



Research Article

OPTIMIZATION OF GREEN LC-MS METHOD FOR ROSUVASTATIN AND TENELIGLIPTIN USING AQbD CHEMOMETRIC APPROACH

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ABSTRACT

Background: Rosuvastatin combined with Teneligliptin formulation is commonly used in the treatment of Diabetic dyslipidaemia. However, only a few analytical methods have been published for the examination of this drug in a synthetic mixture or in a pharmaceutical dosage form. This study demonstrates the successful application of AQbD principles in developing a reliable, efficient, and environmentally friendly LC-MS method. **Methodology:** Box-Behnken Model: Design of experiment (DoE) strategy to identify and optimize critical method parameters using systematic risk assessment. Methanol: 10mM potassium dihydrogen orthophosphate [pH:6.0] (35:65v/v) was the mobile phase employed in the final optimized LC method, and this was using a Waters LC Xbridge C18 Column 5µm (4.6x250mm) as the stationary phase. The experiment was conducted at a flow rate of 1 mL/min, with a 10 µL injection volume, and an ESI-MS-QDA Detector for detection. An analytical method for validation was applied in accordance with ICH Q2 (R1) guidelines. **Results and Discussion:** The retention times of Rosuvastatin and Teneligliptin were observed at 4.392 and 3.202 minutes, respectively. The optimized technique showed excellent linearity, accuracy, precision, and robustness. Additionally, AGREE metrics and AES were used for assessing the eco-friendly nature of the developed method. **Conclusion:** The combination of green principles with AQbD experimental design ensures the robustness of the method. This combined framework is used for the first time in method development for the analysis of rosuvastatin with teneligliptin in formulations. Based on the above facts, an economical, robust, and time-saving method has been developed for assessing the quality control of rosuvastatin with teneligliptin in both pharmaceutical and pure forms.

INTRODUCTION

Diabetic dyslipidaemia is a disease characterized by low HDL levels, high triglycerides, and postprandial lipoproteins. This condition is most common in type II diabetes mellitus (T2DM) and is associated with cardiovascular risk [1]. Rosuvastatin &

Teneligliptin were FDA-approved drugs in 2021 for dyslipidemia associated with T2DM [2]. The combination is available in fixed-dose tablets such as Cedaglip-R 10 & Tenetin-R, which contain 20mg of teneligliptin and 10mg of Rosuvastatin. Rosuvastatin (ROS), a competitive inhibitor of

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"hepatic HMG-CoA reductase," is an enzyme that converts HMG-CoA to mevalonate [3]. So, reduce hepatic cholesterol and enhance uptake of LDL cholesterol [4]. Rosuvastatin calcium is calcium salt of "[*(E,3R,5S)*-7-[4-(4-Fluorophenyl)-2-[methyl (methylsulfonyl) amino]-6-propan-2-ylpyrimidin-5-yl]-3,5-dihydroxy hept-6-enoic acid]" [5]. It is a white powder that is soluble in acetonitrile (ACN) and methanol (CH₃OH), and is slightly soluble in water and ethanol. It has a molecular weight of 481.5g/mol, a molecular formula of C₂₂H₂₈FN₃O₆S, and a pKa of 4.8 [5]. The chemical structure of Rosuvastatin is shown in Figure 1.

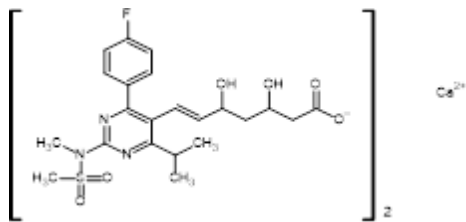


Figure 1: Chemical Structure of Rosuvastatin

Teneligliptin (TEN), a novel "dipeptidyl peptidase-4 inhibitor" which improves glucose levels in patients, reducing risk of hypoglycemia and obesity [6]. "Chemically, teneligliptin hydrobromide hydrate is the {(2*S*, 4*S*)- 4-[4-(3-methyl -1-phenyl -1-*H*-pyrazol-5-yl) piperazine-1-yl] pyrrolidine-2-yl} (1,3-thiazolidine -3-yl) methanone hemi-penta hydrobromide hydrate".

It is a crystalline solid with a molecular weight of 426.58g/mol and a molecular formula of C₂₂H₃₀N₆O₂ · 5HBr · [xH₂O]. It has an aqueous solubility of approximately 1.7mg/mL and is soluble in ethanol, DMSO, and dimethylformamide. The pKa value is 8.70 [7]. The chemical structure of teneligliptin is shown in Figure 2.

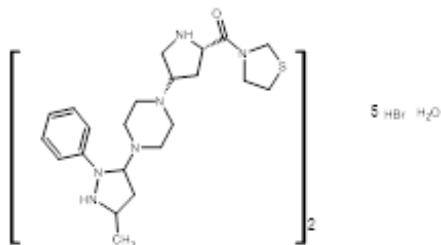


Figure 2: Chemical Structure of Teneligliptin

Liquid chromatography hyphenated with Mass Spectrometry is a highly sensitive and specific method for determining drug formulations in the pharmaceutical industry. Optimization by the Conventional LC-MS method involves analyzing one factor

at a time, while other factors remain constant. Therefore, it produces various experiments, resulting in the wastage of time & solvent consumption, as well as an inadequate understanding of the factors involved. Analytical Quality by Design (AQbD) will prevent problems faced by the conventional LC-MS method. In AQbD, the interaction of various factors with response simultaneously at a time produces high robustness and improves method performance. AQbD promotes product quality through risk assessment and design space definition, involving steps such as analytical target profile (ATP), critical method attributes (CMA), and essential parameters of method (CMP), among others. CCD as well as BBD is the most commonly used model for HPLC method screening and optimization [8, 9].

Green chemistry principles can minimize environmental pollution by preventing waste generation, using safer solvents, reducing chemical accidents, and reducing energy consumption in analytical research and validation studies. To assess the eco-friendly nature of the method, different greenness assessment tools are currently available, ie, "Green analytical procedure index (GAPI), National Environmental Method Index (NEMI), Analytical Greenness(AGREE) & Analytical Eco-Scale (AES)".

