*ejpmr, 2025, 12(7), 196-205*

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN (O): 2394-3211

ISSN (P): 3051-2573

# DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TENELIGLIPTIN AND PIOGLITAZONE HCL IN BULK AND THEIR PHARMACEUTICAL DOSAGE FORM

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**Article Received on 08/05/2025** **Article Revised on 29/05/2025** **Article Accepted on 18/06/2025**

## ABSTRACT

Teneligliptin and Pioglitazone hydrochloride are prescribed in a combination dose form for the treatment of type II diabetes mellitus. A high-performance liquid chromatography method was developed and validated for the simultaneous quantification of teneligliptin and pioglitazone HCl that are supplied in a combination tablet form. A shimpack solar C18 column of (250mm x 4.5mm x 5 µm) is employed, with an injection loop capacity of 10 µl and an ultraviolet detection at 238 nm, and flow rate of 1.0 ml/min. A low-pressure gradient mobile phase of acetonitrile:5 mM phosphate buffer (pH 3.5) in a ratio of (90:10 v/v) was used to perform the RP-HPLC separation. Studies on forced degradation were conducted in thermal, photolytic, peroxide, acid, and base media. Pioglitazone and teneligliptin were shown to have retention times of 3.81 and 6.90 minutes, respectively. Teneligliptin and pioglitazone HCl were shown to have a linearity range of 2–12 µg/ml. Method validation followed ICH guidelines. The developed RP-HPLC method was proven to be sensitive, specific, accurate, and precise. It may be applied to routinely analysis of pharmaceutical formulations containing teneligliptin and pioglitazone HCl. The investigation on forced degradation came to the conclusion that the developed method successfully separated medicinal compounds from degradation products produced under different stress conditions.

**KEYWORDS:** Teneligliptin; Pioglitazone HCl; RP-HPLC; Stability, Validation.

## INTRODUCTION

Teneligliptin (TEN) is chemically known as [(2S,4S)-4-[4-(5-methyl-2-phenylpyrazol-3-yl) piperazin-1-yl] pyrrolidin-2-yl] -(1,3-thiazolidin-3- yl) methanone **(Figure 1),** belonging to dipeptidyl peptidase-4-inhibitors category of antihyperglycemic agents. It is used in the treatment of type 2 diabetes mellitus by lowering blood glucose levels.[1,2]

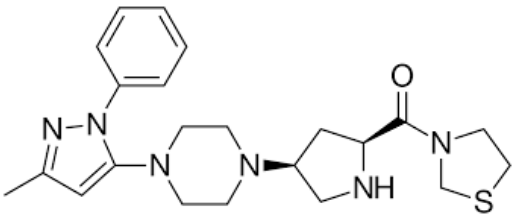


**Figure 1: Chemical structure of teneligliptin.**

Pioglitazone (PIO) is chemically known as 5-[[4-[2-(5-ethylpyridin-2-yl) ethoxy] phenyl] methyl]-1,3-thiazolidine-2,4-dione; hydrochloride (Figure 2), belonging to thiazolidinediones category of antihyperglycemic agents. It is used to reduce higher blood glucose levels caused due to type-2 diabetes mellitus.[3,4]

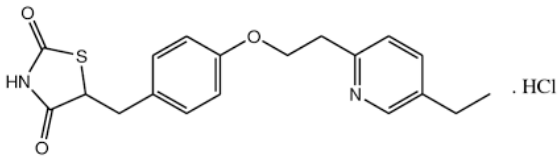


**Figure 2: Chemical structure of pioglitazone HCL.**

Pioglitazone hydrochloride is recognised by IP, BP, and the EU. The article has explained the RP-HPLC method for measuring pioglitazone HCl in tablet form and bulk. In IP, USP, and BP, teneligliptin is not authorised. A review of the literature revealed that a number of analytical techniques, such as UV-visible spectroscopy,[5-7] RP-HPLC,[8-16] RP-UHPLC,[17] stability-indicating HPLC,[18-20] RP-UFLC,[21] HPTLC,[22-24] and LC/MS/MS,[25-28] have been reported thus far for the measurement of TEN and PIO alone or in combination with other medications. The proposed effort aims to

create a sensitive, specific, accurate, and precise RP-HPLC method for the simultaneous quantification of PIO and TEN in pharmaceutical dosage forms and in bulk.

