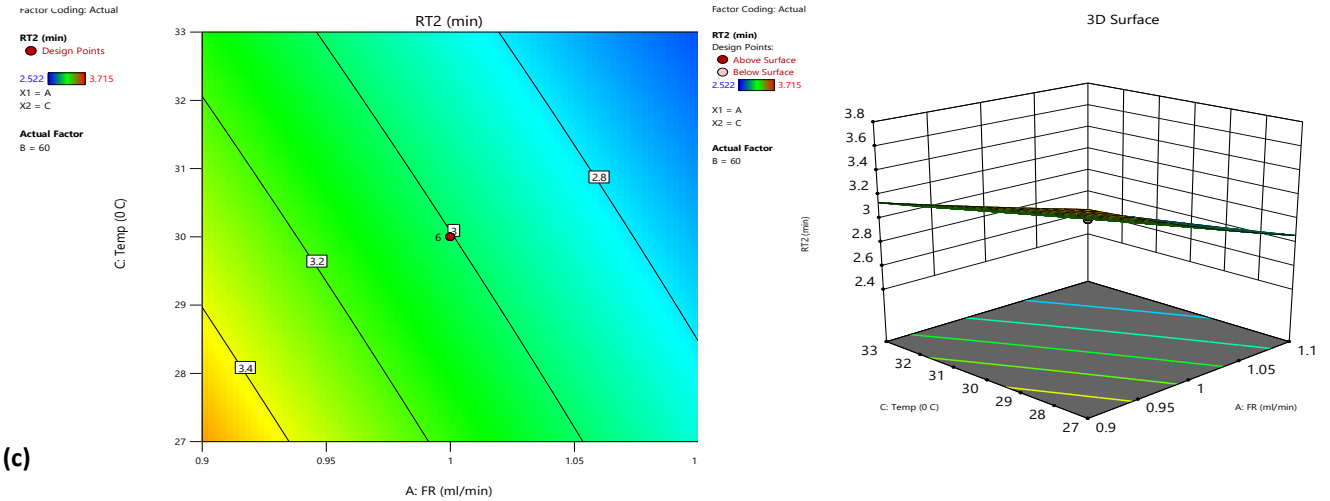

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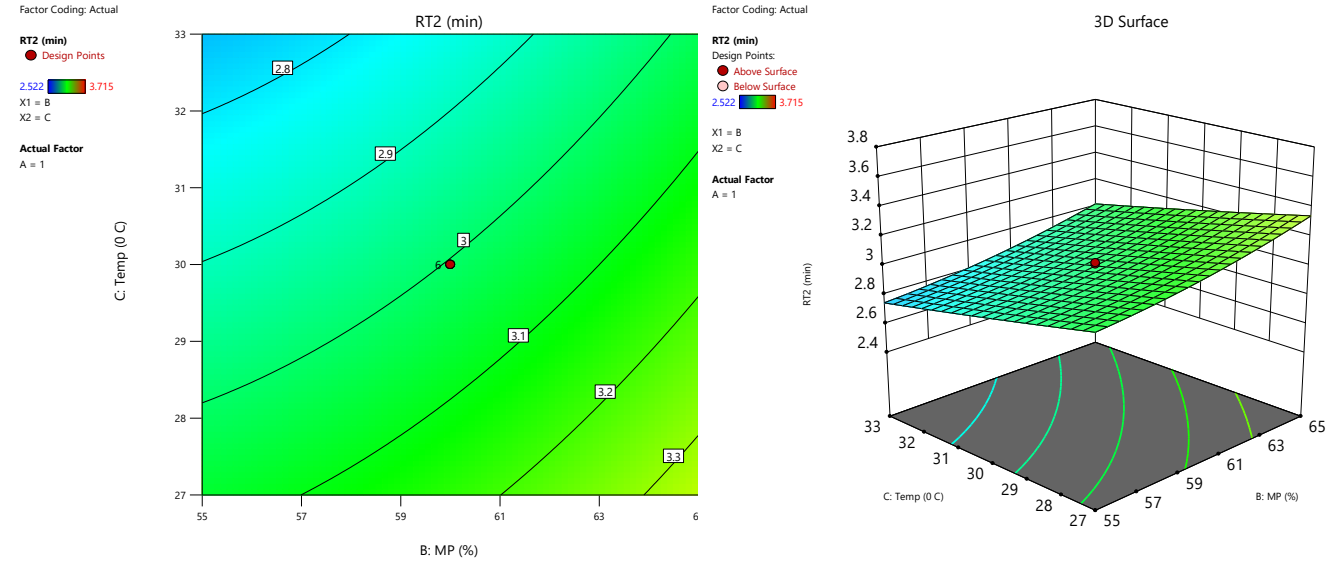

(a)

Fig. 3. 2D-contours and 3D-response surface plots showing the influence of CMPs: (a) flow rate (A) and organic phase content (%) (B); b) organic phase content (%) (B) and oven temperature (C); (c) flow rate (A) and oven temperature (C) on retention time (RT2) as the Critical Analytical Attribute(CAA).