The AGREE calculator provides numeric results, facilitating the easy evaluation of the method's greenness, and encompasses 12 Green Analytical Principles (GAPs). GAPI is another tool, which is represented by a red-green-yellow colour-coded pictogram. AES has an Eco score, which identifies penalty scores based on the solvent/chemical used, environmental, health risks, waste generation, and energy consumption [10-15].

Literature surveys reveal that many publications report different analytical techniques for detecting ROS and TEN, either separately or in combination with other medications or their impurities. Reported methods are mainly focused on determination by UV spectrophotometry, HPLC, UPLC, LC-MS, GC-MS & volumetric methods of analysis. During the method development stages, the consumption of toxic reagents or the production of hazardous waste is considered a serious environmental problem. As per the above facts, green principles in method development ensure the safety of analytical methods. Green principles combined with AQbD experimental design guarantee the robustness of the process. Validation of the developed analytical approach was done as per the guidelines of "International Conference of Harmonisation (ICH) Q2 R1" [16].

MATERIALS AND METHODS

Chemicals and Reagents

Rosuvastatin calcium and Teneeligliptin Hydrobromide Hydrate were purchased from Carbanio Chemicals (Hyderabad). Methanol and Potassium dihydrogen orthophosphate (HPLC Grade) were bought from Thermo Fischer Scientific (Hyderabad, India). Milli-Q water was utilised for dilution.

Instrumentation

The quantification and detection of rosuvastatin and teneeligliptin were determined using Alliance LC-MS-ESI-QDA Techniques. Alliance Liquid chromatographic system equipped with a binary gradient pumping system, autosampler LC injector, online degassing unit, as well as a PDA detector. In a mass spectrophotometer equipped with an electrospray ion source (ESI) for ionizing the sample and an ACQUITY QDA detector for analyzing the mass of separated compounds. Empower-3 software was used for interpreting the chromatographic data. Various instruments are utilized for the estimation of rosuvastatin and teneeligliptin. A Digital Sartorius model electronic balance was employed for weighing samples. The PEAK Scientific Genius Ultra-sonicator is used for sonicating the mobile phase and the sample solution. A micropipette of 100-1000 μ l was used for measuring the sample. Merck produced purified water for the Millipore Milli-Q Water system.

Chromatographic condition

Chromatographic condition optimized through Analytical Quality by Design (AQbD) approach. Various variables are tested to determine the optimal condition for better performance. Finalized conditions: Stationary Phase-Waters Xbridge C18 column (5 μ m, 4.6 \times 250mm), solvent system: methanol and 10 mM Potassium. Dihydrogen orthophosphate [pH 6.0 with acetic acid] in a ratio of 35:65 (v/v) at a flow rate of 1.0 mL/min, the temperature of the column is maintained at 25°C. The injection volume was set at 10 μ L, the detection wavelength was fixed at 247nm & total run time was 10 minutes.

Mass spectrometric condition

The Mass Spectrometry system utilized a Waters LC-MS, comprising a heated ESI probe and a high-temperature gas-supplementing nebulizer gas, which improved desolvation efficiency. ESI operated in both positive (15V) and negative (30V) modes. Nitrogen was used as the ion source gas to aid in analyte spray formation, and nitrogen gas was also used as the ion source at 45 psi to stabilize the ion spray. The source

temperature was maintained at 5000 °C to assist desolvation of ions and droplets. The starting mass was 50Da and the ending mass was 1200Da.

Software

The data was collected by using Alliance (version 2.6). Acquity QDa mass data in the Empower-3 software accessed the mass analysis window to view both the UV and mass spectra for each integrated peak in a chromatogram. Stat-Ease Design-Expert software (version 1.2) was employed in experimental design to optimize chromatographic conditions by varying various variables, and it is used to create a design space.

Preparation of buffer solution, mobile phase, and standard stock solution

Preparation of Buffer

3.4g of KH₂PO₄ was weighed and transferred to a standard flask. The volume was adjusted to 1000 mL using Milli-Q water, and then the solution was sonicated for 15 minutes to degas.

Preparation of the Mobile phase

The mobile phase used in this study was prepared by mixing 65% 10 mM KH₂PO₄ buffer and 35% methanol. The buffer was prepared by mixing potassium dihydrogen orthophosphate in Milli-Q water. Use a pH modifier, such as acetic acid, to maintain the buffer pH at 6.0. Combine the measured solvent in a flask and, after mixing, filter the buffer through a 0.22 μ m filter and sonicate the mobile phase using an ultrasonicator. The final ratio of the mobile phase was observed to be 35:65 % (v/v).

Preparation of Standard

10mg of Rosuvastatin and 20mg of Teneeligliptin were prepared in a separate 10 mL volumetric flask using methanol and suitable dilutions of the mobile phase to achieve concentrations of rosuvastatin at 10 μ g/mL and teneeligliptin at 20 μ g/mL. The standard solution was then sonicated for 15 minutes, and it was ready for analysis.

AQbD- Box-Behnken Design guided chromatographic optimization

LC-MS method development for the determination of ROS, as well as TEN, was conducted by applying the AQbD approach to identify critical method parameters that affect method performance. A literature survey investigation provides insight into the independent and dependent variables for ROS and TEN. The design expert software Stat-Ease was utilized for the current

investigation. BBD-optimized chromatographic conditions and ANOVA statistical analysis provide data on the interaction of variables and response from the simultaneous analysis of ROS and TEN. ANOVA results and model diagrams from 17 trials aid in optimization. Optimized chromatographic conditions from AQBd suggest three independent variables or factors, such as

pH (A), mobile phase ratio (B), and flow rate (C), as well as retention time and resolution, as responses. The Ishikawa fishbone diagram (Figure 3) was employed to assess the interactions of independent and dependent variables that contribute to the risk in method performance. BBD trials are listed in Table 1.

