Method Development and Validation for Simultaneous Estimation of Teneligliptin and Pioglitazone in Pure and Pharmaceutical Dosage Form by Using Rp-Hplc and Uv Spectroscopy

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ABSTRACT:
Ananalytical simple, reproducible and efficient reverse phase high performance liquid chromatographic method was developed for simultaneous determination of Teneligliptin and Pioglitazonein bulk and marketed pharmaceutical dosage forms. This Separation was carried out onSymmetry C_{18} (250 x 4.6mm, 5µm particle size) column in isocratic mode with mobile phase containing Methanol and Phosphate Buffer were taken in proportion of 60:40 v/v adjusted to pH 3.6 using ortho phosphoric acid. The flow rate was 1.0 ml/min and effluent was monitored at 330 nm. The retention time and linearity range for Teneligliptin and Pioglitazonewere 2.131 and 3.056 min respectively. The developed method was found to be accurate, precise and selective for simultaneous determination of Teneligliptin and Pioglitazone in bulk and marketed pharmaceutical dosage forms.
Keywords: Teneligliptin and Pioglitazone, RP-HPLC, Accuracy, Precision, Robustness.

I. INTRODUCTION

Teneligliptin is an amino acid amide. Teneligliptin has been investigated for the treatment of Type 2 Diabetes Mellitus. Teneligliptin is a long-acting, orally bioavailable, pyrrolidine-based inhibitor of dipeptidyl peptidase 4 (DPP-4), with hypoglycemic activity. Teneligliptin may also reduce plasma triglyceride levels through a sustained increase in GLP-1 levels [1]. Teneligliptin (INN; trade name Tenelia) is a pharmaceutical drug for the treatment of type 2 diabetes mellitus. It belongs to the class of anti-diabetic drugs known as dipeptidyl peptidase-4 inhibitors or "gliptins".Teneligliptin has unique J shaped or anchor locked domain structure because of which it has a potent inhibition of DPP 4 enzyme. Teneligliptin significantly controls glycemic parameters with safety [2]. No dose adjustment is required in renally impaired patients.Teneligliptin is a third generation DPP-4 inhibitor approved for treatment of type 2 diabetes. The mechanism of Teneligliptin is to increase incretin levels (GLP-1 and GIP), which inhibit glucagon release, which in turn increases insulin secretion, decreases gastric emptying, and decreases blood glucose levels [3]. The IUPAC name of Teneligliptin is [(2S, 4S)-4-[4-(5-methyl-2-phenyl pyrazol-3-yl) piperazin-1-yl] pyrrolidin-2-yl]-(1, 3-thiazolidin-3-yl) methanone. The Chemical Structure of Teneligliptin is shown in follows

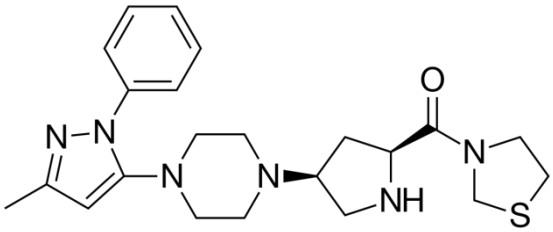

Fig-1: Chemical Structure of Teneligliptin

Pioglitazone is a member of the class of thiazolidenediones that is 1,3-thiazolidine-2,4-dione substituted by a benzyl group at position 5 which in turn is substituted by a 2-(5-ethylpyridin-2-yl)ethoxy group at position 4 of the phenyl ring [4]. It exhibits hypoglycemic activity. It has a role as an insulin-sensitizing drug, an EC 2.7.1.33 (pantothenate kinase) inhibitor, a xenobiotic, an EC 6.2.1.3 (long-chain-fatty-acid--CoA ligase) inhibitor, a ferroptosis inhibitor, a cardioprotective agent, a PPARgamma agonist, an antidepressant, a geroprotector and a hypoglycemic agent [5]. It is a member of thiazolidinediones, aromatic ether and a member of pyridines.Pioglitazone enhances cellular responsiveness to insulin, increases insulin-dependent glucose disposal, and improves impaired glucose homeostasis. In patients with type 2 diabetes mellitus, these effects result in lower

plasma glucose concentrations, lower plasma insulin concentrations, and lower HbA1c values [6]. The IUPAC name of Pioglitazone is 5-[[4-[2-(5-ethylpyridin-2-yl) ethoxy] phenyl] methyl]-1, 3-thiazolidine-2, 4- dione. The Chemical Structure of Pioglitazone is shown in follows