## MATERIALS AND METHODS

### Reagents and Materials

The standard drug substance of teneligliptin was gifted from Live-more Pharmaceuticals Ltd., Vadodara, India, and pioglitazone hydrochloride was gifted from Torrent Pharmaceuticals, Ahmedabad, India. The marketed formulation Zita-Pio by Glenmark Pharmaceuticals, containing a labelled amount of 20 mg of teneligliptin and 15 mg of pioglitazone hydrochloride tablets, was procured from the pharmacy. We obtained methanol, acetonitrile, and HPLC-grade water from Finer Chemicals Ltd. in Ahmedabad.

### Instruments

The Shimadzu Co. HPLC instrument of the LC-2030 Plus model was used for development and validation of method. Data acquisition and integration were carried out by Lab Solutions HPLC software. Shimadzu Co. UV spectrophotometer (1800), Sartorius analytical balance (CP 124S), Electroquip pH metre (PHCAL), and Frontline ultrasonicator bath were used.

## METHODS

### Preparation of a standard stock solution of teneligliptin and pioglitazone hydrochloride

Teneligliptin (5 mg) and pioglitazone hydrochloride (5 mg) were weighed precisely and added to a 25 ml volumetric flask. Methanol was added to the flask to get the volume up to the required level, resulting in a 200 µg/ml concentration.

### Preparation of diluent

The diluent was made by combining acetonitrile and 5 mM phosphate buffer in a 90:10 v/v ratio. 0.1% orthophosphoric acid was added to set the pH to 3.5.

### Preparation of a working stock solution of teneligliptin and pioglitazone hydrochloride

Teneligliptin (0.5 ml) and pioglitazone hydrochloride (0.5 ml) were taken from the aforesaid standard stock solution and added to a 10 ml volumetric flask. The concentrations of the two substances were then adjusted with diluent to obtain 10 µg/ml of teneligliptin and 10 µg/ml of pioglitazone HCL.

### Preparation of calibration curves of teneligliptin (2–12 µg/ml) and pioglitazone hydrochloride (2–12 µg/ml)

Teneligliptin (200 µg/ml) standard solution (0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml, and 0.6 ml) and pioglitazone HCL (200 µg/ml) standard solution (0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml, and 0.6 ml) were added to a 10 ml volumetric flask, and volume was adjusted to the mark using diluents. The peak area was measured after the solutions were injected into a chromatographic system. A plot of the calibration curve for peak area versus concentration was made.

### Analysis of the marketed formulation

Twenty Zita-Pio tablets were precisely weighed and ground into powder. A 50-ml volumetric flask was filled with a tablet powder that contain 20 mg of TEN and 15 mg of PIO, and the tablet powder was dissolved using diluent. The solution was sonicated for fifteen minutes using an ultrasonicator. The resulting solution was filtered through 0.45 µm Whatman filter paper, A 0.2 ml aliquot from the aforementioned solution was collected, diluted with diluent to make 10 ml, and then injected into an HPLC.

### Chromatographic Condition

RP-HPLC was carried out on a Shimpack solar C18 column (250mm x 4.5mm x 5 µm) using an isocratic mobile phase comprising of acetonitrile and 5 mM phosphate buffer (90:10, V/V) pH 3.5, adjusted with 0.1% orthophosphoric acid. The flow rate was adjusted to 1.0 ml/min, and detection was carried out at 238 nm. The sample was injected into a 10 µL volume, and the column's temperature was maintained at room temperature.