(b)

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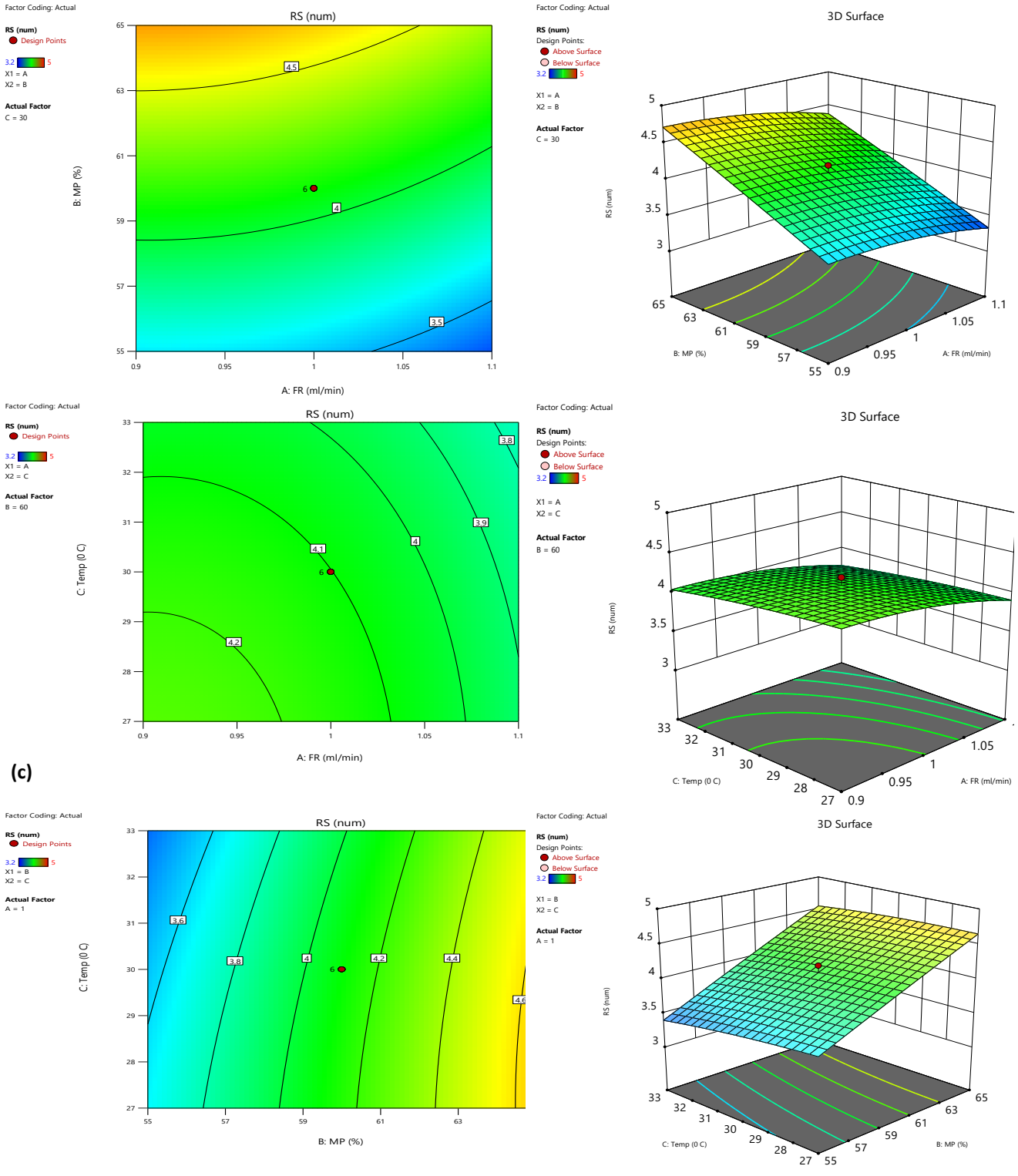
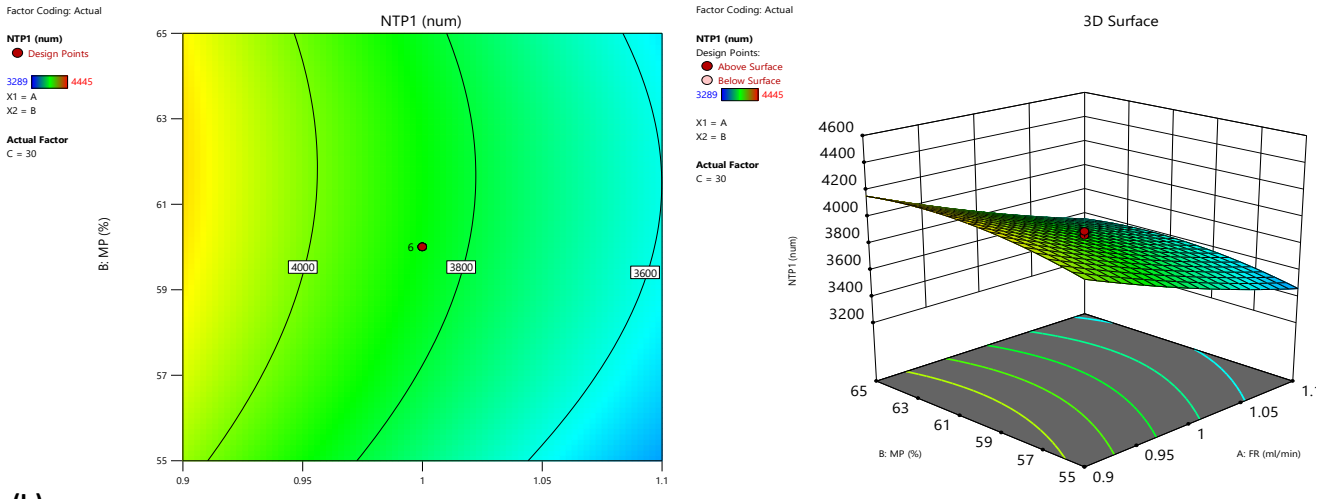