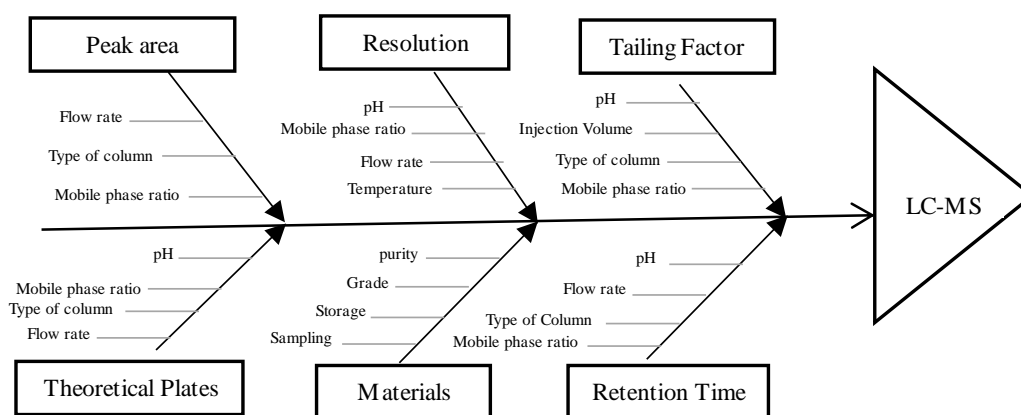


Figure 3: Ishikawa fishbone diagram

Method validation

The developed method for estimating ROS and TEN was verified in accordance with the guidelines of ICH Q2 R1. System suitability studies, linearity, Limit of Quantification (LOQ), Limit of Detection (LOD), Accuracy, and Precision were various parameters done according to guidelines.

System suitability

A standard solution of rosuvastatin (10 µg/ml) and teneligliptin (20 µg/ml) was injected six times & the percentage relative standard deviation (SD) was evaluated to determine system suitability.

Linearity

Linearity was done by preparing a calibration plot of concentration versus peak area of ROS and TEN, over a range of 50-300ng/ml for ROS and 50-300ng/ml for TEN. Six different concentrations of the standard were injected into the chromatographic system.

LOD and LOQ

LOD as well as LOQ analysis are employed for determining the sensitivity of the developed method. Different methods are available in the ICH guidelines for determining LOD and LOQ. These include visual assessment and response standard deviation calculations, signal-to-noise (S/N) ratio calculations & calibration curve slope calculations. From these calibration

curve slope calculations, LOD as well as LOQ were assessed by using $3.3 \times (\sigma/s)$ as well as $10 \times (\sigma/s)$, respectively. 'σ' for standard deviation, and 's' for slope, of the calibration curve.

Precision

Precision studies help determine the closeness of results from the same homogeneous sample. Intra-day precision was assessed by six determinations of a 100 ng/mL standard solution on the same day. Similarly, for inter-day precision, six determinations of a 100 ng/mL standard solution were taken on three successive days, and the %RSD of intra-day and inter-day precision was calculated.

Accuracy

Accuracy studies were performed by adding a known quantity of standard stock solution at various levels, i.e., 50%, 100%, and 150% to a fixed concentration of the sample solution. Three determinations at each level were prepared for accuracy studies, and the percentage recovery was calculated along with the percentage relative standard deviation (RSD).

Application of developed method to marketed formulation

Twenty tablets of rosuvastatin and teneligliptin fixed-dose formulation were accurately weighed and powdered using a mortar and pestle. An equivalent amount of 10mg rosuvastatin and 20mg Teneligliptin was transferred to a 10 mL standard flask and then diluted to 10ml using mobile phase and suitable

dilution to attain a concentration of 10 µg/ml rosuvastatin along with 20µg/ml teneligliptin and sonicated for 15 minutes, then injected into the LC-MS system for determination of its drug content.

RESULTS AND DISCUSSION

Mathematically processed, accurate, precise LC-MS analysis of ROS & TEN was developed & evaluate the method's greenness. Without the AQbD approach, the method performance quality and continuous re-validation may be sought. Combining the hybrid principle of "GAC & AQbD" enhances the robustness of

the method. A DoE-aided Box-Behnken (BBD) statistical screening design was employed to optimize critical process parameters in this study.

AQbD approach implemented in method development

The primary factor in the method development process is Analytical Target Profiling (ATP). In experimental design, the method was optimized using BBD, with three factors and seventeen runs. Lower and higher values for critical method factors of ROS and TEN are shown in Table 2.

Table 1: Design Expert aided Box-Behnken Design

| | Factor I | Factor II | Factor III | Response I | Response II |
|-----------|----------|-------------------|--------------|------------|-------------|
| Trail run | A: pH. | B: Methanol Ratio | C: Flow rate | Rt. of TEN | Resolution |
| | | % | ml/min | Min. | |
| 1 | 6 | 30 | 0.8 | 5.654 | 1.5 |
| 2 | 4 | 40 | 1 | 4.546 | 1.5 |
| 3 | 4 | 35 | 0.8 | 5.657 | 1.5 |
| 4 | 8 | 40 | 1 | 4.534 | 1.5 |
| 5 | 4 | 35 | 1.2 | 3.578 | 2.35 |
| 6 | 4 | 30 | 1 | 4.634 | 1.6 |
| 7 | 6 | 35 | 1 | 3.202 | 3.13 |
| 8 | 6 | 40 | 0.8 | 5.435 | 1.53 |
| 9 | 6 | 35 | 1 | 3.243 | 3.1 |
| 10 | 6 | 35 | 1 | 3.234 | 3.1 |
| 11 | 6 | 30 | 1.2 | 3.484 | 2 |
| 12 | 6 | 35 | 1 | 3.207 | 3.2 |
| 13 | 8 | 35 | 0.8 | 5.675 | 1.6 |
| 14 | 8 | 30 | 1 | 4.564 | 1.3 |
| 15 | 6 | 40 | 1.2 | 3.487 | 2.1 |
| 16 | 6 | 35 | 1 | 3.209 | 3.2 |
| 17 | 8 | 35 | 1.2 | 3.561 | 1.9 |

Table 2: Lower and higher values for critical method factors of ROS and TEN

| Factor | Unit | Type | Minimum | Maximum | Coded Low | Coded High |
|-------------------------------|-----------|----------|---------|---------|-----------|------------|
| pH | | Numeric, | 4.000 | 8.000 | 4.000 | 8.000 |
| Mobile phase Ratio:[Methanol] | % | Numeric | 30.00 | 40.00 | 40.00 | 50.00 |
| Flow rate | ml/minute | Numeric | 0.8000 | 1.20 | 0.80 | 1.20 |

METHOD OPTIMIZATION

Method Screening

In this study, three factors, pH, Methanol ratio, and flow rate, were employed as independent variables. Based on these variables, 17 trial runs were carried out & feed responses (Table 1). The optimized pH value was 6.0, and a mobile phase ratio of 35% (v/v) along with a flow rate of 1.0 mL/min was confirmed.

