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Stability Indicating Method Development and Validation of Teneligliptin and Pioglitazone using UHPLC in Pure and Pharmaceutical Formulation.

Sameer Narayan Godase[1], Mahesh Hari Kolhe[1], Shubham Balasaheb Mhaske[1], Kavita Vitthalrao Dhamak[1], Rohit Jaysing Bhor[2].

[1]Department of Quality Assurance Techniques, Pravara Rural College of Pharmacy, Pravaranagar, Loni, Maharashtra, India. 413736.

[2]Department of Pharmaceutical Chemistry, Pravara Rural College of Pharmacy, Pravaranagar, Loni, Maharashtra, India 413736.

Correspondence:
Mrs. K.V. Dhamak
Department of Pharmaceutical Quality Assurance, Pravara Rural College of Pharmacy, Pravaranagar, Loni-413736, Maharashtra, INDIA.

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KEYWORDS:

Teneligliptin, Pioglitazone, Method Development, Validation, Stability Study.

ABSTRACT:

Introduction: Teneligliptin and Pioglitazone in pharmaceutical combined form can use to treat and control the type 2 diabetes mellitus.

Objective: The objective is to develop a novel, easy, fast, accurate UHPLC method and validate it as per the ICH guidelines. This method aims to determine both Teneligliptin and Pioglitazone in pure and pharmaceutical formulation.

Method: Separation of analyte was carried out using an Agilent C18 (2.5µm; 4.6 x 100 mm ID) column connected to a PDA detector. The moving phase consists of methanol and TEA in (60:40) with flow rate 0.9 ml per minute. Analytes were detected at 241 nm wavelength.

Result: Under optimized condition the retention time was found to be 2.382 for Teneligliptin and 3.315 for Pioglitazone with sharp peak. The linearity of a method was found to be 2-10µg/ml and 1.5-7.5µg/ml for Teneligliptin and Pioglitazone respectively having a coefficient of correlation (R2) 0.999. Teneligliptin's Limit of Quantification and Limit of Detection were determined to be 0.0843 and 0.255 and 0.0084 and 0.025 for Pioglitazone. The procedure is accurate, as demonstrated by the founded good percent recovery.

Conclusion: The method was developed and validated as per guidelines of International Conference on Harmonization. Forced degradation study of both the drugs was done in controlled acidic, basic, peroxide and hydrolysis condition. The develop method shows its suitability for the regular quantitative analysis of both Teneligliptin and Pioglitazone in their pure form or in pharmaceutical formulation. This application of method serves for the purpose of quality control.

INTRODUCTION:

Endocrinological disorder, particularly Diabetes MellitusType 2 (DMT2) possess an escalating health challenge globally.Study shows that a high

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rise with diabetic cases anticipated to surpass 500 million by 2030 and exceeds 700 million by 2045.This heterogeneous metabolic disorder disrupts the carbohydrate, protein and lipid metabolism.Treatment approaches often involve the combination of oral hypoglycemic drugs with diverse mechanism to achieve better glycemic control preferred over monotherapy. Type 2 diabetes arises due to insulin resistance or insufficient insulin production in body. Type I, type II, and gestational diabetes are the three main categories of diabetes. The majority of people with diabetes (90%) have type II diabetes.[1-2] When monotherapy for type II diabetes does not work, combined treatment is often recommended. The FDA has authorized the use of teneligliptin (TEN) and pioglitazone (PIO) together to treat type II diabetes.[3]

Dipeptidyl peptidase-4 inhibition done by the gliptins, include the antidiabetic medication teneligliptin. It is {(2S,4S)-4-[4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-1-piperazinyl]-2-pyrrolidinyl}(1,3-thiazolidin-3-yl) methanone (fig.1). Teneligliptin suppresses postprandial hyperglycemia after meals, which elevates activated glucagonlike peptide-1 (GLP-1) levels and acts for 24 hr. [4-5] Teneligliptin's distinct structure, which is made up of five successive rings, gives it a lasting and powerful impact.Patients with renal impairment do not require a particular dosage modification because the drug's metabolites are excreted through the liver and kidneys.[6]

