RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF PEMAFIBRATEUSING DESIGN OF EXPERIMENTS APPROACH

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Abstract

The present work objective is to develop & validate a simple, accurate & economical RP-HPLC practice for the estimation of Pemafibrate by means of Design of Experiments Approach, which was suitable for multivariate optimization of method. The multinomial equation is a valuable analytical instrument used to investigate the connection among measured reactions and independent factors, and it is especially useful for charting responses across an experimental space to establish the most effective approach. The critical method parameters (CMPs) were optimized using the Box-Behnken Design. Design expert software was equipped for the study. Chromatographic separation was done on Inertsil ODS column with specifications 150mm x 4.6mm, 3.5µm at ambient temperature. The predicted and optimized data from the software consisted of Acetonitrile as an organic phase with a proportion of 59.99% at pH 4.007 with 0.9 ml/min flow rate brought the desirability function of 1. The UV detector was adjusted at 239.4 nm. Validation and Stability Assessment of an Optimized Chromatographic Method with High Linearity and Correlation Coefficient.

Keywords: Pemafibrate, Design of Experiments Approach, Box-Behnken design, RP-HPLC and ICH Q2 (R1).

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1. INTRODUCTION

Pemafibrate available under the brand name Parmodia, which is peroxisome proliferator-activated receptor alpha (PPARα) agonist used in the treatment of hyperlipidemia, used for dropping LDL cholesterol levels in obese patients. By acting on PPARα, the substance significantly lowers remnant lipoprotein cholesterol (RemL-C), non-HDL-C, apo B, apo B-48, and apo C-III levels. The IUPAC name of the compound is ((2R)-2-[3-({(1,3-benzoxazol-2-yl)[3-(4-methoxyphenoxy)propyl]amino}methyl) phenoxy]butanoic acid and the chemical structure of the compound is shown in Fig.1.

After detailed literature review, no HPLC method is reported for determination of Pemafibrate using statistical optimization technique. The aim of research is to develop & validate Novel RP-HPLC technique for determination of Pemafibrate by statistical optimization technique. The current study utilized the Response Surface Methodology (RSM) to create and enhance, through optimization an analytical method for Pemafibrate. RSM is a useful tool for examining the bond between measured responses and self-governing variables through a multinomial equation. This method helps to map out the responses within the experimental area, leading to the development of an optimized approach.

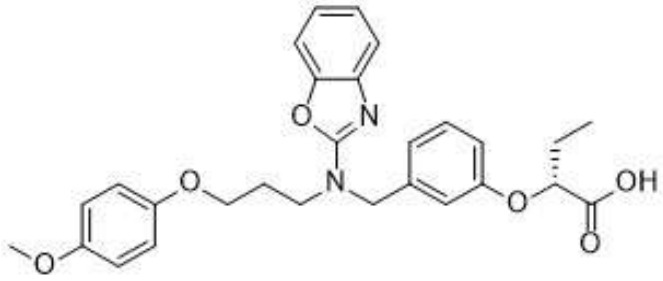


Fig. 1 Chemical Structure of Pemafibrate

2. MATERIALS AND METHODS

HPLC grade formic acid and triethyl amine is acquired from Rankem, HPLC grade water brought from Merck milli-Q. Pemafibrate is gifted from Kowa Pharmaceuticals. The formulation Parmodia (Pemafibrate 0.1 mg) was brought from the international market. In the realm of HPLC study, the Water HPLC 2695 system equipped with a photodiode array detector and integrated with Empower 2 software is utilized for analysis purposes. Shimadzu-1800 UV-VIS Spectrometer is utilized for spectral analysis. All the stress tests were done by radley apparatus in present research work. Design Expert® (13.0.5.0x64) software was worn in the study.

A. Preparation of Stock Solutions (Standard)

In order to make a stock solution of standard, a precise amount of 10 mg of Pemafibrate was carefully weighed & relocated into volumetric flasks of capacity 100 ml. To dilute the solution, 3/4 of the required diluents were poured in volumetric flask & sonicated for ten mins. The additional diluents were added to the flasks in order to bring them up to the required volume. One milliliter of the above preparation was shifted to a volumetric flask of capacity ten milliliters, & then final adjustment of the volume is done with a diluent.