ANOVA Statistical Analysis

The ANOVA results (Table 3) confirmed that the quadratic model was statistically significant for both resolution ($F = 1344.11, p < 0.0001$) and retention time of TEN ($F = 2865.51, p < 0.0001$). Lack of Fit value suggesting it is not significant ($p > 0.05$), indicating that the model adequately described the experimental data. In terms of resolution, the flow rate (C),

$p < 0.0001$, was the most significant factor, followed by pH (A), $p < 0.0001$, and the methanol ratio (B), $p = 0.0200$. Notable interaction effects were detected between AB (0.0009) and AC ($p < 0.0001$), while BC (0.2377) showed no significance. All quadratic terms (A^2 , B^2 , C^2) were very significant ($p < 0.0001$), validating the existence of curvature in the response surface. Regarding retention time, the flow rate (C) was the most influential factor ($p < 0.0001$), while the methanol ratio (B) also had a significant impact on the response ($p = 0.0018$). Conversely, pH(A) $p = 0.2752$ had no significant effect on

retention. Among the interaction terms, only BC ($p = 0.0025$) was significant, while AB ($p = 0.2697$) and AC ($p = 0.4930$) were not significant. The quadratic impacts of A^2 , B^2 , and C^2 were again highly significant ($p < 0.0001$), suggesting nonlinear relationships. In general, flow rate proved to be the key factor for both responses, while pH affected resolution but not retention time. The predicted R^2 and adjusted R^2 values exhibited a difference of 0.2, and the Adequate Precision value exceeded 4, confirming the model's suitability for exploring the design space to achieve robust chromatographic conditions.

Table 3: ANOVA results of Resolution and Retention time of Tenueligiptin

| Source | Resolution | | Retention time of Tenueligiptin | | |
|-----------------------------------|------------|----------|---------------------------------|----------|-----------------|
| | F-value | p-value | F-value | p-value | |
| Model | 1344.11 | < 0.0001 | 2865.51 | < 0.0001 | Significant |
| A - pH | 71.85 | <0.0001 | 1.40 | 0.2752 | |
| B - Mobile phase Ratio [Methanol] | 9.00 | 0.0200 | 23.81 | 0.0018 | |
| C -Flow rate | 838.16 | <0.0001 | 14745.63 | < 0.0001 | |
| AB | 30.61 | 0.0009 | 1.44 | 0.2697 | |
| AC | 102.89 | <0.0001 | 0.5230 | 0.4930 | |
| BC | 1.67 | 0.2377 | 21.04 | 0.0025 | |
| A^2 | 3927.44 | < 0.0001 | 3796.68 | < 0.0001 | |
| B^2 | 4466.53 | < 0.0001 | 2798.84 | < 0.0001 | |
| C^2 | 1552.00 | <0.0001 | 3248.50 | < 0.0001 | |
| Lack of Fit | 0.4167 | 0.7510 | 2.76 | 0.1755 | Not significant |

Numerical optimization graphs

The 3D response surface, along with the related 2D contour plot, was created to examine the interaction between variables and responses. In resolution [Figure 4(a)], the 3D surface plot displays a curved surface, indicating the presence of significant quadratic effects, as demonstrated by the ANOVA.

The contour plot [Figure 4(b)] shows that higher resolution values occurred at intermediate levels of pH and methanol ratio, with resolution decreasing as either parameter shifted to very low or very high values. This suggests that peak separation is influenced interactively by both pH and the composition of the organic phase. Regarding retention time, the 3D surface [Figure 4(c)] shows a sharp slope along the flow rate axis, emphasizing its significant influence.

The contour map [Figure (d)] further confirms that retention time significantly decreased with higher flow rate, while the methanol ratio also had a notable but less intense effect. In contrast, pH exhibited little impact, aligning with the ANOVA

findings. The graphical analysis shows that flow rate is the key factor affecting retention time, whereas pH and methanol ratio are more influential in determining resolution.

Numerical Optimization Report

The optimized values of variables taken from Design Expert software and the criterion of desirability. A graphical model provides information regarding the interaction of factors and responses. The desirability value was found to be 1.0000, as shown in the design space area in Figure 5. Experimental data obtained from the design expert software gives a 95% confidence level. A desirability of 1.00 indicates that better results should be achievable.

Optimized chromatographic conditions and mass spectra of ROS and TEN

Optimized Chromatographic system shown in Table 4. It consists of Waters X-bridge C_{18} column $5\mu\text{m}$, ($4.6 \times 250\text{mm}$) as the stationary phase and Methanol along with 10mM Potassium dihydrogen orthophosphate [pH-6.0 with acetic acid], "35:65v/v

as the mobile phase at 1.00 ml/min flow rate. Wavelengths of ROS and TEN were analyzed using a photodiode array detector (PDA) with a fixed wavelength of 247nm. The injection volume was fixed at 10 µL, and the total run time was fixed at 10 minutes. Figure 6 demonstrates an optimized chromatogram for ROS (4.392min) & TEN (3.202 min). A mass spectrometer was

used for the detection of a separated sample from the chromatographic system, equipped with an ESI-QDA detector. The mass spectra of ROS & TEN are shown in Figures 7 and 8. The developed method was confirmed in accordance with ICH Q2 R1 guidelines, and all reports were in compliance with these guidelines.

Table 4: Optimized chromatographic conditions for ROS and TEN

| | |
|---------------------------|---|
| Column | Waters Xbridge C ₁₈ Column 5µm(4.6x250mm) |
| Mobile Phase | Methanol: 10mM Potassium dihydrogen orthophosphate[pH:6.0 with acetic acid] |
| Mobile Phase Ratio | 35:65v/v |
| Injection Volume | 10.0µl |
| Flow Rate | 1.0.ml/min. |
| pH | 6.0 |
| Detection | ESI-MS-QDA Detector |

Factor Coding: Actual

Retention time of Teneligliptin (Min.)