Fig.4. 2D-contours and 3D-response surface plots showing the influence of CMPs: (a) flow rate (A) and organic phase content (%) (B); b) organic phase content (%) (B) and oven temperature (C); (c) flow rate (A) and oven temperature (C) on resolution (RS) as the Critical Analytical Attribute(CAA).

(a)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NTP1 (num)
Design Points
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4445
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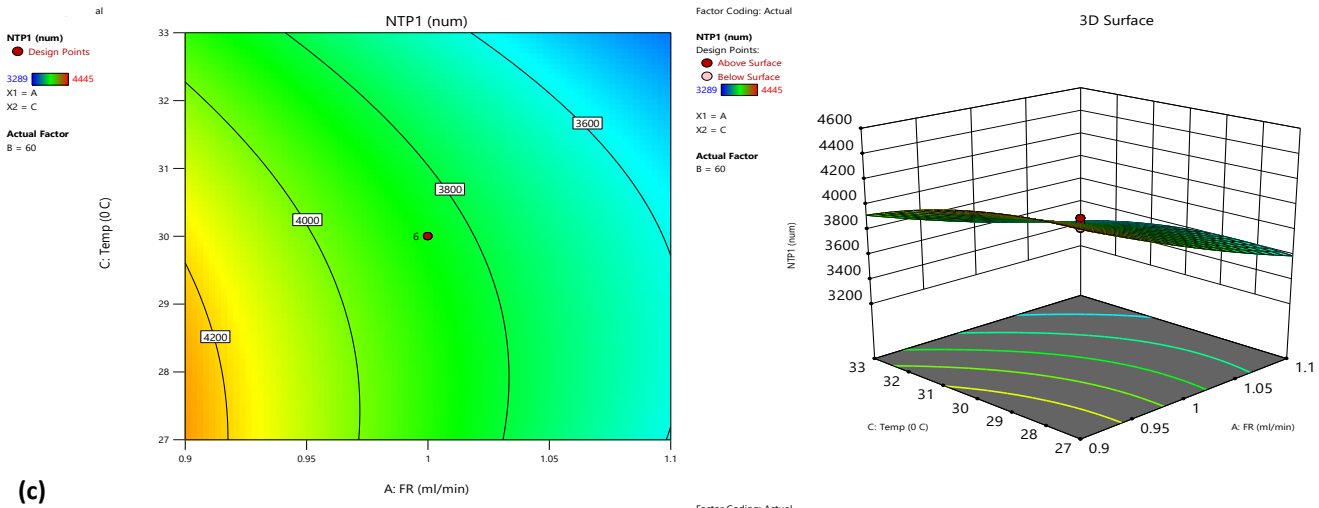
X2 = B
Actual Factor
C = 30
NTP1 (num)
B: MP (%)
A: FR (ml/min)
4000
3800
3600
Factor Coding: Actual
NTP1 (num)
Design Points:
Above Surface
Below Surface
3289
4445
X1 = A
X2 = B
Actual Factor
C = 30
3D Surface
NTP1 (num)
B: MP (%)
A: FR (ml/min)

(b)