B. Preparation of Sample Solution

The mean weight of five tablets was determined. Then, a single tablet's weight was measured and dissolved, made the volume to 10 ml with diluent. Prepared mixture was sonicated for 25 minutes, after which it was filtered using filters, and was diluted further to meet specific requirements.

C. Initial HPLC Trails

These are performed by using different buffers like formic acid and triethyl ammonium acetate and organic modifiers like methanol and acertonitrile. Various preliminary trials are done to finalize the initial chromatographic condition.

D. Optimization of Method by RSM [3]

BBD is adopted for optimization of the method. 4 factors i.e., pH, flow rate, % organic content, and organic modifier were optimized. Hence, , these parameters were optimized as like 3 numerical factors, i.e., pH, flow rate and % organic content at 3 levels (high, mid, and low) and one categorical variable i.e., organic modifier at 2 levels by Box-Behnken design. Different ranges of three parameters 2.5-3 pH, 0.9-1.1 flow rate, 40-60 % organic composition, and methanol & acetonitrile as organic modifiers were considered.

E.Method Validation [4]

The validation procedure for the ultimate refined analytical method was conducted based on the ICH Q2 (R1) guidelines, which covered specificity, system suitability, linearity, accuracy, precision, detection and quantification limits, and robustness.

E. Forced Degradation Studies [5]

The developed method was evaluated for stability-indicating properties by subjecting the drug to different stress conditions as specified in ICH Q1A (R2) guidelines.

3. RESULTS AND DISCUSSION

Box-Behnken design was created with 4 factors at different levels. This design comprises of 26 experimental runs, which were established and studied by Design-Expert software, Table 1 displays the relevant data associated with it.

TABLE I BBD STUDY DESIGN ALONG WITH REPORTS

S. No.	Run	pH	Flow Rate	% Organic Content	Organic Modifier	Retention time	Theoretical Plates
1	18	4	1	40	Acetonitrile	3.789	4396
2	14	4	0.9	50	Acetonitrile	3.354	4398
3	23	6	1.1	40	Acetonitrile	3.547	4405
4	11	6	0.9	60	Methanol	3.358	4379
5	16	4	1.1	50	Acetonitrile	3.215	4239
6	12	6	1.1	60	Methanol	3.125	4123
7	7	4	1	60	Methanol	2.9	4105
8	9	6	0.9	40	Methanol	3.548	4387
9	13	6	1	50	Methanol	3.478	4381
10	22	6	0.9	40	Acetonitrile	3.678	4387
11	4	8	1.1	50	Methanol	3.354	4368
12	19	8	1	40	Acetonitrile	3.923	4485
13	8	8	1	60	Methanol	3.561	4386
14	2	8	0.9	50	Methanol	3.657	4398
15	5	4	1	40	Methanol	3.648	4391
16	15	8	0.9	50	Acetonitrile	3.785	4398
17	25	6	1.1	60	Acetonitrile	3.253	4289

18	21	8	1	60	Acetonitrile	3.646	4417
19	20	4	1	60	Acetonitrile	3.102	4211
20	3	4	1.1	50	Methanol	3.198	4217
21	1	4	0.9	50	Methanol	3.259	4352
22	6	8	1	40	Methanol	3.921	4399
23	10	6	1.1	40	Methanol	3.467	4376
24	24	6	0.9	60	Acetonitrile	3.421	4398
25	17	8	1.1	50	Acetonitrile	3.547	4401
26	26	6	1	50	Acetonitrile	3.536	4399

A. Statistical Analysis Data

It was possible to develop mathematical models to determine the correlation between the four factors and their impact on the responses and evaluations based on the observed effects. The ANOVA method was utilized to evaluate the significance of the model, and associated data are provided in Tables 2-5.

