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Design Approach in RP-HPLC Method Development and Validation of Nirogacestat in its Pure and Pharmaceutical Dosage Form

Chandrasekar R*[1], Sivagami B[2], Roshini M[2], Kumanan R[3], Sudha S[3], Sai Koteswar Sharma [4]

[1]Associate Professor, Faculty of Pharmacy, Department of Pharmacognosy, Seven Hills College of Pharmacy, Tirupati, Andhrapradesh, India.

[2]Associate Professor, Faculty of Pharmacy, Department of Pharmaceutical Analysis, Seven Hills College of Pharmacy, Tirupati, Andhrapradesh, India.

[2]PG Student, Dept of Pharmaceutical Analysis, Seven Hills College of Pharmacy, Tirupati, Andhrapradesh, India.

[3]Professor, Faculty of Pharmacy, Department of Pharmacognosy, Devaki Amma Memorial College of Pharmacy, Malappuram, 673634, Kerala, India.

[3]Professor, Faculty of Pharmacy, Department of Pharmaceutical Biotechnology, Devaki Amma Memorial College of Pharmacy, Malappuram, 673634, Kerala, India.

[4]Professor, Faculty of Pharmacy, Department of Pharmacognosy, Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupati, Andhrapradesh, India.

Corresponding author: Chandrasekar Raju [1]Associate Professor, Faculty of Pharmacy, Department of Pharmacognosy, Seven Hills College of Pharmacy, Tirupati, Andhrapradesh, India.

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KEYWORDS

Box Behnken Design, Experimental design, Dependent variables, Nirogacestat; Responses; Factors.

ABSTRACT:

Aim: Nirogacestat is a selective γ-secretase inhibitor approved by the USFDA for the treatment of Desmoid Tumour.

Objective: In this study, a reversed-phase high performance liquid chromatography (RP-HPLC) method was designed to analyze the concentration of Nirogacestat in both bulk and dosage forms.

Method: With a 30 % Trifluoroacetic acid pH3:70 % Acetonitrile (30:70 ml) mobile phase, 1.2 ml/min flow rate, 338 nm detection wavelength, 10 µl injection volume, and 12-minute run time, a SPURSIL C18-EP (4.6 x 250mm, 5µm) column was utilized. The experiment was designed using the Box-Behnken design (BBD), and the chromatographic conditions flowrate, buffer pH and buffer ratio were optimized by applying the response surface methodology (RSM). After evaluating separation response metrics such tailing factor and retention time to develop an optimization model, the desirability was assessed.

Result: The model indicated that a composite desirability of 0.9943 could be attained with a flowrate of 0.80 mL/min-1, a buffer pH of 4, and a buffer ratio of 30. The theoretical plate and tailing factor of the improved HPLC settings were all within acceptable limits.

Conclusion: The current research was found to be observed with less Rt and this method can be a suggested approach for the development of new techniques in pharma industries.

1. Introduction

The USFDA has approved nirogacestat, a selective γ-secretase inhibitor, to treat desmoid tumors.[1]An estimated 3 to 5 people per million are diagnosed with desmoid tumors, also known as aggressive fibromatosis, a rare kind of soft tissue tumor.[2] Desmoid tumors that compress important tissues can cause severe pain, functional impairment, nerve damage, and perforation or obstruction of the colon.[3] The way that Nirogacestat, an oral, small-molecule, selective γ-secretase inhibitor under research, works.[4]

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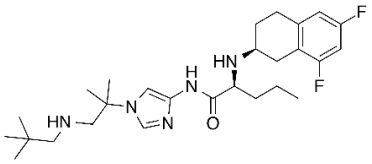

Fig. 1 Structure of Nirogacestat

However, there were few publications outlining Nirogacestat's contents. [5,6] There are no papers that report on reversed phase high-performance liquid chromatography (RP-HPLC). [7,8] To achieve a satisfactory separation, it is necessary to evaluate the suitable RP-HPLC conditions. [9,8]

The Box-Behnken design (BBD) for pharmaceutical dosage forms can be used in combination with response surface methodology (RSM), an experimental design for optimization purposes. [11,12] The ideal conditions for compound separation can be predicted through computational optimization of RP-HPLC settings. [13,14] Experimental design study can benefit from using the desire functions produced by the desirability analysis stage to improve the prediction quality.[15] With the use of the RSM, this study attempted to design an RP-HPLC method for achieving the right chromatographic conditions needed for Nirogacestat analysis. In order to obtain several responses, namely retention time and tailing factor, experimental parameters including flowrate, buffer pH, and buffer ratio were investigated in this study.