NTP1 (num)
Design Points
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4445
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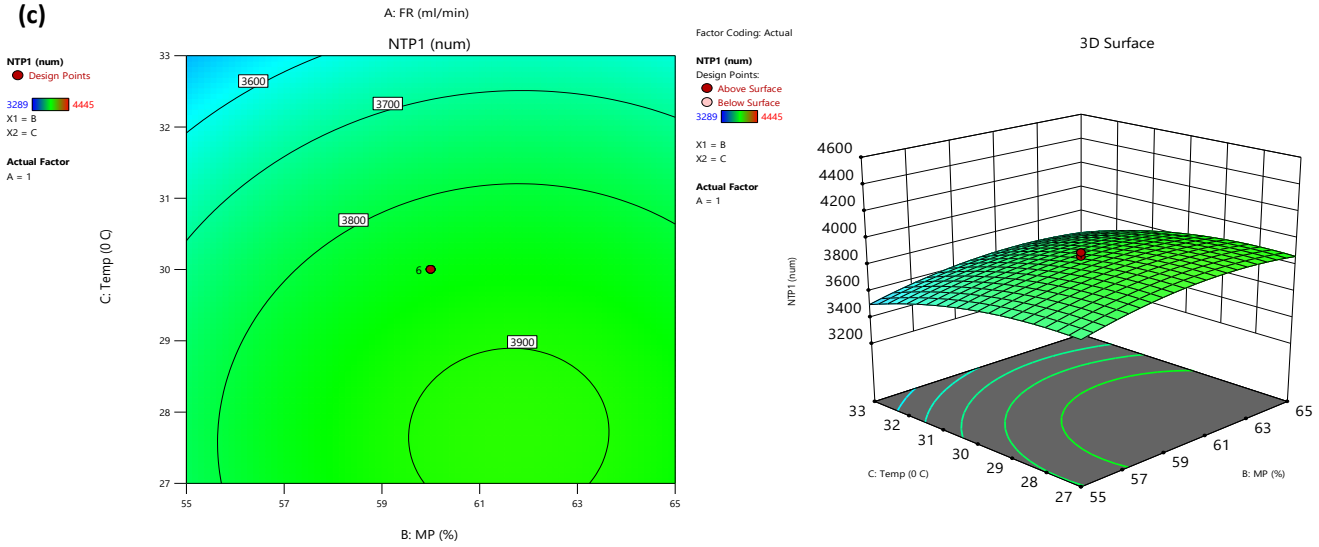
X2 = C
Actual Factor
B = 60
C: Temp (0 C)
A: FR (ml/min)
4200
4000
3800
3600
Factor Coding: Actual
NTP1 (num)
Design Points:
Above Surface
Below Surface
3289
4445
X1 = A
X2 = C
Actual Factor
B = 60
3D Surface
NTP1 (num)
C: Temp (0 C)
A: FR (ml/min)

(c)


NTP1 (num)
Design Points
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4445
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X2 = C
Actual Factor
A = 1
C: Temp (0 C)
B: MP (%)
3600
3700
3800
3900
Factor Coding: Actual
NTP1 (num)
Design Points:
Above Surface
Below Surface
3289
4445
X1 = B
X2 = C
Actual Factor
A = 1
3D Surface
NTP1 (num)
C: Temp (0 C)
B: MP (%)

Fig.5. 2D-contours and 3D-response surface plots showing the influence of CMPs: (a) flow rate (A) and organic phase content (%) (B); b) organic phase content (%) (B) and oven temperature (C); (c) flow rate (A) and oven temperature (C) on No of Theoretical Plates (NTP1) as the Critical Analytical Attribute(CAA).