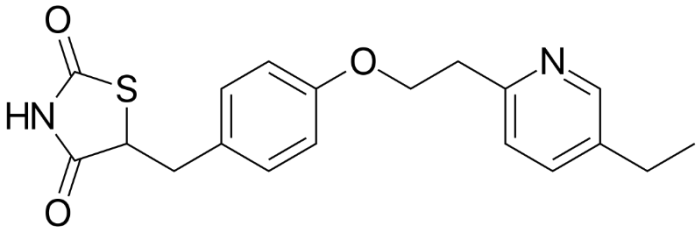

Fig-2: Chemical Structure of Pioglitazone

II. MATERIALS AND METHODS

Table-1: Instruments used

Sr. no.	Name of Instrument	Instrument Model	Name of manufacturer
1	UV-Visible double beam spectrophotometer	UV 1800	Elico
2	HPLC	717	Waters
3	Ultra Sonicator	--------	Entrech electronics limited
4	Vaccum filtration kit	---------	Labindia
5	pH Meter	pH-7000	Labindia

Table-2: Chemicals used

S.No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
3.	Potassium dihydrogen ortho phosphate	96%	A.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
5.	Hydrochloric acid	99.9%	A.R.	A.R Chemicals Pvt.Ltd
6.	Sodium Hydroxide	99.9%	A.R.	A.R Chemicals Pvt.Ltd
7.	3% Hydrogen Peroxide	99.9%	A.R.	A.R Chemicals Pvt.Ltd

Development and Validation of a Method forthe Simultaneous Estimation of Teneligliptin and Pioglitazone by UVin Bulk and Pharmaceutical Dosage Form

Selection of Solvent:
Ethanol was chosen as the typical solvent to prepare the solution. Based on the solubility studies [7].

Solubility Studies:

Table-3: Solubility Studies

S.No.	Solvent	Teneligliptin	Pioglitazone
1	0.1N HCL	Slightly Soluble	Soluble
2	Ethanol	Soluble	Soluble
3	n-butanol	Soluble	Slightly Soluble

Preparation of Working Standard Stock Solution:

Solutions containing individual drugs of teneligliptin and pioglitazone were prepared by taking 1mg of Teneligliptin and 1mg of Pioglitazone and weighed accurately and transferred into 100ml volumetric flask. The drugs were dissolved in methanol up to 100 ml to get a concentration of 1000 µg/ml of Teneligliptin and 1000 µg/ml of Pioglitazone.

Preparation of Working Standard Solution:

From the stock solution above, 5 mL solution was taken and diluted up to 100 ml with ethanol to get working standard solution of 50ug/ml Teneligliptin and 50µg/ml Pioglitazone. The solution was further used to prepare various dilutions having concentration range 10-50µg/ml using ethanol [8].

Selection of Wavelength:

For selection of λ max Standard drug solutions of Pioglitazone and Teneligliptin having concentration 10 µg/mL were scanned separately in double beam UV Visible spectrophotometer using ethanol as blank in the range of 200 - 300nm. Data was obtained by overlay spectra of Pioglitazone and Teneligliptin [9]. Data was obtained as 228nm λ max for Pioglitazone and 250nm λ max for teneligliptin.

