

Integrated Design of Experiments Approach for a Stability-indicating Rp-hplc Method of Fexuprazan Hydrochloride

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Research Article

Keywords: Fexuprazan hydrochloride, RP-HPLC, Method validation, Design of Experiments, Stability-indicating method

Posted Date: June 25th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9946357/v1>

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Additional Declarations: No competing interests reported.

INTEGRATED DESIGN OF EXPERIMENTS APPROACH FOR A STABILITY-INDICATING RP-HPLC METHOD OF FEXUPRAZAN HYDROCHLORIDE

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ABSTRACT

Background: Fexuprazan hydrochloride is a novel potassium-competitive acid blocker requiring reliable analytical methods for quality and stability evaluation.

Aim: To develop and validate a rapid, robust, and stability-indicating RP-HPLC method using a Design of Experiments (DoE) approach.

Methods: Separation was achieved on a C18 column using Methanol: phosphate buffer (70:30, pH 3.5) at 1.0 mL/min with detection at 256 nm. Central Composite Design (CCD) was applied for optimization. Validation was performed as per ICH guidelines, and forced degradation studies were conducted.

Results and Discussion: The method showed a retention time of ~4.5 min with good peak symmetry and no interference. Linearity was observed over 10–60 µg/mL ($R^2 = 0.998$). Accuracy (99.54–99.75%) and precision (%RSD < 2%) were within acceptable limits. LOD and LOQ were 4.88 µg/mL and 14.79 µg/mL, respectively. DoE identified methanol composition and pH as significant factors. Degradation products were well resolved, confirming stability-indicating capability.

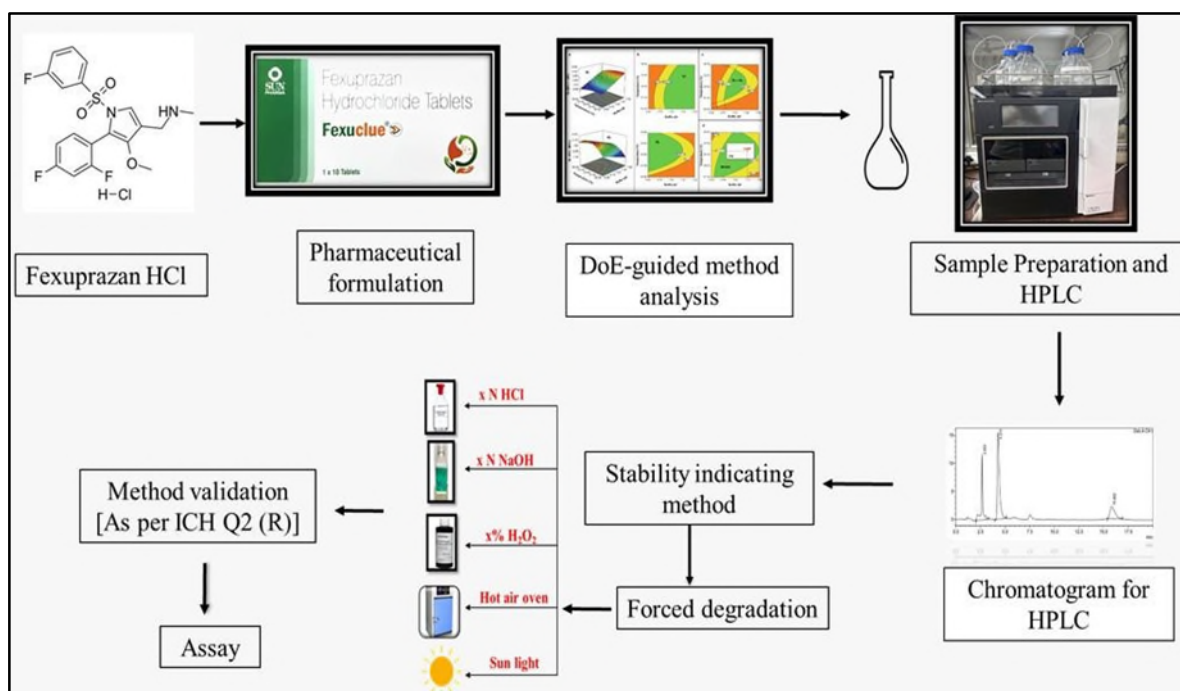
Conclusion: The developed RP-HPLC method is simple, precise, and reliable, suitable for routine quality control and stability analysis of fexuprazan hydrochloride.

keywords: Fexuprazan hydrochloride, RP-HPLC, Method validation, Design of Experiments, Stability-indicating method

Article Highlights:

- Developed a novel stability-indicating RP-HPLC method for quantitative analysis of Fexuprazan Hydrochloride.
- Optimized critical chromatographic parameters using Central Composite Design for enhanced method performance.
- Achieved excellent linearity, accuracy, precision, specificity, and robustness in accordance with ICH Q2(R2) guidelines.
- Successfully resolved Fexuprazan Hydrochloride from degradation products under multiple stress conditions.
- Established a reliable and cost-effective analytical method suitable for routine quality control and stability studies.

GRAPHICAL ABSTRACT



Abbreviations:

ACN	Acetonitrile
ANOVA	Analysis of Variance
CCD	Central Composite Design
DoE	Design of Experiments
HPLC	High-Performance Liquid Chromatography
ICH	International Council for Harmonisation
LOD	Limit of Detection
LOQ	Limit of Quantification
PDA	Photodiode Array Detector
PBUH	Potassium Buffer
QbD	Quality by Design
RSD	Relative Standard Deviation
RP-HPLC	Reverse-Phase High-Performance Liquid Chromatography
R _t	Retention Time
SD	Standard Deviation
USP	United States Pharmacopeia
UV	Ultraviolet

1 INTRODUCTION

Fexuprazan hydrochloride is a novel potassium-competitive acid blocker (P-CAB) developed for rapid and sustained suppression of gastric acid secretion. Unlike conventional proton pump inhibitors (PPIs), which require activation in an acidic environment, fexuprazan directly inhibits the gastric H^+/K^+ -ATPase enzyme, resulting in rapid onset and prolonged acid suppression [1,2]. This pharmacological advantage makes it effective in the management of gastroesophageal reflux disease (GERD) and other acid-related disorders. The chemical structure of fexuprazan hydrochloride is shown in **Figure 1**.