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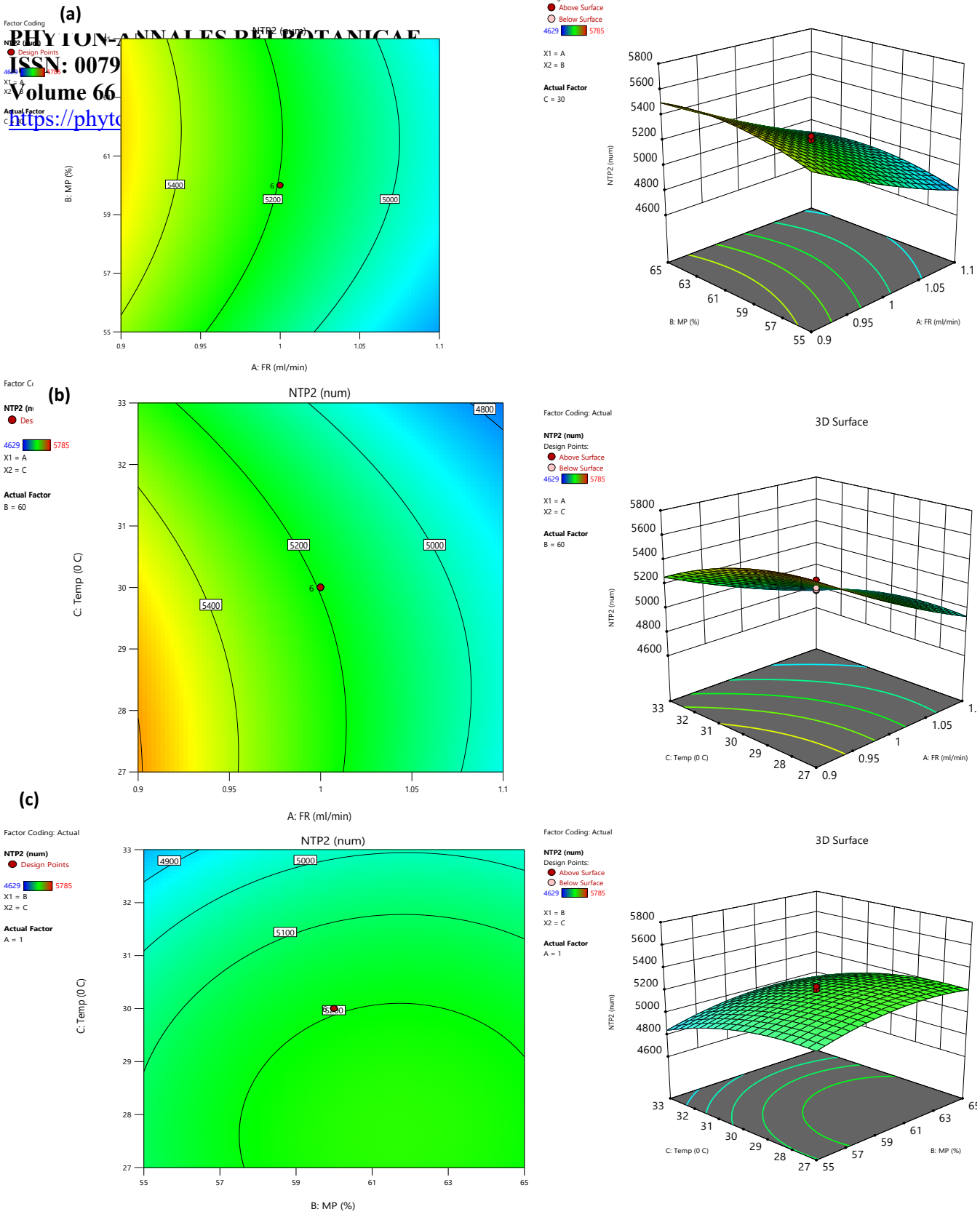

Fig.6. 2D-contours and 3D-response surface plots showing the influence of CMPs: (a) flow rate (A) and organic phase content (%) (B); b) organic phase content (%) (B) and oven temperature (C); (c) flow rate (A) and oven temperature (C) on No of Theoretical Plates (NTP2) as the Critical Analytical Attribute(CAA).