2. Methods

2.1. Materials

Nirogacestat was obtained as a gift sample from MSN Labs Pvt. Ltd. Hyderabad. KH_2PO_4, FINAR chemical LTD, Solvents of Acetonitrile for HPLC, water and methanol gradient grade for liquid chromatography (Standard solutions Ltd) and redistilled water were used in this study. HCl, H_2O_2, NaOH was purchased from Merckmillipore.

2.2. Instrumentation and Software

A system of WATERS HPLC, software: Empower, 2695 separation module. 2487 UV detector, accompanied with a column of SPURSIL C18-EP (4.6 x 250mm, 5□m) column was used in this study. Other instrumentation was listed as follow: UV/VIS spectrophotometer T460 LABINDIA UV 3000^+ pH meter Adwa – AD 1020, analytical balance Afcoset ER-200A, ultrasonicator, sterile syringe filter with a 0.2 µm pore size hydrophilic PTFE membrane (Merckmillipore), and a set of Borosil ® Pipettes and Burettes.

2.3. Methods

2.3.1. Standard and sample preparation

A precisely weighted 10 mg Nirogacestat standard was added to a 10 mL volumetric flask. Each volumetric flask containing nirogacestat standard was diluted into the volume using diluent. Before being injected into the HPLC system, these solutions were filtered using a sterile syringe filter membrane. Transfer 0.3 ml of the previously mentioned stock solution into a 10 ml volumetric flask and use diluents to dilute it to the appropriate level. (30 ppm)

2.3.2. Experimental design

Three factors, three levels, and four central points were used to develop the BBD. The buffer pH, buffer ratio and flowrate percentage were chosen as factors (independent variables). Conversely, separation attributes like tailing factor and retention time were chosen as responses (dependent variables). Table 1 displayed the experimental levels in combination with observational independent variables. Seventeen experimental runs were accomplished. The RP-HPLC equipment was utilized for these runs, with a 10 µL volume injection and UV detection at 338 nm. [3,4]

2.3.3. RSM observation

The seventeen experimental runs were produced by the BBD software, which were performed and observed. To develop the RSM model, each response for each compound was observed and recorded. The second-order polynomial models were developed by taking full advantage of all the variables and responses. Significant models were chosen for the purpose of producing the desire function during the desirability analysis phase of the study once all functions for each drug had been achieved. To visualize the RSM models, perspective plots for each response were also depicted. [5,6]

2.3.4. Desirability analysis

The desirability function has been generated according to the previous study. Each response can be set for minimum, maximum, or specific target value along with upper and lower value estimation.[3]

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2.3.5. System suitability test

The system suitability test was performed by injecting standards and samples solution containing Nirogacestat. These solutions were filtered using sterile syringe filter membrane before injection into HPLC system. These solutions were injected in six replications.

The BBD for optimization of independent variables and experimental dependent variables of Nirogacestat separation using RP-HPLC. The experimental design for optimization of independent variables and dependent variables of Nirogacestat separation using RP-HPLC was presented in Table 1.

3. Results

Table 1: Experimental Levels in Observational Independent and Dependent Variables

