

# CCD-Based Quality by Design Strategy for RP-HPLC Method Development and Optimization for Quantifying Pioglitazone and Tenueligliptin

Devi Swapna PV<sup>1</sup>, Dr. G. Saravanan<sup>2\*</sup>

<sup>1</sup>Research Scholar, Faculty of Pharmacy, Karpagam Academy of Higher Education, Coimbatore-641032, Tamil Nadu, India

<sup>2</sup>Professor, Faculty of Pharmacy, Karpagam Academy of Higher Education, Coimbatore-641032, Tamil Nadu, India

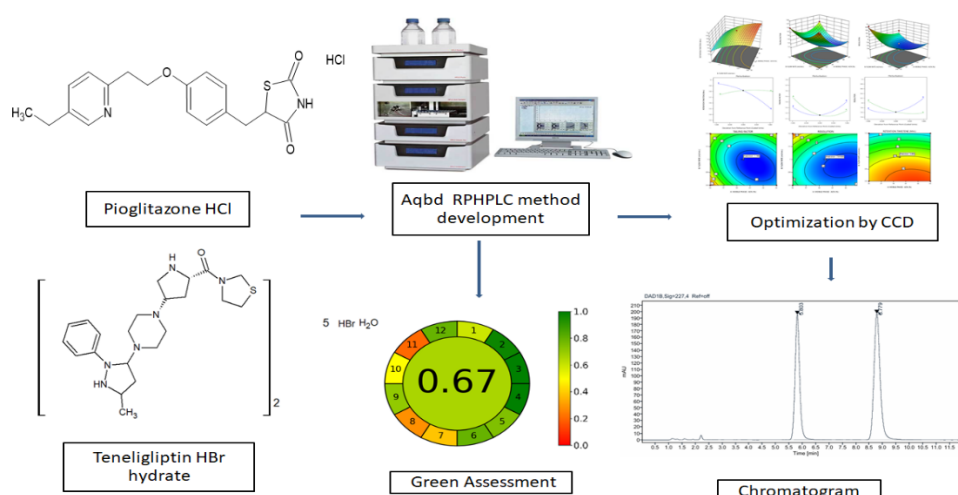
Received: 16th Dec, 2025; Revised: 8th Feb 2026; Accepted: 12th Feb, 2026; Available Online: 28th Feb, 2026

## ABSTRACT

A Novel Robust Design of Experiments (DoE) based RPHPLC technique was established for quantifying pioglitazone and tenueligliptin in dosage form. By using statistical models, a methodical and structured approach is used to determine the relationships between independent variables that affect one or more dependent variables. Using a quadratic model with 13 experimental runs, optimisation was done and using the “Central Composite Design” (CCD) response surface methodology. This led to analysis and optimisation of dependent variable's correlation with the independent factors. Retention time, tailing factor, and resolution are response variables, while mobile phase ratio, flow rate are the chosen input components. Graphical optimisation was carried out using 3-D surface plots, perturbation, and contour plots. A PDA-detector, an automated device injector, a binary solvent delivery pump, and an HPLC Agilent 1220 Infinity II were used in the development. An Agilent Inertsil ODS 3 (250mm x 4.6mm, 5µm) was used to achieve separation. Pioglitazone and tenueligliptin were shown to have retention time of 5.794 and 8.764 minutes, respectively. Using mobile phase, acetonitrile and phosphate buffer pH 3 in a ratio of 60:40% at a flow rate of 1ml/min produced better peak resolution. The UV detection was set at 247nm, and injection volume was 9µl. Good precision, accuracy, and improved peak resolution were achieved when the method was validated based on ICH recommendations. Furthermore, assessment using AGREE and MoGAPI green metrics confirmed the method's sustainability and minimal environmental impact. The validated RP-HPLC method is well-suited for consistent quality evaluation of pioglitazone and tenueligliptin in combined pharmaceutical products, making it reliable for routine analysis in quality control laboratories

**Keywords:** AQBD; Agree; ICH guidelines; Method development; RPHPLC; Validation

## GRAPHICAL ABSTRACT



**How to cite this article:** Devi Swapna PV, Dr. G. Saravanan, CCD-Based Quality by Design Strategy for RP-HPLC Method Development and Optimization for Quantifying Pioglitazone and Tenueligliptin..Int J Drug Deliv Technol. 2026; 16(2): 699-710; DOI: 10.25258/ijddt.16.2.75

**Source of support:** Nil.

**Conflict of interest:** None

## INTRODUCTION

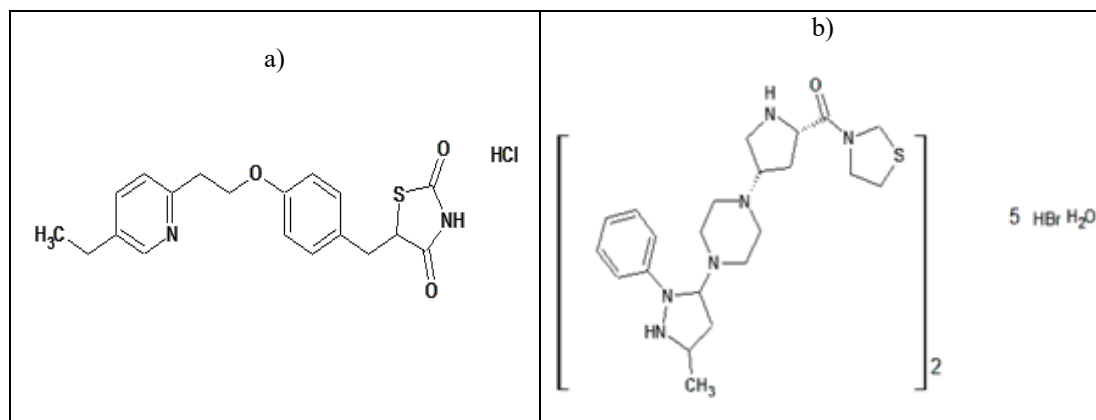
A fixed medication combination is a dosage form that contains two or more active pharmaceutical substances that have been approved by the US FDA. Each ingredient meets

a distinct therapeutic need, and the combination provides more therapeutic advantages than each medicine alone [1]. The fixed drug combinations of Pioglitazone and

## CCD-Based Quality by Design Strategy for RP-HPLC Method Development and Optimization for Quantifying Pioglitazone and Tenzeligiptin

teneligiptin were approved by the CDSCO in the year 2022, and Glenmark is the first pharmaceutical company to launch these combinations into the market.

Pioglitazone, an oral antidiabetic drug that belongs to the thiazolidinedione (TZD) group, is chemically 5-[4-[2-(5-ethylpyridin-2-yl) ethoxy] phenyl] methyl]-1, 3-thiazolidine-2,4-dione (Fig 1) [2].