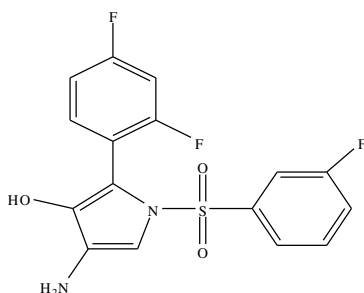


Figure 1. Structure of Fexuprazan Hydrochloride

Fexuprazan acts through reversible and potassium-competitive inhibition of the proton pump, blocking acid secretion irrespective of enzyme activation. Pharmacokinetic studies indicate rapid absorption with peak plasma concentration within 2–3 hours and sustained pharmacodynamic activity. The drug is primarily metabolized by CYP3A4 and shows minimal food interaction [3,4]. Clinical studies have demonstrated comparable or improved efficacy over conventional PPIs [5].

Robust analytical approaches are vital to assure the quality and stability of pharmaceutical formulations. Reverse-phase high-performance liquid chromatography (RP-HPLC) is broadly utilized owing to its accuracy, sensitivity, and applicability for complex matrices [6]. Method validation as per ICH guidelines ensures reliability and reproducibility [7].

Design of Experiments (DoE) provides a systematic approach for method optimization by evaluating multiple variables simultaneously. Central Composite Design (CCD) enables efficient modeling of linear and quadratic effects with a limited number of experiments [8]. The application of DoE improves method robustness and provides better understanding of factor–response relationships [9].

Despite its clinical importance, limited analytical methods are available for fexuprazan hydrochloride, particularly stability-indicating methods developed using DoE. Therefore, the present study aims to develop and validate a robust and stability-indicating RP-HPLC method using a DoE-based optimization approach.

2 MATERIALS AND METHODS

2.1 Materials

Fexuprazan hydrochloride reference standard was obtained from a certified pharmaceutical supplier (India). Commercial tablet formulation containing 40 mg of fexuprazan hydrochloride was procured from the local market. Methanol (HPLC grade) was purchased from Merck Ltd. (India). Potassium dihydrogen phosphate and orthophosphoric acid were obtained from Rankem (India). Ultrapure water was prepared using a Milli-Q system. All reagents used were of analytical grade.

2.1.1 Chromatographic Conditions

Chromatographic analysis was performed using a Shim-pack C18 column (250 mm × 4.6 mm, 5 µm). The mobile phase consisted of methanol and phosphate buffer (pH 3.5) in the ratio of 70:30 (v/v). The flow rate was maintained at 1.0 mL/min, and detection was carried out at 256 nm. The injection volume was 20 µL, and column temperature was maintained at 25°C. Under these conditions, fexuprazan hydrochloride eluted at approximately 4.5 min.

2.1.2 Preparation of Standard and Sample Solutions

A standard stock solution (1000 µg/mL) was prepared by dissolving an accurately weighed quantity of fexuprazan hydrochloride in methanol: phosphate buffer (70:30 v/v). Working standard solutions (10–60 µg/mL) were prepared by appropriate dilution.

Tablet powder equivalent to 40 mg of drug was weighed, transferred to a volumetric flask, and extracted using diluent with sonication for 10 min. The solution was filtered and suitably diluted for analysis.

2.2 Design of Experiments (DoE)

Method optimization was carried out using Central Composite Design (CCD). Three independent variables—methanol composition, buffer pH, and flow rate—were selected. A total of 20 experimental runs, including factorial, axial, and center points, were performed. Retention time and peak area were selected as responses. Data were fitted to a quadratic model, and statistical significance was evaluated using ANOVA. Response surface and contour plots were used to identify optimal conditions. Statistical analysis was performed using DesignExpert® software, and significance was considered at $p < 0.05$.

2.3 Method Validation

The developed RP-HPLC method was validated in accordance with internationally accepted ICH Q2(R2) guidelines to ensure its suitability for routine analytical application [7].

2.3.1 System Suitability:

System suitability tests were performed prior to analysis to ensure adequate chromatographic performance. Parameters such as theoretical plates, tailing factor, retention time, and %RSD of peak area were evaluated as per compendial recommendations [8], and the results were within acceptable limits.

2.3.2 Specificity:

Specificity was assessed by analyzing blank, standard, and sample solutions to confirm the absence of interference at the retention time of fexuprazan hydrochloride, demonstrating method selectivity [7].

2.3.3 Working Range:

Linearity was established over the concentration range of 10–60 µg/mL by plotting peak area versus concentration. The method exhibited a strong correlation, confirming proportional detector response [7].

2.3.4 Accuracy:

Accuracy was evaluated using the standard addition method at 80%, 100%, and 120% levels. The percentage recovery values were within acceptable limits, confirming method trueness [7].

2.3.5 Precision:

Precision was assessed in terms of repeatability and intermediate precision. The %RSD values were found to be within acceptable limits, indicating good method reproducibility [7].

2.3.6 Robustness:

Robustness was evaluated by introducing small deliberate variations in chromatographic parameters such as flow rate, wavelength, and temperature. The method remained unaffected, demonstrating its reliability [7].

2.3.7 Limit of Detection and Limit of Quantification:

LOD and LOQ were determined based on the standard deviation of the response and the slope of the calibration curve, indicating adequate sensitivity of the method [7].

2.4 Forced Degradation Studies

Forced degradation studies were performed under acidic, basic, oxidative, thermal, and photolytic conditions to evaluate the stability-indicating capability of the developed method in accordance with ICH guidelines [12] and established stress testing approaches [13,14]. Acidic and basic degradation were carried out using 0.1 N HCl and 0.1 N NaOH at 40°C for 60 min, respectively. Oxidative degradation was performed using hydrogen peroxide, while thermal degradation was conducted at 80°C and photolytic degradation under sunlight exposure for 24 h. Percentage degradation was calculated by comparing the peak areas of stressed and unstressed samples, confirming the extent of degradation and method selectivity [13,14].

3 RESULTS AND DISCUSSION

3.1 Chromatographic Performance and System Suitability

The developed RP-HPLC method demonstrated efficient and reproducible separation of fexuprazan hydrochloride under optimized chromatographic conditions. The analyte produced a sharp, symmetrical, and well-resolved peak at a retention time of approximately 4.5 min, indicating appropriate selection of mobile phase composition and stationary phase interaction.

