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Optimization and Validation of Stability-Indicating RP-HPLC Method for Teneligliptin and Pioglitazone using Response Surface Methodology

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Abstract

The research work discloses establishment & thorough validation of method for stability-assessing RP-HPLC system designed for concurrent assessment of Teneligliptin & Pioglitazone in both bulk drug and tablet formulations. Response Surface Methodology was applied to optimize key chromatographic variables, including acetonitrile concentration in mobile phase (ranging from 70% to 80%), rate of flow (0.9 to 1.1mL/minute), & temperature of column (30°C to 40°C), in order to meet predefined critical quality attributes (CQAs) as peak retention time, resolution, & tailing factor. HPLC system of Waters Alliance make, operating with UV detector set to 229nm, was employed for separation process. Optimized composition of mobile phase was combination of 71.9:29.1 (v/v) acetonitrile & HSA buffer, with buffer pH attuned to 2.5 with orthophosphoric acid. Established retention times for Teneligliptin & Pioglitazone were 5.615 and 2.258 minutes, correspondingly. Stress testing confirmed that technique effectively separated APIs from their degradation products, validating its stability-indicating nature. Method validation, conducted in harmony with ICH guidelines, confirmed strong linearity, high precision, accuracy, and robustness. Recovery values were within 98-102% range, with no observable interference from excipients or degradation products. Furthermore, low LOD and LOQ demonstrated method's suitability for sensitive & routine pharmaceutical analysis. In summary, RP-HPLC method developed and optimized through RSM offers robust, cost-effective, and reproducible analytical approach, well-suited for quality control & routine assessment of Teneligliptin & Pioglitazone in commercial pharmaceutical preparations.

Keywords: Teneligliptin, Pioglitazone, HPLC, Response Surface Methodology, Validation, Forced Degradation Studies.

Introduction

Accurate measurement of APIs (active pharmaceutical ingredients) in combinational dosage form is vital for maintaining therapeutic effectiveness & meeting regulatory standards. Co-treatment with Teneligliptin, classified as DPP-4 inhibitor (1-4), & Pioglitazone, from thiazolidinedione group (5-8), is recognized strategy for type 2 diabetes mellitus, is commonly practiced due to their complementary mechanisms in controlling blood glucose levels (Fig. 1) (9,10). As result, creation of dependable analytical technique for their concurrent quantification is of considerable pharmaceutical relevance.

RP-HPLC is widely recognized as dominant & preferred analytical technique for evaluating multi-drug formulations, offering high levels of specificity, accuracy, & rapid throughput (11,12). Its adaptability in analyzing diverse pharmaceutical

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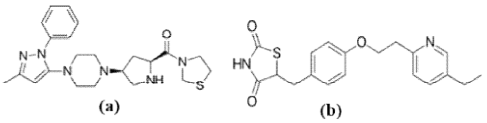

Fig. 1: Chemical structures of (a) Teneligliptin & (b) Pioglitazone

compounds-including active drugs & their potential degradation products has made RP-HPLC critical tool in both R&D & quality assurance settings (13-19).

To improve method robustness & analytical performance, statistical optimization approaches such as Response Surface Methodology (RSM) have become increasingly important (20-22). RSM enables systematic investigation of key chromatographic parameters, including altering mobile phase's organic solvent content, adjusting flow rate, & controlling column temperature, optimal chromatographic results-such as desirable retention times, enhanced resolution, & symmetrical peaks can be obtained. Moreover, experimental design techniques facilitate identification of interactions among variables, contributing to method robustness & consistency (23-26).

Stability-indicating assays play crucial role in pharmaceutical quality assurance by distinguishing APIs from their degradation products generated under stress conditions like hydrolysis, oxidation, photo degradation, & heat exposure. These methods ensure long-term efficacy & safety of drug formulations throughout their shelf life.

Given this background, present research is dedicated to establishment, refinement, & validation of simple, robust & stability-monitoring RP-HPLC system for concurrent quantification of Teneligliptin & Pioglitazone in both bulk substances & dosage forms (27,28). Method was validated following ICH strategy Q2(R1), confirming its appropriateness for routine quality control & potential regulatory applications (29,30).

Materials and Methods

Chemicals & Reagents

Certified pharmaceutical manufacturers supplied analytical-grade reference standards of Teneligliptin & Pioglitazone. Marketed tablet formulations were sourced from licensed pharmacies for analysis. HPLC-grade acetonitrile (ACN) & orthophosphoric acid (OPA) were procured from Merck (India). Hexane sulfonic acid (HSA), of analytical grade, was used for preparation of buffer solution. Ultrapure water was prepared using Millipore purification system. All solvents & chemicals utilized throughout study were either of HPLC or analytical grade to ensure compatibility & reliability in chromatographic procedures.