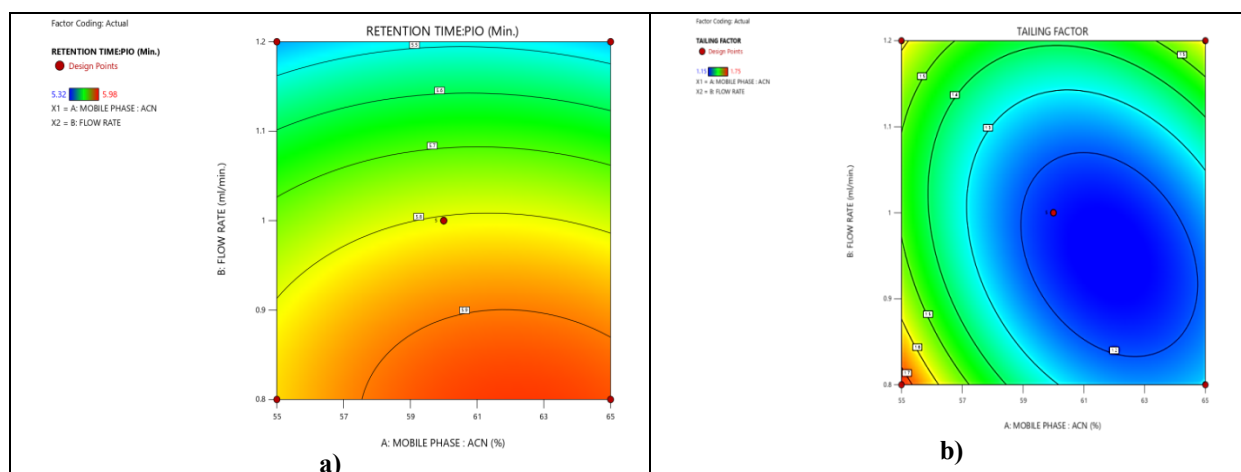


Thiazolidinediones improve the absorption of glucose by peripheral tissues and also somewhat reduce the liver's synthesis of glucose. During this activity, the nuclear receptor's gamma form, called PPAR-gamma is active. The coding of many genes involved in energy balance, lipid as well as glucose metabolism, and other processes is changed when this nuclear receptor is activated. While some genes are triggered, others are suppressed. These comprise the genes for the lipoprotein lipase, GLUT4 glucose transporter, fatty acid transporter protein and the glucokinase. TZDs aid in lowering liver, adipose tissue, and

muscle insulin resistance. Due to the high concentration of PPAR-gamma in adipose tissue, adipokines' endocrine signaling from adipocytes appears to be the mechanism affecting muscle and liver [3].

Tenzeligiptin is an antidiabetic drug that acts as a dipeptidyl peptidase-4 (DPP-4) inhibitor, primarily prescribed for managing diabetes mellitus. Its IUPAC name is [(2S,4S)-4-[4-(5-methyl-2-phenylpyrazol-3-yl)piperazin-1-yl]-(1,3-thiazolidin-3-yl)methanone];hydrate; penta-hydrobromide (Fig 2) [4].

**Fig. 2:** Structure of a) Pioglitazone, b) Tenzeligiptin Hydrobromide hydrate



It inhibits the level of incretin hormone, glucagon like peptide 1, also inhibits the hormone glucagon, which causes an increase in insulin, which in turn causes gastric emptying and decreases the level of glucose in blood [5].

“Quality by design is a deliberate approach to development that begins with specified goals and emphasizes understanding products and processes, regulating them using scientific knowledge, controlling risks, and assuring their safety” [6]. Analytical Quality by Design (AQbD) starts

by defining Analytical Target Profile (ATP), clarifying purpose and required performance of RP-HPLC method (such as sensitivity, accuracy, and selectivity for the target analytes). Key method attributes—like peak area, retention time, resolution, and robustness—are identified as Critical Quality Attributes (CQAs), while method variables such as flow rate, column temperature, mobile phase composition, and pH become Critical Method Parameters (CMPs) [7]

Compared to conventional trial-and-error procedures, Analytical Quality by Design offers a methodical, scientific framework that improves the resilience, precision, and dependability of RP-HPLC techniques [8, 9, 10]. To mimic their effect on the method's performance, it first establishes an Analytical Target Profile (ATP) and pinpoints Critical Quality Attributes (CQAs) and Critical Method Parameters (CMPs). These are then improved using Design of Experiments (DoE). This minimizes out-of-specification findings and method failures by creating a Method Operable Design Region (MODR), which is a diverse area that ensures the method works well even in the face of slight deviations in analytical circumstances by recording the method's control approach and enabling ongoing monitoring and lifecycle management, AQbD further promotes regulatory flexibility [11-12]. The analytical methods developed by implementing the AQbD, integrating with the Green Analytical Chemistry (GAC) principles, suggest that the method is robust and eco-friendly and has more advantages than the traditional method development, which utilizes more solvent usage, which is more harmful to the environment as well as the analyst.

A critical component of pharmaceutical quality control is establishment of reliable analytical techniques that makes sure both the safety and efficacy of drug formulations. For simultaneously quantifying teneligliptin and pioglitazone in combination, only a limited number of analytical approaches have been reported, including HPTLC [13], UV[14], UHPLC[15], and RP-HPLC[16]. A white analytical chemistry-based RP-HPLC technique using ethanol and water as mobile phase has also been described in recent literature [17]. The use of UV detection at 210 nm in this procedure, however, has a significant drawback since ethanol absorbs strongly in this range, which might compromise sensitivity and selectivity [18]. Using a central composite design (CCD) for optimization, the current work aims to create a novel, robust RP-HPLC technique coupled with response surface approach in order to solve these constraints. The method further emphasizes the green analytical chemistry (GAC) principles to provide an eco-friendly and reliable approach for quantifying teneligliptin and pioglitazone in dosage form.

**MATERIALS AND METHODS**

Pure samples of pioglitazone and teneligliptin were procured from Sigma Aldrich. The tablet Zita Pio-plus was brought from the nearby market. HPLC grade solvents were employed throughout the study.

**Instrument and Chromatographic condition**

A binary solvent delivery pump with an insertil ODS C-18 column, (250 x 4.6cm, 5µm) is employed in Agilent 1220 Infinity II HPLC system. Acetonitrile and phosphate buffer solution, in 60:40 ratios, pH 3 adjusted with orthophosphoric acid, made up with the mobile phase.

**Preparation of standard stock solution**

Stock solutions were prepared by accurately weighing 20mg of teneligliptin and 15mg of pioglitazone and are dissolved in diluent, the volume was made up to 100ml to yield a concentration of 200µg/ml of teneligliptin and

150µg/ml of pioglitazone. These stock solutions were then further diluted to get the working standard solutions containing 15µg/ml of pioglitazone and 20µg/ml of teneligliptin.

**AQbD Method development**

The AQbD approach was employed to create the RPHPLC technique. Design space was established as the range of chromatographic conditions that ensured satisfactory method performance. Risk assessment was done by finding the independent variables; mobile phase ratio and flow rate; that have the more effects upon important quality features like retention duration, tailing factor, and resolution. To assess the effect of “CMP”'s on the method's performance, DoE was carried out.

The CCD experimental design, which uses response surface methodology to generate quadratic simulation in the optimisation step. Flow rate of 0.8 to 1.2ml/min and the amount of organic phase was kept between 55 and 65%, 13 experimental runs were therefore carried out, and the results were noted and tallied [Table no. 1].

