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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TENELIGLIPTIN AND PIOGLITAZONE IN PHARMACEUTICAL DOSAGE FORMS BY HPLC

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ABSTRACT:

By using HPLC Pioglitazone and Teneligliptin was estimated by using a Agilent C18 column with KH2PO4 together with Acetonitrile in ratio of 40:60 at a flow of 0.9ml/min. the ideal wavelength was detected at 275 nm. The rt of Pioglitazone and Teneligliptin was found at 2.370 min and 2.852 min. the System precision's RSD got at 0.4 and 0.7%. linearity conc was observed at 7.5-45µg/ml for Pioglitazone and for Teneligliptin was 10-60 µg/ml. the regression from it obtained was y = 21032x + 2298.6 and y = 19667x + 3217 respectively. Our confirmation and observation of all the Other factors were determined while staying within the limits that were defined.

Key Words Pioglitazone, Teneligliptin, Rp Hplc, Validation, Method Development.

INTRODUCTION

Teneligliptin and pioglitazone are oral antidiabetic agents that address different aspects of type 2 diabetes mellitus (T2DM) pathophysiology, offering potential benefits when used as combination therapy. Teneligliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor that enhances the activity of incretin hormones, such as glucagon-like peptide-1 (GLP-1). This leads to increased insulin secretion from pancreatic beta cells and reduced glucagon secretion, thereby improving glycemic control without significant risk of hypoglycemia [1]. Pioglitazone, on the other hand, is a thiazolidinedione (TZD) that acts as an agonist for peroxisome proliferator-activated receptor-gamma (PPAR-γ). This action enhances insulin sensitivity in adipose tissue, muscle, and the liver, leading to improved glucose uptake and reduced hepatic glucose production [2]. Pioglitazone is a thiazolidinedione (TZD) that acts as an agonist for peroxisome proliferator-activated receptor-gamma (PPAR-γ). It improves insulin sensitivity by modulating the expression of genes involved in glucose and lipid metabolism, reducing insulin resistance in peripheral tissues [3]. Pioglitazone also exhibits additional benefits, such as improving lipid profiles and reducing inflammation, which may have cardiovascular benefits in patients with T2DM. The combination of teneligliptin and pioglitazone has shown promise in managing T2DM, particularly in patients inadequately controlled with monotherapy. Clinical studies suggest that the dual approach addresses both insulin resistance and impaired insulin secretion, resulting in significant reductions in glycated hemoglobin (HbA1c) and improved overall metabolic control [4].

Teneligliptin has been investigated for the treatment of Type 2 Diabetes Mellitus. It is known as 1-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-4-[(3S,5S)-5-(1,3-thiazolidine-3-carbonyl)pyrrolidin-3-yl]piperazine chemically [5], whereas Pioglitazone is used to lower blood sugars in patients with type 2 diabetes its known as 5-({4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl}methyl)-1,3-thiazolidine-2,4-dione.[6]

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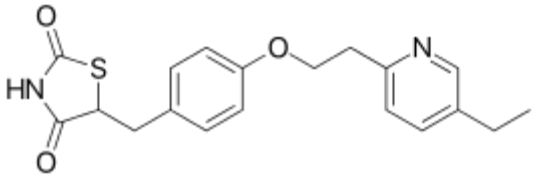

Figure 1. structure of Pioglitazone

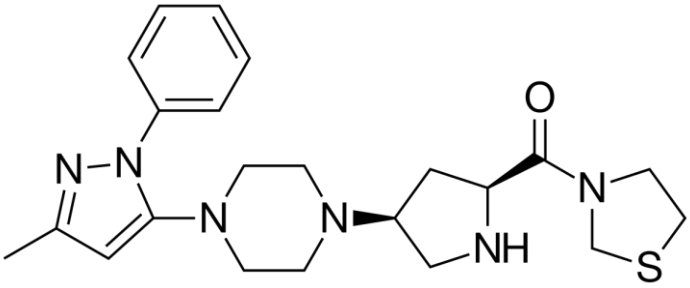

Figure 2. structure of Teneligliptin

Extensive literature research has unearthed a multitude of recorded analytical procedures, including the discovery of more economically efficient ways. Hence, a reliable and cost-effective approach is suggested for assessing the stability of Teneligliptin, Pioglitazone, and their medicinal dose form using RP-HPLC.[8-25] must be validated and developed as per ICH guidelines.