### Method Validation

The International Council on Harmonisation Guidelines (ICH Q2 (R1)) were followed in the validation process.

### Linearity

Six distinct levels of the calibration curve in the range of 2–12 µg/ml for TEN and PIO were analysed in order to ascertain the linear response. Triple analysis was performed on each solution. Peak area was measured, and peak area vs. concentration was plotted to create the calibration curve. The correlation coefficient should be used to express the linearity range.

### Precision

For the standard solution of TEN and PIO (6, 8, and 10 µg/ml), intraday and interday precision were measured three times on the same day and three times on different days, respectively. The relative standard deviation is used to express precision results.

### Accuracy

A previously examined test sample was spiked with three distinct standard concentrations—80%, 100%, and 120%, respectively—to assess the method's accuracy. By using a conventional drug assay and adding a known amount, accuracy can be reported as a percentage of recovery.

### Limit of Detection and Limit of Quantification

The slope and standard deviations (SD) of intercepts were computed after the calibration curve was run five times. LOD and LOQ were calculated using the following mathematical formulas: LOQ = 10 δ/S, and

LOD = 3.3 δ/S, where S is the regression line's slope and δ is standard deviation at the y-intercept.

**Robustness**

Robustness measures the method's reliability under a variety of conditions. Robustness was typically carried out by deliberately varying critical method parameters such as the flow rate, pH, mobile phase composition, and detection wavelength. The method's robustness is evaluated based on its ability to produce results within acceptable ranges of accuracy, precision, and other performance characteristics, despite the variations.

**Specificity**

Specificity focuses on demonstrating that an analytical method can accurately identify and quantify the analyte in the presence of other substances. It helps in distinguishing the analyte from other components and ensures that the measurement is not confounded by external factors. Specificity was determined by injecting sample, standard and blank solution and resultant chromatograms were compared to check the interference.

**System Suitability**

System suitability was performed to check that the analytical method is suitable for the intended analytical procedure and to check that the method is performing as expected before analyzing actual samples. The parameters used in these were asymmetry of the chromatographic peak, theoretical plates, retention time, and resolution.

## RESULTS AND DISCUSSION

**Optimization of HPLC method**

Several chromatographic conditions were used in order to develop an effective RP-HPLC method for the analysis of teneligliptin and pioglitazone hydrochloride. After experimenting with various ratios of methanol to water, acetonitrile to water, and acetonitrile-methanol to water, no effective separation could be achieved. In order to get a successful separation, phosphate buffer pH 3.5 with acetonitrile was utilised. Following the optimisation of separation parameters, the separation was performed using a shimpack solar C18 column (250 mm x 4.5 mm x 5 µm), with a pH 3.5 mobile phase (acetonitrile: 5 mM phosphate buffer, 10:90 V/V), a flow rate of 1.0 ml/min, and a detection wavelength of 238 nm. Fig. 3 displays the chromatogram of the PIO and TEN standard solutions. PIO and TEN were shown to have retention time of 3.81 and 6.90 minutes, respectively.