Instrumentation & Chromatographic Conditions

The analysis was done using HPLC system of Waters Alliance make equipped supported with UV detector, which was set to monitor at wavelength of 229nm. Empower software was utilized for system control, data acquisition, & processing. Reverse Phase C18 column (250 milli meter×4.6 milli meter, particle size 5 micro meter) was engaged, chosen after initial evaluations due to its high separation efficiency & favorable peak shapes. Optimized movable phase consisted of ACN & HSA buffer (pH adjusted to 2.5 with OPA) in 29.1:71.9 v/v ratio. Chromatographic procedure was carried out at 1.0mL/min flow rate, with volume of each sample injection was fixed at 10µL. Column temperature was adjusted at 35°C throughout analysis to ensure reproducibility.

Preparation of Solutions

Preparation of Buffer Solution: To prepare buffer solution, 1.8grams of hexane sulfonic acid were accurately measured & dissolved in 1000mL HPLC-grade water. pH of resultant solution was attuned to 2.5 using orthophosphoric acid. Following pH adjustment, solution was filtered using membrane filter with orifice 0.45 µm to eliminate any particulate matter & ensure clarity for chromatographic use.

Preparation of Mobile Phase: It was arranged by combination of previously prepared buffer with acetonitrile in 71.9:29.1 (v/v) ratio. This solution was then filtered &

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degassed using an ultrasonic bath to remove any entrapped or dissolved gases that might affect detector sensitivity or chromatographic performance.

Standard Stock Solution: An accurately measured quantity of approximately 40mg of Teneligliptin & 30mg of Pioglitazone was moved into volumetric flask of capacity 100mL. Compounds were dissolved using suitable diluent, sonicated for 10mins, & then volume was adjusted with same diluent.

Standard Working Solution: An aliquot of 1mL from stock solution was moved into volumetric flask of capacity 10mL & diluted to indicated volume with diluent for obtaining standard working solution.

Sample Preparation: Twenty tablets were weighed together to establish average weight. An amount equivalent to 40mg of Teneligliptin & 30mg of Pioglitazone was accurately moved into volumetric flask of capacity 100mL.Contents were sonicated with diluent for 30 minutes to ensure complete dissolution, followed by filtration using membrane filter with orifice 0.45µm. Subsequently, 1mL aliquot of filtrate was diluted to 10mL with diluent to prepare final sample solution for analysis.

Method Development Utilizing RSM

This approach was adopted to methodically optimize key chromatographic parameters, including composition of mobile phase, rate of flow and temperature of the column. Three-level, three-factor factorial design was implemented to assess their impact on critical quality attributes such as retention time, resolution, & tailing factor. Optimization process was done with employing Design-Expert software (version 11.0; Stat-Ease Inc., USA), facilitating identification of an optimal method operating space through comprehensive statistical evaluation.

Method Validation

Generated RP-HPLC technique was validated according to ICH Q2(R1). Following validation parameters were assessed:

System Suitability: Key metrics were evaluated to confirm system performance.

Specificity: Method's ability to distinctly analyze Teneligliptin & Pioglitazone without interference from excipients or degradation products was verified by analyzing blank, placebo, & forced degradation samples.

Linearity: Calibration curves were constructed over concentration range appropriate for each analyte, & correlation coefficients were determined.

Precision: Intra & inter-day precision were assessed by monitoring %RSD from multiple injections conducted at different time intervals.

Accuracy: Method's accuracy was confirmed through recovery experiments performed at 3 concentration levels-80, 100 & 120%-using standard addition technique.

Limits of Detection (LOD) & Quantitation (LOQ): These were generated based on S/N ratios, with approximate values of 3:1 for LOD & 10:1 for LOQ.

Robustness: This was assessed by introducing intentional variations in chromatographic parameters, including changes in flow rate & ratio of organic solvent.

Forced Degradation Studies: The analytes were subjected to different stress conditions to evaluate the method's ability to indicate stability. Resulting degradation products were effectively separated from primary analyte peaks.

Results

Method Development Utilizing RSM

A structured optimization strategy based on RSM was implemented to refine chromatographic conditions for concurrent analysis of Teneligliptin & Pioglitazone. three-factor, three-level factorial design was utilized to investigate influence of mobile phase composition (ranging from 70% to 80% acetonitrile), flow rate (0.9-1.1mL/min), &

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column temperature (30-40°C) on essential analytical parameters, including retention time, resolution, & tailing factor.