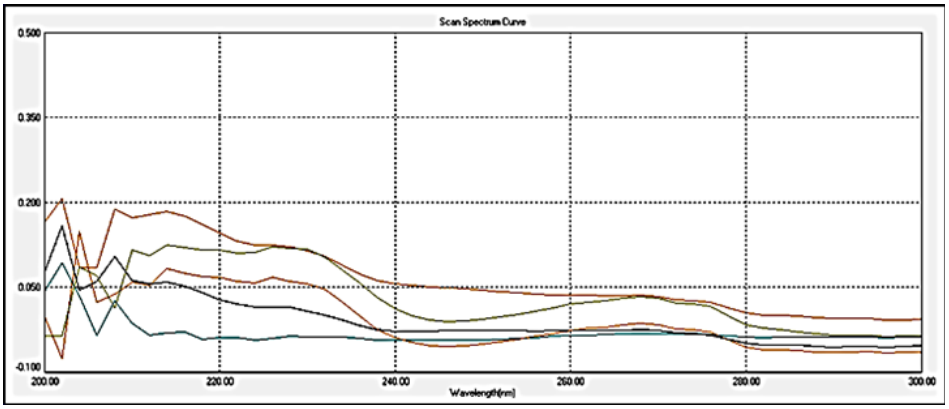

Fig-3: Overlay Spectra of Pioglitazone in Ethanol, 228nm

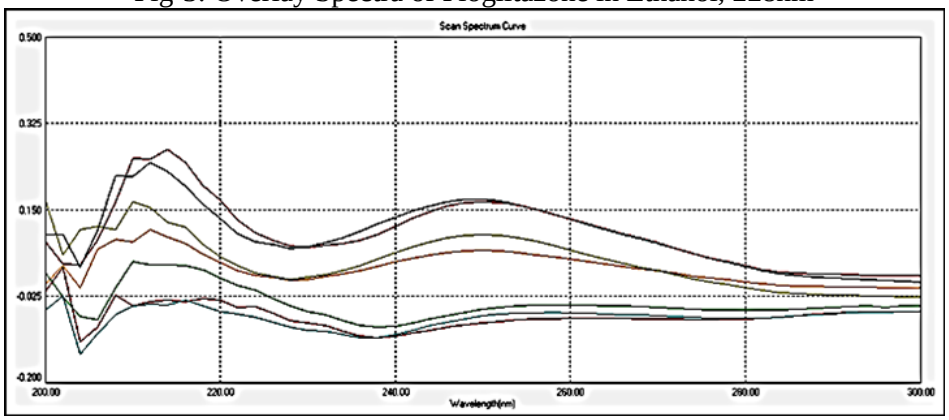

Fig-4: Overlay Spectra of Teneligliptin in Ethanol, 250nm

UV analysis for Development of Method and validation of developed method for Simultaneous determination of Teneligliptin and Pioglitazone:

Preparation of Standard Stock Solution of Teneligliptin

Accurately weighed 10mg of Teneligliptin and it was transferred to clean and dry 100 ml of volumetric flask and dissolved in Methanol : buffer (60:40) and made-up the volume to 100 ml with same solvent system. The final solution contained 100µg per ml of Teneligliptin solution.

Preparation of Standard Stock Solution of Pioglitazone

Accurately weighed Pioglitazone (10mg) was transferred to 100ml volumetric flask, dissolved in Methanol: buffer (60:40) and made-up the volume to 100 ml with same solvent system. The final solution contained 100 µg per ml of Pioglitazone solution.

Determination of Wavelength of Maximum Absorbance for Teneligliptin

Standard Teneligliptin solution (1ml) was transferred to separate 10 ml volumetric flask. The final volume was adjusted to 10 ml with the same mobile phase. The absorbance of the final resulted solution was scanned in the range 400 to 220 nm against mobile phase as blank [10].

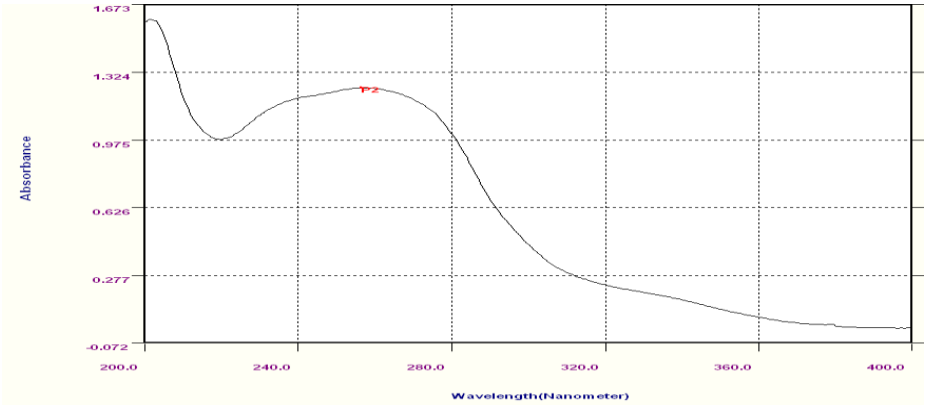

Fig-5: UV Spectrum of Teneligliptin (261 nm)

Estimation of Maximum Wavelength for Pioglitazone

First of all take 1ml of standard Pioglitazone solution from the above standard solution (1 ml) was transferred to separate clean and dry of 10 ml volumetric flask. The final volume was adjusted to 10ml with same mobile phase (Solvent). The absorbance of the final resulted solution was scanned in the range 400 to 220 nm against solvent mixture as blank. The results are shown in following figure-6.

