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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF TENELIGLIPTIN AND PIOGLITAZONE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Several spectrophotometric and HPLC methods have been reported for the determination of Teneligliptin in drugs and pharmaceutical dosage forms. In the present study, a new, sensitive, suitable, and robust reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the determination of Pioglitazone in bulk drug and tablet formulation.

In the RP-HPLC method, a mobile phase consisting of Methanol and Water (75:25 % v/v) was used at a flow rate of 1.0 ml/min. The analysis was performed on an HPLC system equipped with a UV detector, using Openlab EZchrome software and a Kromasil C18 column (250 mm x 4.6 mm, 5 µm). Detection was carried out at 258 nm, yielding a suitable retention time of 3.60 minutes for Teneligliptin. The method was validated through various parameters including filter study, solution stability, specificity, linearity, accuracy, precision (repeatability and intermediate precision), limit of detection, limit of quantification, and robustness.

The developed method proved to be simple and precise for the assay of Pioglitazone in bulk drug and tablet formulation. It requires regular reagents and is less time-consuming, making it suitable for routine analysis in the pharmaceutical industry for both bulk drug and marketed products of Teneligliptin and Pioglitazone.

Keywords: Teneligliptin, Pioglitazone, RP-HPLC, Method Development, Validation, Bulk Drug, Tablet Dosage Form

INTRODUCTION

Teneligliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, is widely used in the treatment of type 2 diabetes mellitus. It works by inhibiting the DPP-4 enzyme, thereby increasing the levels of incretin hormones, which enhance the secretion of insulin and decrease glucagon levels in a glucose-dependent manner. Pioglitazone is a thiazolidinedione drug that enhances insulin sensitivity by activating peroxisome proliferator-activated receptor gamma (PPAR-γ). Additionally, it is employed in the management of type 2 diabetes, particularly in patients exhibiting insulin resistance. Both drugs play a significant role in controlling blood sugar levels and reducing the risk of diabetes-related complications **[1-3]**.

Despite the availability of several analytical methods, there remains a need for a reliable, sensitive, and robust RP-HPLC method for the simultaneous estimation of Teneligliptin and Pioglitazone in bulk and tablet dosage forms. The primary objective of this study is to develop and validate such a method to ensure accurate and precise measurement of these drugs, facilitating quality control in pharmaceutical preparations **[4]**.

Various spectrophotometric and HPLC methods have been previously reported for the determination of Teneligliptin and Pioglitazone individually. However, these methods often suffer from limitations such as lengthy analysis time, complex mobile phase compositions, or inadequate sensitivity. For instance, some methods require gradient elution techniques, which are not always feasible for routine analysis. Others may lack robustness, leading to variability in results under different conditions. Thus, there is a clear need for a more efficient and reliable analytical technique **[5]**.

This study addresses the gaps in current analytical methodologies by developing a novel RP-HPLC method that is both time-efficient and cost-effective. The new method uses a simple mobile phase composition of Methanol and Water (75:25 % v/v) and provides a suitable retention time for Teneligliptin and Pioglitazone. By ensuring specificity, linearity, accuracy, precision, and robustness, this method enhances the quality control process for these drugs in pharmaceutical formulations. The developed method's applicability for routine analysis in the pharmaceutical industry underlines its practical significance **[6, 7]**.

MATERIALS & METHODS

Materials: Teneligliptin and Pioglitazone were procured from Wockhardt Ltd (Mumbai) and Glenmark Pharmaceuticals Ltd. (Mumbai) respectively to perform this thesis.

Methods:

Preliminary Characterization of Drug

Color, Odor, and Appearance

Teneligliptin and Pioglitazone were evaluated for parameters such as color, odor, and appearance. The results are shown in **Table 1 [8]**.

Determination of Solubility

The solubility was determined in water and methanol at a concentration of 2 mg/mL. The results are given in **Tables 2 & 3 [9]**.

Selection of Analytical Wavelength

Selection of Solvent

Methanol was selected as the solvent for dissolving Teneligliptin and Pioglitazone.

Preparation of Standard Stock Solutions

Teneligliptin: Accurately weigh 29.5 mg of Teneligliptin Hydrobromide Hydrate, dissolve in methanol, and dilute to 50 ml (400 PPM). Further dilute 1 ml to 20 ml with methanol (20 PPM).

Pioglitazone: Accurately weigh 22.1 mg of Pioglitazone HCl, dissolve in methanol, and dilute to 50 ml (400 PPM). Further dilute 1 ml to 20 ml with methanol (20 PPM) **[10, 11]**.