Std	Run	Block	Factor 1 a. Flow rate	Factor 2 b. Buffer ph	Factor 3 c. Buffer ratio	Response 1(RT)	Response 2 (Tailing factor)
17	1	Block 1	1.00	4.50	40.00	8.35	0.97
9	2	Block 1	1.00	4.00	30.00	5.303	0.96
5	3	Block 1	0.80	4.50	30.00	6.547	1
16	4	Block 1	1.00	4.50	40.00	8.35	0.97
3	5	Block 1	0.80	5.00	40.00	8.52	1.06
10	6	Block 1	1.00	5.00	30.00	5.228	1.07
11	7	Block 1	1.00	4.00	50.00	4.115	0.89
2	8	Block 1	1.20	4.00	40.00	7.053	0.95
15	9	Block 1	1.00	4.50	40.00	8.35	0.97
4	10	Block 1	1.20	5.00	40.00	6.717	1.07
6	11	Block 1	1.20	4.50	30.00	4.334	1.02
8	12	Block 1	1.20	4.50	50.00	6.655	0.98
13	13	Block 1	1.00	4.50	40.00	8.35	0.97
1	14	Block 1	0.80	4.00	40.00	10.2	0.98
14	15	Block 1	1.00	4.50	40.00	8.35	0.97
7	16	Block 1	0.80	4.50	50.00	7.879	1
12	17	Block 1	1.00	5.00	50.00	5.294	1.01

Seventeen experimental runs were carried out and observed. Retention time and tailing factor of Nirogacestat was evaluated. Each response for each analyte was modelled to obtain RSM model equations along with RSM properties such as multiple R^2, adjusted R^2, and p-value. The RSM model equations of retention time and tailing factor for Nirogacestat is presented in Table 2.

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Table 2: RSM model of Retention Time and Tailing Factor for Nirogacestat

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	< 0.0001		0.9071	0.8520	
2FI	0.9786		0.8815	0.6607	
Quadratic	< 0.0001		0.9943	0.9602	Suggested
Cubic			1.0000		Aliased

With a lower and higher value estimate of 4.1 and 10 minutes, respectively, the target value of the nirogacestat retention time was chosen at 8 minutes. A maximum value of 1.07 and a lower value prediction of 0.89 were chosen for the Nirogacestat tailing factor. A minimum value of 4.115 minutes and an upper value estimate of 10.2 minutes were specified for the nirogacestat retention time. To evaluate the Nirogacestat standard and dosage form containing Nirogacestat, the HPLC system was configured with the appropriate conditions derived from the RSM model, which were then followed by the desirability function (Figure 2-6).