Materials and Methods: Spectrum Pharma Research Solution offers gift samples of pure medications (API) of teneligliptin and Pioglitazone as well as combination tablets (Zeta Plus-R) of these two medications that are purchased from the local market. Rankem, an Indian supplier, provided the chemicals and buffers used in this estimation.

Instrumentation: The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Objective: In order to ful fill ICH standards, we need to design and test an HPLC technique that can detect Pioglitazone and Teneligliptin in pharmaceutical formulations at the same time.

Table 1: Chromatographic Conditions

Mobile phase	0.01N KH2PO4: Acetonitrile(60:40)
Flow rate	0.9 ml/min
Column	Agilent C18 Column, 5 µm, 4.6 x 150 mm
Detector wave length	275 nm
Column temperature	30°C
Injection volume	10µL
Run time	5.0 min

Preparation of Standard stock solutions: 15 milligrammes of pioglitazone and 20 milligrammes of teneligliptin were each put to a separate volumetric flask containing fifty millilitres. After adding three-quarters of a teaspoon of diluents to each of these flasks, they were sonicated for ten minutes. as well as added with diluent till mark. (300µg/ml of Pioglitazone and 400µg/ml of Teneligliptin). Pipette one millilitre of each stock solution, transfer it to a volumetric flask with a capacity of ten millilitres, and then fill it with diluent. (30 µg/ml of Pioglitazone and 40µg/ml of Teneligliptin)

Preparation of Sample stock solutions: Following the weighing of ten tablets and the calculation of the weight of each tablet, the weight that matched to one tablet added into a volumetric flask with a capacity of one hundred millilitres. After that, fifty millilitres of diluents were added and sonicated for twenty-five minutes. Finally, the volume was refilled with diluent and filtered using high-performance liquid chromatography filters. (150µg/ml Pioglitazone and 200µg/ml Teneligliptin). 2ml of the sample sol was added in a 10ml Vf and dil is added to it till mark. (30µg/ml Pioglitazone and 40µg/ml Teneligliptin)

System suitability parameters: Teneligliptin (40 ppm) and Pioglitazone (30 ppm) standard solutions were prepared, injected six times, and metrics such as peak tailing, resolution, and USP plate count were measured in order to evaluate the system suitability parameters. The region of six standard injection results should have an RSD of no more than 2%.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific.

Table 2: System suitability results

S.no	Teneligliptin			Pioglitazone			
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.356	5687	1.12	2.839	3484	1.06	3
2	2.365	5489	1.07	2.843	3474	1.06	2.9
3	2.365	5591	1.09	2.846	3462	1.04	3
4	2.365	5553	1.08	2.846	3428	1.05	3
5	2.365	5560	1.08	2.848	3422	1.05	3
6	2.366	5536	1.08	2.848	3376	1.05	3