TABLE II CREATED ANOVA DATA FOR RETENTION TIME

Source	Sum of Squares	df	Mean Square	F-value	p-value	Inference
Model	0.0016	6	0.0003	11.03	0.0004	significant
A-pH	0.0004	1	0.0004	18.00	0.0014	
B-% Organic Composition	0.0003	1	0.0003	11.76	0.0056	
C-Column	0.0006	1	0.0006	25.30	0.0004	
AB	0.0000	1	0.0000	1.83	0.2034	
AC	0.0002	1	0.0002	8.11	0.0159	
BC	0.0000	1	0.0000	1.22	0.2931	
Residual	0.0003	11	0.0000			

The significance of the model in relation to retention time is demonstrated by the high F-value of 16.17, indicating that the chances of such a large F-value being a result of noise are very low, at only 0.01 %. Additionally, significance of model terms is established by the value of p i.e., less than 0.0500, with terms A, B, C, D, AC and B^2 being deemed significant in this particular instance. The adjusted R^2 of 0.8875 is significantly higher than the predicted R^2 of 0.7464, indicating a low variation of < 0.2. The adequate precision helps in finding signal to noise ratio, and a ratio > four is required. The S/N is 13.7050 as presented in Table 3, which implies that there is sufficient signal present. Using Design Expert® software, contour & surface plots were studied to identify factors that impact the responses and how they do so. The dark blue regions correspond to lower values, while the dark red regions signify higher values. While the other colors represent transitional values. This developed model is useful in exploring design space.