No interference from excipients or baseline noise was observed at the retention time of the drug, confirming the specificity of the method.

The chromatographic behavior and peak purity are clearly illustrated in **Figure 2**, which shows a distinct and isolated peak corresponding to fexuprazan hydrochloride.

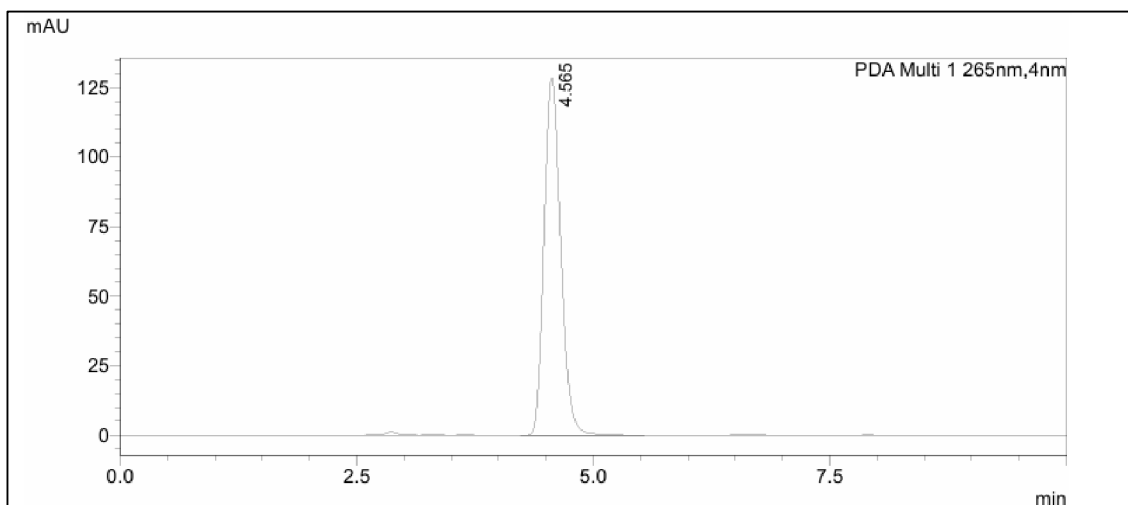


Figure 2. Chromatogram of Fexuprazan Hydrochloride under optimized chromatographic conditions.

System suitability results demonstrated that theoretical plates exceeded 2000, tailing factor was below 2, and %RSD for peak area was within acceptable limits, confirming adequate chromatographic performance.

3.2 Design of Experiments (DoE) Optimization

3.2.1 Experimental Design and Response Analysis

A Central Composite Design (CCD) was employed to systematically evaluate the influence of methanol composition (A), buffer pH (B), and flow rate (C) on chromatographic responses.

The experimental design matrix along with observed responses is presented in **Table 1**.

Table 1. Experimental design matrix and observed responses for CCD runs.

		Factor 1	Factor 2	Factor 3	Response 1	Response 2
Run	Space Type	A: Volume of Methanol	B: pH	C: Flow Rate	RT	Peak area
		ml		ml/min		
1	Axial	70	4.3409	1	5.225	1.48563E+06
2	Factorial	60	3	1.2	8.813	1.81493E+06
3	Axial	70	3.5	1.33636	4.216	1.99267E+06
4	Factorial	80	4	0.8	5.703	1.48292E+06
5	Center	70	3.5	1	4.555	640322
6	Factorial	80	3	0.8	5.235	2.00236E+06

7	Axial	70	3.5	0.663641	4.452	2.19186E+06
8	Center	70	3.5	1	4.555	640322
9	Factorial	80	4	1.2	5.334	813813
10	Factorial	60	4	0.8	5.917	898916
11	Center	70	3.5	1	4.555	640322
12	Factorial	60	4	1.2	5.359	1.64808E+06
13	Factorial	60	3	0.8	10.165	1.19636E+06
14	Center	70	3.5	1	4.555	640322
15	Axial	86.8179	3.5	1	4.913	1.2061E+06
16	Axial	53.1821	3.5	1	8.832	1.2236E+06
17	Center	70	3.5	1	4.555	640322
18	Axial	70	2.6591	1	10.443	1.09593E+06
19	Center	70	3.5	1	4.555	640322
20	Factorial	80	3	1.2	5.863	897585

The variation in retention time and peak area across different experimental runs (Table 1) clearly indicates that the selected variables significantly influence chromatographic performance, thereby justifying the application of a multivariate optimization approach.

3.2.2 ANOVA and Model Significance

The statistical significance of the developed quadratic model was assessed using ANOVA, and the combined results for retention time and peak area are summarized in **Table 2**.

Table 2. Combined ANOVA summary for retention time and peak area responses.

Source	RT Fvalue	RT pvalue	Peak Area F-value	Peak Area pvalue	Significance
Model	47.88	<0.0001	22.10	<0.0001	Significant
A (Methanol)	94.13	<0.0001	0.3126	0.5856	Significant (RT only)
B (pH)	118.98	<0.0001	—	—	Significant (RT only)
C (Flow Rate)	1.82	0.2066	1.12	0.3086	Not significant
AB	43.36	<0.0001	—	—	Significant (RT)
AC	3.49	0.0911	34.44	<0.0001	Significant (Peak Area)
BC	0.0306	0.8646	—	—	Not significant
A ²	60.27	<0.0001	9.29	0.0093	Significant
B ²	119.00	<0.0001	12.86	0.0033	Significant
C ²	0.2929	0.6002	85.94	<0.0001	Significant (Peak Area)

The model exhibited high F-values with corresponding p-values less than 0.0001 (Table 2), confirming that the model is statistically significant. Methanol composition (A) and buffer pH (B) were identified as critical factors affecting retention time, whereas peak area was

significantly influenced by interaction (AC) and quadratic terms. The presence of significant second-order terms further confirms curvature in the response surface, validating the suitability of the quadratic model.

3.2.3 Model Fit and Statistical Validation

The adequacy and predictive capability of the developed models were further evaluated using statistical parameters summarized in **Table 3**.

Table 3. Model fit statistics for retention time and peak area.