A total of 32 experimental runs were carried out in randomized sequence, & resulting data were evaluated using Design-Expert software. Regression analysis was employed to establish relationships between independent variables & each response. Among models tested, quadratic model provided best fit, as indicated by statistical parameters such as adjusted R^2, predicted R^2, & lack-of-fit assessments (Table 1 and Fig. 2).

Regression Analysis & Model Equations: The final polynomial equations (in terms of coded factors) were,

Table 1: Statistical significance of models summary

Response	Model type	Adjusted R^2	Predicted R^2	Adequate precision	p-value	Model status
Retention time	Quadratic	0.822	0.689	14.2	0.00106	Significant
Resolution	Quadratic	0.681	0.474	7.58	0.0316	Significant
Tailing factor	Quadratic	0.534	0.300	7.73	0.822	Significant

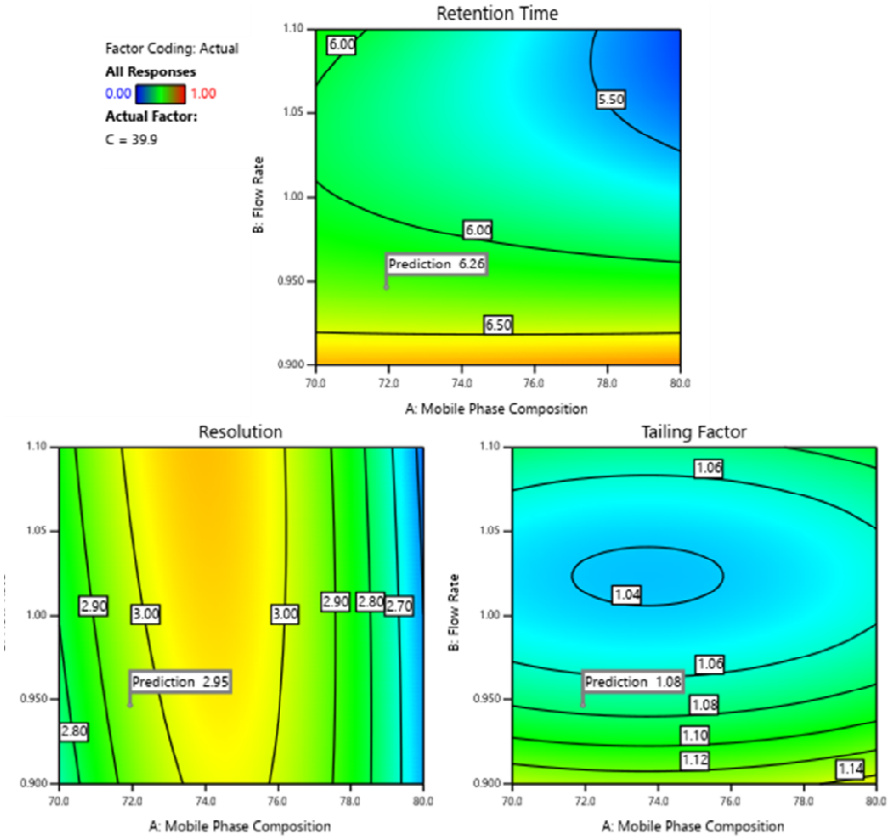

Fig. 2: Plots from response surface methodology highlighting the combined effects of mobile phase components (% v/v acetonitrile) and rate of flow (mL/min) on (a) retention time (min), (b) resolution, and (c) tailing factor. Statistical model significance: RT (R^2 = 0.991, $p < 0.001$), Rs (R^2 = 0.992, $p < 0.001$), Tf (R^2 = 0.881, $p = 0.032$). Desirability function (D = 0.96) indicated optimal chromatographic conditions at 29.1% acetonitrile, 1.0 mL/min rate of flow, & 35°C temperature of column

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Retention Time (RT):

$$RT = 5.79 - 0.25A - 0.56B - 0.06C - 0.22AB + 0.08AC + 0.06BC + 0.01A^2 + 0.38B^2 + 0.11C^2$$

Resolution (Rs):

$$Rs = 3.01 - 0.06A - 0.006B + 0.02C - 0.04AB - 0.04AC + 0.03BC - 0.34A^2 - 0.009B^2 + 0.008C^2$$

Tailing Factor (Tf):

$$Tf = 1.07 + 0.006A - 0.044B - 0.022C + 0.017BC + 0.061B^2$$

Optimized Conditions

Mobile phase composition:
71.9% buffer: 29.1% acetonitrile (v/v)
Flow rate: 1.0 mL/min
Column temperature: 35°C

Under these conditions, Teneligliptin & Pioglitazone exhibited well-resolved peaks with acceptable retention times (5.615 min & 2.258 min, respectively), resolution (>2.5), & tailing factors close to 1.