Pioglitazone functions as an insulin sensitizer, chemically described as (RS)-5-(4-[2-(5-ethylpyridin-2-yl) ethoxy] benzyl) thiazolidin-2,4-dione, an oral type II diabetes medication (fig.2). This type of diabetes arises from insufficient insulin production in the body. Pioglitazone triggers the ligand-activated transcription factor PPARgamma stimulating cell differentiation while inhibiting cell growth and angiogenesis. Pioglitazone boosts insulin sensitivity by increasing cellular responsiveness.The patients with type II diabetes, pioglitazone primarily enhances peripheral insulin sensitivity for better glycemic control. Additionally, when paired with a sulfonylurea or insulin, it maycause edema and carries potential side effects such as heart failure and respiratory infections.[7-8]

In the literature survey, we found that there are several spectroscopic & liquid chromatographic procedures for the determination of Teneligliptin and Pioglitazone by HPLC,[9] RP-HPLC,[10-14] HPTLC,[15-17] UV,[18-20]and there is no UHPLC method reported.

Hence, our proposal involved creating an efficient UHPLC technique for concurrently determining Teneligliptin and pioglitazone in both pure and pharmaceutical formulations. This research is focused on developing and validating a rapid, sensitive UHPLC method that offers improved resolution and peak symmetry, adhering to ICH guidelines during validation.

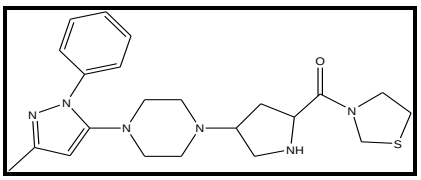

Figure 1: Structure of Teneligliptin.

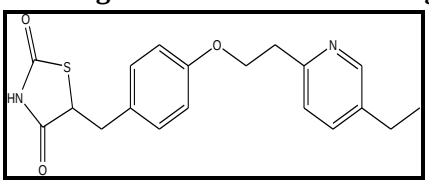

Figure 2: Structure of Pioglitazone.

MATERIALS AND METHODS:

Reagent and Reference Samples:

The standard samples of teneligliptin and pioglitazone obtained as a gift sample from Swapnroop Drugs and Pharmaceuticals, Aurangabad. The chemicals,reagents,double distilled water and Mili-Q water which are used in analysis are of HPLC grade. A combined fixed dose tablet formulation Zita plus Pio (Glenmark

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Pharmaceuticals Ltd.) containing Teneligliptin 20 mg and Pioglitazone 15 mg is used. This medication is using to treat Type II Diabetes Mellitus. Calibrated glassware's and analytical balancewere used during the study. For UHPLC study,were utilized.

Instrumentation:

An Agilent 1100 HPLC system inbuilt with reciprocating pump (HP- 1100) and coupled with UV detector. We utilized the chemstation software to analyse the HPLC reports. An Agilent C18 column (2.5µm; 4.6 x 100 mm ID) was used. The Digital weighing balance (ME-204)and the digital pH meter by Mettler-Toledo was obtained from Mumbai, India. The ultra-sonicator Labman was used during sampling. Nylon membrane filters with pore size 0.20µ and 0.45µ were sourced from Phenomenex, Mumbai, India.

Determination of working Wavelength:

The UV- Spectrophotometric was used to identify the wavelengths at which both the drug shows significant absorption. The wavelength where both the drugsexhibit the highest absorbance or a strong absorption peak,there isobestic pointis typically chosen for HPLC analysis. figure 3.

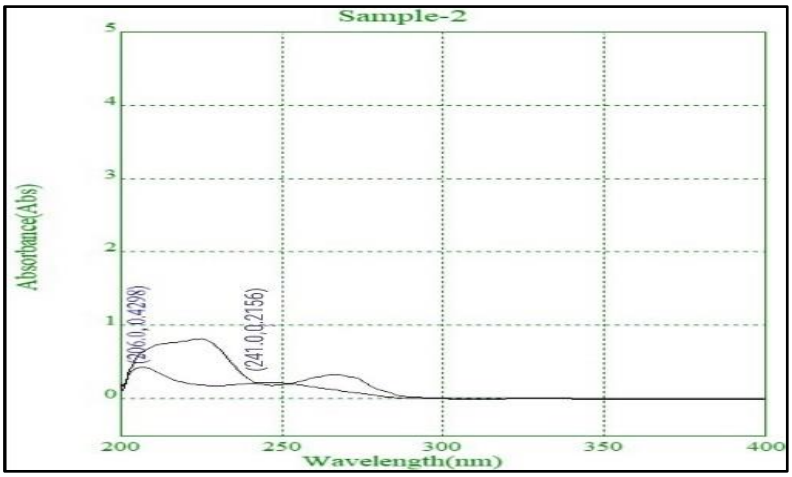

Figure 3.Overlaying Spectrum of TEN and PIO

METHODOLOGY:

Preparation of Stock Standard Solutions:

20mg of Teneligliptin and 15mg of Pioglitazone were weighed and dissolved in 100 ml methanol to get200µg/ml and 150µg/ml respectively. Removal of air bubbles and small particles solubilize by sonicating the both samples for2-5 min. Then serial of homogeneous mixture consisting Teneligliptin (200µg/ml) and Pioglitazone (150µg/ml) were made as needed and used to determine robustness, accuracy, and repeatability and other validation parameters.

Preparation of Sample Solution:

Weigh 20 tablets and determine their average weight. Using a mortar and pestle tablets grinded into powder. The resulting powder was weighed and put into a 100ml volumetric flask, equal to 15mg of Pioglitazone and 20mg of Teneligliptin. After adding 100 mL of methanol diluent, the small particles dissolved by sonicating the sample for 15 min. The volume was further adjusted with diluent in order to achieve a strength of 150µg/ml for Pioglitazone and 200µg/mL for Teneligliptin.

Preparation of Buffer:

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To create a 0.1% triethylamine buffer, 0.1gm of triethylamine was diluted to 100ml using HPLC-grade water and PH is modified to 6 with OPA.

Mobile Phase:

The moving phase consisting of methanol and 0.1 % TEA (PH 6 WITH OPA) having ratio 60:40 v/v.

Optimized Chromatographic Conditions:

The optimized chromatographic conditions yielded results as presented in table 1. All system suitability attributes, including theoretical plates, tailing factor and retention time, have to meet the specified acceptance standards.

Table 1: Optimized chromatographic conditions.

Parameters	Chromatographic Condition
Mode of elution	Isocratic
Mobile Phase	Methanol: 0.1 % TEA (PH 6 WITH OPA); (60:40)
Column	Agilent C18 (ID 2.5µm; 4.6 x 100 mm)
Flow Rate	0.9ml/min
Runtime	10 min
Injection Volume	20µL
Detection Wavelength	241
Temperature	33^0C
Retention Time	2.382 for TEN and 3.315 for PIO

Method Validation:

According to ICH guidelines, method validation was completed.

System Suitability Studies:

A uniform mixture of a recently prepared stock solution containing equal concentrations of Teneligliptin (20 ppm) and Pioglitazone (15 ppm) was subjected to five injections in order to assess the consistency of results with respect to the relative standard deviation (RSD), which should consistently remain below 2%. Furthermore, all system suitability attributes, including theoretical plates, tailing factor and retention time were evaluated. Table 2 summarize of these system suitability parameters.

Table 2: Summary of system suitability parameters.

Parameters	Teneligliptin	Pioglitazone	Acceptance criteria
Tailing factor	0.78	0.57	≤2
Retention time	2.382	3.315	≥2
Theoretical plates	6503	4323	≥2000

Intraday Precision and Interday Precision:

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Peak areas were measured after sample solutions were injected three times on three separate days, all under optimal conditions. It is not acceptable for the RSD% to exceed 2 for the peak areas of the three standard injection results. The method demonstrates satisfactory precision, with a relative standard deviation (RSD) not exceeding 2%. In interdayprecisionthe peak areas of three replicates of the sample solutions were measured the same day they were injected under ideal circumstances. The three replicate injection results' peak areas' RSD% shouldn't be more than 2. The %RSD for both studies was calculated and illustrated in table 3.

Table 3. Intraday and Interday Precision

Concentration (µg/ml)	Teneligliptin %RSD		Concentration (µg/ml)	Pioglitazone %RSD	
	Intraday	Interday		Intraday	Interday
6	0.04	0.02	4.5	0.10	0.05
8	1.41	0.05	6	0.04	0.04
10	1.11	0.01	7.5	0.02	0.11

Accuracy:

Recovery experiments involve the addition of measured quantity of pure standard drug to the sample solution and evaluation of method's accuracy assessed by measuring its recovery from the peak areas. Standard was added to the sample at concentrations of 80%, 100%, and 120% of the test. For every level, the recovery percentage should range from 98% to 102%. Teneligliptin and Pioglitazone recoveries fell within the range of 101.55-99.50% and 101.11-101.42% respectively. In accordance with established guidelines, theresults have been expressed in percentages as illustrated in table 4**.** Therefore, from results the process is said to be accurate.