Parameter	Retention Time	Peak Area
R ²	0.9773	0.9107
Adjusted R ²	0.9569	0.8695
Predicted R ²	0.8192	0.7194
Std. Dev.	0.4103	1.893E+05
C.V. (%)	6.97	15.91
Adeq Precision	20.73	12.49

The high coefficient of determination values ($R^2 > 0.90$) presented in Table 3 indicate strong agreement between experimental and predicted responses. Additionally, the close proximity between adjusted and predicted R^2 values confirms good model predictability. Adequate precision values greater than 4 demonstrate a sufficient signal-to-noise ratio, indicating that the model is reliable for navigating the design space.

3.2.4 Response Surface Analysis and Optimization

The interaction effects of independent variables on chromatographic responses were visualized using contour and response surface plots. The contour plot shown in **Figure 3** demonstrates that increasing methanol composition leads to a decrease in retention time due to increased elution strength, while pH exhibits a non-linear influence on analyte retention.

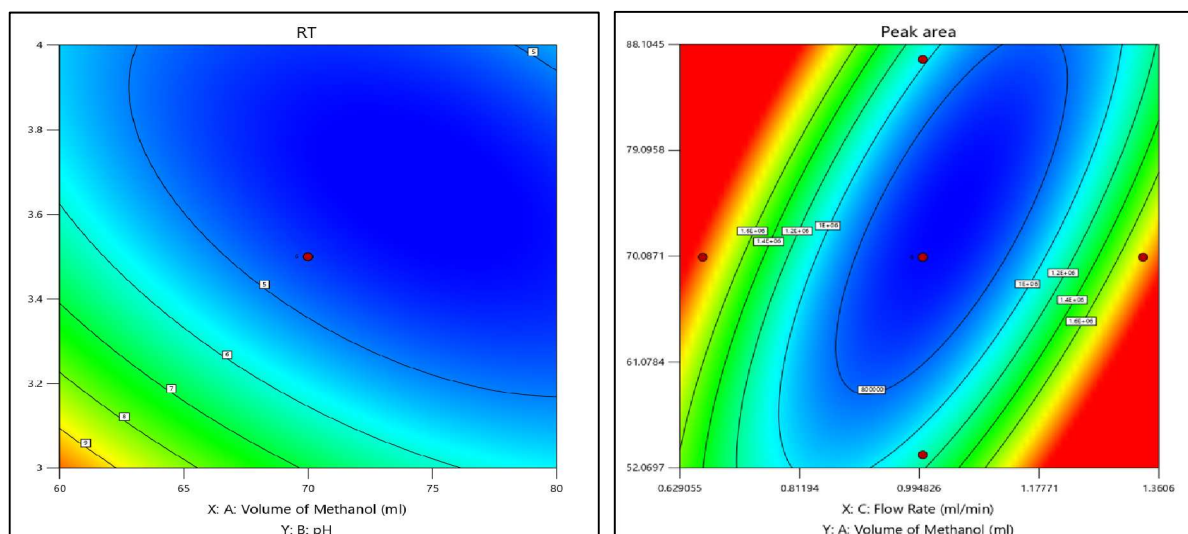


Figure 3. Contour plot showing effect of methanol composition and pH on retention time

Similarly, the three-dimensional response surface plot in **Figure 4** highlights the combined influence of flow rate and pH on chromatographic behavior, confirming the presence of interaction effects and curvature.

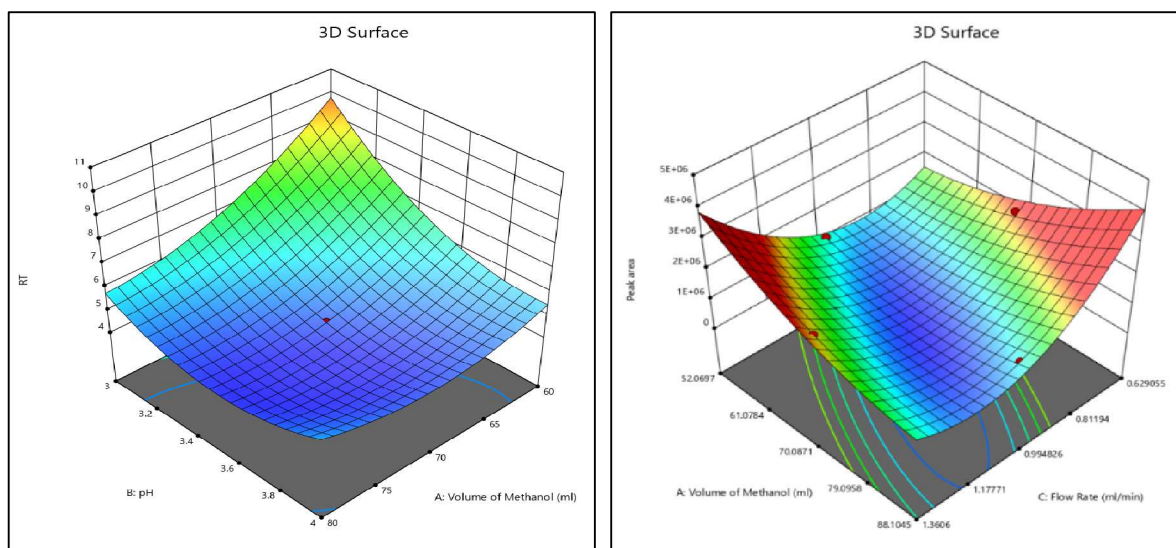


Figure 4. 3D response surface plot showing interaction between flow rate and pH

The optimized chromatographic conditions were determined using a desirability function approach, and the design space is represented in **Figure 5**. The optimized conditions were found to be approximately 70% methanol, pH 3.5, and flow rate of 1.0 mL/min