**Table 1: CCD experimental runs with responses for Optimization by Design Expert software**

Ru n	F1: A: Organic phase ACN( %)	F2: B: Flow rate (ml/min.)	R1: Retention Time of PIO (min.)	R2: Tailing factor	R3: Resolution
1	60	0.717157	5.98	1.51	8.1
2	52.9289	1	5.68	1.75	8.4
3	67.0711	1	5.8	1.41	7.6
4	60	1.28284	5.32	1.62	8.5
5	60	1	5.79	1.15	7.8
6	55	1.2	5.42	1.62	8.9
7	60	1	5.78	1.17	7.8
8	60	1	5.79	1.18	7.5
9	60	1	5.89	1.17	7.5
10	60	1	5.79	1.16	7.5
11	55	0.8	5.81	1.72	8
12	65	1.2	5.41	1.61	8.2
13	65	0.8	5.87	1.25	7.8

ACN: Acetonitrile, PIO: Pioglitazone

**VALIDATION**

The current method was validated in accordance with the ICH Q2 regulations.

**Linearity**

Working standard solutions with concentrations between 5 - 25µg/ml for pioglitazone and 10–50µg/ml for teneligliptin were prepared in order to conduct the linearity investigations. The peaks that resulted from injecting varying volumes of the standard solution mixture into the HPLC system were measured at 247nm.

### Accuracy

The usual addition approach was applied to evaluate the accuracy at three distinct levels: 50%, 100%, and 150%. The sample solution is spiked with the standard at each level in triplicate and chromatogram was run and % recovery was determined.

### Precision

Repeatability, intraday, and inter-day variation were measured to evaluate the method's precision. Six replicate injections of the same concentration into the system were carried out to assess the repeatability. Injecting three distinct concentrations of the material 3 times in a single day and 3 times over 3 days is allowed for the evaluation of the intermediate precision.

### LOD ( Limit of detection) and LOQ ( Limit of Quantification)

Detection limit had been computed from the equation with slope and standard deviation obtained from calibration curve.

$$i. LOD = 3.3 \times \frac{\sigma}{S}$$

$$ii. LOQ = 10 \times \frac{\sigma}{S}$$

### System suitability

It can be assessed by six replicate injections of 20µg/ml concentration of teneiglipitin and 15µg/ml concentrations of pioglitazone and calculated the % RSD for theoretical plates and tailing factor.

### Assay of marketed formulation

Accurately weighed 20 tablets containing pioglitazone 15mg and teneiglipitin 20mg and finely powdered by using mortar and pestle. A 100ml volumetric flask has been loaded with a determined amount of powder, equal to 20mg of teneiglipitin and 15 mg of pioglitazone. After adding 20mL of diluent, a 15minute sonication operation is carried out. After that, the solution is run through a 0.45µ membrane filter. After that, it was diluted until the concentrations of pioglitazone and teneiglipitin were 15 and 20 µg/ml, respectively. Following the solution's introduction into the HPLC apparatus, a chromatogram was produced. Analysis was done in triplicate.

## RESULTS AND DISCUSSION

A previously reported method for quantifying pioglitazone and teneiglipitin together relied on ethanol as organic phase with UV detection wavelength at 210 nm, wavelength close to the cutoff region of ethanol. This overlap presents a fundamental drawback as it compromises sensitivity and may introduce baseline noise or interference. To address this limitation, the present study adopted a mobile phase of acetonitrile and phosphate buffer (pH 3), offering improved stability and broader applicability for routine analysis, at a flow rate of 1ml/min at PDA detection at 247nm. Initial screening process involved testing different ratios of mobile phase, ranging 55 to 65%. The flow rate examined ranged from 0.8ml to 1.2ml at order to obtain a clear peak with excellent resolution and a low tailing factor, the C-18

column was chosen with acetonitrile (ACN) and phosphate buffer in ratio 60:40 based on screening.

### Optimization and screening with Aqbd

The chromatographic conditions have been optimized by employing the DoE tool of Design expert software which utilized a quadratic model. Since the design chosen was CCD; the values beyond the range were also evaluated that include organic phase volume of 52 and 67%, flow rate of 0.7ml/min. Using the design expert program, the current model was assessed using ANOVA, which produced non-significant F values and a significant P-value <0.05. Quadratic model created for the response variable retention time of pioglitazone was determined to be statistically significant with a model F-value of 32.30 (p = 0.0001). This suggests that the retention behaviour in the established RP-HPLC technique is significantly influenced by the independent variables and their interactions. Among the all components, flow rate (Factor B) contributed the most to retention duration, showing a very significant impact (F = 134.49, p < 0.0001). The observed substantial statistical significance is consistent with the fact that an increase in flow rate usually results in a shorter retention time because of quicker analyte elution. The mobile phase composition (Factor A), indicated a small and statistically negligible contribution (F = 2.04, p = 0.1962), indicating that changes in the acetonitrile ratio had a relatively less impact on the retention of pioglitazone within the design space.

The interaction effect (AB) between composition of mobile phase and flow rate was statistically insignificant (F = 0.4144, p = 0.5403), showing that these two parameters influence retention time separately rather than synergistically. Quadratic terms A<sup>2</sup> (p = 0.0461) and B<sup>2</sup> (p = 0.0025) were significant, revealing curvature on the response surface. This shows the nonlinear effects of both the acetonitrile ratio and flow rate, the flow rate is having a greater curvature effect. With a non-significant lack of fit test result (F = 1.76, p = 0.2934), the selected quadratic model was found to match the experimental data well and without systematic variation.

The ANOVA analysis of the quadratic model for resolution, statistically significant with F value and P value (F = 15.17, p = 0.0012), indicating that it was appropriate for expressing the system. Both the mobile phase composition (A) (F = 18.67, p = 0.0035) and the flow rate (B) (F = 15.75, p = 0.0054) were shown to significantly impact on the peak resolution, suggesting their crucial role in attaining successful separation. More over the interaction term (AB) was not significant (F = 2.26, p = 0.1762), showing that the two variables functioned less in affecting resolution. Significant quadratic effects with the p values for A<sup>2</sup>, p = 0.0129 and B<sup>2</sup>, p = 0.0007 indicate a nonlinear relationship among variables and resolution. This curvature effect highlights the importance of optimizing both parameters carefully and precisely within a given experimental range, rather than relying solely on linear changes. The lack of fit (F = 1.05, p = 0.4610) is non-significant which confirmed

that the model accurately reflected the observed data. The data of Anova analysis were illustrated in table no. 2