Table 4: Recovery values of Teneligliptin and Pioglitazone.

Drug	Level	Analyte amount (mg)	Recovery amount (mg)	Mean % recovery	RSD %
Teneligliptin	80%	1.6	1.62	101.55	0.08
	100%	2	2.057	102.87	0.04
	120%	2.4	2.38	99.46	0.03
Pioglitazone	80%	1.2	1.21	101.11	0.16
	100%	1.5	1.52	101.61	0.09
	120%	1.8	1.82	101.54	0.08

Linearity:

The preparation of five distinct concentration calibration standards in five replicates allowed for the determination of the method's linearity. Graphs are plotted where the y-axis represents peak areas and the X-axis represents concentrations. The quantification exhibited linearity within the 2-10µg/ml range of concentration for Teneligliptin

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and achieving a 0.9993 correlation coefficient as illustrated in figure 4. Similarly for Pioglitazone, Linearity was noted across the concentration range of 1.5 to 7.5 µg/ml, with correlation coefficient 0.999 as depicted in figure 5. The linearity results have been summarized in table 5.

Table 5: Linearity values of Teneligliptin and Pioglitazone.

Parameters	Teneligliptin	Pioglitazone
Linearity range (µg/mL)	2-10	1.5-7.5
Regression coefficient ± SD	0.999± 13.89	0.999±1.20
Slope ± SD	543.34 ±13.89	472.55 ± 1.20
Intercept ± SD	406.2± 13.89	160.5 ± 1.20

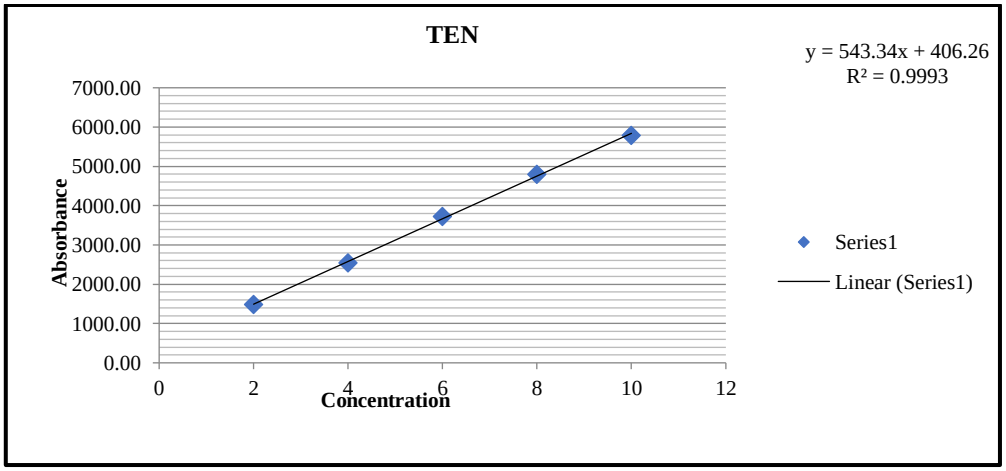

Figure 4: Linearity plot of Teneligliptin.