Design Points:

● Above Surface

○ Below Surface

3.202 5.675

X1 = A

X2 = B

Actual Factor

C = 1

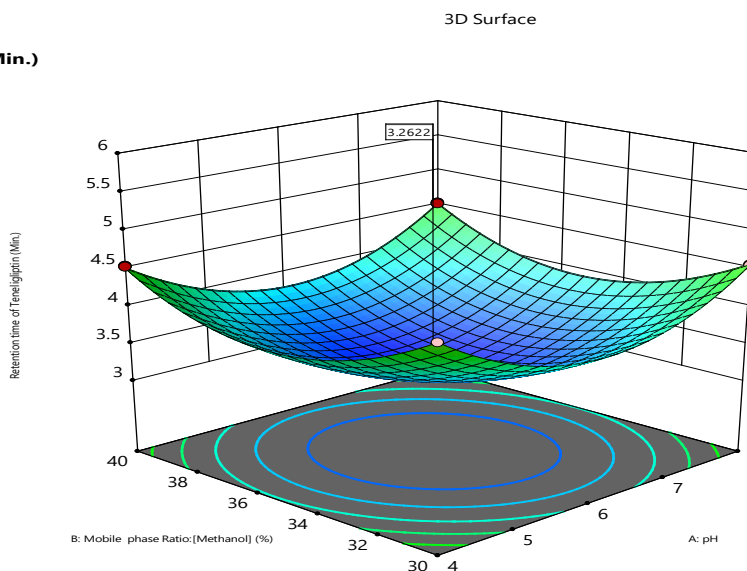


Figure 4(a): 3D response surface plot showing the effect of pH and mobile phase ratio on resolution

Factor Coding: Actual

Retention time of Teneligliptin (Min.)

● Design Points

3.202 5.675

X1 = A

X2 = B

Actual Factor

C = 1

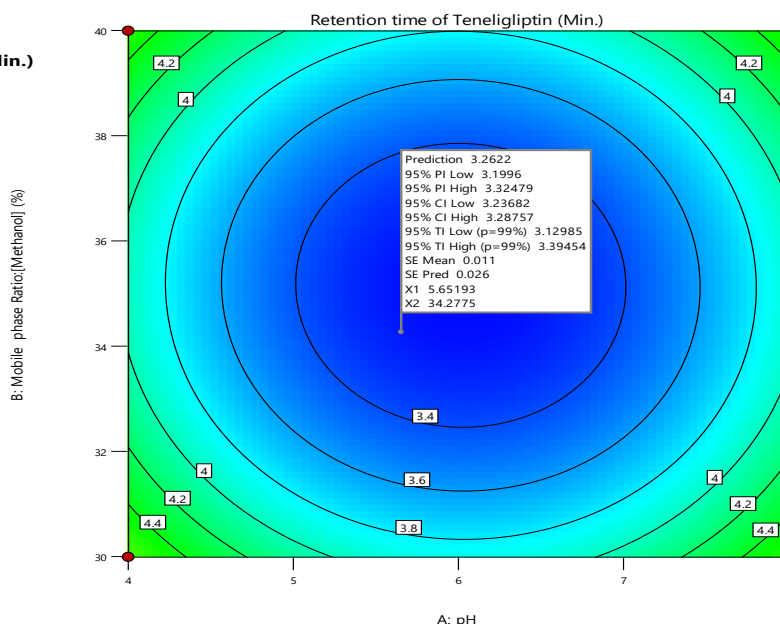


Figure 4 (b): 2D contour plot showing the effect of pH and mobile phase ratio on resolution

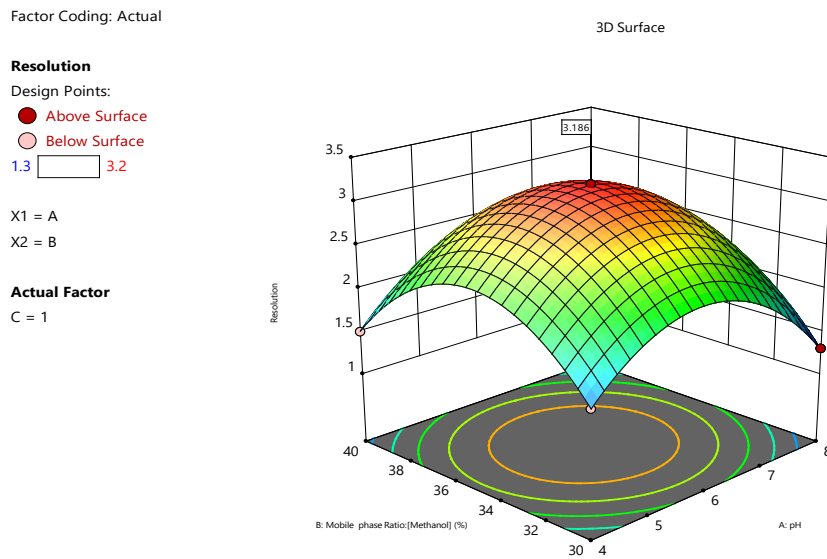


Figure 4(c): 3D response surface plot showing the effect of flow rate and mobile phase ratio on the retention time of TEN

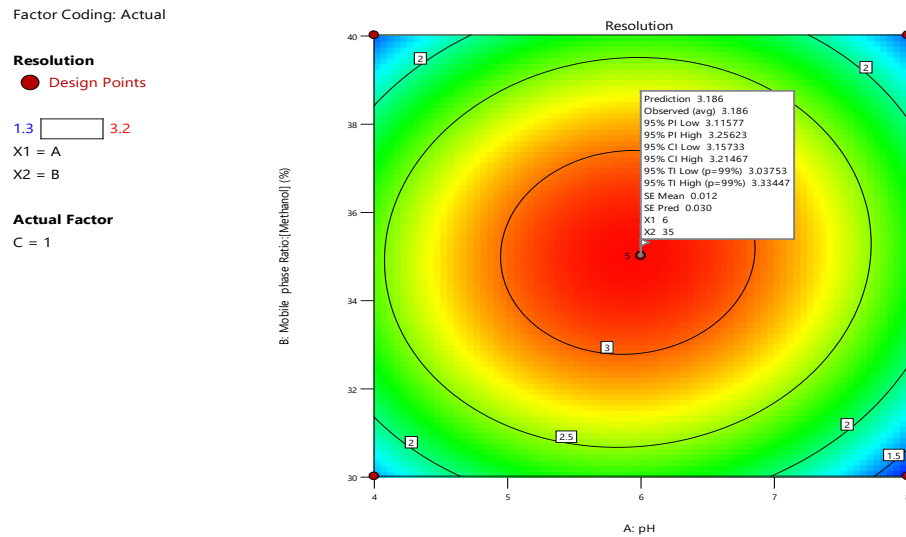


Figure 4 (d): 2D contour plot showing the effect of flow rate and mobile phase ratio on the retention time of TEN