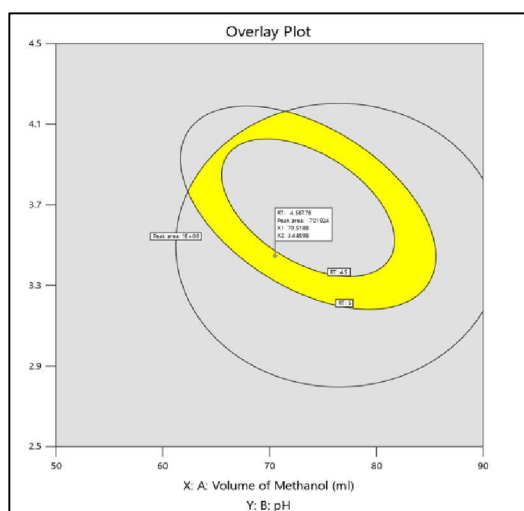


Figure 5. Overlay plot showing optimized chromatographic region

3.3 Method Validation

3.3.1 Working Range and Sensitivity

The method exhibited excellent linearity over the concentration range of 10–60 µg/mL, with a regression equation of $y = 15872x + 12453$ and a correlation coefficient ($R^2 = 0.998$), confirming a strong linear relationship between concentration and peak area. The graphical representation of the calibration curve is illustrated in **Figure 6**, which clearly depicts the linear increase in peak area with concentration. Additionally, the overlay chromatograms corresponding to different concentration levels are presented in **Figure 7**, demonstrating

consistent peak shape, retention behavior, and proportional response without distortion or peak broadening.

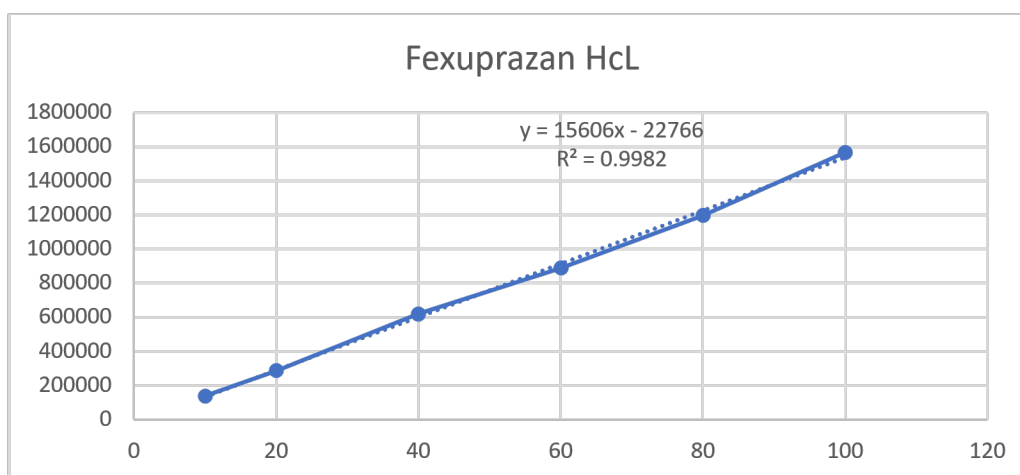


Figure 6. Calibration curve of Fexuprazan Hydrochloride showing linear relationship between concentration and peak area

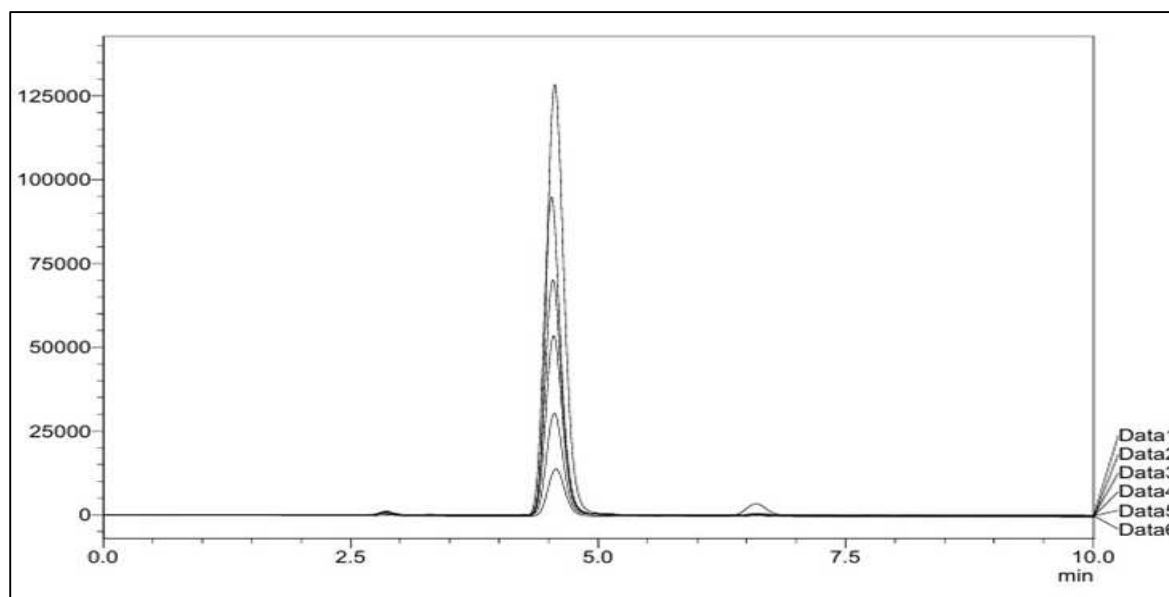


Figure 7. Overlay chromatograms representing linearity across concentration range (10–60 µg/mL)

The method exhibited adequate sensitivity, with a **limit of detection (LOD)** of 4.88 µg/mL and a **limit of quantification (LOQ)** of 14.79 µg/mL, demonstrating its suitability for reliable detection and quantification of fexuprazan hydrochloride within the working range.

3.3.2 Specificity

Specificity of the method was confirmed by analyzing blank and standard solutions, where no interfering peaks were observed at the retention time of the drug. The specificity chromatogram shown in **Figure 8** demonstrates the absence of co-eluting peaks, confirming the selectivity of the method.