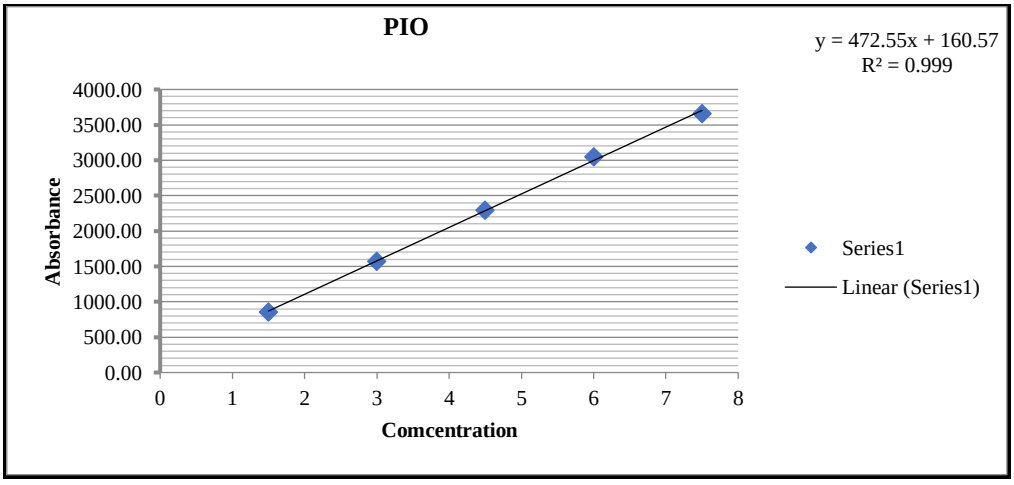

Figure 5: Linearity plot of Pioglitazone.

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Robustness:

The chromatographic methods robustness was evaluated by deliberately introducing minor changes to the chromatographic conditions. Variation in a flow rate, pH, wavelength and mobile phase were specifically evaluated, calculated and summarized in table 6.

Table 6: Robustness values of Teneligliptin and Pioglitazone.

Sr. No	Chromatographic Condition	Changes	%RSD of TEN	%RSD of PIO
1.	Flow (-0.1ml/min)	0.8	0.05	0.47
2.	Flow(+0.1ml/min)	1.0	0.03	0.03
3.	M.P (-0.1ml/min)	59+41	0.29	0.28
4.	M.P (+0.1ml/min)	61+39	0.11	0.17
5.	Wavelength (-1)	240	0.06	0.04
6.	Wavelength (+1)	242	0.08	0.01

LOD AND LOQ:

LOD means limit of detection represents the minimum detectable concentration, although it may not be precisely quantified and LOQ stands for limit of quantification refers to minimum quantity of an analytic agent within the solution that can be precisely and accurately evaluated by the formulas mentioned below,

$LOD = 3.3\ \sigma / S.$

$LOQ = 10\ \sigma / S.$

where σ = Standard Deviation; S = Slope.

To establish the detection limit and quantification limit calculations, depends on the slope and standard deviation (SD) values, and these limits have been documented in Table 7.

Table 7. LOD and LOQ Values of TEN and PIO

Sample	LOD	LOQ
Teneligliptin	0.084358878	0.25563296
Pioglitazone	0.00841159	0.025489666

Forced Degradation Study:

In a force degradation study, the sample is exposed to a variety of stress conditions, including oxidative, neutral, basic and acid degradation for potential degradation that may occur during the storage conditions. The aim is that to understand the how subject reacts, changes or degrades under various stress conditions. Force degradation study mainly conducted in pharmaceutical research to evaluate the stability and degradation pathway of drug and drug products.

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Acid Degradation:

0.3 ml of stock solution was mixed with 5ml of 0.1N HCL was added followed by makeup volume upto 10 ml with mobile phase. The resulting stock solution allowed to remain exposed for 24 hr. After 24 hrs 0.1 N NAOH was used to neutralized the before injection.

Base Degradation:

Mix 0.3 ml of stock solution with 5ml of 0.1N NAOH and adjust the volume upto 10ml with mobile phase. Let the stock solution exposed for 24 hr. Afterward, neutralized the sample with 0.1 N HCL prior injection.

Oxidative (H_2O_2)Degradation:

0.3 ml of stock solution is added in 10ml volumetric flask with 5ml of 6% H_2O_2. Then

volume is adjusted to 10ml with mobile phase and stock solutions is exposed for 24 hr. Then analysis was carried out.

Neutral:

Combine the 0.3 ml of stock solution of with 5ml of water. 10 ml volume is made up with mobile phase in volumetric flask. After exposing the stock solutions for 24 hr proceed for the analysis.

In the forced degradation study standard solution is subjected to different stress conditions as outlined in the procedure. Acidic condition shows 1.60% and 2.07% degradation ofTeneligliptin and Pioglitazone illustrated in figure 6. 3.14% and 4.37% degradation ofTeneligliptin and Pioglitazone occurred in alkaline condition shown in figure 7. Oxidative conditions resulted about 6.02% and 21.15% degradation for teneligliptin and Pioglitazone as in figure 8. Neutral conditions incurred less than 1% degradation forteneligliptin and Pioglitazone shown infigure 9. The comprehensive outcomes of the forced degradation study are showed in table 8.