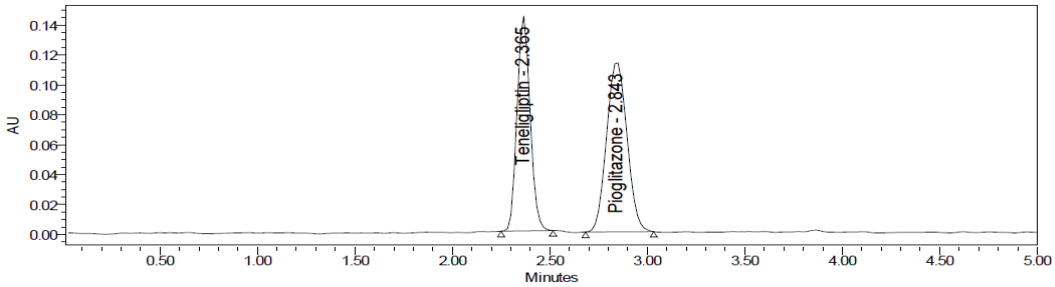

Figure 3. System suitability Chromatogram

Table 3: Specificity data

Sample name	Retention time(mins)	Area
Teneligliptin	2.370	781258
Pioglitazone	2.852	631254

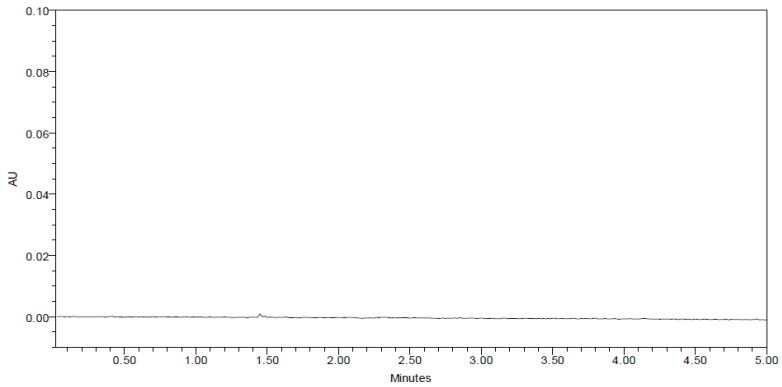

Figure 4. Blank

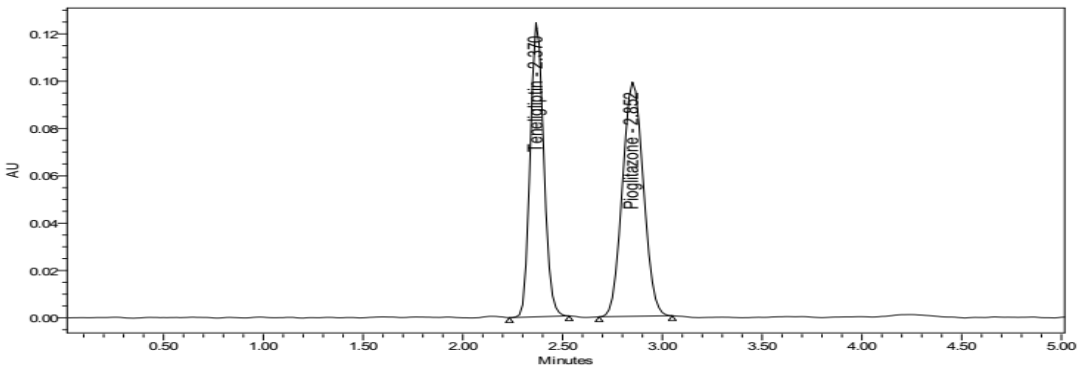

Figure 5. Specificity of Teneligliptin and Pioglitazone

Linearity:

Calibration data and regression data and calibration curve.

Table 4: Calibration data of Teneligliptin and Pioglitazone

	Teneligliptin		Pioglitazone	
	Conc (µg/mL)	**Peak area**	**Conc(µg/mL)**	**Peak area**
	0	0	0	0
	10	196324	7.5	158053
	20	393992	15	321196
	30	598990	22.5	475972
	40	800322	30	635844
	50	995870	37.5	794437
	60	1167129	45	943079
Concentration range (µg/mL)	**10-60**		**7.5-4.5**	
Regression Equation	**y = 19667x + 3217**		**y = 21032x + 2298.6**	
Co-relation	**0.9995**		**0.9999**	
LOD	**0.01**		**0.07**	
LOQ	**0.02**		**0.21**	