**Table no 2:** Anova for quadratic model for the responses

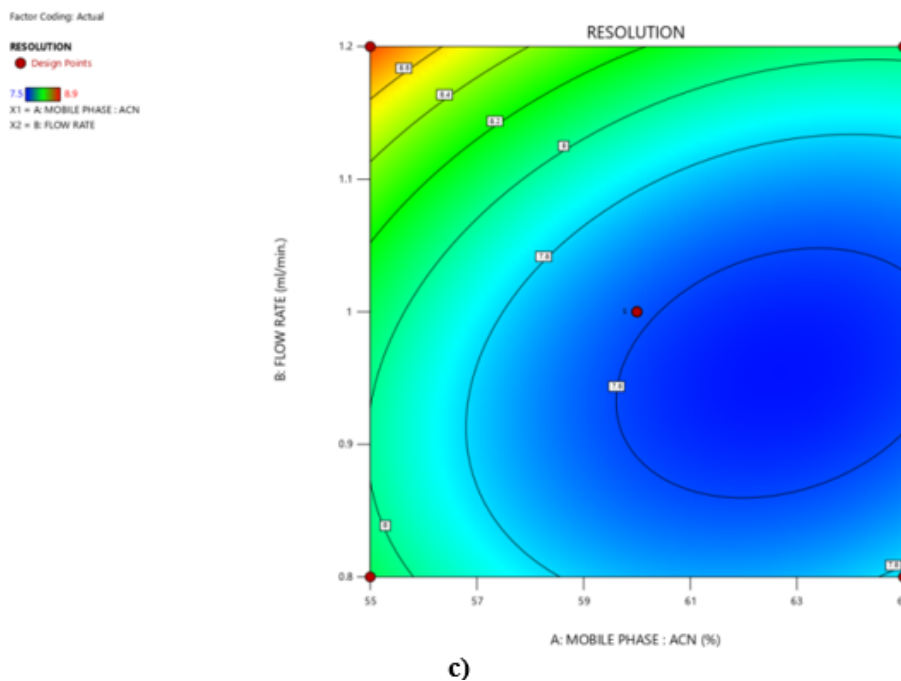
Response Terms	R1		R2		R3		
	F-value	p-value	F-value	p-value	F-value	p-value	
<b>Model</b>	32.30	0.0001	324.28	< 0.0001	15.17	0.0012	<b>Significant</b>
A-MOBILE PHASE : CAN	2.04	0.1962	278.94	< 0.0001	18.67	0.0035	
B-FLOW RATE	134.49	< 0.0001	52.18	0.0002	15.75	0.0054	
AB	0.4144	0.5403	127.87	< 0.0001	2.26	0.1762	
A <sup>2</sup>	5.85	0.0461	681.88	< 0.0001	10.98	0.0129	
B <sup>2</sup>	21.18	0.0025	632.04	< 0.0001	32.41	0.0007	
<b>Residual</b>							
Lack of Fit	1.76	0.2934	6.09	0.0567	1.05	0.4610	<b>not significant</b>

**Table no. 3:** Fit Statistics

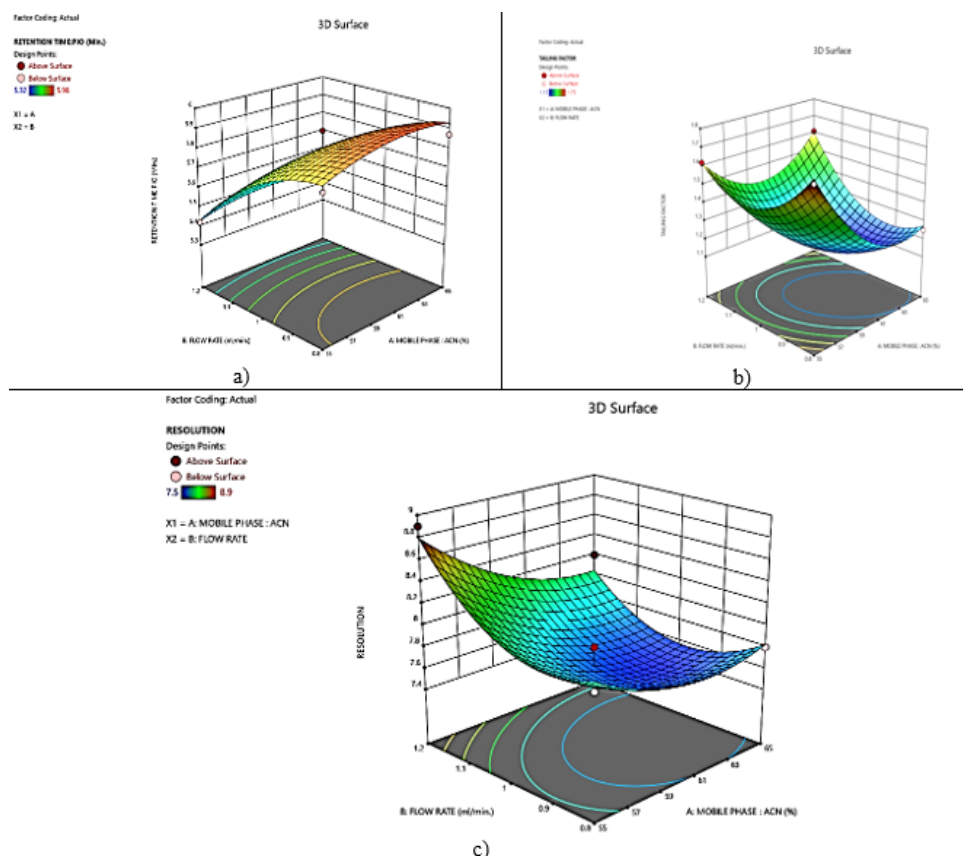
Responses	R1	R2	R3
<b>Std. Dev.</b>	0.0544	0.0203	0.1662
<b>Mean</b>	5.72	1.41	7.97
<b>C.V. %</b>	0.9510	1.44	2.09
<b>R<sup>2</sup></b>	0.9585	0.9957	0.9155
<b>Adjusted R<sup>2</sup></b>	0.9288	0.9926	0.8551
<b>Predicted R<sup>2</sup></b>	0.8039	0.9737	0.6610
<b>Adeq Precision</b>	17.0701	41.8585	10.4478

The reliability of the three quadratic models was validated by the fit statistics. Strong agreement was found for retention, suggesting high predictive power. The model was perfectly fit the tailing factor since it was very robust and had almost perfect correlation with the difference of the predicted and adjusted R2 value less than 0.2. Despite having a somewhat lower predictive power, the model nevertheless displayed respectable reliability for resolution also. Overall, the most accurate modeling was of the tailing factor, which was followed by retention time and resolution, indicating the appropriateness of CCD for method optimization. The information was displayed in Table No. 3.

**The contour graphs (fig. 3a) and 3D surface graphs (fig 4a)**



## CCD-Based Quality by Design Strategy for RP-HPLC Method Development and Optimization for Quantifying Pioglitazone and Tenofovir



**Fig 4:** 3-D surface methodology graph for a) Retention time, b) Tailing factor c) Resolution

shows how organic phase (acetonitrile %) and flow rate affect retention duration of pioglitazone. The 3D surface plot revealed that the retention duration gets decreased as the flow rate gets increased, as predicted due to more rapid elution at higher flow rates. Whereas, when the percentage of acetonitrile concentration in mobile phase rose, retention duration gets increased somewhat, indicating the low mobile phase polarity, causing pioglitazone to interact longer with the stationary phase. The curved curvature of the surface emphasizes the nonlinear interaction between the elements, which is consistent with the quadratic model significance seen in the ANOVA findings.

**Table no. 4:** Optimized Chromatographic condition

Mobile phase	Acetonitrile: 10mM Phosphate buffer [pH:3] ratio [60:40]
Detection	247nm
Injection volume	9 µl
Flow Rate	Rate: 1ml/min
Column	Agilent Inertsil ODS 3 (250mm x 4.6mm, 5µm).
Instrument	HPLC Agilent 1220 Infinity II

The contour map illustrates these trends in two dimensions, where increased levels of acetonitrile are linked to longer retention times, while reduced acetonitrile levels combined

with higher flow rates lead to shorter retention times. These observations imply that flow velocity is the dominant factor influencing retention, although the composition of organic phase has a minor yet important effect. The comparison between the predicted and actual values demonstrates a robust linear correlation of experimental outcomes and model's predictions, with data points aligning closely with the diagonal regression line. This indicates that the model is both accurate and trustworthy in its forecasts, demonstrating that the quadratic model successfully accounts for the variations in pioglitazone retention time. The clustering of data points around the regression line indicates low error and very minimal bias. The graphical optimization confirms the statistical findings, where flow rate is the most critical factor affecting retention time, with acetonitrile percentage contributing modestly.