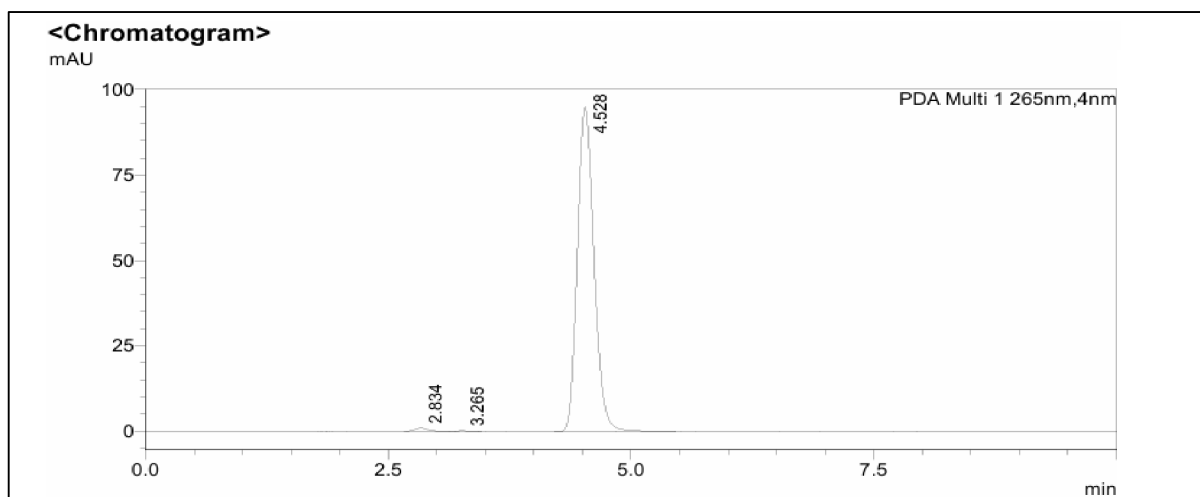
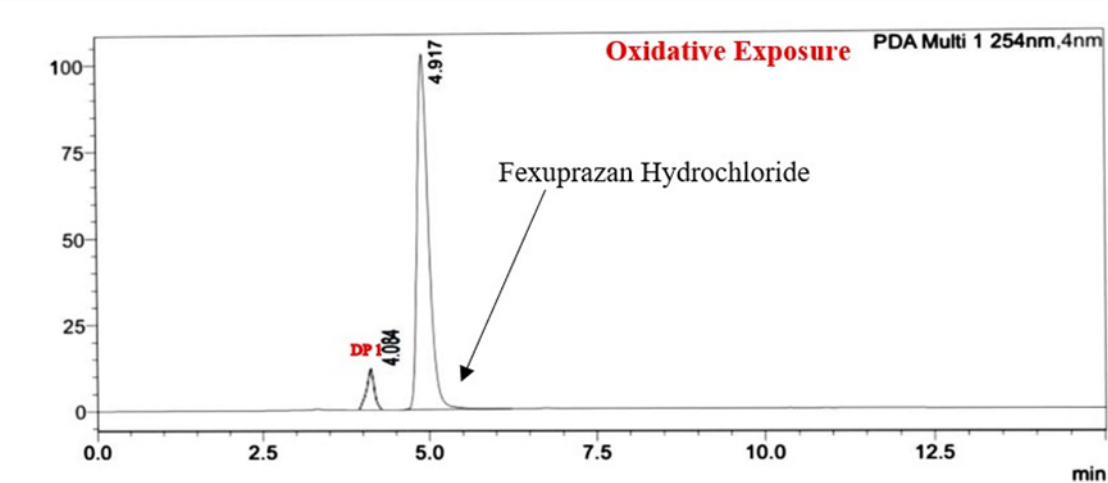
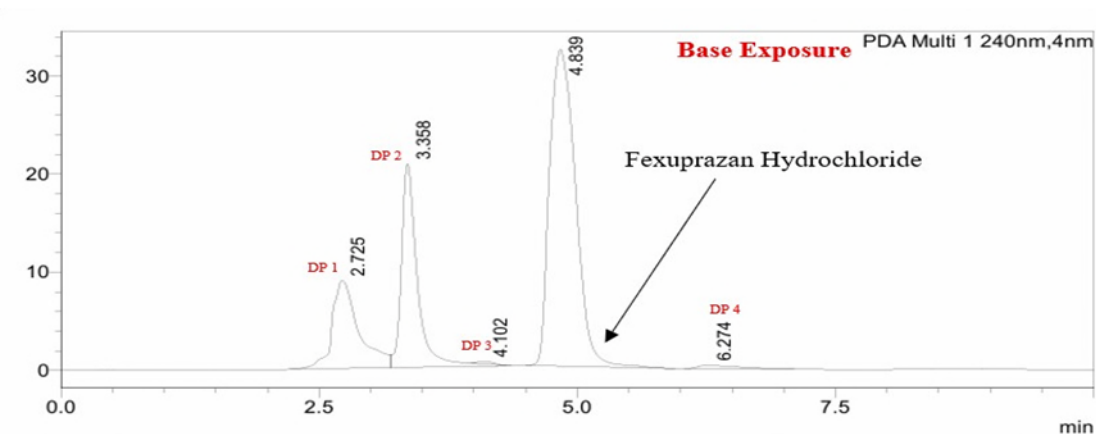
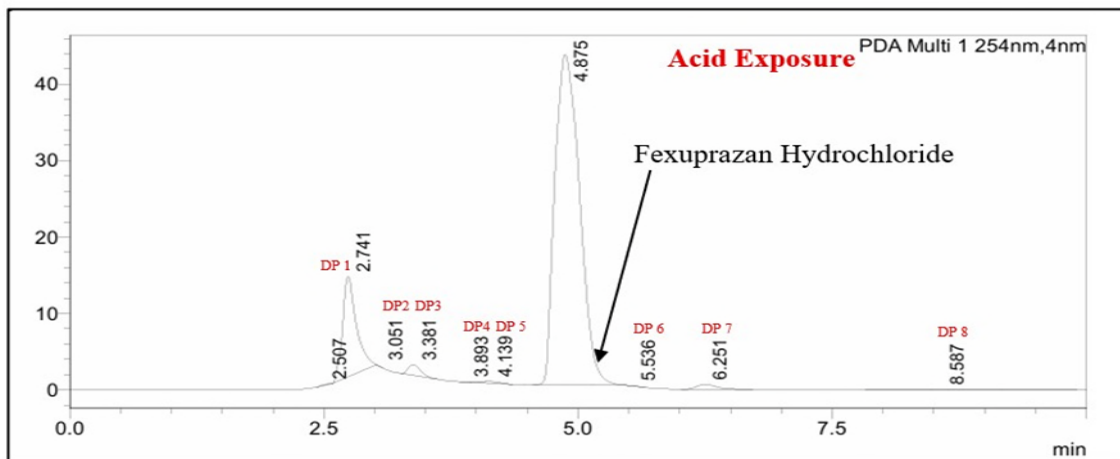


Figure 8. Specificity chromatogram showing absence of interference at retention time of Fexuprazan Hydrochloride.