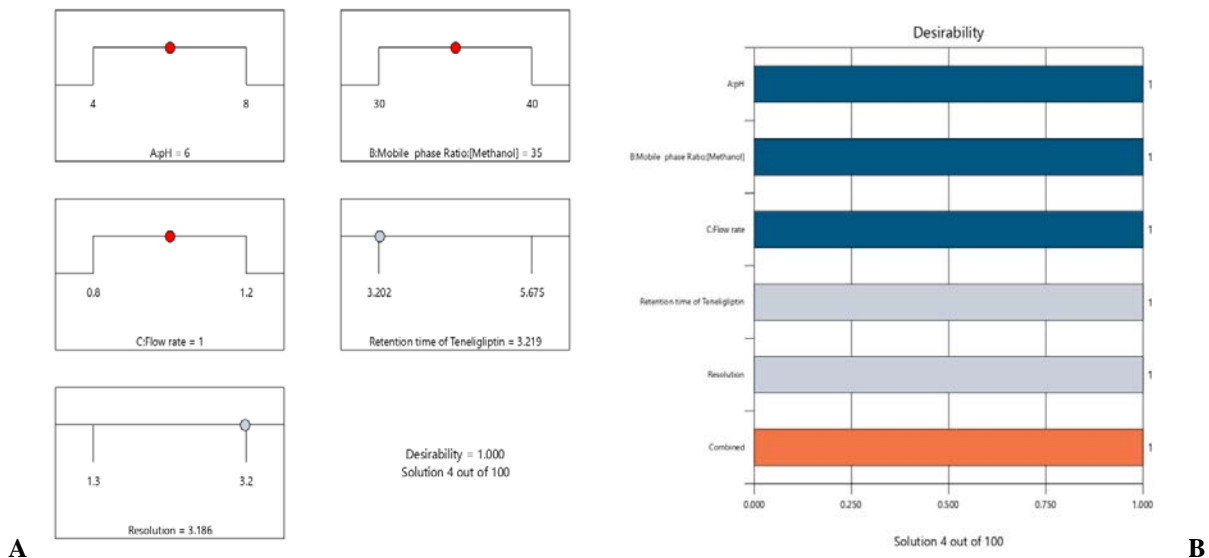


Figure 5: Optimized values for all factors and responses [A]; Numerical optimization Ramps for Desirability study [B].

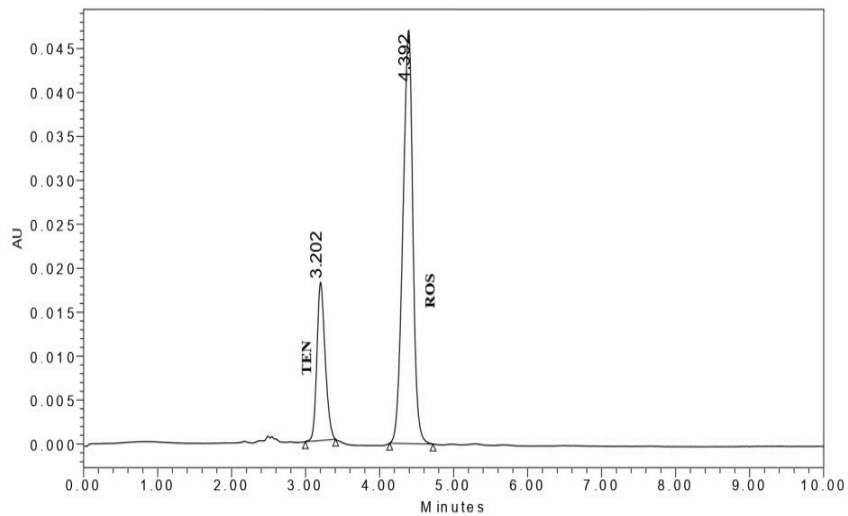


Figure 6: Optimized chromatogram of Rosuvastatin (ROS) and Teneligliptin (TEN) obtained under developed LC conditions