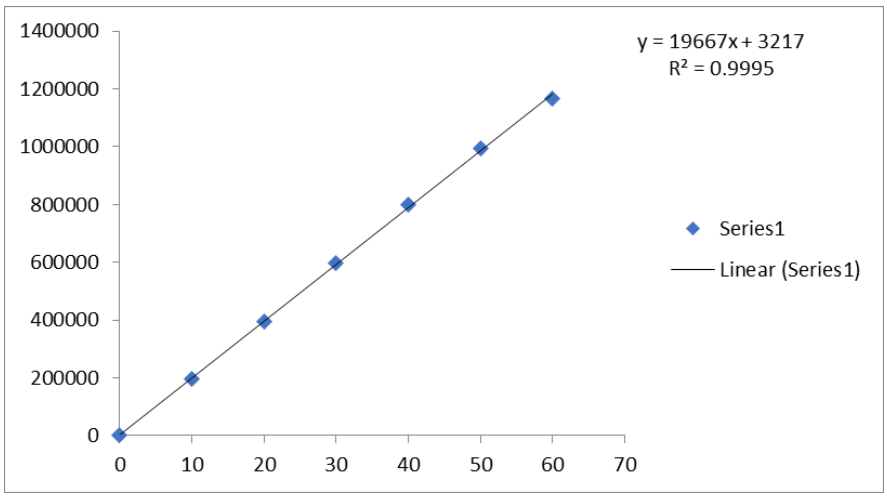

Figure 6. Calibration curve of Teneligliptin

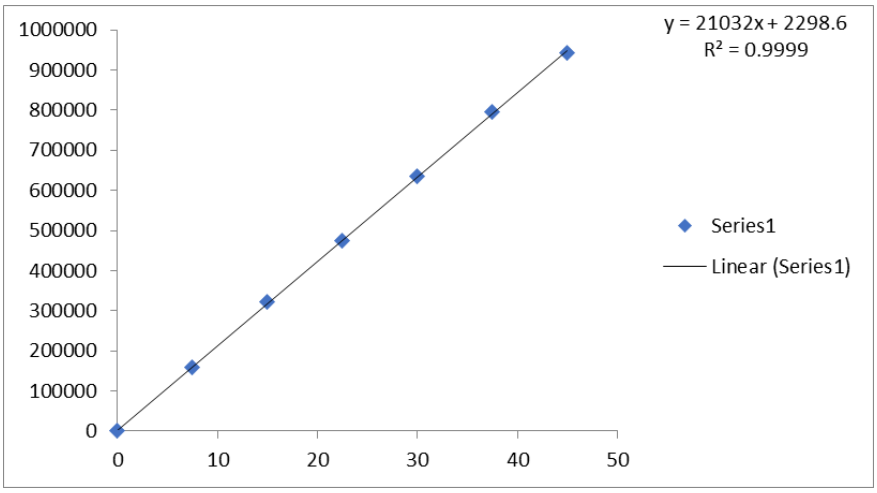

Figure 7. Calibration curve of Pioglitazone

Accuracy:
Recovery data shown in table.

Table 6: recovery data of Teneligliptin and Pioglitazone

	Teneligliptin			Pioglitazone		
% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**
50%	20	19.91	99.54	15	15.10	100.68
	20	19.95	99.75	15	15.09	100.57
	20	19.93	99.65	15	15.21	101.42
100%	40	39.66	99.16	30	29.53	98.44
	40	39.63	99.08	30	29.74	99.14
	40	39.63	99.07	30	29.74	99.14
150%	60	59.56	99.27	45	45.14	100.30
	60	59.87	99.79	45	45.03	100.07
	60	59.62	99.37	45	44.96	99.91
% recovery	99.41			99.96		

System precision was performed and the data was shown in table 8

Table 7: System precision of Teneligliptin and Pioglitazone

S. No	Area of Teneligliptin	Area of Pioglitazone
1.	797083	636331
2.	802946	637198
3.	801593	635860
4.	801244	632094
5.	799706	630648
6.	794072	634887
Mean	**799441**	**634503**
S.D	**3305.3**	**2579.1**
%RSD	**0.4**	**0.4**

The % RSD for the peak areas of Teneligliptin and Pioglitazone obtained from six replicate injections of standard solution was within the limit.