The 3D surface plot (Fig. 3b) illustrating the tailing factor clearly indicates that both factors have substantial nonlinear influences. The lowest values of the tailing factor were observed when the mobile phase composition was approximately 60% ACN and flow rate was near 1.0ml/min. Any deviation from these optimal conditions, whether by reducing the ACN concentration or by raising flow rate beyond the ideal levels, led to an increase in tailing, as demonstrated by the upward curvature of the surface. This finding is consistent with the ANOVA results, which showed that both quadratic terms ( $A^2$  and  $B^2$ ) were significantly impactful, thereby confirming the curvature in the response. Additionally, the contour plot reinforces this conclusion by depicting a central "blue zone" (indicating

minimum tailing) that is encircled by areas of heightened tailing towards the edges. This pattern implies that optimal peak symmetry is attained within a defined design space, while extremes in either factor result in undesirable peak broadening. The significant interaction term (AB), identified in the statistical analysis, is visually apparent in this context, as the influence of one variable varies according to the level of the other.

The 3D surface map (fig 4c) for resolution indicates that resolution improves with intermediate levels of mobile phase composition (~60% ACN) and moderate flow rates (~1.0 mL/min). Extreme circumstances (extremely high or low ACN, or very high flow rates) resulted in diminished resolution, as shown by the curved, sloping response surface. The nonlinear behaviour is consistent with the ANOVA results, which showed substantial quadratic terms ( $A^2$  and  $B^2$ ), indicating that peak resolution is very sensitive

to small experimental adjustments. The contour map supports these findings, with a centre "blue zone" denoting the region with the highest resolution. This shows that both the factors must be carefully managed – greater acetonitrile levels paired with moderate flow rates enable more effective peak separation, but excessive values of either factor reduce resolution.

The Predicted vs. Actual plot (fig. 5) shows a remarkable alignment of data points with the regression line, indicating a robust correlation between experimental results and model forecasts. The minimal scatter and close clustering demonstrate insignificant prediction error, highlighting the exceptional performance of the quadratic model for this response. The graphical analysis reveals that mobile phase composition is the main indicator of tailing factor, although flow rate also plays an essential role.

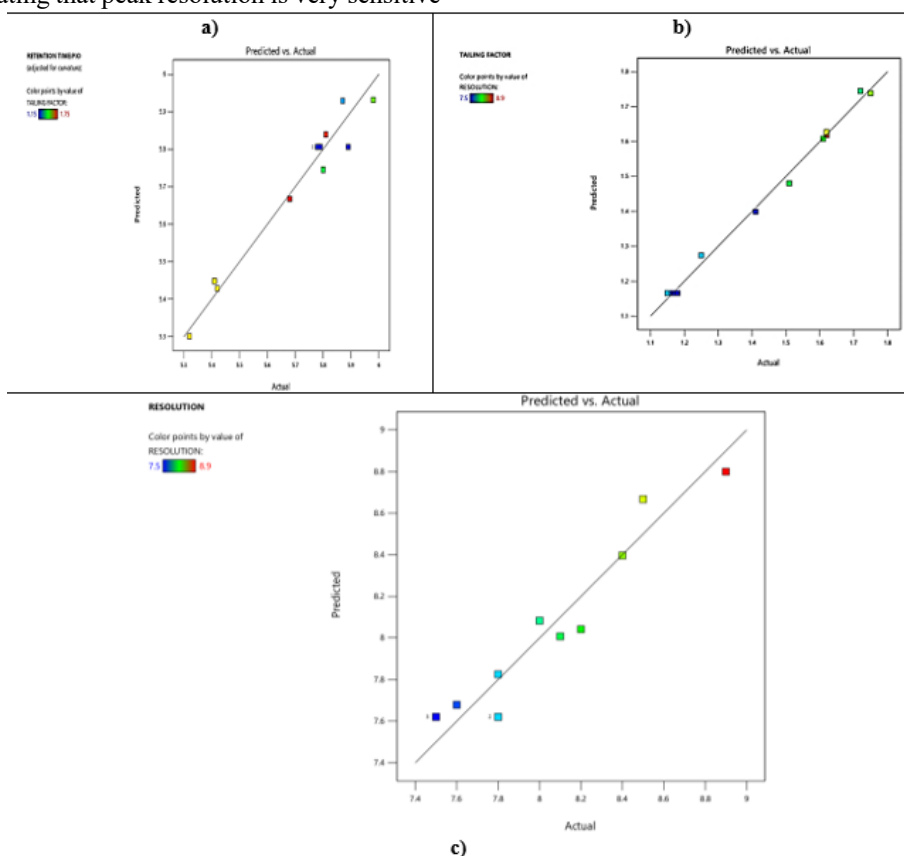
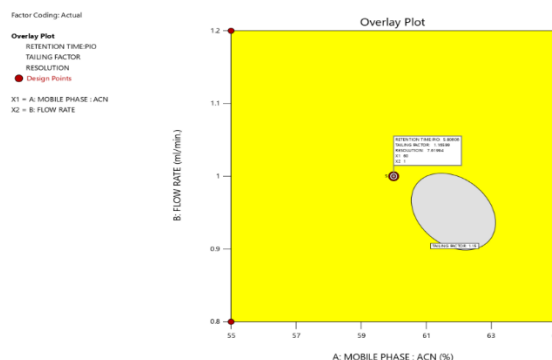


Fig. 5: Prediction vs Actual plot for a) retention time b) Tailing Factor, c) Resolution

The quadratic model's high prediction accuracy is confirmed by close alignment of data in regression line in the Predicted vs. Actual diagram. While there are minor discrepancies observed at the higher resolution levels, the overall correlation between predicted and actual data indicates that the model is suitable for response optimization. These graphical results confirm the statistical model and highlight the optimization of parameters to achieve the robust separation of both the drugs.

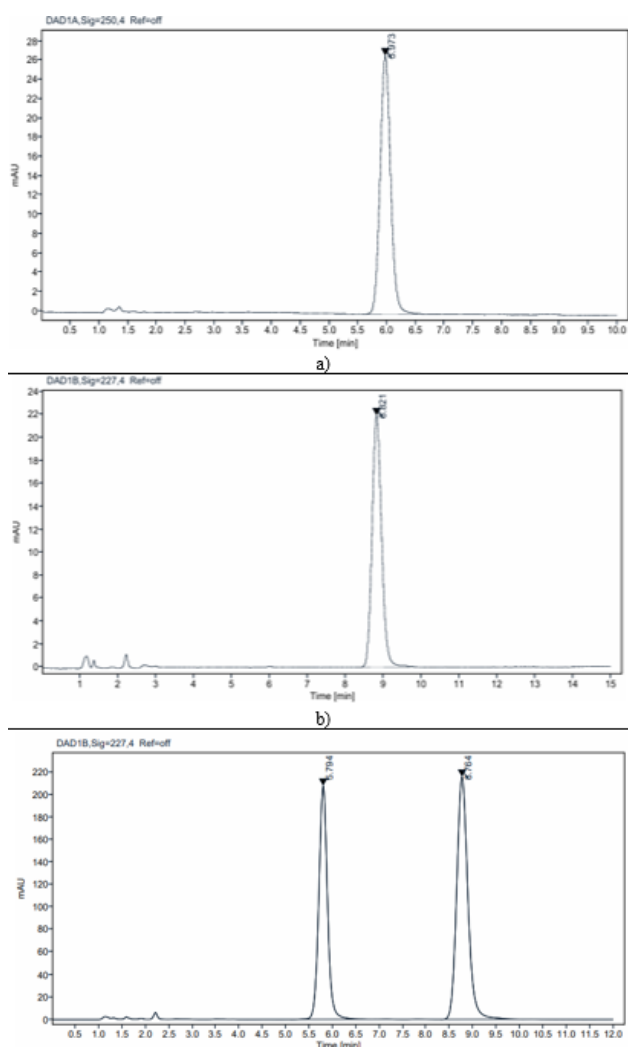
The overlay plot (fig 6.)