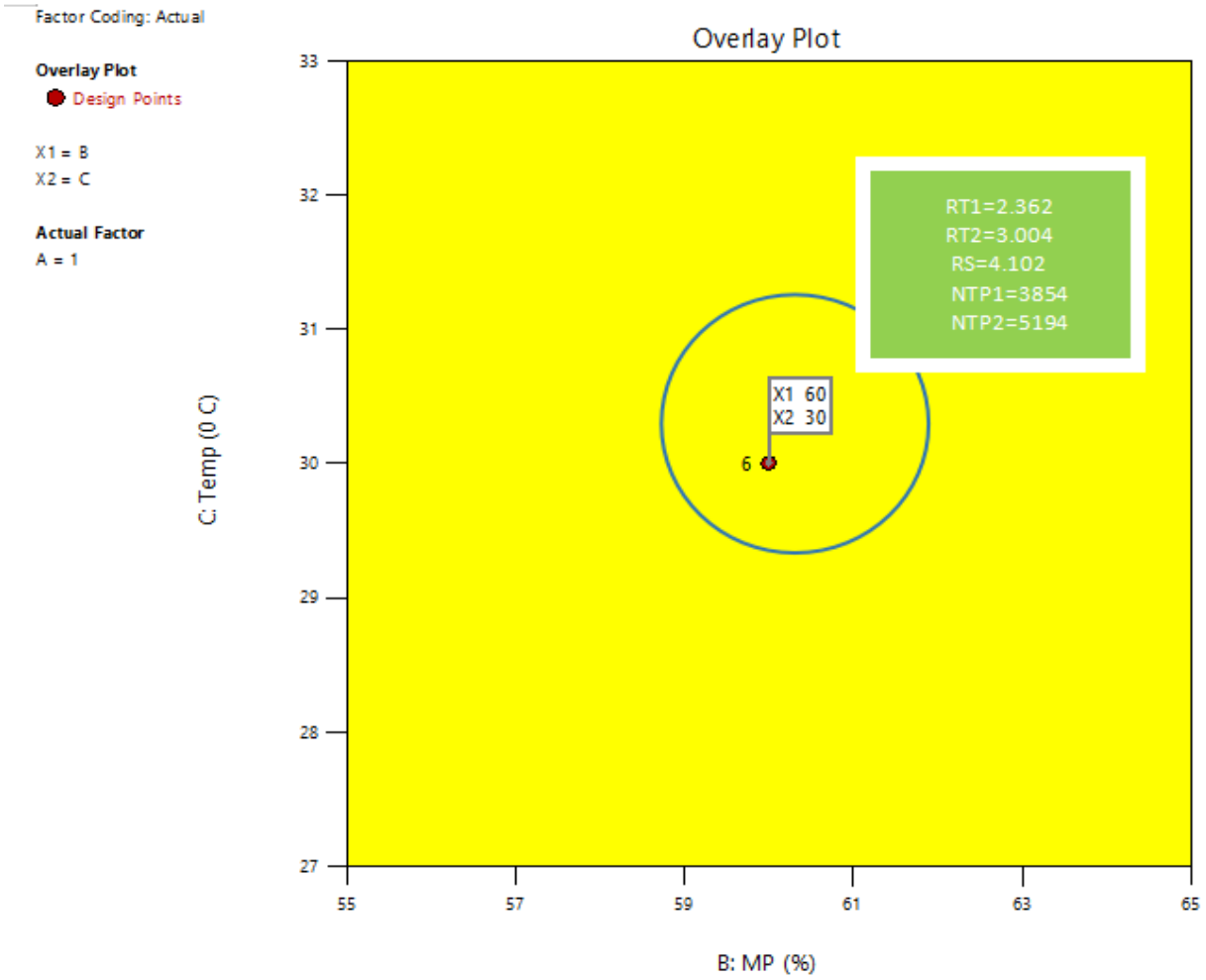

Fig. 7. Illustration of the overlay plot in graphical form displaying the ideal design space or technique operational design region.

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 Figs. 8b, 8c. The chromatogram of a blank obtained by a PDA detector is shown in Fig. 8a.

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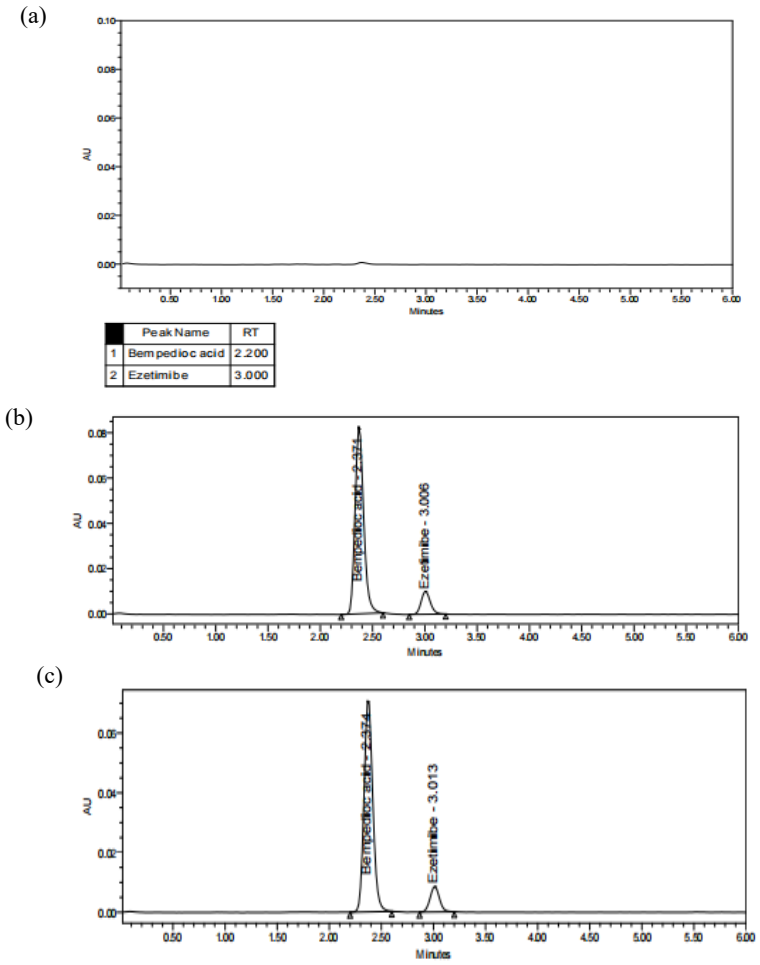

Fig. 8. Chromatograms of (a) blank, (b) standard Bempedoic acid and Ezetimibe, and (c) sample.