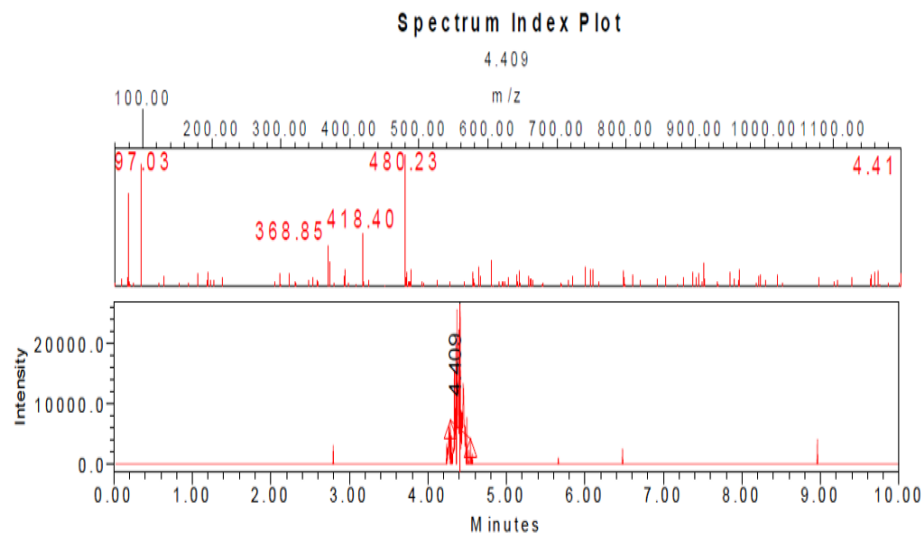


Figure 7: Mass Spectrum of Rosuvastatin

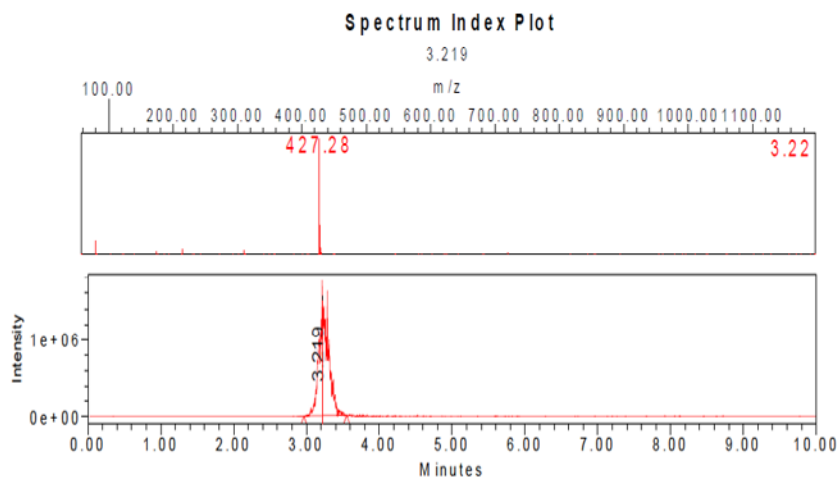


Figure 8: Mass Spectrum of Teneligliptin

Method Validation

Linearity: Linearity of ROS and Tenueligliptin obtained in the range of 50-300ng/ml. Findings are presented in Table 5. Calibration curves were plotted by using concentration on the X-axis and peak area on the Y-axis, as demonstrated in Figure 9.

$$Y = bX + C$$

This equation was used for estimating R^2 values obtained from calibration curves.

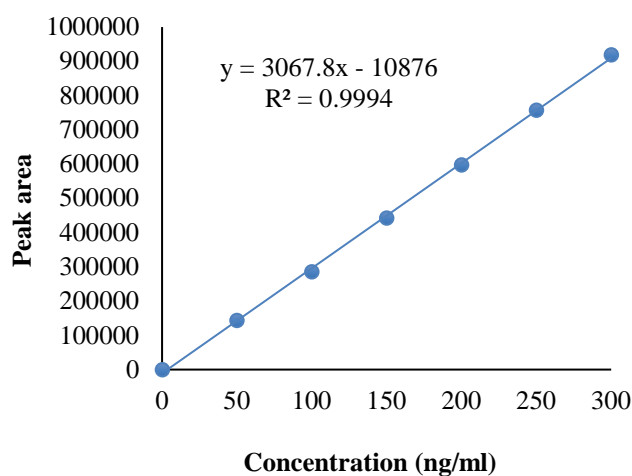


Table 5: Linearity data for ROS and TEN

| Serial No: | Concentration (ng/ml) | | Peak area | |
|------------|-----------------------|-----|-----------|--------|
| | ROS | TEN | ROS | TEN |
| 1 | 50 | 50 | 143453 | 69886 |
| 2 | 100 | 100 | 285681 | 139544 |
| 3 | 150 | 150 | 442907 | 209202 |
| 4 | 200 | 200 | 597126 | 278860 |
| 5 | 250 | 250 | 757359 | 348518 |
| 6 | 300 | 300 | 918571 | 426984 |

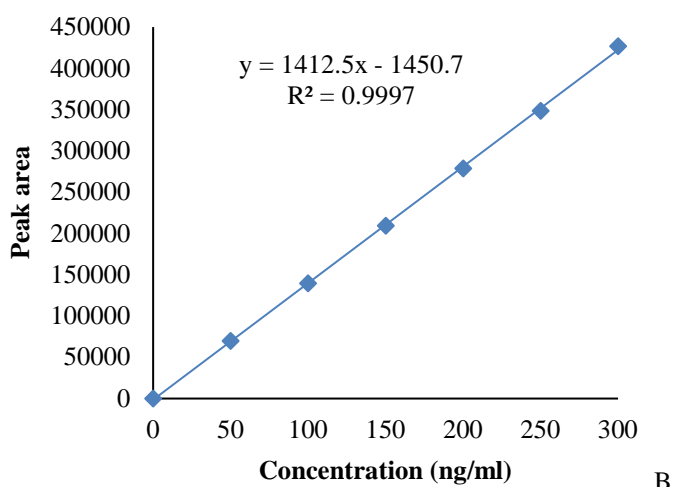


Figure 9: Calibration curve of ROS [A] and TEN [B]

System Suitability

System suitability studies of the method were conducted using various parameters, including a resolution of above 2.0, a tailing factor of below 2.0, and a theoretical plate count of greater than 3000, indicating values within the acceptable limits. System suitability studies are represented in Table 6.

Table 6: System suitability parameters of ROS and TEN

| Parameters | Average \pm SD | |
|-------------------------|-------------------|-------------------|
| | ROS | TEN |
| Resolution | 3.1 \pm 0.05 | |
| Retention time (minute) | 4.392 \pm 0.002 | 3.202 \pm 0.001 |
| Theoretical plates | 5878 \pm 65.32 | 5624 \pm 84.04 |
| Tailing factor of ROS | 0.411 \pm 0.01 | 0.323 \pm 0.01 |

Validation parameters of the optimized method

A DoE-based method development was conducted and validated in accordance with the ICH Q2R1 Guidelines. Various analytical method validation parameters were used. In linearity, ROS and TEN between the range of 50-300ng/ml, and a calibration curve was constructed, and the values of slope, intercept, and regression equation were obtained from the graph. Intra-day and Inter-Precision studies were conducted, and the %RSD was observed to be 0.04 and 0.07 for ROS and 0.03 and 0.06 for TEN, respectively. Accuracy studies were conducted through

three determinations at three concentration levels, i.e., 50, 100, and 150%. The % recovery findings of the sample were observed to be in the range of 99.79-101.29. Table 7 shows a summary of the optimized method validation variables.

Analysis of Marketed Formulation

The ROS and TEN drug formulations were estimated using the LC-MS method. Findings are represented in Table 8, and the chromatogram of ROS & TEN in the marketed formulation is presented in Figure 10.

Evaluation of the green of the developed analytical method

The eco-compatibility of the developed methods was evaluated using greenness evaluation tools, including the Analytical Greenness (AGREE) and Analytical Eco Scale (AES) metrics, as shown in Table 9. AGREE metric is a software-based tool for determining method greenness with a score range of 0-1. It comprises 12 principles of GAC. This principle applied to the proposed method, and the combined result is visualized as a coloured pictogram. The final score is displayed in the center, showing the overall greenness of the technique. An AGREE score of 0.63 indicates the method shows good compliance with greenness principles.