TABLE III FIT STATISTICS

Std. Dev.	0.0050	**R^2**	0.9460
Mean	2.28	**Adjusted R^2**	0.8875

C.V. %	0.2181	**Predicted R^2**	0.7464
		Adeq Precision	11.1461

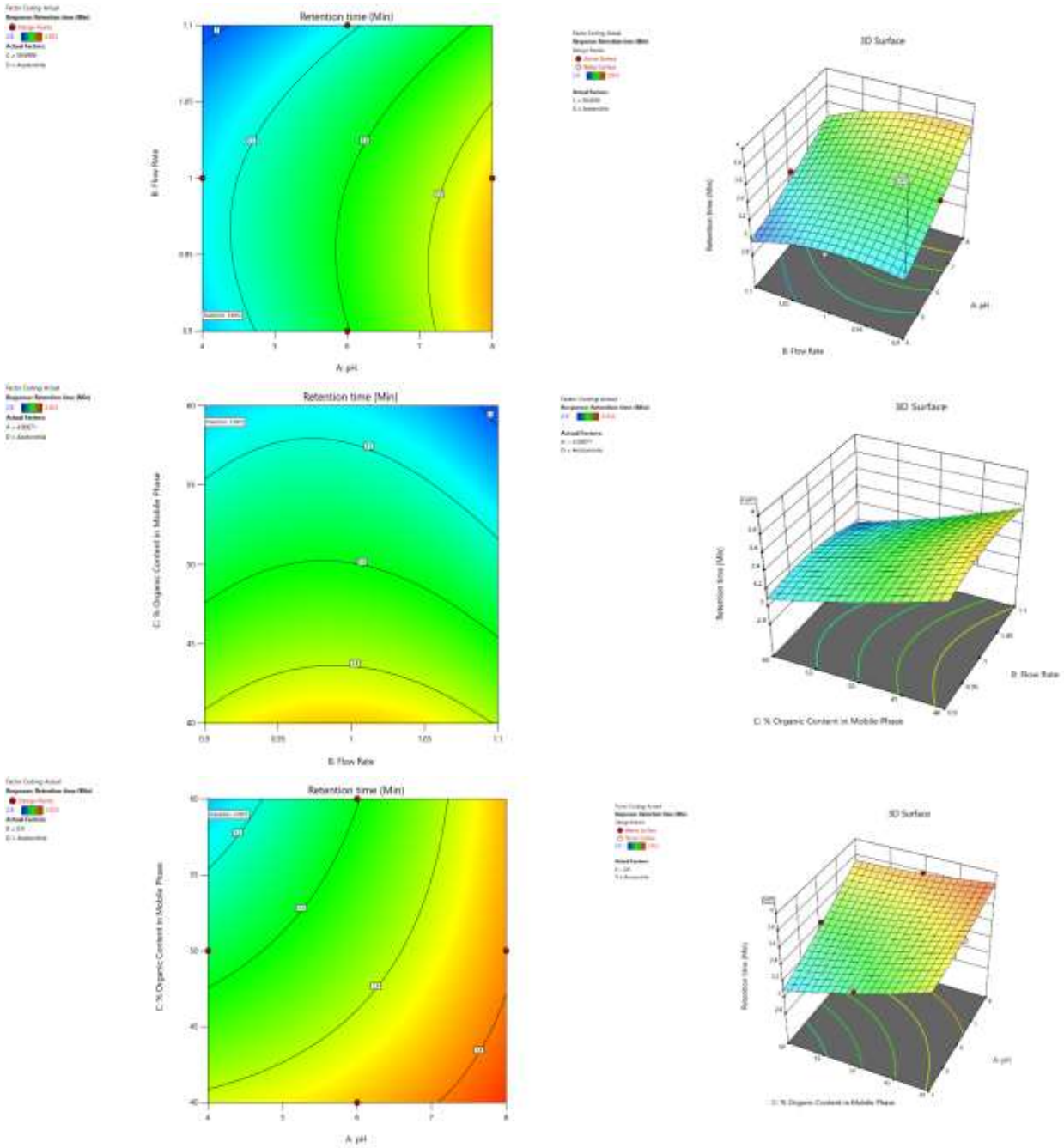


Fig. 2 Retention Time's 2D Contour and 3D Surface Plots

Upon analyzing contour & surface graphs depicting retention time in Fig. 2, it was determined that at median pH, low rate of flow, and median organic phase composition result in lower retention times.

TABLE IV ANOVA TABLE FOR THEORETICAL PLATES

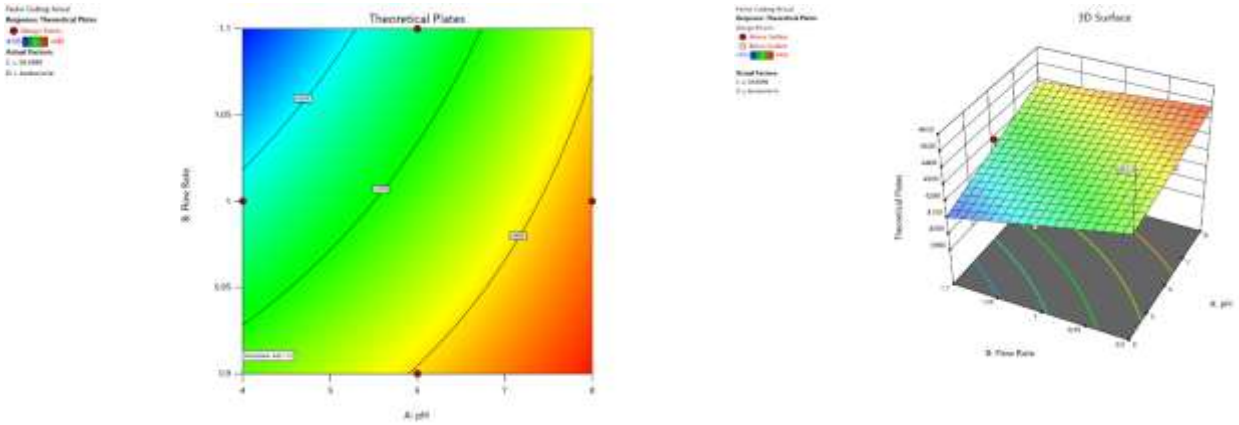
Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value	Inference
Model	1.991E+05	10	19913.15	16.18	< 0.0001	significant