Fig 6: overlay plot of optimized data.



shows a complete picture of the design space in which main chromatographic responses, tailing factor, and resolution meet required parameters. The yellow region depicts the acceptable factor settings in which the combination of organic phase and flow rate which results in the decreased tailing and resolution. The middle region represents the 60% acetonitrile and flow rate 1ml/min with a tailing factor roughly 1.16 and a resolution of 7.97. These values are ideal and acceptable limits with consistent peak shape and efficient separation. The yellow area signifies that the methods operable area with minimal adjustments in either organic phase percentage or flow rate to drive the results beyond system suitability requirements.

Based on the Qbd optimization chromatographic conditions, the drugs were quantified simultaneously using the optimized rphplc method in marketed formulation and both the chromatograms were depicted in fig 7 and 15



**Fig. 7: Chromatogram of Standard solution of a) PIO b) TENE c) Standard mixture of PIO Rt-5.794 and TENE Rt-8.764**

Further validation studies were done in accordance with ICH Q2 guidelines.

**VALIDATION**

**Linearity**

By introducing a working standard solution with concentrations between 5 and 25µg/ml for pioglitazone and 10 and 50µg/ml for teneligliptin, the linearity was demonstrated. A plot of the calibration curve showed in fig 8 and 9. For both medications, the regression square value was calculated and found to be 0.99.

**System Suitability**

Six replicate samples of pioglitazone and teneligliptin were injected as part of the system suitability studies. Response variables that involve retention time, tailing factor, “theoretical plates” and resolution have been noted, and the percentage RSD values obtained are less than 2, it means that the is suitable for the method, tabulated in Table no. 5.

**Table 5. System suitability study data**

Suitability parameters	Pioglitazone	Teneligliptin	Criteria
Tailing factor	0.995	1.14	n=6, TF<2
Theoretical plates	4409	6251	n=6, >2000
Resolution	-	7.8	n=6, >2
Retention time	5.79	8.75	n=6, %RSD<2

Low-tailing factors and theoretical plate counts above 2000 suggest minor chromatographic problems and uniform peak shapes, emphasizing dependability. The assured resolution values enabled distinct separation without interference, thus improving the quantitation specificity for both analytes. A consistently low retention time RSD indicated strong stability of the instrument and precision of the method.

**Precision**

Repeatability, intraday and interday precision assessments had been conducted. The mean standard deviation and RSD were computed after six replicate injections of the standard mixture including pioglitazone (15µg/ml) and teneligliptin (20µg/ml) were performed for repeatability. The %RSD for pioglitazone and teneligliptin were found to be 0.70941 and 0.2493 respectively. The method's precision was demonstrated by the fact that the percentage RSD was within the allowed range after the interday and intraday precision tests were completed during the same day and the following days. The table no. 6 and 7

**Table 6: Repeatability and Interday and Intraday precision data of Pioglitazone**

CCD-Based Quality by Design Strategy for RP-HPLC Method Development and Optimization for Quantifying Pioglitazone and Tenueligliptin

Conc. (µg/ml)	Area (day 1)			Area (day 2)			Area (day 3)		
	11:00am	01:00pm	3:00pm	11:00am	01:00pm	3:00pm	11:00am	01:00pm	03:00pm
15	5189	5180	5178	5178	5179	5175	5186	5179	5178
15	5099	5089	5107	5101	5095	5112	5127	5099	5213
15	5185	5184	5152	5180	5182	5168	5189	5189	5209
15	5126	5119	5170	5122	5118	5179	5122	5119	5199
15	5152	5149	5175	5161	5129	5178	5208	5128	5197
15	5186	5184	5172	5187	5181	5174	5210	5200	5179
Mean(n=6)	5156.167	5150.833	5159	5154.833	5147.333	5164.333	5173.667	5152.333	5195.833
SD	34.14389	36.26484	24.69818	32.19429	34.81698	23.66901	35.9011	38.46066	13.42158
%RSD	0.662195	0.704058	0.47874	0.624546	0.676408	0.458317	0.69392	0.746471	0.258314

SD: standard deviation, RSD: Relative Standard Deviation, n=no. of replicates

**Table 7: Repeatability and interday precision data of tenueligliptin**

Co nc (µg /ml )	Area ( day 1)			Area (day 2)			Peak area (day 3)		
	11 :0 0a m	01 :0 0p m	3: 00 p m	11 :0 0a m	1: 00 p m	3: 00 p m	11 :0 0a m	1: 00 p m	3: 00 p m
30	70 35	70 31	70 41	70 39	70 42	70 39	69 89	70 12	70 10
30	70 25	70 29	70 39	70 08	70 40	70 09	69 90	69 89	69 52
30	69 89	71 00	70 08	69 98	70 09	70 35	70 12	69 99	70 08
30	70 41	69 98	69 99	70 37	70 35	70 33	70 10	70 10	70 13
30	70 37	70 29	70 08	70 25	70 37	70 28	69 87	69 87	69 75
30	70 18	70 41	68 95	70 22	70 36	70 41	69 89	70 12	69 85
Me an( n=6 )	70 24. 17	70 38	69 98. 33	70 21. 05	70 33. 167	70 30. 833	69 96. 167	70 01. 5	69 90. 5
SD	17. 516	30. 724	48. 886	14. 682	11. 067	10. 621	10. 542	10. 532	22. 156
%R SD	0.2 49	0.4 36	0. 698	0.2 09	0.1 57	0.1 51	0.1 507	0. 1504	0. 3169

displayed the data's percentage RSD. The low %RSD values in both repeatability and interday precision experiments demonstrate that the current RP-HPLC technique for pioglitazone is precise and reproducible, suitable for application in quality control environments. The negligible variation in peak areas implies that random error and instrumental drift were minimized. Consistency over

three days further validates the method's robustness against day-to-day laboratory variables, supporting its reliability in routine use.

**Robustness**

The robustness is systematically evaluated by creating central composite design (CCD) to optimize and vary key parameters, namely the organic phase composition and flow rate. The %RSD < 2% resulted in insignificant changes in peak area and retention duration, indicating the analytical method's ability to withstand operational variability. This result shows that small variations in chromatographic settings show little effect, ensuring consistent performance throughout routine analysis and enhancing the method's suitability for pharmaceutical quality control.