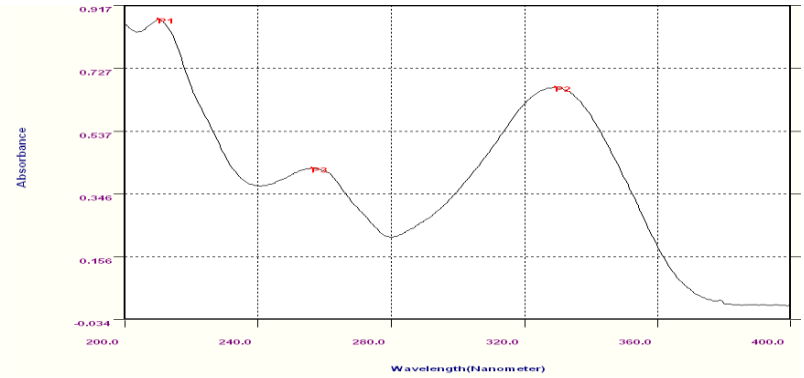

Fig-6: UV Spectrum of Pioglitazone (330 nm)

Method Development byHPLC:

Selection of Wavelength:

The λmax of the two ingredients i.e. Teneligliptin and Pioglitazone, were found to be 261 nm and 330 nm respectively in methanol as solvent system. As two drugs having almost near absorption max & at 261 nm Teneligliptin shows more intense as compare to Pioglitazone at 330 nm, 330 nm has been chosen as common absorption maximum for RP-HPLC analysis [11].

Preparation of Standard Solution of Teneligliptin

Weighed accurately 10mg of standard Teneligliptin and transferred into clean & dry 100 ml volumetric flask. Then 20 ml of mobile phase was added and sonicated to dissolve in 100ml of volumetric flask. The final volume was made up to the mark with same solvent. The final solution contained about 10µg/ml of Teneligliptin.

Preparation of Standard Solution of Pioglitazone

First 10 mg of Pioglitazone was weighed accurately and transferred into clean & dry 100 ml volumetric flask. Then 20 ml of mobile phase was added and sonicated to dissolve it in mobile phase. The final volume was

made up to the mark with same solvent. The final solution contained about 10µg/ml of Pioglitazone.

Initialization of the Instrument

The HPLC instrument was switched on. First the column was washed with the HPLC grade water for 45 minutes. After washing the column the column is saturated with the mobile phase in 45 minutes. The mobile phase was run to find the peaks or identification of peaks [12]. After 20 minutes the standard drug solution was prepared and injected in HPLC system.

Different Chromatographic Conditions used and their Optimizations

The various HPLC chromatographic conditions are used to fin the optimum chromatographic condition for best elution of drugs in the mixture [13].

Preparation of Mobile Phase

The mobile phase can be prepared by taking Methanol: Phosphate Buffer and maintained pH-3.6 with diluted orthophosphoric acid (60:40). The resulted Mobile phase was filtered through 0.45 µm membrane filter and degassed under ultrasonic bath. The final obtained mobile phase was pumped through the selected column and maintained at a flow rate of 1.0 ml/min [14].

Optimized Chromatographic Conditions:

Table-4: Optimized Chromatographic Condition

Mobile phase	**Methanol : Phosphate Buffer pH-3.6 with OPA (60:40% v/v)**
Wavelength	330 nm
Flow rate	1.0 ml/ min.
Auto Sampler Temperature	Ambient
Injection Volume	20µl
Run time	7 min.
Column	Symmetry C_{18} (250 x 4.6mm, 5µm particle size)
Column Temperature	Ambient