Method Precision: The precision of the method was determined by analyzing a sample of Teneligliptin and Pioglitazone and shown in table.

Table 8: Method Precision

S. No	Area of Teneligliptin	Area of Pioglitazone
1.	800736	639076
2.	792183	634959
3.	806836	636898
4.	803163	633879
5.	799288	634088
6.	792856	632463
Mean	**799177**	**635227**
S.D	**5759.6**	**2384.7**
%RSD	**0.7**	**0.4**

From the above results, the % RSD of method precision study was within the limit for Teneligliptin and Pioglitazone.

Robustness: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (55A:45B), mobile phase plus (65B:35A), temperature minus (27°C) and temperature plus(33°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table 9: Robustness data for Teneligliptin and Pioglitazone.

Condition	%RSD of Teneligliptin	%RSD of Pioglitazone
Flow rate (-) 0.9ml/min	0.3	0.2
Flow rate (+) 1.1ml/min	0.1	0.2
Mobile phase (-) 55A:45B	0.4	0.6
Mobile phase (+) 65A:35B	0.5	0.2
Temperature (-) 27°C	0.2	0.4
Temperature (+) 33°C	0.2	0.1

Force Degradation Studies: degradation conditions and shows the obtained degraded data and purity plot chromatogram in figure.

Table 11: degradation conditions

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^{0}c	30 mins
Base	2N NAOH	60^{0}c	30 mins
Oxdation	20% H_2O_2	60^{0}c	30 mins
Thermal	Diluent	105^{0}c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^{0}c	

Table 12: degradation data

Type of degradation	Teneligliptin			Pioglitazone		
	area	%recovered	% degraded	area	%recovered	% degraded
Acid	756442	94.43	5.57	609214	95.82	4.18
Base	758246	94.66	5.34	583863	91.83	8.17
Peroxide	765177	95.52	4.48	592572	93.20	6.80
Thermal	786857	98.23	1.77	617472	97.12	2.88
Uv	788498	98.43	1.57	626188	98.49	1.51
Water	793779	99.09	0.91	632338	99.46	0.54