**Specificity**

Specificity studies revealed that when solutions containing exclusively excipients were injected into the RP-HPLC system, no peaks emerged throughout retention duration of pioglitazone or tenueligliptin, and there were no additional interfering peaks or degradation products were observed. This demonstrates that inclusion of excipients in the formulation won't affect in quantifying pharmaceuticals, therefore confirming method's specificity and selectivity for the analytes in the presence of common formulation constituents.

**LOD AND LOQ**

The slope of the calibration curve and the response's standard deviation were used to compute the limits of detection (LOD) and quantification (LOQ). The approach accurately detects and quantifies low drug concentrations (LOD = 0.36 µg/mL, LOQ = 1.119 µg/mL for pioglitazone and LOD = 0.132µg/ml , LOQ = 0.402µg/ml for tenueligliptin). The RP-HPLC method's sufficient sensitivity is confirmed by its comparatively low LOD and LOQ values, making it suited for trace-level detection and exact quantification in pharmaceutical analysis.

**Accuracy**

The percentage recovery was calculated for both pioglitazone and tenueligliptin, at three concentration levels (50%, 100%, and 150%) to illustrate the accuracy of the established RP-HPLC technique. With percentage RSD values continuously below 0.75%, the mean recoveries for pioglitazone and tenueligliptin obtained within 99.86% to 100.29% and 99.38% to 100.32%, respectively. The results presented support the method's remarkable accuracy in measuring these drugs in presence of the excipients. The recovery data shows that pioglitazone and tenueligliptin may be reliably measured by the devised RP-HPLC technique, free from sample matrix effects and formulation excipient interference. Low %RSD scores at all stages attest to method's accuracy in repeated quantitation. The recovery levels near 100% validate method's trueness and usefulness for quality control and release testing of pharmaceutical dosage forms. These findings align with recommended ICH guidelines and support the method's applicability and reported in table no. 8 and 9 for both pioglitazone and tenueligliptin.

**Table 8:** % recovery studies of Pioglitazone

LEVEL	Conc. (µg/ml)	Conc. before addition (µg/ml)	Conc. of std added (µg/ml)	Conc. after addition (µg/ml)	% recovery	Mean	SD	%RSD
50%	15	14.9942	5	19.997	100.056	100.2893	0.413223	0.41203
	15	15.0029	5	20	99.942			
	15	14.9625	5	20.006	100.87			
100%	15	14.9942	10	25	100.058	100.1613	0.158308	0.158053
	15	15.0029	10	25.007	100.041			
	15	14.9625	10	25.001	100.385			
150%	15	14.9942	15	30.006	100.0787	99.99657	0.096574	0.096577
	15	15.0029	15	29.982	99.86067			
	15	14.9625	15	29.97	100.05			

**Table 9:** Percentage recovery studies of Teneligliptin

LEVEL	CON.(µg/ml)	conc before addition (µg/ml)	conc of std added (µg/ml)	conc after addition (µg/ml)	% recovery	AVG	SD	%RSD
50%	20	19.9718	5	24.941	99.384	99.66267	0.197074	0.197741
	20	20.0141	5	25.004	99.798			
	20	20.0047	5	24.995	99.806			
100%	20	19.9718	10	30.004	100.322	100.0147	0.229934	0.2299
	20	20.0141	10	29.991	99.769			
	20	20.0047	10	30	99.953			
150%	20	20.0141	15	35.004	99.9326	100.0073	0.056482	0.056478
	20	19.9718	15	34.982	100.068			
	20	20.0047	15	35.008	100.022			

**Application of Developed Method to Marketed Formulation**

The assay of the marketed formulation for pioglitazone and teneligliptin yielded % assay results within the permissible range, validating the method's applicability in actual samples. The assay results for pioglitazone varied from 99.93% to 101.66%, while the teneligliptin ranged from 99.98% to 101.20%, suggesting close agreement with the

labelled claims and proving the method's applicability for regular quality control testing. Table 10 and fig 10 shows the results of marketed formulation.

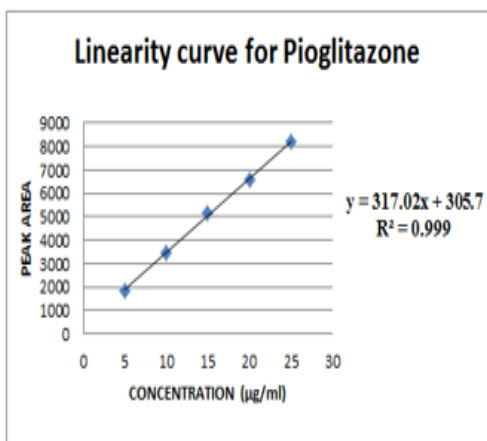


Fig 8: Linearity curve of Pioglitazone HCl

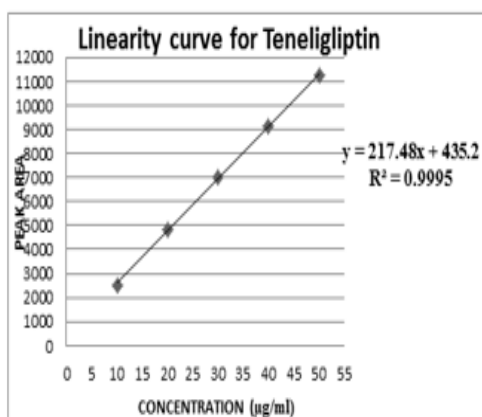


Fig 9: Linearity curve of tenoeligiptin Hydrobromide hydrate

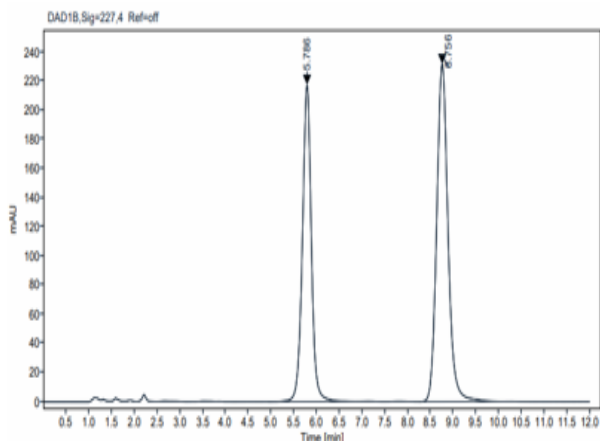


Fig 10: Chromatogram of marketed formulation Table

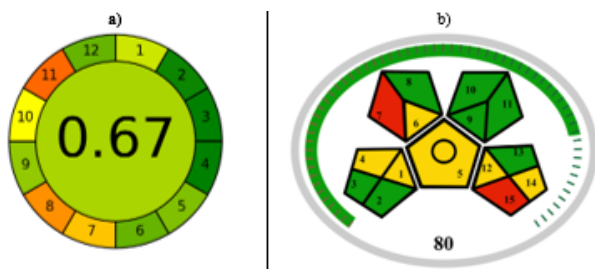


Fig 11: a) AGREE and b) MoGAPI Pictogram showing the green assessment score of the developed method

**10: Assay of marketed formulation**

Pioglitazone			Tenoeligiptin		
Labelled claim (mg)	Amount of drug obtained (mg)	% assay	Labelled claim (mg)	Amount of drug obtained (mg)	% assay
15	14.99	99.93	20	19.996	99.98
15	15.16	101.66	20	20.15	100.75
15	15.02	100.13	20	20.24	101.20

**ASSESSMENT OF GREENNESS OF THE DEVELOPED METHOD**

Developed RP-HPLC method was evaluated for its environmental greenness using both AGREE and MoGAPI tools, yielding a green assessment score of 0.67 on AGREE and a MoGAPI score of 80, reflecting robust compliance with eco-friendly analytical principles. The AGREE pictogram demonstrates that the technique meets the majority of green analytical chemistry (GAC) criteria, as indicated by the predominance of green and yellow sectors in the outer circle and an overall score of 0.67. This score signifies an “acceptable” level of method greenness, with strengths in reagent safety, waste minimization, and operational simplicity. Areas identified for improvement include aspects marked in orange—chiefly instrumentation energy consumption and sample. The fig 16 a and b shows the pictogram for AGREE and MoGAPI score.