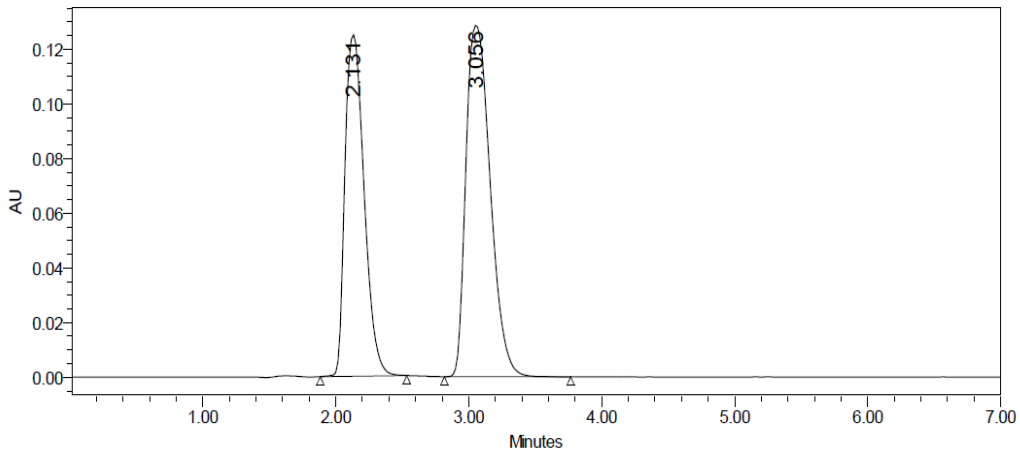

Fig-7: Optimized Chromatographic Condition

Analytical Method Validation:

Linearity and Range:

Standard solutions ofTeneligliptin in the concentration range of 0 µg/ml to 16 µg/ml were obtained by transferring (0.6, 0.8, 1.0, 1.2, 1.4, 1.6ml) of Teneligliptin stock solution (1000ppm) to the series of 10 ml volumetric flasks and standard solutions of Pioglitazone in the concentration range of 0 µg/ml to 35 µg/ml were obtained by transferring (0.6, 0.8, 1.0, 1.2, 1.4, 1.6ml) of Pioglitazone stock solution (1000ppm) to the separate series of 10ml volumetric flasks. The volumetric flasks were made up to the mark with methanol. The solutions were filtered through a 0.45 µm membrane filter and degassed under ultrasonic bath. The final resulted solutions were injected into HPLC the system. The run time/stop time maintained was 6 min and the various types of peak areas were measured [15]. The calibration data are shown in Table 5 and 6 and calibration curve data are shown in figure 8 and 9.

Table-5: Calibration Data for Teneligliptin

S. No.	Conc. (µg/ml)	Mean Peak Area
1	0	0
2	6	641233
3	8	844610
4	10	1052647
5	12	1250435
6	14	1465354
7	16	1662043

*Mean of three triplicate determinations

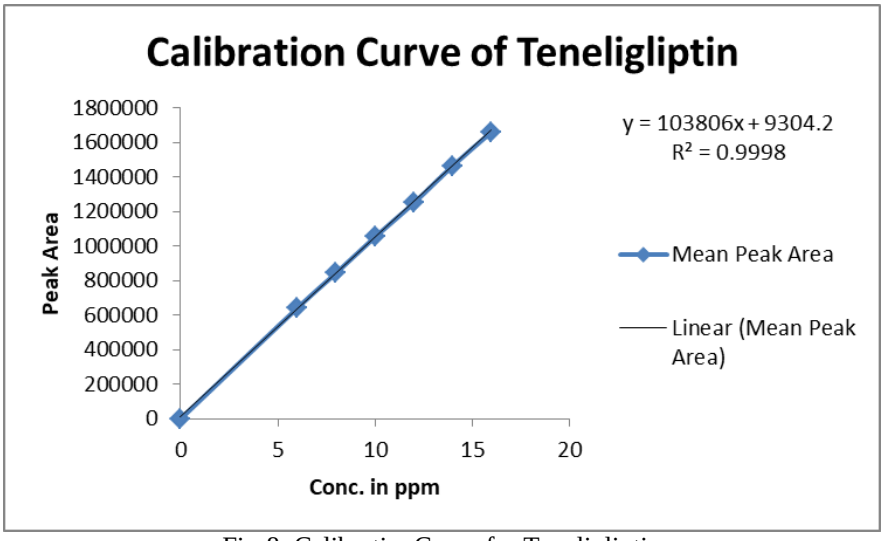

Fig-8: CalibrationCurve for Teneligliptin

Table-6: Calibration Data for Pioglitazone

Sr. No.	Conc. (µg/ml)	Mean Peak Area
1	0	0
2	6	628423
3	8	835412
4	10	1045742
5	12	1254033
6	14	1452471
7	16	1653504