Method Validation

Generated RP-HPLC technique was validated in accordance with ICH Q2(R1).

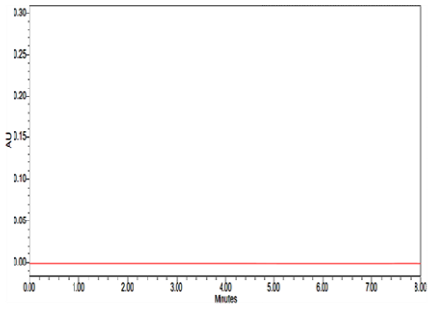
System Suitability: It was investigated through 6 consecutive injections of standard solution, and all measured parameters for both analytes complied with the established acceptance criteria (Table 2).

Specificity: No peaks were observed at retention times of analytes in blank & placebo chromatograms, confirming method specificity (Fig. 3).

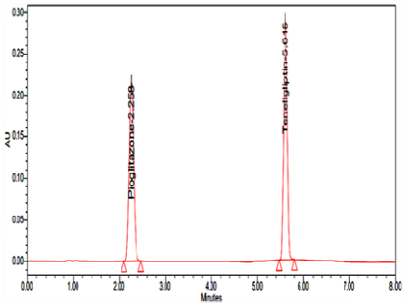
Linearity: Linearity was assessed overconcentration range of 10-60 & 7.5-45 µg/mL for Teneligliptin & Pioglitazone correspondingly (Table 3).

Precision: Intra- & Inter-day Precision studies were performed, & %RSD values were within acceptable limits (Table 4).

Accuracy: Recovery studies were performed at 80%, 100%, & 120% levels (Table 5).



Chromatogram of blank



Optimized chromatogram

Fig. 3: Overlaid chromatograms of blank & standard solution

Table 2: System suitability results

Parameter	Teneligliptin	Pioglitazone	Acceptance criteria
Retention time (min)	5.615	2.258	Consistent within ±2%
Theoretical plates	7865	6349	> 2000
Resolution	3.0	—	> 2
Tailing factor	1.10	1.12	< 2

Table 3: Linearity data

Drug	Range of concentration (µg/mL)	Regression equation	R^2
Teneligliptin	10–60	y = 63858x + 43067	0.999
Pioglitazone	7.5–45	y = 66069x + 1794	0.999

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Table 4: Precision data		
Drug	Intra-day (%RSD)	Inter-day (%RSD)
Teneligliptin	0.27%	0.63%
Pioglitazone	0.27%	0.69%

Table 5: Accuracy data	
Drug	% Recovery (Mean ± SD)
Teneligliptin	99.9 ± 0.5%
Pioglitazone	100.4 ± 0.6%

Table 6: LOD & LOQ results		
Drug	LOD	LOQ
Teneligliptin	0.12	0.40
Pioglitazone	0.09	0.30

LOD & LOQ: These values demonstrate method's high sensitivity (Table 6).

Robustness: Minor deliberate changes in flow rate (±0.1mL/min) & organic phase ratio (±3%) did not significantly affect system suitability parameters. %RSD remained below 2%, indicating robustness.

Forced Degradation Studies: These studies demonstrated that method was capable of distinguishing degradation products from active ingredients under different stress conditions (Table 7 and Fig. 4).