A-pH	55578.06	1	55578.06	45.16	< 0.0001	
B-FR	28815.06	1	28815.06	23.41	0.0002	
C-% OC	52670.25	1	52670.25	42.80	< 0.0001	
D-OM	12104.65	1	12104.65	9.84	0.0068	
AB	8911.13	1	8911.13	7.24	0.0168	
AC	19012.50	1	19012.50	15.45	0.0013	
AD	52.56	1	52.56	0.0427	0.8391	
BC	17298.00	1	17298.00	14.06	0.0019	
BD	2139.06	1	2139.06	1.74	0.2072	
CD	2550.25	1	2550.25	2.07	0.1706	
Residual	18460.82	15	1230.72			

The significance of the model in relation to theoretical plates is demonstrated by the high F-value of 16.18, indicating that the chances of such a large F-value being a result of noise are very low, at only 0.01 %. Additionally, Additionally, significance of model terms is established by the value of p i.e., less than 0.0500, with terms A, B, C, D, AB, AC & BC being deemed significant in this particular instance. The adjusted R^2 of 0.8586 is significantly higher than the predicted R^2 of 0.7533, indicating a low variation of < 0.2. The adequate precision helps in finding signal to noise ratio, and a ratio > four is required. The S/N is 12.436 as presented in Table 5, which implies that there is sufficient signal present. Using Design Expert® software, contour & surface plots were studied to identify factors that impact the responses and how they do so. The dark blue regions correspond to lower values, while the dark red regions signify higher values. While the other colors represent transitional values. This is useful in exploring design space.

TABLE V FIT STATISTICS

Std. Dev.	**35.08**	**R^2**	**0.9152**
Mean	4349.42	**Adjusted R^2**	0.8586
C.V. %	0.8066	**Predicted R^2**	0.7533
		Adequate precision	12.436