* Mean of three triplicate determinations

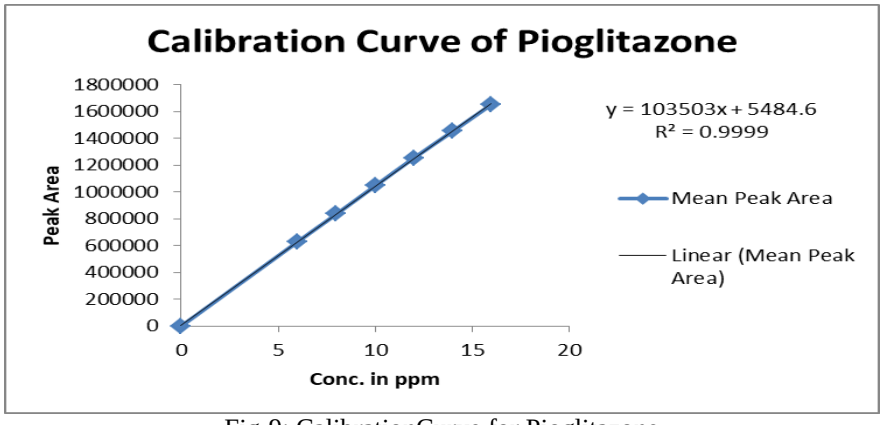

Fig-9: CalibrationCurve for Pioglitazone

Accuracy

Accuracy: Recovery Study of Teneligliptin

The accuracy of the proposed developed method the % recovery studies were carried out by adding different quantities (80%, 100%, and 120%) of pure drug of TENELIGLIPTIN was taken and added to the prepared pre-analyzed formulation of concentration 10µg/ml. From that % recovery values were measured [16-17]. The results were shown in Table-7.

Table-7: Data of Recovery Studies for Teneligliptin

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	8	8.105	93435	101.312	Mean= 100.0163% S.D. = 1.293505 % R.S.D.= 1.293294
S_2 : 80 %	8	7.898	91287	98.725	
S_3 : 80 %	8	8.001	92356	100.012	
S_4 : 100 %	10	10.195	115135	101.95	Mean= 101.4033% S.D. = 0.613379 % R.S.D.= 0.60489
S_5 : 100 %	10	10.152	114687	101.52	
S_6 : 100 %	10	10.074	113879	100.74	
S_7 : 120 %	12	12.171	135647	101.425	Mean= 100.6053% S.D. = 0.730041 % R.S.D. = 0.725649
S_8 : 120 %	12	12.044	134324	100.366	
S_9 : 120 %	12	12.003	133897	100.025	

Accuracy: Recovery Study of Pioglitazone

To determine the accuracy of the given developed method the percentage recovery studies were carried out by adding different quantities (80%, 100%, and 120%) of pure drug of Pioglitazone was taken and added into the pre-analyzed formulation of concentration 10µg/ml. From that % recovery values were determined [18]. The results were shown in Table-8.

Table-8: Data of Recovery Studies for Pioglitazone

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	8	8.100	89325	101.25	Mean= 100.1207% S.D. = 1.251602 % R.S.D.= 1.250093
S_2 : 80 %	8	8.027	88569	100.337	
S_3 : 80 %	8	7.902	87279	98.775	
S_4 : 100 %	10	10.122	110254	101.22	Mean= 101.44% S.D. = 0.330454% R.S.D.= 0.325763
S_5 : 100 %	10	10.128	110312	101.28	
S_6 : 100 %	10	10.182	110874	101.82	
S_7 : 120 %	12	12.147	131215	101.225	Mean= 101.444% S.D. = 0.284828 % R.S.D. = 0.280774
S_8 : 120 %	12	12.161	131356	101.341	
S_9 : 120 %	12	12.212	131879	101.766	

Precision

Repeatability

Repeatability was assessed using six time repetition of working concentration [19-20]. The results are shown in Tables 9 and 10.

Table-9: Data Showing Repeatability Analysis for Teneligliptin

HPLC Injection Replicates of Teneligliptin	Area Under the Curve
Replicate – 1	1013546
Replicate – 2	1025824
Replicate – 3	1012351

Replicate – 4	1036584
Replicate – 5	1015419
Replicate – 6	1008572
Average	**1018716**
Standard Deviation	**10495.73**
% RSD	**1.03029**

Table-10: Data Showing Repeatability Analysis for Pioglitazone

HPLC Injection Replicates of Pioglitazone	**Area Under the Curve.**
Replicate – 1	1035681
Replicate – 2	1065897
Replicate – 3	1078953
Replicate – 4	1058748
Replicate – 5	1078754
Replicate – 6	1065871
Average	**1063984**
Standard Deviation	**15986.99**
% RSD	**1.50256**

Intermediate Precision:

Intra-Assay & Inter-Assay:

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Teneligliptin and Pioglitazone revealed that the proposed method is precise [21-22].