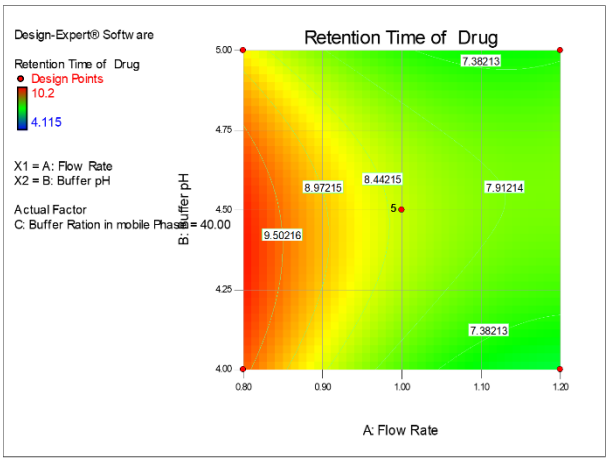

Fig. 2. Retention time for Nirogacestat

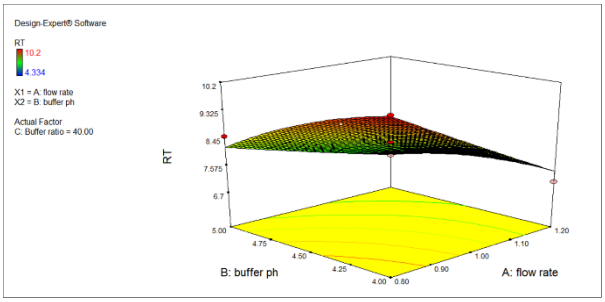

Fig. 3. 3D Graph of Retention time for Nirogacestat

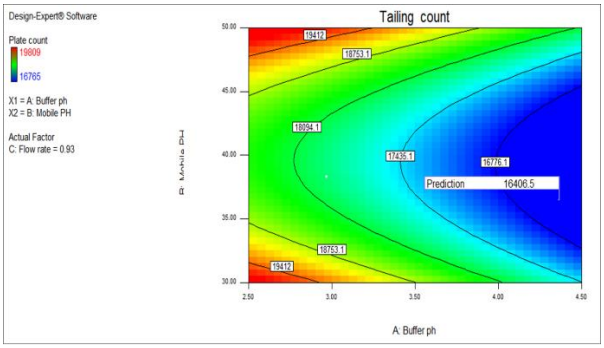

Fig. 4. Tailing factor for Nirogacestat

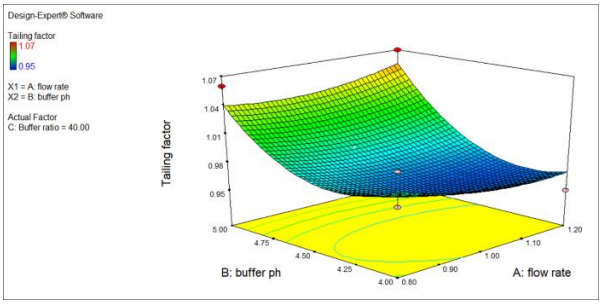

Fig. 5. 3D Surface for Nirogacestat

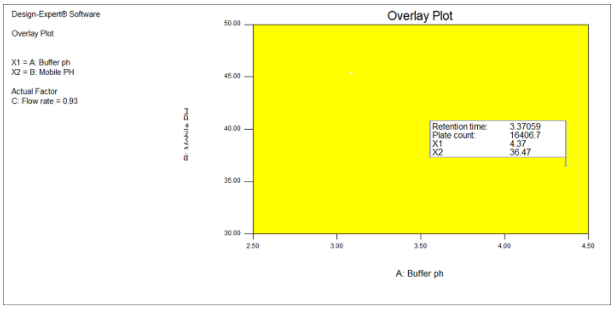

Fig. 6. Overlay plot for Nirogacestat

3.1. Experimental design

The present investigation involved the optimization of the RP-HPLC conditions for the purpose of separating Nirogacestat in dosage form. RSM was used to

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implement the BBD model in the development of the experimental design. Flowrate, buffer pH, and buffer ratio three independent variables or factors were noted in this investigation. Nirogacestat retention time and tailing factor were identified as the dependent variables or responses. R statistical software was used to create the BBD model. Three elements, three levels, and four central points were effectively used to create a BBD model. In order to create an RSM model for every response, this model was used to improve the RP-HPLC settings and then response observation.

3.2. RSM observation

Retention time and tailing factor were successfully generated in the RSM model for Nirogacestat. The multiple determination coefficient (R^2), adjusted R^2, and p-value can be used to assess the quality of the RSM model of Nirogacestat. Only when the multiple $R^2 \geq 0.9$ and adjusted $R^2 > 0.8$ can it be concluded that the experimental factors have had a significant impact on the responses. Moreover, the fact that the Adjusted R^2 and the Predicted R^2 deviate by less than 0.2 suggests that the second-order polynomial models adequately capture the real data. [16] With a value of less than 0.0001, the model's p-value suggests a decent predictive model. [17] The results showed that, for Nirogacestat, the Adjusted R^2 and the Predicted R^2 were, respectively, 0.9943 and 0.9602 (p-value = < 0.0001). The RSM model for Nirogacestat was effectively generated for retention time and tailing factor, Similar to the Nirogacestat models. The results showed that Nirogacestat multiple R^2 and adjusted R^2 were, respectively, 0.9931 and 0.9826. The RSM model was further examined to produce a desirability function since only the Nirogacestat retention time model met the requirements of multiple R^2, modified R^2, and p-value.

3.3. Desirability analysis

To acquire the chosen condition for optimization, RSM might be built in combination with the desirability analysis. It is possible to choose multiple RSM response using the significant model for desirability consideration. The Nirogacestat retention time and Nirogacestat tailing factor were selected for this investigation and used in the desirability analysis. A computational calculation was used to determine the composite desirability. It was found that with the flowrate of 1 mL.min-1, buffer pH of 4.5, and buffer ratio of 40 can obtain the overall desirability of 0.9778, predicted by the model. The desirability functions yield desirability values that fall between 0 and 1. The undesirable response derived from the predictive factors is represented by a desirability value of 0. However, the desirability value of 1 is associated with the most expected responses. [16,17]