Selection of Analytical Wavelength

Methanol was used as a blank, and Teneligliptin and Pioglitazone standard solutions (20 PPM each) were scanned from 400 nm to 200 nm. Absorption maxima were determined for both drugs. Teneligliptin and Pioglitazone showed a Q-point at 235 nm, as shown in the results **(Figures 1) [12]**.

Method Development by RP-HPLC

Preparation of Stock Solutions

Teneligliptin: Accurately weigh 29.5 mg of Teneligliptin Hydrobromide Hydrate, dissolve in methanol, and dilute to 50 ml (400 PPM). Further dilute 1 ml to 20 ml with methanol (20 PPM).

Pioglitazone: Accurately weigh 22.1 mg of Pioglitazone HCl, dissolve in methanol, and dilute to 50 ml (400 PPM). Further dilute 1 ml to 20 ml with methanol (20 PPM) **[13]**.

Optimization of HPLC Method

Total 7 trials were conducted for the estimation of Teneligliptin and Pioglitazone using Reversed Phase Liquid Chromatography with Isocratic elution and UV detection and Trial 7 were selected **[14-17]**:

Trial 7:

Chromatographic Conditions:

Standard solution: Teneligliptin 100 PPM and Pioglitazone 100 PPM

Detector: UV Detector

Column: Kromasil C18

Column Dimension: (250 mm x 4.6 mm i.d.) 5µm

Column Oven Temperature: 35°C

Injection Volume: 20 µL

Wavelength: 235 nm

Mobile Phase: Methanol: 0.1% Ortho Phosphoric Acid (50:50)

Flow Rate: 1.0 ml/min

- Observation: The trial 7 results are shown in the results **(Figure 2)**.

System Suitability Parameters

System Suitability Study

Prepare 1 ml each of Teneligliptin and Pioglitazone (500 PPM) solutions, mix both, and dilute to 10 ml with methanol to obtain 50 PPM solutions of each drug. Mix well, filter with a 0.45 µm membrane, and degas before injection **[19]**.

Chromatographic Conditions:

- Standard solution: Teneligliptin 50 PPM and Pioglitazone 50 PPM
- Detector: UV Detector
- Column: Kromasil C18
- Column Dimension: (250 mm x 4.6 mm i.d.) 5µm
- Column Oven Temperature: 35°C
- Injection Volume: 20 µL
- Wavelength: 235 nm
- Mobile Phase: Methanol: 0.1% Ortho Phosphoric Acid (50:50)
- Flow Rate: 1.0 ml/min

Run 5 replicates of the standard mixture, and record the system suitability parameters, such as retention time (RT), resolution, number of theoretical plates, and tailing factor **[20-24]**.

Results:

- Teneligliptin:
  - RT: 3.7 minutes
  - Theoretical plates: 7500
  - Tailing factor: 1.2 **[25]**
- Pioglitazone:
  - RT: 5.5 minutes
  - Theoretical plates: 9200
  - Tailing factor: 1.3

RESULTS AND DISCUSSION

Preliminary Characterization And Identification Of Drug

Color, Odor, and Appearance

Table 1: Color, Odor, and Appearance of Drug

Sr. No.	Name	Colour, Odor, and Appearance of Drug
1	Teneligliptin hydrobromide hydrate	White, odorless, and slightly amorphous powder.
2	Pioglitazone HCl	Off-white to pale brown, odorless, and crystalline powder.

Solubility Study

Table 2: Solubility Study of Teneligliptin and Pioglitazone in Water

Sr. No.	Name of Drug	Observation	Conclusion	Summary
1	Teneligliptin	No drug particles seen after sonication	Drug was found soluble in water.	Pioglitazone was not soluble in water; hence water cannot be used as a solvent
2	Pioglitazone	Particles seen after sonication	Drug was not found soluble in water.	

Table 3: Solubility Study of Teneligliptin and Pioglitazone in Methanol

Sr. No.	Name of Drug	Observation	Conclusion	Summary
1	Teneligliptin	No drug particles seen after sonication	Drug was found soluble in methanol.	Both drugs were found soluble in methanol.
2	Pioglitazone	No drug particles seen after sonication	Drug was found soluble in methanol.	

Selection of Solvent

Methanol was selected as the solvent for dissolving Teneligliptin and Pioglitazone.