Table-11: Results of Intra-Assay & Inter-Assay Teneligliptin

Conc. of Teneligliptin (API) (µg/ml)	**Observed Conc. of Teneligliptin (µg/ml) by the Proposed Method**			
	Intra-Day		**Inter-Day**	
	Mean (n=6)	**% RSD**	**Mean (n=6)**	**% RSD**
8	8.03	0.25	9.95	0.23
10	10.05	0.36	10.02	0.32
12	11.14	0.14	12.06	0.19

Table-12: Results of Intra-Assay & Inter-Assay for Pioglitazone

Conc. of Pioglitazone (API) (µg/ml)	**Observed Conc. of Pioglitazone (µg/ml) by the Proposed Method**			
	Intra-Day		**Inter-Day**	
	Mean (n=6)	**% RSD**	**Mean (n=6)**	**% RSD**
8	8.05	1.02	9.59	0.98
10	9.94	0.74	10.09	0.56
12	11.97	0.35	12.04	0.32

Method Robustness:

The influence of small changes in optimized chromatographic conditions such as changes in flow rate (± 0.1ml/min), Temperature (±2^0C), Wavelength of detection (±2nm) & Acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table-13, RSD (%) < 2%) the proposed RP-HPLC method was used for the

analysis of Teneligliptin (API) [23-25].

Table-13: Result of Method Robustness Test

Change in Parameter	% RSD
Flow (1.1 ml/min)	1.06
Flow (0.9 ml/min)	0.69
Temperature (27^0C)	0.45
Temperature (23^0C)	0.56
Wavelength of Detection (332 nm)	0.28
Wavelength of detection (298 nm)	0.14

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^0C), Wavelength of detection (±2nm) & Acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table-14, % RSD < 2%) the developed RP-HPLC method for the analysis of Pioglitazone (API) [26].

Table-14: Result of Method Robustness Test

Change in Parameter	% RSD
Flow (1.1 ml/min)	0.03
Flow (0.9 ml/min)	0.08
Temperature (27^0C)	0.19
Temperature (23^0C)	0.73
Wavelength of Detection (330 nm)	0.82
Wavelength of detection (298 nm)	0.46

Limit of Detection and Limit of Quantification

The limit of detection and limit of quantization (LOD and LOQ) can be determined by the following equations. These equations are based on the signal to noise ratio. These two equations are useful for the determination of LOD and LOQ [27-29].

L.O.D. = 3.3 (SD/S).
L.O.Q. = 10 (SD/S)

Where,

SD = Standard deviation Response

S = Slope of the Calibration curve

The slope S and standard deviation response values are obtained from the calibration curve of the analyte (Drug) [30].

III. RESULT & DISCUSSION

The LOD was found to be 0.607 µg/ml and 1.821 µg/ml and LOQ was found to be 0.451 µg/ml and 1.353 µg/ml for Teneligliptin and Pioglitazone respectively which represents that sensitivity of the method is high.

System Suitability Parameter

This includes the type of equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be examinated. The following system suitability test parameters were determined [31]. The obtained data are shown in Table 15.

Table-15: Dataof System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Resolution	Rs > 2	3.56
2	Asymmetry	T ≤ 2	Teneligliptin =0.17 Pioglitazone =0.61
3	Theoretical plate	N > 2000	Teneligliptin =3698 Pioglitazone= 4926

Determination of Teneligliptin and Pioglitazone in Pharmaceutical Dosage form

Each tablet contains: 20/15mg. 20 tablets were taken and the I.P. method was followed to measure the average weight. Above weighed tablets were finally powdered and triturated well by using mortar and pestle. A quantity of powder equivalent to 100 mg of drug were calculated and transferred to clean & dry 100ml volumetric flask, and add 70 ml of HPLC grade methanol and solution was sonicated for 15 minutes by using Sonicator. Then the volume was made up to 100 ml with same solvent. Then finally 10ml of the above solution was diluted up to 100ml with HPLC grade methanol or same solvent. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From this stock solution (0.1 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system [32].