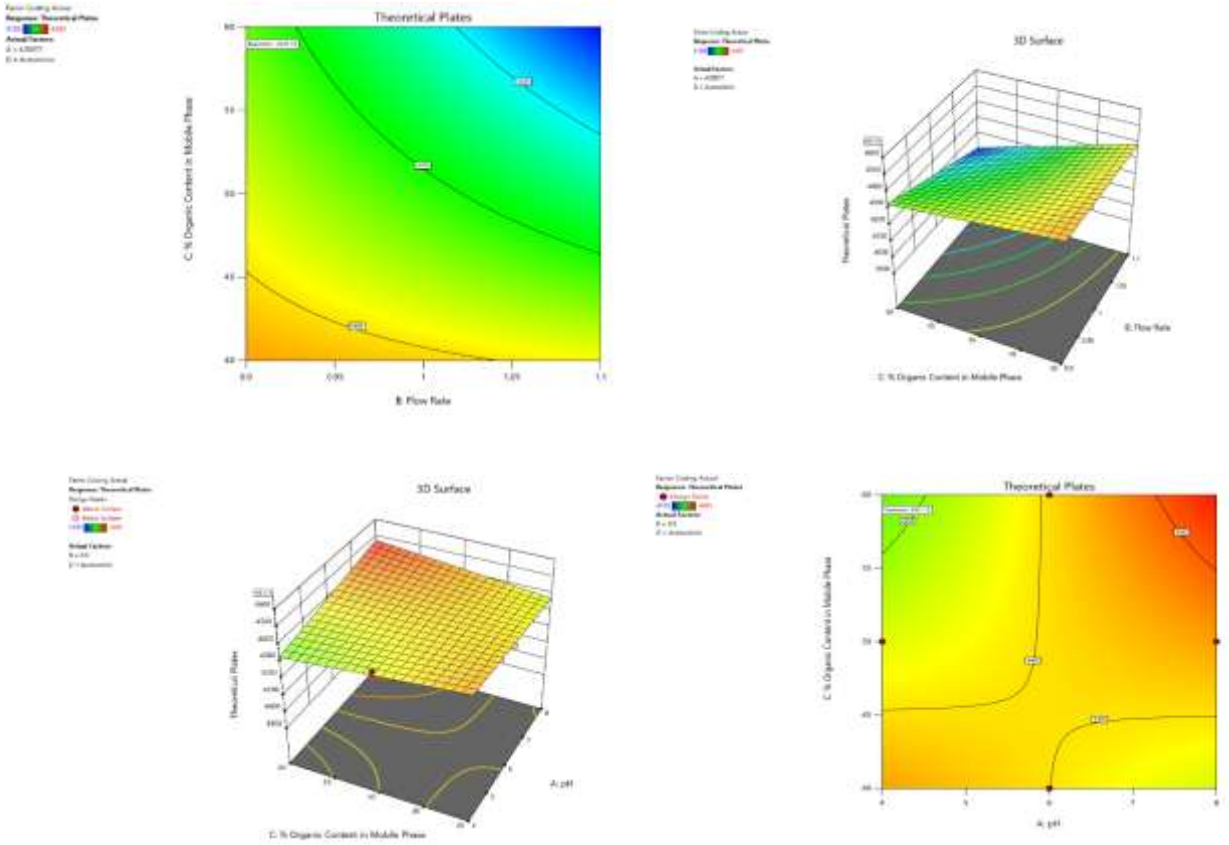


Fig. 3 Contour and Surface Plots of Theoretical Plates

Based on the contour & surface plots presented in Fig. 3, it has been observed that at a median pH, low flow rate, and median organic phase composition leads to a higher value of theoretical plates.

1) Design Validation: Upon visualizing studentized residuals plot for 2 responses portrayed in Fig. 4, that deduced created models aimed at each response was appropriate for chosen design by way of the associated graphs reflected a straight line. Subsequently, Tables 3 and 5 confirmed the significance of the selected models with the value of $p < 0.05$. Therefore, it was concluded that the designated models fitted well for design hired in current study.

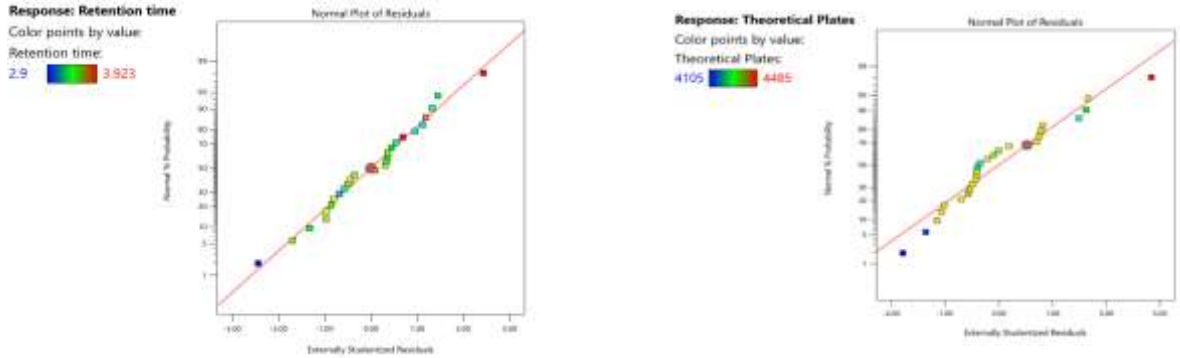


Fig. 4 Normal Plot of Studentized Residuals for Retention Time and Theoretical Plates

Optimization by Desirability Function: The process of Desirability was utilized in determining the best circumstances grounded on specific aims & limitations intended for individual response. In this

approach, a numerical scale ranging from d=0 to d=1 is utilized to evaluate the quality of results, where d=1 represents desirable outcomes, and d=0 represents undesirable ones. After considering the minimum retention time and maximum theoretical plates required, a total composite desirability score (D) of 1 was attained. An overlay counter plot was utilized to showcase the QbD design space, where the best combination of the three independent factors was determined based on the desirability of maximum theoretical plates and minimum retention time. By using this method, the desired goal of mean performance and robustness criteria can be achieved effectively, as illustrated in Fig. 5. To confirm that the ideal conditions outlined in Table 6 are accurate, the drug was tested by analyzing three separate injections. The objective was to establish whether the retention times and theoretical plates obtained in their experiment were consistent with the prescribed ranges presented in Table 7. Visual representations of the optimized sample and standard chromatograms were also included in Fig. 6 and 7.