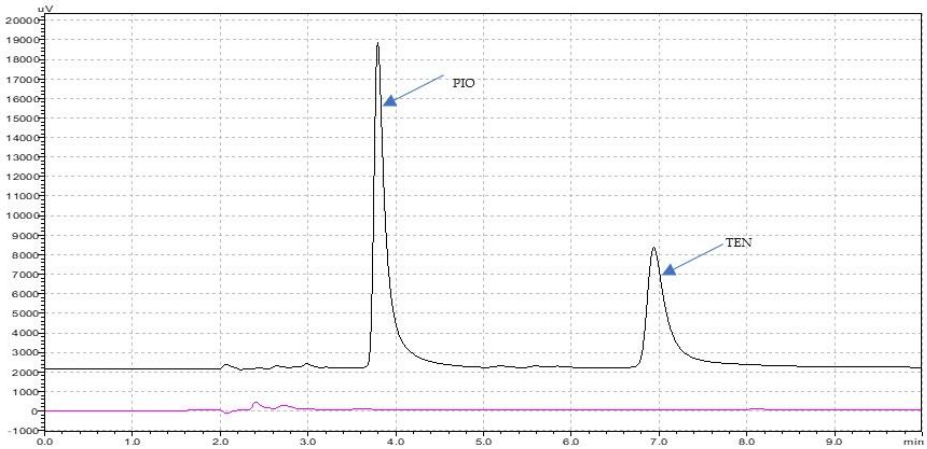



**Figure 3: Overlaid HPLC Chromatogram of blank and standard of TEN and PIO (10 µg/ml).**

**Method Validation results**

For both medications, linear responses were seen in the concentration range of 2–12 µg/ml. It was discovered that the calibration curves of TEN and PIO had correlation values of 0.999. In Fig. 4 and 5, the calibration curve is displayed. Table 1 includes the linearity data for TEN and PIO. The precision study, which is typically represented as a percentage of RSD. TEN and PIO were found to have mean percentage RSD values for intra-day precision of 0.298 and 0.295, respectively, and mean percentage RSD values for inter-day precision of 0.339 and 0.342, respectively. The low RSD values show that the proposed method is precise. Tables 2 and 3 show the precision study's results. TEN and PIO were discovered to have LOD values of 0.089 µg/ml and 0.052 µg/ml, respectively, and LOQ values of 0.269 µg/ml and 0.157 µg/ml, respectively. The developed method is sensitive enough to identify and quantify smaller concentrations of drugs, as evidenced by the lower values of LOD and LOQ. The accuracy study results are represented as a percentage of recovery and are obtained by adding various concentrations of a standard solution to a test solution. It was discovered that TEN and PIO had recovery rates of 99.95% and 99.72%, respectively. The accuracy of the suggested procedure is demonstrated by the recovery data. Table 4 presents the accuracy study's results.

This method was specific because no interfering peaks were observed, and the retention time of the test sample was the same as that of standard teneligliptin and pioglitazone hydrochloride. The results of the robustness study can be expressed as % RSD for peak area for TEN and PIO. The results indicate an insignificant difference

in results, indicating that the proposed method is robust. The results of the robustness study are shown in Table 5. Table 6 presents the findings of the system suitability analysis and makes it abundantly evident that the suggested method is appropriate for the effective separation of TEN and PIO with good peak symmetry, number of HETP, and resolution.