The solution prepared was injected in five replicates into the HPLC system and the observations were recorded.

A duplicate injection of the standard solution (without drug) was injected into the HPLC system and the peak areas were recorded [33]. The data are shown in Table-16.

ASSAY:

Assay % =

$$\% \text{ Assay}=AT/AS\times WS/DS\times DT/WT\times P/100\times AW/LC\times 100$$

Where:

AT = Peak Area of sample obtained with sample preparation
WS = Weight of working standard taken in mg
AS = Peak Area of standard obtained with standard preparation
WT = Weight of sample taken in mg
DS = Dilution of Standard solution
P = Percentage purity of working standard
DT = Dilution of sample solution

The assay was performed explained in above chapter. Results obtained are tabulated in below:

Table-16: Recovery Data for Estimation Teneligliptin and Pioglitazone in Pharmaceutical Dosage form

Brand name of Tablets	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	Assay + % RSD
Zita Plus-Pio Tablet (Glenmark Pharmaceuticals Ltd)	20/15	19.569 (± 0.268) /14.396 (± 0.274)	99.637/99.578 (± 0.635)

Result & Discussion: The assay of Zita Plus-Pio Tablet containing TENELIGLIPTIN AND PIOGLITAZONE was found to be 99.637% and 99.578% respectively.

Stability Studies:

The results of the forced degradation studies indicated the specificity of the developed method that has been developed. Teneligliptin and Pioglitazone were stable in thermal and photolytic stress conditions [34]. The results of stability studies are given in the following Table-17.

Table-17: Results of Stress Studies of Teneligliptin and Pioglitazone API

Stress Condition	Time (hours)	Assay of Active Substance	Assay of Degraded Products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	93.05	6.95	100.00
Basic Hydrolysis (0.IN NaOH)	24Hrs.	97.11	2.89	100.00
Thermal Degradation (50 ^{0}C)	24Hrs.	63.22	36.78	100.00
UV (254nm)	24Hrs.	87.65	12.35	100.00
3% Hydrogen peroxide	24Hrs.	96.44	3.56	100.00

SUMMARY AND CONCLUSION

Summary:

From the results shown in system suitability the %RSD for retention times, peak areas and number of theoretical plates and tailing factor were found to be within limits i.e.,%RSD for retention times not more than 2.0%, peak areas not more than 2.0% and number of theoretical plates not less than 2000 and tailing factor for not more than 2.0, so they had method passed system suitability.

From the results shown in precision tables it was found that % RSD is not more than 2%; which indicates that the proposed method has good reproducibility.

In the case of accuracy 80%, 100% and 120% of solutions with respect to target assay concentrations the percentage recovery for each levels are between 98.0 % to 102%. It indicates the method was accurate and also reveals that the commonly used excipients and additives present in the pharmaceutical formulations were not interfering the proposed method.

From the results shown in Linearity table it was found that the method was linear and the correlation coefficient is not less than the 0.9999.

In case of the LOD and LOQ the S/N ratios are within the limits for Teneligliptin / Pioglitazone.

Conclusion:

A RP-HPLC method is developed and validated as per ICH guidelines for simultaneous estimation of Teneligliptin and Pioglitazone in bulk form and Marketed Pharmaceutical Dosage forms.

In present study an attempt has been made to modify experimental condition, in order to estimate simultaneously the drugs in combination. The mobile phase was selected after trying various combinations of polar solvents. The proportion of solvents and variation of buffers was found to be quite critical as slight variation in it adversely affected the resolution of peaks. Considering all the fact the following parameter were finally fixed for this method:

Equipment : High performance liquid chromatography equipped with Auto Sampler and UV detector.
Column : Symmetry C18 (4.6 x 150mm, 5µm, Make: XTerra) or equivalent
Mobile phase : Phosphate buffer (pH-3.6) 40%: Methanol 60%.
Mode : Isocratic
Flow rate : 1.0 mL per min
Wavelength : 330 nm
Injection volume : 20 µl
Column oven : Ambient
Run time : 7min

The proposed method was found to be rapid, accurate, precise, specific, robust and economical. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. This method is also having an advantage than reported method that the retention time of both the drugs is below 4 mins and both the drugs can be assayed with the short time. Thus the method is not time consuming and can be used in laboratories for the routine analysis of combination drugs.

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