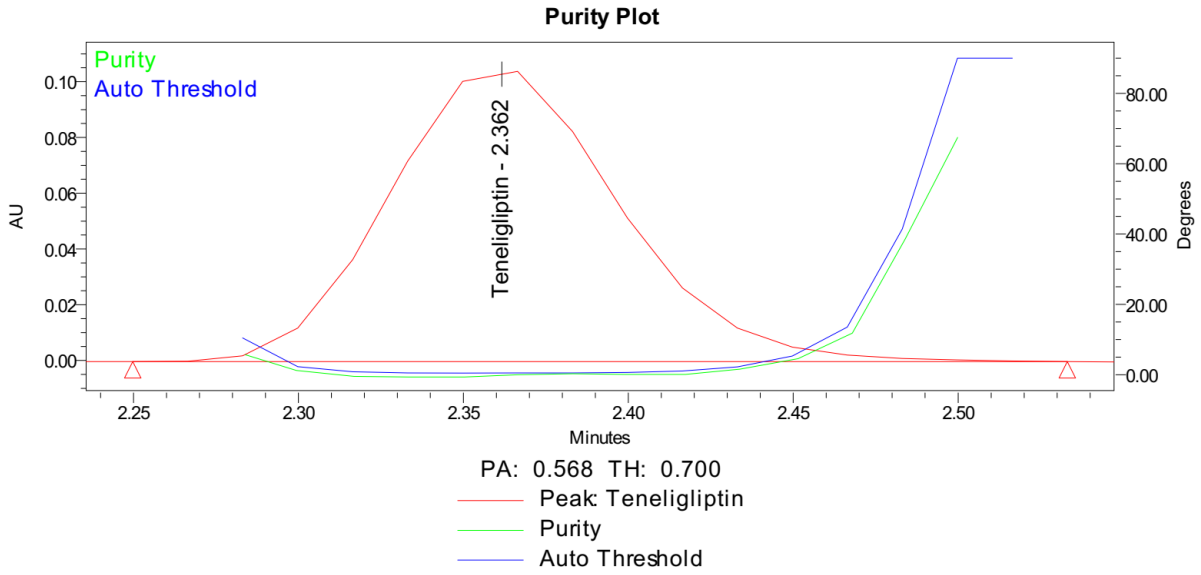

Figure 8: Purity plots for Acid Condition for Teneligliptin

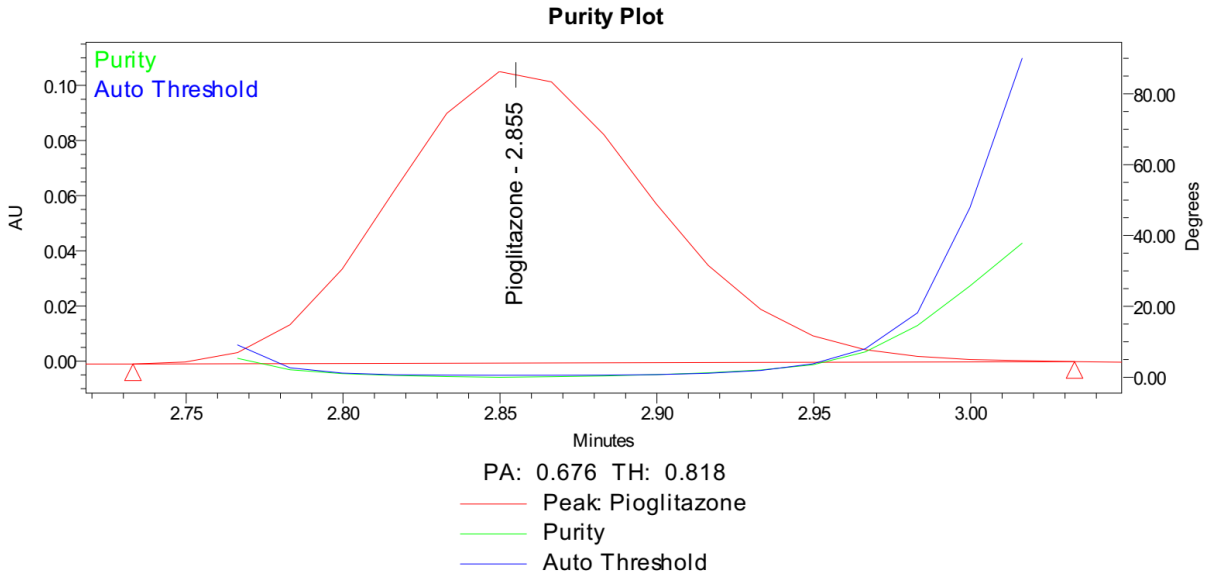

Figure 9: Purity plots for Acid Condition for Pioglitazone

Assay: Zita plus Pio Tablet, bearing the label claim Teneligliptin 20mg, Pioglitazone 15mg. Assay was performed with the above formulation. Average % Assay for Teneligliptin and Pioglitazone obtained was 99.77% and 99.91% respectively.