Forced Degradation Studies

To further establish the stability-indicating capability, forced degradation studies were performed under various stress conditions. The representative chromatograms of degraded samples are shown in **Figure 9**, which clearly demonstrate that degradation products are well separated from the main analyte peak.



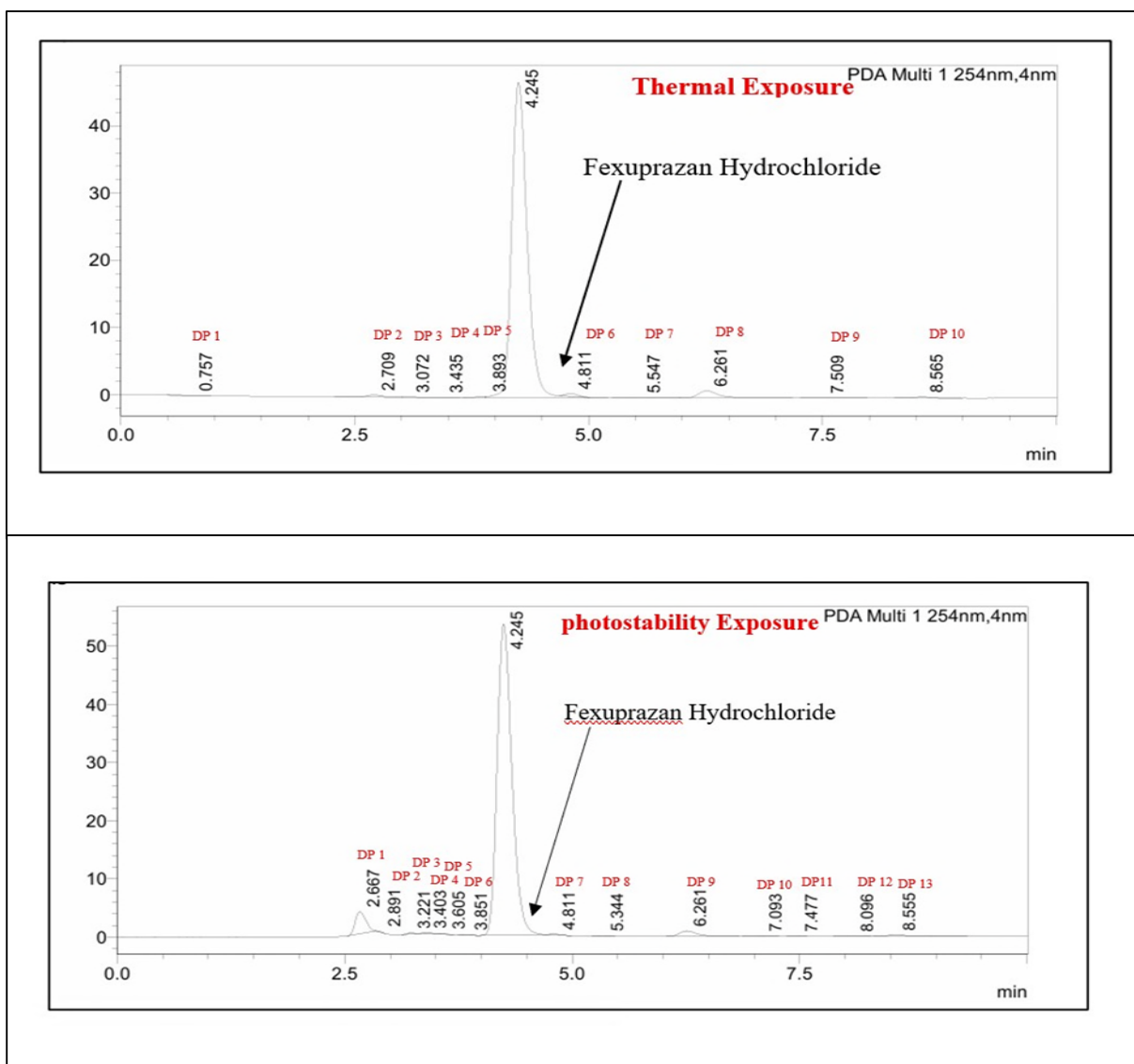


Figure 9. Chromatograms of Fexuprazan Hydrochloride under forced degradation conditions.

The percentage degradation observed under different stress conditions is summarized in **Table 4**. Among the tested conditions, maximum degradation was observed under thermal stress, followed by acidic and oxidative conditions, whereas photolytic degradation showed comparatively lower impact (Table 4). The absence of interfering peaks in Figure 8, along with clear separation of degradation products in Figure 9, confirms the specificity and stability indicating capability of the method.

Table 4. Forced degradation behavior of Fexuprazan Hydrochloride under stress conditions.

Degradation Type	Stress Condition	% Degradation of Fexuprazan Hydrochloride
Acidic	0.1 N HCL, 40°C for 60 min	14.49%
Basic	0.1 N NaOH, 40°C for 60 min	12.07%
Oxidative	3% H ₂ O ₂ , Room Temperature, 3Hr	11.66%
Thermal	Hot air oven, 80 °C for 6 hrs	16.05%

Photolytic	Sunlight exposure, 24 hrs	5.8%
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The absence of co-elution in Figure 8 confirms that the developed method is capable of selectively quantifying the drug in the presence of its degradation products.

3.3.3 Accuracy

The method demonstrated high accuracy, with percentage recovery values ranging between 99.54% and 99.75%, as presented in **Table 5**, indicating minimal deviation and high reliability.

Table 5. Accuracy results of Fexuprazan Hydrochloride.

Drug	Spiking Level (%)	Initial Amount (µg/mL)	Amount Added (µg/mL)	Total Amount added (µg/mL)	Total Amount Found (µg/mL)	% Recovery Sd (n=3)	% RSD
Fexuprazan HCl	80	4	3.2	7.2	7.18	99.72	0.005
	100	4	4	8	7.98	99.75	0.010
	120	4	4.8	8.8	8.76	99.54	0.010

3.3.4 Precision

The precision of the method was confirmed with %RSD values less than 2%, demonstrating excellent repeatability. The detailed precision results are summarized in **Table 6**.