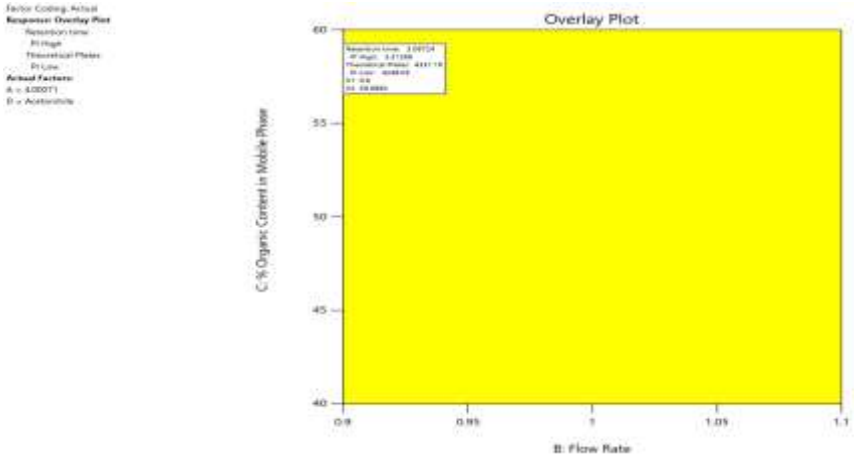


Fig. 5 Overlay Plot

TABLE VI OPTIMIZED CONDITIONS

Chromatographic Condition	Value
pH	4.0071
Flow Rate	0.9
% Organic Content	59.99
Organic Modifier	Acetonitrile