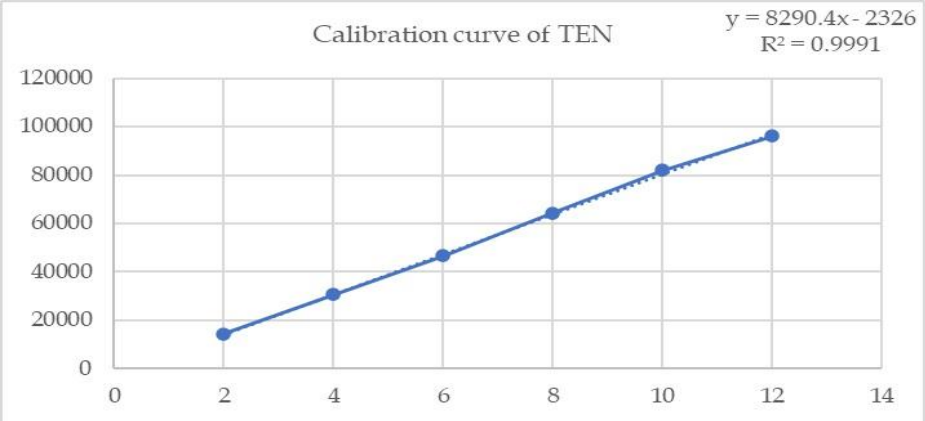



**Figure 4: Calibration curve of Teneligliptin (2-12µg/ml).**

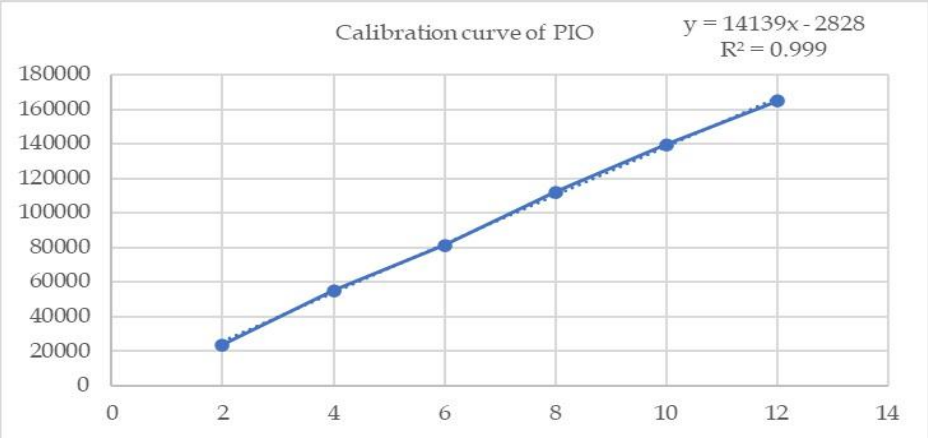



**Figure 5: Calibration curve of Pioglitazone (2-12µg/ml).**

**Table 1: Result of Linearity.**

| Conc. (µg/ml) | Conc. (µg/ml) | Peak area (mean ± S.D, n=3) | Peak area (mean ± S.D, n=3) | %RSD | %RSD |
|---|---|---|---|---|---|
| **TEN** | **PIO** | **TEN** | **PIO** | **TEN** | **PIO** |
| 2 | 2 | 14479.6 ± 29.194 | 23703.4 ±214.623 | 0.201 | 0.905 |
| 4 | 4 | 30662 ± 167.255 | 55159.3 ± 78.545 | 0.546 | 0.142 |
| 6 | 6 | 46601.6 ± 111.505 | 81379.3 ± 42.004 | 0.239 | 0.051 |
| 8 | 8 | 64332.3 ± 24.785 | 112172 ± 23.459 | 0.038 | 0.020 |
| 10 | 10 | 82084 ± 102.898 | 139678 ± 271.846 | 0.125 | 0.194 |
| 12 | 12 | 96122.2 ± 779.553 | 164780 ± 17.673 | 0.811 | 0.010 |
| | | | **Mean %RSD** | **0.191** | **0.220** |

**Table 2: Result of Intraday Precision.**

| Conc. (µg/ml) | Conc. (µg/ml) | Peak area (mean ± S.D, n=3) | Peak area (mean ± S.D, n=3) | %RSD | %RSD |
|---|---|---|---|---|---|
| **TEN** | **PIO** | **TEN** | **PIO** | **TEN** | **PIO** |
| 6 | 6 | 46700 ± 67.34 | 81719.3 ± 62.34 | 0.325 | 0.647 |
| 8 | 8 | 64240 ± 87.56 | 111972 ± 52.58 | 0.452 | 0.154 |
| 10 | 10 | 82304 ± 97.21 | 139632 ± 123.14 | 0.118 | 0.084 |
| | | | **Mean %RSD** | **0.298** | **0.295** |

**Table 3: Result of Interday Precision.**

| Conc. (µg/ml) | Conc. (µg/ml) | Peak area (mean ± S.D, n=3) | Peak area (mean ± S.D, n=3) | %RSD | %RSD |
|---|---|---|---|---|---|
| **TEN** | **PIO** | **TEN** | **PIO** | **TEN** | **PIO** |
| 6 | 6 | 46750 ± 40.78 | 81200 ± 84.98 | 0.450 | 0.545 |
| 8 | 8 | 64500 ± 66.78 | 112300 ± 44.67 | 0.350 | 0.325 |
| 10 | 10 | 82310.5± 82.86 | 139785.48 ± 78.05 | 0.217 | 0.155 |
| | | | **Mean %RSD** | **0.339** | **0.342** |