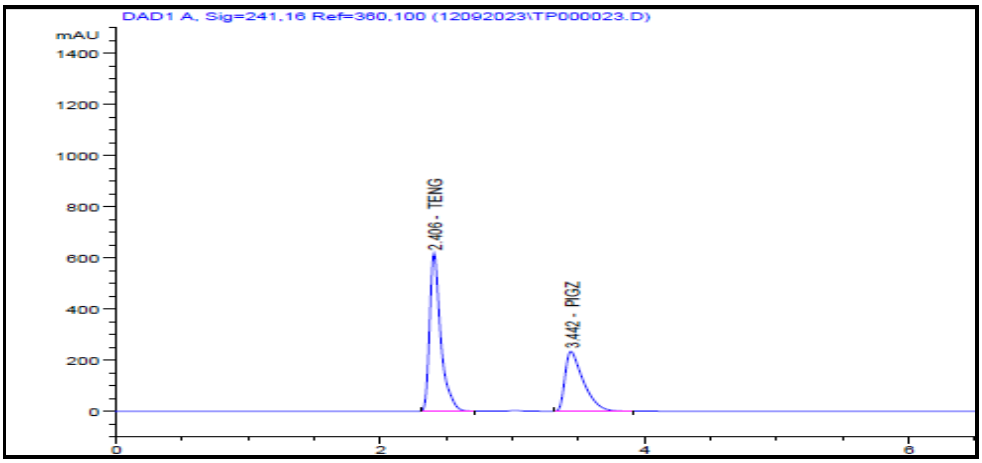

Fig 6. HCL Degradation

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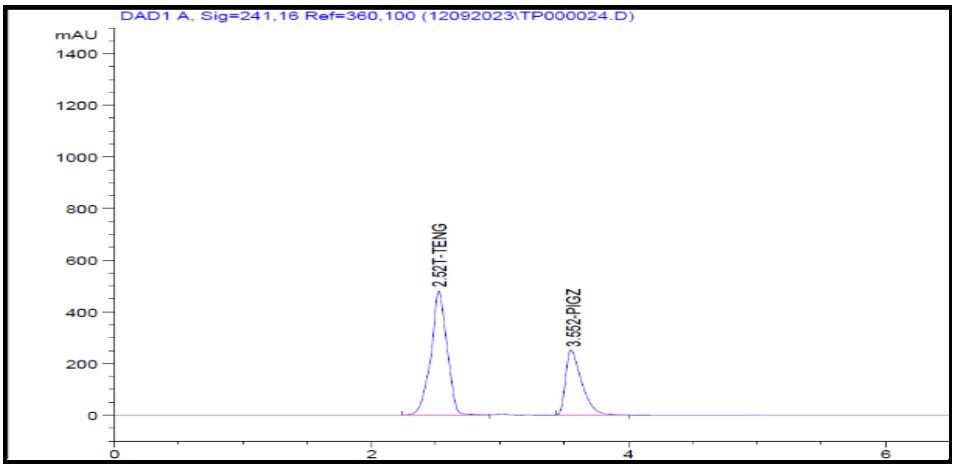