3.4. Validation

3.4.1 System suitability test

HPLC separation properties including retention time, area, resolution, tailing factor, and theoretical plates number were evaluated to ensure the appropriateness of the analytical method. According to the results, it can be found that the optimized HPLC conditions met the acceptance criteria for the system suitability test with minimum RSD of retention time and area (RSD 2.0), tailing factor of less than 2.0 (TF≤2.0), and theoretical plates number of more than 3900 (N>2000). Results of system suitability parameters are shown in Table 3 & Figures 7-9 represents blank, standard and sample chromatograms.

Table 3: Results of system suitability parameters

S.No	Name	RT (min)	Area (µV sec)	Height (µV)	USP tailing	USP plate count
1	Nirogacestat (Standard)	4.746	3248855	211234	1.15	2205
2	Nirogacestat (Sample)	4.75	3158850	201231	1.18	2115

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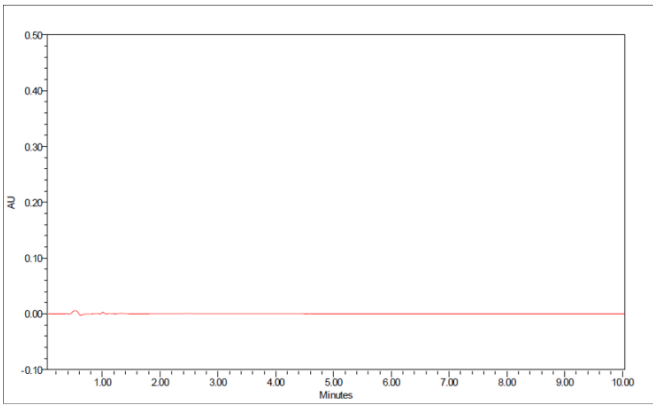

Fig. 7. Blank Chromatogram

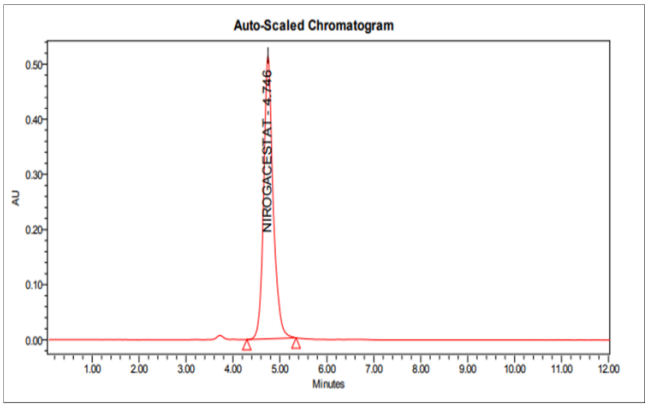

Fig. 8. Chromatogram for Standard

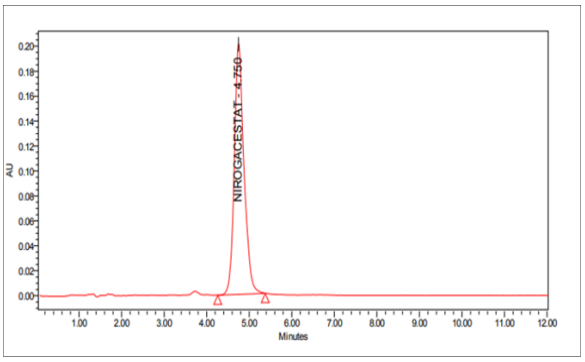

Fig. 9. Chromatogram for Sample

3.4.2 Linearity:

The linearity range was found to lie from 10 µg/ml to 50 µg/ml of Nirogacestat and chromatograms are shown in Table 4 & the calibration curve for Nirogacestat is depicted in Figure 10.