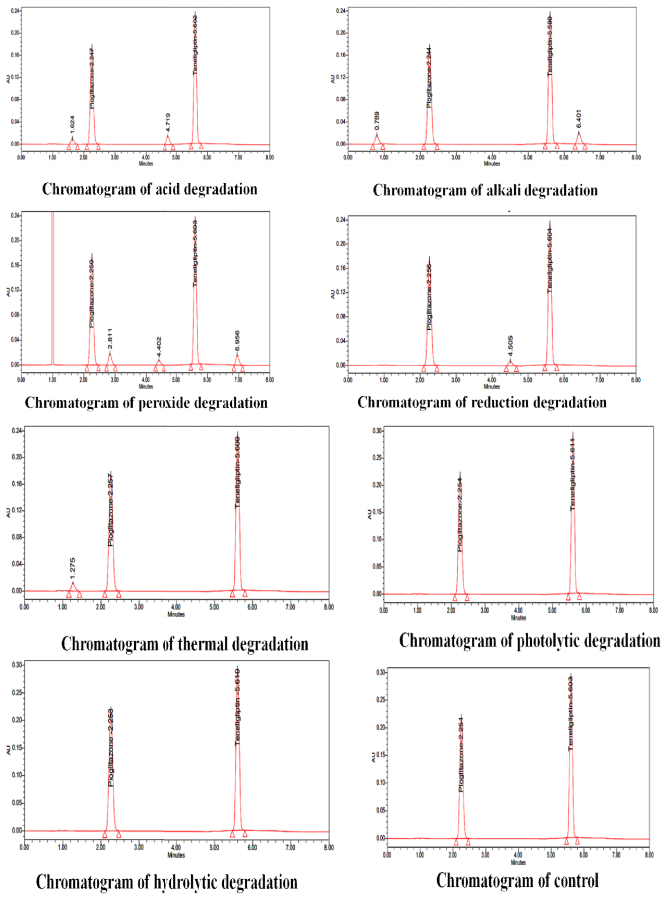

Fig. 4: Representative chromatograms showing degradation peaks under stress conditions

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Table 7: Results of forced degradation studies

Degradation conditions	Teneligliptin		Pioglitazone	
	Peak area	% Degradation	Peak area	% Degradation
Control	2641873	0	1935654	0
Acid	2319234	12.2	1702178	12.1
Alkali	2297134	13.1	1671236	13.7
Peroxide	2245165	15.0	1659782	14.2
Reduction	2351783	11.0	1903205	1.7
Thermal	2593481	1.9	1689782	12.7
Photolytic	2571821	2.7	1889452	2.4
Hydrolytic	2618748	0.9	1852838	4.3

Discussion

The principal purpose of this work was to generate & validate robust RP-HPLC technique for simultaneous evaluation of Teneligliptin & Pioglitazone, employing scientific Quality by Design (QbD) framework integrated with Response Surface Methodology (RSM). Unlike previously reported conventional methods, the present study adopted statistically guided optimization strategy that offers superior sensitivity, shorter runtime, and broader linear range, thus ensuring enhanced regulatory and industrial applicability.

The optimization process systematically evaluated influence of mobile phase composition, rate of flow, & temperature of column on critical chromatographic responses, including retention time, resolution, and tailing factor. The RSM models yielded statistically significant regression equations ($R^2 > 0.98$, $p < 0.05$), confirming their predictive reliability. The RSM-derived regression models for RT, Rs, and Tf demonstrated excellent predictive accuracy ($R^2 > 0.98$), confirming model adequacy & robustness in predicting chromatographic outcomes. Among the studied factors, mobile phase composition exerted the most pronounced influence, followed by flow rate. Higher buffer ratio increased retention times, while marginal reduction in organic content (29.1% acetonitrile vs. 30%) enhanced peak sharpness and resolution without substantially prolonging analysis. The optimized mobile phase ratio of 71.9:29.1 (buffer:acetonitrile, v/v) provided efficient separation, achieving retention times of approximately 5.6 minutes for Teneligliptin and 2.2 minutes for Pioglitazone.

The generated technique was established in compliance with ICH Q2(R1). System suitability parameters consistently met acceptance limits, demonstrating excellent column performance. Specificity studies confirmed absence of interference from excipients and degradation products. The method exhibited outstanding linearity with regression value > 0.999), high precision (%RSD < 2%), & accuracy with recoveries close to 100%. Sensitivity was reflected by low LOD and LOQ values, while robustness testing under deliberate parameter variations further confirmed method reliability.

Comprehensive stress at various conditions demonstrated stability-indicating character of the method. Degradation elements were well resolved from peaks of analyte, with peak purity analysis confirming absence of co-elution. Quantitative degradation values were included to strengthen reliability of stress testing outcomes.

In comparison with earlier trial-and-error approaches, the RSM-assisted QbD approach provided statistically validated, efficient, and reproducible optimization pathway.

Conclusion

A novel & stability-monitoring RP-HPLC technique was generated & confirmed for concurrent quantification of Teneligliptin & Pioglitazone. This method was designed within

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Quality by Design (QbD) framework utilizing Response Surface Methodology (RSM) for systematic optimization. Finalized chromatographic conditions-comprising mobile phase ratio of 71.9:29.1 (v/v, buffer to acetonitrile), 1.0 mL/min as rate of flow, & temperature of column on 35°C-enabled rapid & reliable separation with clearly resolved peaks.

Validation data confirmed that method is specific, linear, precise, accurate, sensitive, & robust. Additionally, it effectively separated degradation products, underscoring its stability-indicating capability. As such, developed technique is suitable for routine QC, & regulatory applications involving Teneligliptin & Pioglitazone combination formulations.

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