Table 7: Optimized validation parameters of the method.

| Validation parameters | | ROS | TEN |
|---|------------|-------------------|--------------------|
| Precision (%RSD) | Intra-Day | 0.04 | 0.03 |
| | Inter- Day | 0.07 | 0.06 |
| Linearity range (ng/ml) | | 50-300 | 50-300 |
| Regression Equation | | $y=3067.8x-10876$ | $y=1412.5x-1450.7$ |
| CorrelationCoefficient(R ²) | | 0.9994 | 0.9997 |
| LOQ (ng/ml) | | 35.45 | 10.27 |
| LOD (ng/ml) | | 11.69 | 3.38 |
| Accuracy (%Recovery) | 50 | 99.87 | 101.29 |
| | 100 | 99.25 | 100.97 |
| | 150 | 99.79 | 100.92 |

Table 8: Analysis of ROS and Ten in Marketed formulation

| Tablet brand name | Amt.Taken (ng/ml) | Amt. found (mean \pm SD,g) | % Label claim | % RSD |
|------------------------|-------------------|------------------------------|---------------|-------|
| Brand A (Tenlifast RV) | 150 | 150.007 \pm 0.004726 | 100.07% | 0.047 |
| Brand B (Tenetin R) | 150 | 150.009 \pm 0.004509 | 100.09% | 0.045 |

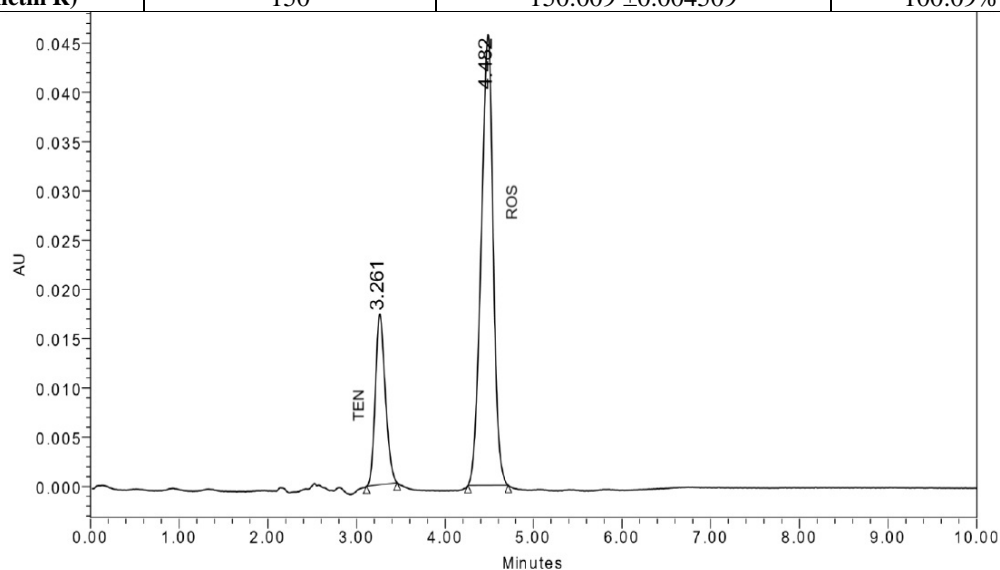





Figure 10: Chromatogram of Teneligliptin (TEN) & Rosuvastatin (ROS) and in the marketed tablet formulation

Table 9: Greenness Evaluation of existing and Present methods based on AGREE and Analytical Eco-Scale Metric

| SL No: | Chromatographic conditions | AGREE pictogram [AGREE Score:0-1] | AES Score [0-100] | Rationale | Ref. |
|--------|--|---|-------------------|---|----------------|
| 1 | RP-HPLC, Methanol: water [40:45:15] C18 (4.6 \times 250 mm, 5 μ m); flow \approx 1.0 mL/min; UV detection. |  | 58 | High fraction of toxic chemicals [ACN/methanol] consumption increases waste generation and low greenness and high reagent penalty | [17] |
| 2 | RP-HPLC, Methanol: Water [85:15], C 18, Flow rate 1.0 ml/min, UV detection |  | 60 | Heavy use of toxic reagent, [methanol] and significant wastage reflecting the average greenness of the method. | [18] |
| 3 | LC-MS, Methanol: 10mM dihydrogen orthophosphate (35:65), ESI-MS-QDA Detector |  | 72 | Minimizes hazardous reagent use and low waste generation indicates significantly greener and more eco-friendly method | Present method |

Scores in the range of about 0.5-0.7 are considered moderate to good greenness. AES is another widely used tool for evaluating the environmental impacts of analytical methods. It is a score-based system that encompasses various factors, including waste generation, solvent consumption, energy consumption, and the utilisation of hazardous chemicals. AES score is calculated as:

$$\text{AES Score} = 100 - (\text{Reagent PP} + \text{Waste PP} + \text{Energy consumption PP})$$

For the developed method, the AES scores were found to be 72. According to the established scale, AES scores above 75 indicate excellent green analysis, scores between 50 and 75 signify acceptable green analysis, and scores below 50 are considered poor greenness. Therefore, the AES score of 72 classifies the developed method as an acceptable green analytical method, demonstrating a good balance between analytical performance and Eco-sustainability.

CONCLUSION

Eco-compatible-based study designed for analysis of Rosuvastatin along with Teneigliptin in pharmaceutical formulations. The proposed method includes a small volume of organic solvents & small amount of waste produced. Therefore, relatively little harm is done to aquatic animals. A green analytical method was employed, utilizing the Ishikawa fishbone diagram, followed by a BBD for optimization. This combined framework is used for the first time for the method development stage in rosuvastatin, along with teneigliptin, in a pharmaceutical dosage form. The developed method was verified in accordance with the ICH Q2 R1 guidelines. All validation parameters were within acceptable limits. The %RSD is less than 0.1% indicating extremely high precision and very low variability in the method. Such strong precision supports that the method is not only precise but also robust. Furthermore, the method was developed using the AQbD approach, which involves a systematic understanding & control of sources of variability. Hence, the developed method is robust, accurate & precise for the simultaneous estimation of rosuvastatin and teneigliptin in the routine analysis of pharmaceutical formulations.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Aswathy SR performed the experimental work, analyzed the data, interpreted the results, and prepared the original draft. Hemnath Elango provided supervision and contributed to the review and editing of the manuscript.

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