Table 6. Precision results of Fexuprazan Hydrochloride.

Drug	Precision Type	Concentration (µg/mL)	Mean Peak Area (n=6)	Standard Deviation	%RSD
Fexuprazan Hydrochloride	Repeatability	40	739820	7612.79	1.03
Fexuprazan Hydrochloride	Intraday Precision	20	365420	4215.32	1.15
		40	741250	8125.64	1.09
		60	1123650	12456.33	1.10
Fexuprazan Hydrochloride	Interday Precision	20	358960	4982.15	1.39
		40	732415	8456.32	1.15
		60	1112480	13652.74	1.22

3.3.5 Robustness

The low %RSD values obtained under varied chromatographic conditions (**Table 7**) indicate that the method performance is not significantly affected by small deliberate changes, confirming its robustness and operational reliability.

Table 7. Robustness study results of Fexuprazan Hydrochloride.

Parameter	Condition	Area (Mean $\pm$ SD)	% RSD	RT (Mean $\pm$ SD)	% RSD	NTP (Mean $\pm$ SD)	% RSD	TF (Mean $\pm$ SD)	% RSD
Flow Rate	0.8 mL/min	4.907 $\pm$ 0.006	0.135	1206 $\pm$ 48.19	0.039	4227 $\pm$ 2.64	0.062	1.281 $\pm$ 0.002	0.162
	1.0 mL/min	5.355 $\pm$ 0.004	0.092	1646 $\pm$ 300.5	0.182	2385 $\pm$ 2.64	0.110	1.471 $\pm$ 0.002	0.171
	1.2 mL/min	5.199 $\pm$ 0.002	0.038	8989 $\pm$ 12.66	0.001	2523 $\pm$ 5.57	0.220	1.557 $\pm$ 0.001	0.064
Temperature	23°C	3.858 $\pm$ 0.019	0.494	8005 $\pm$ 5177	0.646	2542 $\pm$ 8.08	0.317	1.295 $\pm$ 0.008	0.628
	25°C	4.571 $\pm$ 0.007	0.173	1579 $\pm$ 21.45	0.001	4077 $\pm$ 17.67	0.433	1.295 $\pm$ 0.004	0.312
	27°C	3.226 $\pm$ 0.016	0.502	1791 $\pm$ 412.6	0.230	2129 $\pm$ 7.37	0.346	1.364 $\pm$ 0.011	0.849
Wavelength	263 nm	4.321 $\pm$ 0.020	0.468	8133 $\pm$ 453.3	0.055	3151 $\pm$ 2.51	0.079	1.277 $\pm$ 0.005	0.045
	265 nm	4.555 $\pm$ 0.003	0.066	6403 $\pm$ 21.12	0.003	2540 $\pm$ 5.56	0.219	1.301 $\pm$ 0.004	0.310

	267 nm	4.818	0.090	1614	0.004	3212	0.078	1.282	0.196
		±		±		±		±	
		0.004		6.55		2.51		0.002	

Compared to conventional RP-HPLC methods, the developed method offers reduced analysis time, improved sensitivity, and enhanced robustness due to the application of DoE-based optimization.

4 DISCUSSION

The present study successfully demonstrates the development of a statistically optimized RPHPLC method for fexuprazan hydrochloride using a DoE-based approach. The chromatographic system provided consistent peak symmetry, high efficiency, and reproducible retention behavior, confirming the suitability of the selected conditions. The absence of interfering peaks at the analyte retention time, as evidenced in Figure 2 and Figure 8, establishes the high specificity of the method. The application of Central Composite Design enabled systematic evaluation of variable interactions, offering a significant advantage over traditional trial-and-error approaches. Methanol composition and buffer pH were identified as dominant factors influencing chromatographic behavior, whereas flow rate showed secondary influence through interaction effects. The statistical model exhibited strong predictive capability, supported by high R^2 values and adequate precision. The method demonstrated excellent analytical performance, including strong linear response (Figure 6 and Figure 7), acceptable sensitivity, and minimal variability. The obtained LOD and LOQ values indicate that the method is sufficiently sensitive for low-level detection. Accuracy and precision results confirm the reliability of the method for routine analysis. Forced degradation studies (Figure 9 and Table 4) revealed that the drug is more susceptible to thermal and acidic conditions, while photolytic degradation was comparatively minimal. Importantly, all degradation products were well resolved from the main peak, confirming the stability-indicating capability of the method. Overall, the integration of DoE significantly enhanced method understanding, robustness, and efficiency.

5 CONCLUSION

A reliable, rapid, and stability-indicating RP-HPLC method for the quantification of fexuprazan hydrochloride was successfully developed and validated using a Design of Experiments-based strategy. The method demonstrated excellent specificity, linearity, precision, accuracy, robustness, and sensitivity in accordance with ICH guidelines. The application of Central Composite Design enabled efficient optimization of chromatographic conditions while providing comprehensive insight into factor–response relationships. The method effectively separated the drug from its degradation products, confirming its suitability for stability studies. Due to its simplicity, reproducibility, and analytical performance, the method is highly suitable

for routine quality control, stability testing, and pharmaceutical analysis. The study highlights the effectiveness of integrating statistical design with analytical validation to develop high-quality, regulatory-compliant methods.

Declarations

Funding

The authors declare that no specific funding was received for this work.

Author Contributions

Saish Mhaske and Prexa Tandel contributed to method development, data interpretation, and manuscript drafting. Dr. Hiralben Mehta supervised the study and critically reviewed the manuscript. All authors approved the final version.

Competing Interests

The authors declare that they have no competing interests.

Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval Not

applicable.

This study did not involve human participants, human tissues, animals, or clinical investigations.

Consent to Participate Not

applicable.

Consent for Publication Not

applicable.

Acknowledgement

We express our gratitude to Parul University, Parul Institute of Pharmacy and Research, for offering laboratory facilities, encouragement, and continuous support throughout the research process. Finally, we appreciate all individuals who contributed to this work for their cooperation and valuable assistance.

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