Fig 7. NaOH Degradation

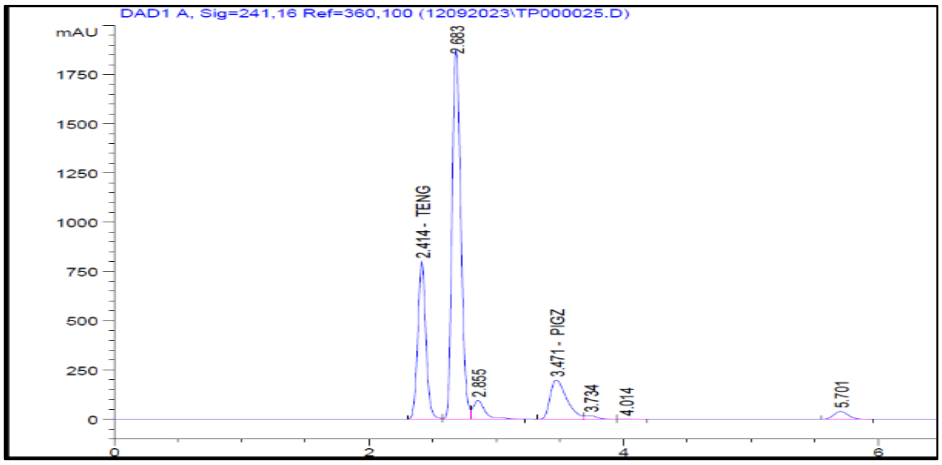

Fig 8. H_2O_2 Degradation

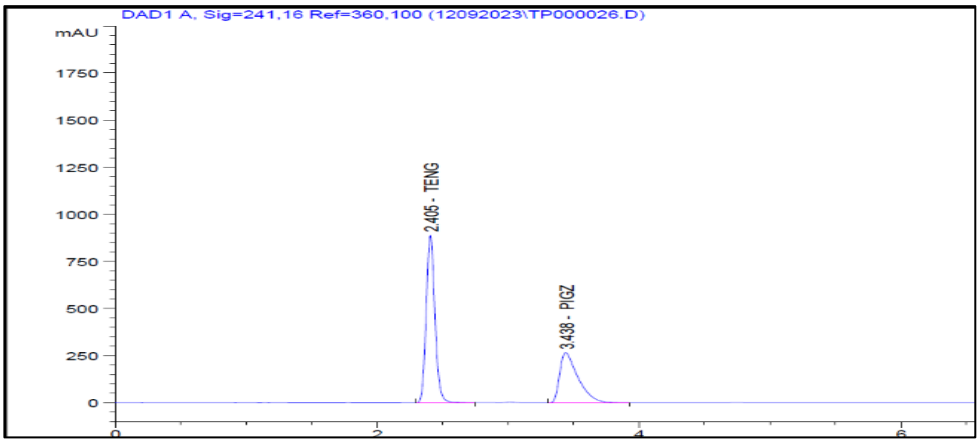

Fig 9. Neutral

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Table 8. Degradation study of Teneligliptin and Pioglitazone.

Degradation Condition	Retention Time		% Recovery		%Degradation	
Name	**TEN**	**PIO**	**TEN**	**PIO**	**TEN**	**PIO**
0.1N HCL	2.406	3.442	98.40	97.93	1.60	2.07
0.1N NaOH	2.522	3.552	96.86	95.63	3.14	4.37
3%H_2O_2	2.414	2.414	93.98	78.85	6.02	21.15
Neutral	2.405	3.438	99.42	99.39	0.58	0.61

DISCUSSION:

This linearity of this UHPLC method demonstrated between the range of 2-10µg/ml and 1.5-7.5µg/ml for teneligliptin and pioglitazone respectively. The method validation is carried out successfully in the optimized conditions and validation results for various parameters were within the acceptable limits. Sample preparation done with the dilution and filtration of sample. The determination was achieved by using column Agilent C18, 4.6 x 100mm; 2.5µm consisting an isocratic mobile phase of Methanol: 0.1% TEA (PH-6 WITH OPA) 60: 40v/v at a rate of flow 0.9ml/min. Retention time of teneligliptin is 2.382 min and for pioglitazone is 3.315 min. The lower quantification limit (LOQ) was 0.255µg/ml, 0.025µg/ml for teneligliptin and pioglitazone respectively and lower limit of detection (LOD) were found to be 0.084µg/ml, 0.0084µg/ml for both teneligliptin and pioglitazone respectively. Validated was conducted by following the ICH guidelines. Determination of teneligliptin and pioglitazone has been successfully done by this method for pure drug and tablet formulations.

CONCLUSION:

The UHPLC method was developed and validated for the simultaneous determination of teneligliptin and pioglitazone in pure form and formulation form offering the speed, convenience, precision and accuracy. This method considered more cost effective as compared to other reported method. It is suitable for the tablet analysis, making it a viable option routine quality control of teneligliptin and pioglitazone in these pharmaceutical formulations.

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FUNDING:

None.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest.

ABBREVIATIONS:

ICH: International Council for Harmonisation; UHPLC: Ultra High-Performance Liquid Chromatography; TEA: Triethylamine; OPA: Ortho-Phosphoric Acid, DMT2: Diabetes Mellitus Type 2; FDA: Food and Drug Administration; TEN – Teneligliptin; PIO- Pioglitazone; HPLC: High Performance Liquid

Chromatography; HPTLC: High-Performance Thin-Layer Chromatography; RP-HPLC: Reverse Phase High Performance Chromatography; LOD: Limit of Detection; LOQ: Limit of Quantification; RSD: Relative Standard Deviation.; S.D: Standard Deviation; M.P: Mobile Phase.

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