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%.

Linearity, working range, and accuracy. The *Linearity, working range, and accuracy.* The nominal standard concentration in the current validation procedure was 90 µg/mL of Bempedoic acid 5 µg/mL of Ezetimibe. Plotting the peak areas against the corresponding concentration, we prepared standard solutions at 25, 50, 75, 100, 125, and 150% of the nominal concentration. Both 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 22.5 – 135 µg/mL for Bempedoic acid and Ezetimibe. The regression equations were as follows (Eqs. (6) and (7)):

Bempedoic Acid :$y = 5435.1x + 1324.3$ ($r^2 > 0.999$), (6)

Ezetimibe: $y = 5433.1x + 1378.9$ ($r^2 > 0.999$) (7)

Table 7. Linearity data for Bempedoic acid and Ezetimibe

Bempedoic acid		Ezetimibe	
concentration, µg/mL	average area ratio	concentration, µg/mL	average area ratio
22.5	125036	1.25	15645
45	245798	2.5	30562
67.5	369393	3.75	46342
90	487184	5	62611
112.5	617031	6.25	77578
135	732911	7.5	91403

Table 8. Accuracy data for Bempedoic acid and Ezetimibe

% of nominal	Amount spiked, µg/mL		Amount recovered, µg/mL		Recovery, µg/mL	
	Bempedoic Acid	Ezetimibe	Bempedoic Acid	Ezetimibe	Bempedoic Acid	Ezetimibe
50%	45	2.5	44.6	2.51	99.0	98.5
	45	2.5	45.3	2.50	100.7	100.3
	45	2.5	45.2	2.48	100.4	98.9
100%	90	5	90.0	4.98	100.1	98.9
	90	5	89.3	4.97	99.2	99.8
	90	5	89.9	4.99	99.8	99.7
150%	135	7.5	135.0	7.49	100.0	99.3
	135	7.5	133.7	7.44	99.1	98.7
	135	7.5	135.8	7.47	100.6	99.3
Mean recovery, %					99.9	99.3

Table 9. Summary of precision

Injection	Bempedoic acid		Ezetimibe	
	method precision	inter-day precision	method precision	inter-day precision
1	485010	485994	61416	60695
2	492424	479983	60862	60312

3	486961	477543	61262	60120
4	491336	484844	61142	60395
5	486561	486262	60984	60349
6	491169	486133	60647	60743
Mean	488910	483460	61052	60436
SD	3094.0	3753.2	279.1	239.1
RSD, %	0.6	0.8	0.5	0.4

* Peak area values are given.

Table 10. Analysis of system suitability

Parameter	Bempedoic acid	Ezetimibe	Limit
RSD of area[a], %	0.8	0.3	RSD ≤ 2%
RSD of retention[b], %	1.2	1.03	RSD ≤ 2%
Tailing factor	1.40	1.422	USP *Tf* ≤ 2.0
Resolution	–	5.5	RS ≥ 2.0
LOD, µg/mL	0.21	0.02	*S/N* = 3 : 1
LOQ, µg/mL	0.65	0.06	*S/N* = 10 : 1

Abbreviations: RSD—relative standard deviation, LOD—limit of detection, LOQ—limit of quantification. a b
N = 6, based on visual detection.

Table 11. Robustness study

S. No.	Variation of parameter	Peak area		RSD, %	
		Bempedoic acid	Ezetimibe	Bempedoic acid	Ezetimibe
1	Flow rate minus (–10%)	815322 ± 3058	161818 ± 665.8	0.9	0.8
2	Flow rate plus (+10%)	684657 ± 6094.1	137757 ± 915.8	0.2	0.9
3	Mobile phase minus (–10%)	735565.2 ± 1273.2	148441 ± 987.3	0.3	1.0
4	Mobile phase plus (+10%)	745145 ± 3096.7	150183± 1474.1	0.3	0.3
5	Temperature minus (–5°C)	729114± 4693.6	147131± 671.6	1.3	0.4

6	Temperature plus (+5°C)	732783.2 ± 5548.3	146722 ± 1496.5	0.3	1.4

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 7, 8 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 9 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.