Table 4: Area of different concentration of Nirogacestat

S. No.	Nirogacestat	
	Concentration (µg/ml)	Area
1	10	1052950
2	20	2105900
3	30	3158850
4	40	4111800
5	50	5264750

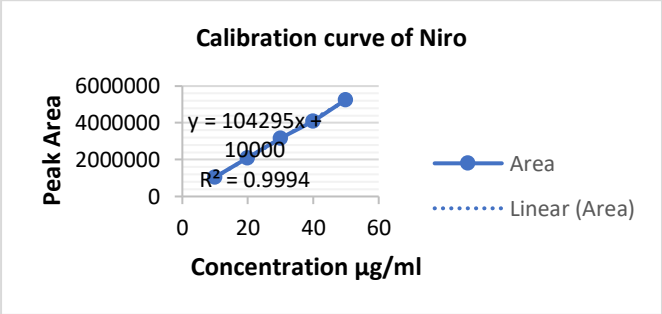

Fig. 10. Calibration curve of Nirogacestat

3.4.3 Precision:

Precision of the method was carried out for both sample solutions as described under experimental work. The corresponding chromatograms and results are shown below in Table 5.

Table 5: Results of Precision for Nirogacestat

Injection	Area
Injection-1	3237324
Injection-2	3254564
Injection-3	3225848
Injection-4	3233044
Injection-5	3181991

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Injection-6	3187586
Average	3220060
Standard Deviation	28963.65
% RSD	0.8

3.4.4 Intermediate Precision (Ruggedness)

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation. Table 6 Represents the Results of Intermediate precision for Nirogacestat.

3.4.5 Accuracy:

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated. The Accuracy results are displayed in Table 7.

Table 6: Results of Intermediate precision for Nirogacestat

Injection	**Area**
Injection-1	3237324
Injection-2	3244564
Injection-3	3225848
Injection-4	3233044
Injection-5	3241991
Injection-6	3287586
Average	3245060
Standard Deviation	21869.87
% RSD	0.6

3.4.5 Accuracy:

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated. The Accuracy results are displayed in Table 7.

Table 7: Accuracy (recovery) data for Nirogacestat

% Concentration (at specification Level)	**Area***	**Amount Added (mg)**	**Amount Found (mg)**	**% Recovery**	**Mean Recovery**
50 %	1579425	5	4.88	97.6	98.6
100 %	3158850	10	9.92	99.2	
150%	4738275	15	14.85	99	

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3.4.6 Limit of Detection and Quantification for Nirogacestat

The lowest concentration of the sample was prepared with respect to the base line noise and the signal to noise ratio was measured. The results for LOD & LOQ for Nirogacestat are displayed in Table 8.

Table 8: Results of LOD & LOQ

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio	Conc.
Nirogacestat	55	162	2.94	0.16 µg/ml
Results of LOQ				
Drug name	**Baseline noise (µV)**	**Signal obtained (µV)**	**S/N ratio**	**Conc.**
Nirogacestat	55	545	9.90	0.5 µg/ml

3.4.7 Robustness:

The standard and samples of Nirogacestat were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. The results for robustness are shown in Table 9 & Table 10.

Table 9: Results for variation in flow for Nirogacestat

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	1.4	2212	1.1
2	1.2	2115	1.18
3	1	2234	1.2

Table 10: Results for variation in mobile phase composition for Nirogacestat

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10 % less (63 ml)	2145	1.10
2	*Actual (70 ml)	2115	1.18
3	10 % more (77 ml)	2129	1.19

4. Conclusion

An analytical method of RP-HPLC for separating Nirogacestat has been successfully developed. Optimization has been performed by applying the response surface methodology of the Box-Behnken design. The desirability functions have been successfully generated to strengthen the quality of the RSM. It was found that the optimized HPLC conditions were buffer

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pH of 4.50, flowrate of 1mL.min-1, and buffer ratio of 40. These conditions were set for the HPLC system followed by the system suitability test. Several separation properties such as retention time, area, resolution, tailing factor, and theoretical plates number were reported to meet the acceptance criteria of the system suitability test. However, the optimized analytical method can be developed further. In the future, it is recommended to perform the analytical method validation to empirically demonstrate if the method is appropriate to be applied for the intended purposes.

Competing interests

Authors have declared that no competing interests exist.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Data and material are available upon request.

Funding

Not applicable

Authors' contributions

All the authors have equally contributed to the article.

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