The MoGAPI pictogram substantiates the AGREE findings, assigning a score of 80 and displaying most pentagon sectors in green, with a few yellow and red alerts where moderate environmental risks remain. Key strengths include reduced chemical hazards, limited volume of organic solvents, and favorable waste management. However, slight deficits were noted in sections involving energy usage and specific reagent hazards, as shown by the isolated red sectors. Both AGREE and MoGAPI metrics consistently indicate that the method is having a strong environmental profile, with MoGAPI providing a little more detailed information on specific aspects of the method's greenness.

**CONCLUSION**

The quality by design concepts, which include risk assessment, optimization, and creating design space for the experiment, served as the foundation for the development of the current RPHPLC process. Because the CCD model is used as a statistical tool for the method, deliberate changes in the experimental conditions have not had a significant impact on the response parameters. AGREE measures are additionally employed to evaluate the analytical method's greenness. Twelve GAC are covered. It displays a 0.67 score.

The validation of the method revealed that it is highly accurate and selective. For the estimate of these medications in combination dosage form, an extremely effective, well-optimized, exact, sensitive, dependable, and durable approach has been created. This method can be used on a daily basis in analytical laboratories.

#### ACKNOWLEDGEMENT

The authors were truly grateful to the staffs of Karpagam Academy of Higher Education and KTN college of Pharmacy for their support and cooperation for providing the facilities do the entire work.

#### CONFLICT OF AUTHORS

There was no conflict of interest between the authors

#### REFERENCE

- Gautam CS, Saha L. Fixed dose drug combinations (FDCs): rational or irrational: a view point. *Br J Clin Pharmacol*; 2008; 65(5): 795-796. Doi:10.1111/j.1365-2125.2007.03089.x.
- PubChem [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 4829, Pioglitazone; [cited 2024 Oct. 4]. Available from: <https://pubchem.ncbi.nlm.nih.gov/compound/Pioglitazone>.
- Singh G, Can AS, Correa R. Pioglitazone. In: *Stat Pearls* [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan- Available from: <https://www.ncbi.nlm.nih.gov/books/NBK544287/>.
- PubChem [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 53297474, Teneigliptin Hydrobromide Hydrate; [cited 2024 Dec. 6]. Available from: <https://pubchem.ncbi.nlm.nih.gov/compound/Teneigliptin-Hydrobromide-Hydrate>.
- Holst JJ. The physiology of glucagon-like peptide 1. *Physiol Rev*. 2007; 87(4):1409-1439. Doi:10.1152/physrev.00034.2006.
- Yu LX, Amidon G, Khan MA, Hoag SW, Polli J, Raju GK, Woodcock J. Understanding pharmaceutical quality by design. *AAPS J*; 2014; 16(4):771-783. Doi: 10.1208/s12248-014-9598-3.
- Randive KH, Maste MM, Kempwade AA. Exploring analytical quality by design (AQbD) enabled RP-HPLC method for carvedilol. *Indian J. Pharm. Educ. Res*. 2024 Apr; 58:651-60.
- Peraman R, Bhadraya K, Reddy YP, Reddy CS, Lokesh T. Analytical Quality by Design Approach in RP-HPLC Method Development for the Assay of Etofenamate in Dosage Forms. *Indian J Pharm Sci*; 2015; 77(6):751-757. doi: 10.4103/0250-474x.174971. PMID: 26997704; PMCID: PMC4778236
- Bossunia MT, Urmi KF, Chironjit KS. Quality-By-Design Approach to Stability Indicating RP-HPLC Analytical Method Development for Estimation of Canagliflozin API and Its Validation. *Pharmaceutical Methods*. 2017 Jul 1; 8(2): 92-101. DOI:10.5530/phm.2017.8.15
- Suresh P, Jadon G, Theivendren P. Development and validation of a RP-HPLC method for Milrinone quantification using analytical quality by design principles: stability, precision, and method optimization. *Essential Chem*; 2025; 31;2(1):2502833.
- Akabari AH, Patel SK, Shah KV. et al. Analytical quality by design (AQbD) methodology for concurrent determination of perindopril erbumine and moxonidine hydrochloride using RP-HPLC with an eco-friendly evaluation. *Discov. Chem*; 2025; 2(1). <https://doi.org/10.1007/s44371-024-00067-x>.
- Caro YS, Cámara MS, De Zan MM. Quality by design applications to development of analytical methods for quality control of pharmaceuticals. *Introduction to Quality by Design in Pharmaceutical Manufacturing and Analytical Development*. Cham: Springer International Publishing; 2023 :pp221-244.
- Sen AK, Bhimani N, Sen DB, Maheshwari RA, Gohil D, Koradia SK. Densitometric simultaneous assessment of teneigliptin hydrobromide and pioglitazone hydrochloride in combined tablet. *Separation Science Plus*; 2024; 7(1): 2300139. <https://doi.org/10.1002/sscp.202300139>
- Gupta RS, Tiwari A, Patel SK, Karthikeyan C, Gupta DK, Soni P, Joshi M. Development and validation of a UV spectrophotometric method for simultaneous estimation of teneigliptin hydrobromide hydrate and pioglitazone hydrochloride in pharmaceutical dosage form. *Future Journal of Pharmaceutical Sciences*; 2024; 10(1):172. <https://doi.org/10.1186/s43094-024-00745-8>.
- Kolhe MH, Godase SN, Vikhe KB, Bhor RJ. Method Development and Validation for Simultaneous Estimation of Teneigliptin and Pioglitazone by UHPLC Method. *International Journal of Pharmaceutical Investigation*; 2024; 14(2): 552. Doi: 10.5530/ijpi.14.2.69.
- Akabari AH, Gajiwala H, Patel SK, Surati J, Solanki D, Shah KV, Patel TJ, Patel SP. Stability-Indicating TLC-Densitometric and HPLC methods for simultaneous determination of teneigliptin and pioglitazone in pharmaceutical dosage forms with eco-friendly assessment. *Journal of Chromatographic Science*; 2025; 63(2). <https://doi.org/10.1093/chromsci/bmae038>.
- Prajapati P, Rana B, Pulusu VS, Shah S. Method operable design region for robust RP-HPLC analysis of pioglitazone hydrochloride and teneigliptin hydrobromide hydrate: incorporating hybrid principles of white analytical chemistry and design of experiments. *Future Journal of Pharmaceutical Sciences*; 2023; 9(1): 93. <https://doi.org/10.1186/s43094-023-00546-5>.
- Veronika R. M. *Practical High-Performance Liquid Chromatography*. John Wiley & Sons: 4th edition; New York, 2004, Ltd. ISBN: 0-470-09377-3, pp. 74..