System suitability. All the system suitability parameters like peak area, USP plate count, tailing, and resolution met the desired level. Results are presented in column temperature were examined in our robustness Table 10. Investigation. The RSDs for peak areas were found to be less than 2%. Table 11 displays the outcomes. *Robustness.* The robustness was studied by the slight but deliberate change in intrinsic method

Forced degradation study. These experiments were parameters. The flow rate, buffer concentration, and 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/m^2. 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. Bempedoic acid was found to be significantly stable to all environmental factors, degrading the most in peroxide media. In contrast, Ezetimibe displayed sensible stability under all circumstances, degrading the most in acid media. Marked degradation took place, and it was found that 6.09% of Bempedoic acid and 6.92% of Ezetimibe degraded at low acid concentrations (0.1 M HCl) and relatively low percent of drug, i.e., 4.62% of Bempedoic acid and 4.45% of Ezetimibe, degraded at low concentrations of the base (0.1 M NaOH), when compared to acidic conditions. As the percentage of degradation is within the limits (5–20%), it has be proven that Bempedoic acid and Ezetimibe are stable. These data can be used for the analytical method development for the determination of Bempedoic acid and Ezetimibe in tablet preparations.

The results are given in Table 12.

Table 12. Forced degradation study

S. No.	Degradation parameter	Bempedoic acid		Ezetimibe	
		drug undegraded, %	drug degraded, %	drug undegraded, %	drug degraded, %
1	Acid degradation (0.1 N HCl/60°C/30 min)	93.91	6.09	93.08	6.92
2	Alkaline degradation (0.1 N NaOH/60°C/30 min)	95.38	4.62	95.55	4.45
3	Oxidative degradation (20% (w/v) H_2O_2/60°C/30 min)	92.32	7.68	93.22	6.78
4	Photo degradation (UV radiation at 200 W h/m^2)	95.90	4.10	95.13	4.87
5	Temperature degradation (105°C/1 day)	97.33	2.67	97.36	2.64
6	Neutral degradation (water/60°C/6 h)	99.15	0.85	99.47	0.53

CONCLUSIONS

It has been discussed to use a QbD technique to design an analytical method for HPLC. The method aims are clearly stated based on the analytical target product profile. the primary experimental parameters of the HPLC technique, including the organic phase composition, flow rate, and column temperature. A multivariant study of several critical process parameters (CPP), including the combination of three factors—the mobile phase composition, flow rate, and column temperature at three different levels—was conducted to determine the best-performing system and the final design space in order to develop the HPLC method for Bempedoic acid and Ezetimibe. At several levels, their interrelationships were analyzed and optimized using central composite design. The variables influencing chromatographic separation and the efficacy of techniques are better understood in this context. This approach offers practical knowledge that supports chromatographic optimization and can be used in the future. All validated parameters were found to meet the acceptance criteria. The validated method was found to be linear, precise, accurate, and robust for the determination of bempedoic acid and ezetimibe. The use of the QbD methodology in method development has enhanced comprehension of the method variables and enabled more successful validation and transfer of methods. The automated QbD method development methodology employing the Design Expert software has created a method that is more robust and performs better in a shorter amount of time than manual method creation. This technique will be used by the pharmaceutical sector for routine analysis

and quality control of Ezetimibe and Bempedoic acid in a variety of pharmaceutical formulations.

SUMMARY

For the simultaneous measurement of bempedoic acid and ezetimibe in tablet dosage form, an RP-HPLC technique based on Quality by Design (QbD) was created and refined. In order to attain the best separation with short retention durations and good resolution, critical method parameters were methodically examined using a central composite design. The technique showed good linearity, precision, accuracy, and resilience after being validated in accordance with ICH requirements. The stability-indicating nature of the approach for regular quality control analysis was established by forced degradation tests, which verified that degradation products were well resolved from the analyte peaks.

ABBREVIATIONS

- **QbD:** Quality by Design
- **HPLC:** High Performance Liquid Chromatography
- **RP-HPLC:** Reverse Phase High Performance Liquid Chromatography
- **LDL-C:** Low-Density Lipoprotein Cholesterol
- **ATP:** Adenosine Triphosphate
- **NPC1L1:** Niemann-Pick C1-Like 1
- **BEM:** Bempedoic acid
- **EZ:** Ezetimibe
- **OPA:** Orthophosphoric acid
- **PDA:** Photo Diode Array
- **CCD:** Central Composite Design
- **RSM:** Response Surface Methodology
- **QTPP:** Quality Target Product Profile
- **CQA:** Critical Quality Attributes
- **ANOVA:** Analysis of Variance
- **RSD:** Relative Standard Deviation
- **RT:** Retention Time
- **NTP:** Number of Theoretical Plates
- **MODR:** Method Operable Design Region
- **LOD:** Limit of Detection
- **LOQ:** Limit of Quantification
- **ICH:** International Council for Harmonisation
- **FDA:** Food and Drug Administration
- **USP:** United States Pharmacopeia
- **2FI:** Two Factor Interaction

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AUTHOR CONTRIBUTIONS

Miss. H. Neelofar: Conceptualization, Methodology, Experimental work, Data curation, Formal analysis, Software (Design-Expert), Validation, Visualization, Writing – original draft.

Prof. D. Ranganayakulu: Supervision, Resources, Project administration, Investigation, Writing – review and editing, Critical revision of the manuscript.

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