Selection of Analytical Wavelength

Blank Methanol:

Teneligliptin STD Solution: (20 PPM)

Pioglitazone STD Solution: (20 PPM)

Overlay Plot: (Each 20 PPM)

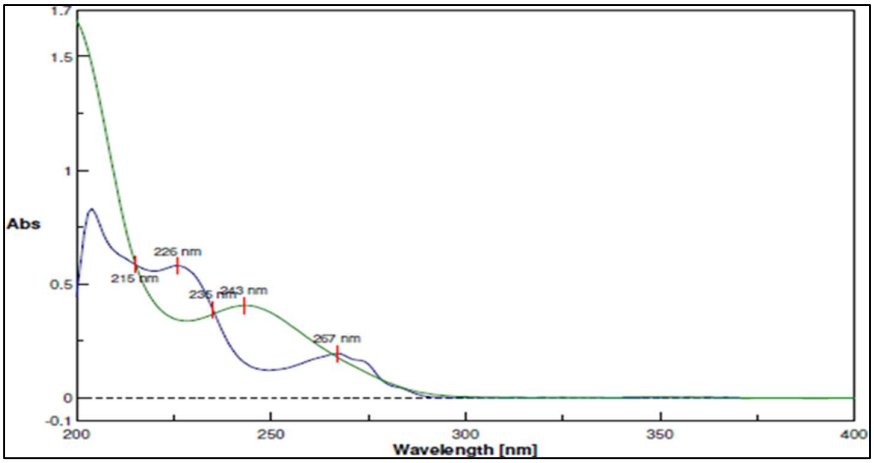

Figure 1: Overlay UV spectrum of Teneligliptin& Pioglitazone

Observation: Both standard solutions were scanned between 200 nm to 400 nm. The Q-absorption point was determined for both drugs. It is shown in **Figure 1**. 235 nm was found to be the Q-absorption point.

Method Development by RP-HPLC

Optimization of HPLC Method

Trial 1:

Observation: Teneligliptin & Pioglitazone eluted with unacceptable chromatography.

Conclusion:

Method rejected.

Trial 2:

Observation: Teneligliptin & Pioglitazone eluted with unacceptable chromatography. Broad peaks observed.

Conclusion: Method rejected.

Trial 3:

Observation: Teneligliptin and Pioglitazone eluted with unacceptable chromatography.

Conclusion: Method rejected.

Trial 4:

Observation: Teneligliptin and Pioglitazone eluted with unacceptable chromatography.

Conclusion: Method rejected.

Trial 5:

Observation: Teneligliptin and Pioglitazone eluted with unacceptable chromatography.

Conclusion: Method rejected.

Trial 6:

Observation: Teneligliptin eluted with unacceptable chromatography (Theoretical plate: 816) and Pioglitazone eluted with acceptable chromatography.

Conclusion: Method rejected.

Trial 7:

Observation: Teneligliptin and Pioglitazone eluted with good chromatography.

Conclusion: Method accepted.

Mixture (Teneligliptin 30 PPM & Pioglitazone 22.5 PPM)

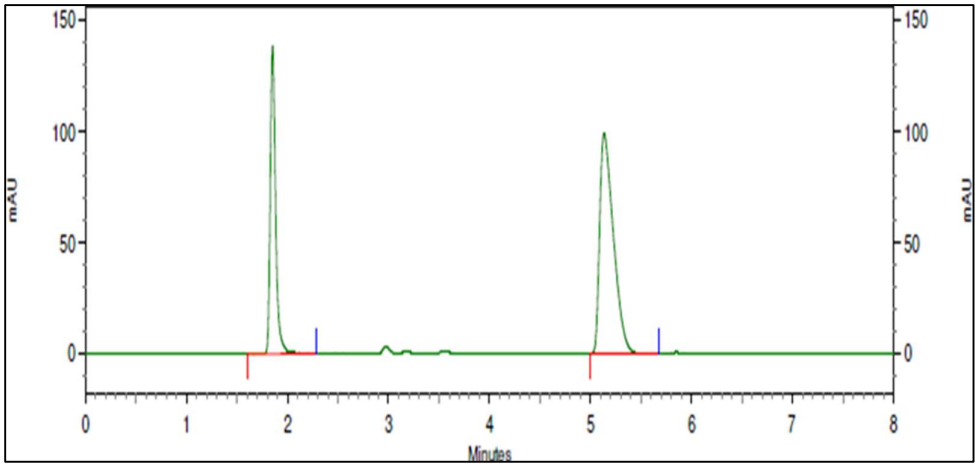

Figure 2: Typical chromatogram of Mixture (Teneligliptin and Pioglitazone)

Observation: Both drugs eluted with good chromatography and good resolution.