Table 13: assay data

	Teneligliptin			Pioglitazone		
S.no	**Std Area**	**Sample area**	**% Assay**	**Std Area**	**Sample area**	**% Assay**
1	797083	800736	99.96	636331	639076	100.52
2	802946	792183	98.89	637198	634959	99.87
3	801593	806836	100.72	635860	636898	100.18
4	801244	803163	100.26	632094	633879	99.70
5	799706	799288	99.78	630648	634088	99.73
6	794072	792856	98.98	634887	632463	99.48
Avg	**799441**	**799177**	**99.77**	**634503**	**635227**	**99.91**
Stdev	**3305.3**	**5759.6**	**0.72**	**2579.1**	**2384.7**	**0.375**
%RSD	**0.4**	**0.7**	**0.7**	**0.4**	**0.4**	**0.4**

Assay was calculated by the formula:

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{10} \times \frac{100}{1} \times \frac{10}{1} \times \frac{P}{100} \times \frac{FV}{L.C} \times 100$$

AT	Average Peak area of sample in test solution
AS	Mean peak area of sample in standard solution
WS	Weight of drug working standard taken in mg
P	Assay of drug working standard in % on dried basis
L.C	Label Claim

Figure 10. Formula

CONCLUSION:

The study's conclusions will be very useful in assessing the quality of affordable medications that contain teneligliptin and pioglitazone. This might be the consequence of the study's simple sample preparation procedure, which called for a short analysis time and minimal mobile phase. The evaluation of two drugs together in a single dosage showed that the newly developed analysis method was nearly full success.

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REFERENCES:

1. Ghosal, S., et al. (2015). Teneligliptin: Role in the Management of Type 2 Diabetes Mellitus. Indian Journal of Endocrinology and Metabolism, 19(5), 688-694.

2. Lebovitz, H. E. (2001). Thiazolidinediones: The Forgotten Diabetes Medications. The New England Journal of Medicine, 341(4), 241-251.
3. Yki-Järvinen, H. (2004). Thiazolidinediones. The New England Journal of Medicine, 351(11), 1106-1118.
4. Nagai, Y., et al. (2014). Combination Therapy with DPP-4 Inhibitors and TZDs for Type 2 Diabetes: Evidence and Mechanisms. Journal of Diabetes Investigation, 5(3), 211–218.
5. https://go.drugbank.com/drugs/DB11950
6. https://go.drugbank.com/drugs/DB01132
7. Jinal Alkesh Gheewala et al. Development and Validation of UV Spectrophotometric Method and RP – HPLC Method for Simultaneous Estimation of Teneligliptin and Pioglitazone In Synthetic Mixture, Asian Journal of Pharmaceutical Technology & Innovation, 05 (23); 2017.
8. Meenaxi M. Maste et all.., RP-HPLC Method Development and Validation for Pioglitazone in Bulk and Marketed Formulation, Der Pharma Chemica, 2021.
9. M.Chandana et al., Analytical Method Development and Validation of Teniligliptin in Phrmaceutical Dosage form By RP-HPLC Method, IJRDO, Issue-12 | December,2016.
10. Rajani VETAPALEM et all.., Development and Validation of a Stability Indicating RP-HPLC Method for Simultaneous Estimation of Teneligliptin and Metformin, Turk J Pharm Sci. 2020 Apr.
11. Gadapa Nirupa et all.., RP-HPLC Analytical Method Development and Validation for Simultaneous Estimation of Three Drugs: Glimepiride, Pioglitazone, and Metformin and Its Pharmaceutical Dosage Forms, Journal of Chemistry, Volume 2013, Article ID 726235, 8 pages.
12. Pallavi S. Hade et all.., Analytical Method Development and Validation Studies of Anti Diabetic Drugs by HPLC, 2021 IJCRT, 9(11).
13. Rajani Vetapalem et al , Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Teneligliptin and Metformin, Turk J Pharm Sci 2020;17(2):141-147.
14. Ashok H Akabari et al., Stability-Indicating TLC-Densitometric and HPLC Methods for Simultaneous Determination of Teneligliptin and Pioglitazone in Pharmaceutical Dosage Forms with Eco-Friendly Assessment, researchsquare, version 1, 2023.