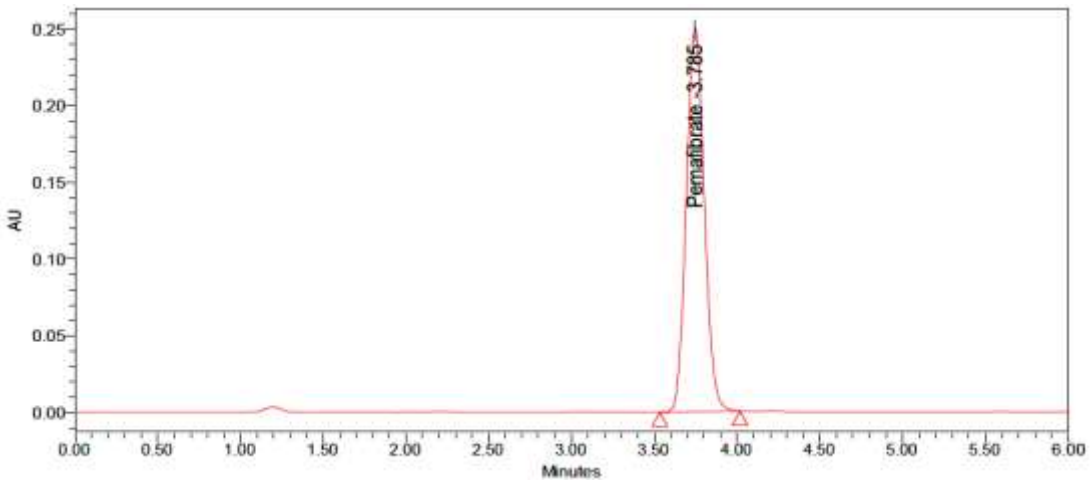


Fig. 6 Standard Chromatogram

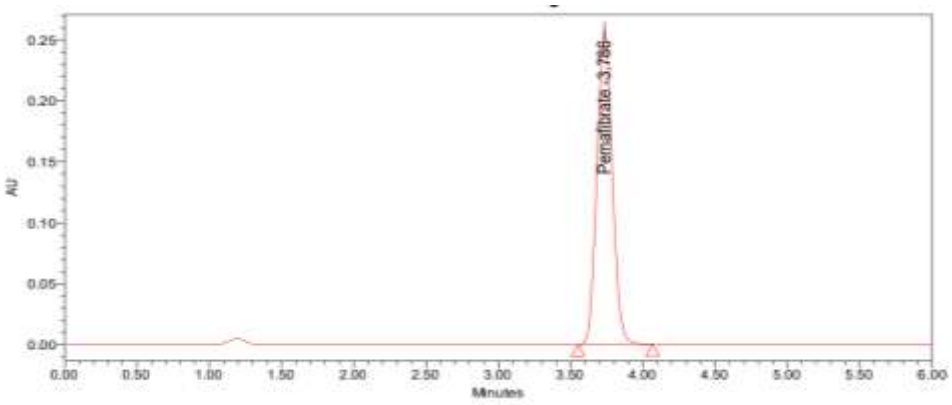


Fig. 7 Chromatogram of Sample

TABLE VII OPTIMIZED METHOD RESPONSES

S. No.	Response Variables	Predicted Value	Actual Value	Desirable Range
1	Retention time (min)	3.3972	3.785	3.1532-3.821
2	Theoretical plates	4331.15	4234	4230.09-4432.21

B. Method Validation

The validation of the optimized analytical method was done as per the guidelines of International Conference on Harmonization Q2(R1).

S. No.	Parameters	Results
1	**Linearity**	
	Linearity range (µg/ml)	2.5-15
	Correlation coefficient	0.9998
	Regression equation	y = 355841.39x + 43603.46
2	**Accuracy (% recovery)**	
	50%, 100%, 150% levels	Between 98.8 to 100.2
3	**Precision (% RSD of peak area)**	
	System precision	0.733
	Repeatability	0.716
	Intermediate precision	0.815
4	**Sensitivity**	
	LOD (µg/ml)	0.3
	LOQ (µg/ml)	1.0
5	**Robustness (% RSD of peak area)**	
	Flow rate (±0.1 ml/min)	1.6
	Organic phase (±10%)	0.19
	Temperature (±5°C)	0.64
6	**System suitability**	
	Retention time (min)	3.728
	Tailing factor	1.10
	Plate count	4220

C. Forced Degradation Studies

The degradation analysis of the drug has been extensively researched by subjecting the substance to different pressure circumstances, given in the guidelines set forth in the ICH Q1A (R2) standard. Forced degradation experiments were carried out to affirm the method that was created demonstrated stability.

Drug	Degradation Condition	% Recovery	% Drug Degraded
Pemafibrate	Acid	99.9	0.1
	Alkali	87.2	12.8
	Oxidation	86.2	13.7
	Reduction	85.9	14
	Thermal	90.3	9.6
	UV	89.2	10.7
	Water	95.7	4.2

4. CONCLUSIONS

The current study found that the newly developed process for estimating Pemafibrate using Response Surface Methodology is simple, accurate, and cost-effective, as well as sensitive and linear, which involved selecting the CMPs of pH, flow rate, % of organic content in the mobile phase, and organic modifier to ensure retention time and theoretical plates met precise Critical Quality Attributes. BBD was utilized to analytically adjusted these constraints and the final optimized chromatographic conditions used acetonitrile:0.1% triethyl amine (60:40 v/v), driven at 0.90 ml/min flow rate. Utilizing RSM led to a better understanding of method development, as shown through 2D contour and 3D surface plot analysis that revealed the significant factors impacting each response. Developed method was confirmed using ICH Q2 (R1) guidelines, and stress studies were conducted under numerous conditions, with the substance found to have the least degradation under acidic and neutral conditions yet more degradation occurred under reductive conditions due to the reduction of the drug. 3.785 mins is the retention time for the drug, and theoretical plates were within acceptable limits.

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