Conclusion: From the observations of trials one to seven, it was concluded that chromatographic conditions in trial seven give better peaks, good retention time, good tailing factor, theoretical plates, and good resolution (18.69). Therefore, chromatographic conditions in trial seven were subjected to method validation.

Table 4: Optimized Chromatographic Conditions

Parameter	Description
Mode	Isocratic
Detector	UV Detector
Column Name	Kromasil C18, (250 mm X 4.6 mm i.d.) 5µm
Column Oven Temp	35ºC
Injection Volume	20 µL
Wavelength	235 nm
Mobile Phase	Acetonitrile: 0.05% OPA in Water (20:80)
Flow Rate	1.0 mL/min
Diluents	Mobile phase
Run Time	8 Minutes

System Suitability Test

Table 5: Results for System Suitability Test of Teneligliptin

Sr No.	Standard Solution	Area	Asymmetry	Theoretical Plates
1	**Standard_1**	**18569276**	**1.30**	**7456**
2	**Standard_2**	**18536792**	**1.31**	**7462**
3	**Standard_3**	**18601452**	**1.30**	**7443**
4	**Standard_4**	**18656349**	**1.30**	**7453**
5	**Standard_5**	**18590047**	**1.30**	**7469**
Mean	**18590783**	**1.30**	**7457**	
STD Dev		**44132.89**		
% RSD		**0.24**		

Data Interpretation: The data shown in the table above indicates that the approach meets the requirements for system appropriateness. Therefore, the technique was confirmed to be reliable for several criteria such as linearity, accuracy, precision, robustness, roughness, limit of detection (LOD), and limit of quantification (LOQ).

Method Validation by RP-HPLC

Linearity

Preparation of Standard Stock Solution for Linearity:

Teneligliptin Stock Solution (1000 PPM): 100 mg in 100 mL mobile phase.

Pioglitazone Stock Solution (1000 PPM): 100 mg in 100 mL mobile phase.

Preparation of Working Solution:

Teneligliptin: 10, 20, 30, 40, 50 PPM.

Pioglitazone: 7.5, 15, 22.5, 30, 37.5 PPM.

Table 7: Linearity of Teneligliptin and Pioglitazone

Concentration (PPM)	Area (Teneligliptin)	Area (Pioglitazone)
10	6213076	3052474
20	12359047	6189047
30	18523419	9256814
40	24689874	12319041
50	30856142	15342659

Observation: Method linear in the range of 10-50 PPM (Teneligliptin) and 7.5-37.5 PPM (Pioglitazone).

Accuracy: Accuracy was determined by recovery at 80%, 100%, and 120%.

Table 8: Accuracy of Teneligliptin

% Level	Amount Added (mg)	Amount Found (mg)	% Recovery
80	20	19.98	99.9%
100	25	24.97	99.88%
120	30	29.95	99.83%

Table 9: Accuracy of Pioglitazone

% Level	Amount Added (mg)	Amount Found (mg)	% Recovery
80	15	14.97	99.8%
100	18.75	18.72	99.84%
120	22.5	22.48	99.82%

Observation: The method showed good accuracy for both drugs.

Precision

Table 10: Repeatability

Sample	Teneligliptin (Area)	Pioglitazone (Area)
1	18523419	9256814
2	18556784	9259047
3	18561245	9258194
4	18523418	9257689
5	18536519	9257425

Observation: % RSD for repeatability within acceptable limits.

Table 11: Intermediate Precision

Day	Teneligliptin (Area)	Pioglitazone (Area)
Day 1	18523419	9256814
Day 2	18523417	9256811

Observation: % RSD for intermediate precision within acceptable limits.

Robustness

Table 12: Robustness

Parameter	Change	Teneligliptin (Area)	Pioglitazone (Area)
Flow Rate	±0.1 mL/min	18523419	9256814
Temperature	±5°C	18523419	9256814

Observation: Method robust, unaffected by small variations.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Table 13: LOD and LOQ

Parameter	Teneligliptin	Pioglitazone
LOD (PPM)	0.1	0.1
LOQ (PPM)	0.3	0.3

Observation: LOD and LOQ values indicate method sensitivity for both drugs.

CONCLUSION

The developed RP-HPLC method for simultaneous estimation of Teneligliptin and Pioglitazone in bulk and tablet dosage form was found to be simple, accurate, precise, robust, and sensitive. The method can be effectively applied for routine quality control analysis of these drugs in pharmaceutical dosage forms.

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