**Table 4: Result of Accuracy study.**

| Drug | % Level | Test Conc. (µg/ml) | Amount of standard drug added (µg/ml) | Total Conc. (µg/ml) | Found Conc. (µg/ml) | % recovery ± S.D. |
|---|---|---|---|---|---|---|
| TEN | 80 | 4 | 3.2 | 7.2 | 7.19 | 99.99 ± 0.17 |
| | 100 | 4 | 4 | 8 | 7.99 | 99.88 ± 0.55 |
| | 120 | 4 | 4.8 | 8.8 | 8.79 | 99.99 ± 0.47 |
| PIO | 80 | 4 | 3.2 | 7.2 | 7.14 | 99.26 ± 0.24 |
| | 100 | 4 | 4 | 8 | 7.99 | 99.95 ±0.65 |
| | 120 | 4 | 4.8 | 8.8 | 8.79 | 99.95 ± 0.14 |

**Table 5: Result of System Suitability study.**

| Parameter | TEN | PIO |
|---|---|---|
| Retention time (min) | 3.81 ± 0.03 | 6.90 ± 0.05 |
| Theoretical plates | 4569.86 ± 12 | 5150.29 ± 24 |
| Asymmetry | 1.49 ± 0.12 | 1.59 ± 0.1 |
| Resolution | - | 10.69 ± 0.3 |

**Table 6: Result of robustness study.**

| Parameter | TEN (% RSD) | PIO (% RSD) |
|---|---|---|
| Flow rate | 0.237 | 0.373 |
| Mobile phase composition | 0.151 | .088 |
| Wavelength | 0.149 | 0.123 |
| pH | 0.446 | 0.646 |

**Analysis of the marketed formulation by the proposed method**

Teneligliptin (6 mg) and pioglitazone HCl (8 mg) in marketed tablets were analysed using assays, and the results showed good agreement with the contents as stated. The marketed formulation contained 99.87% pioglitazone HCl and 99.63% teneligliptin. Since there was no evidence of excipient interaction with the peaks of interest, and the suggested method might be effectively used to estimate teneligliptin and pioglitazone HCL simultaneously in a combination tablet dose form. Table 7 presents the assay findings.

**Table 7: Estimation of TEN and PIO in marketed formulation.**

| Drug | Label claim | Amount Found | % Assay ± S.D. |
|---|---|---|---|
| PIO | 8 mg | 7.99 | 99.87 ± 0.22 |
| TEN | 6 mg | 5.98 | 99.66 ± 0.17 |

**Forced Degradation Studies**

**Acid Hydrolysis**

Acid hydrolysis was performed in 0.1N HCL. The samples were subjected to a solution containing 10 µg/ml pioglitazone and 10 µg/ml teneligliptin for 30 minutes at 40 °C. The drugs peak areas were found to be smaller than those of the standard chromatogram. PIO was shown to be more susceptible to acid hydrolysis than TEN, with degradation rates of 9.75% and 15.0% for TEN and PIO, respectively. In Fig. 6A, the chromatogram is displayed.

**Base Hydrolysis**

Base hydrolysis was carried out with 0.1N NaOH and subjected to a solution containing 10 µg/ml pioglitazone and 10 µg/ml teneligliptin for 30 minutes at 40 °C. It was discovered that both drugs peak areas decrease in comparison to the standard chromatogram, with TEN and PIO showing degradation rates of 5.0% and 13.0%, respectively. In Fig. 6B, the chromatogram is displayed.

**Oxidative Degradation**

In a solution containing 10 µg/ml teneligliptin and 10 µg/ml pioglitazone, oxidative degradation was carried out using 3% $H_2O_2$ at 40 °C for 30 minutes. Comparing

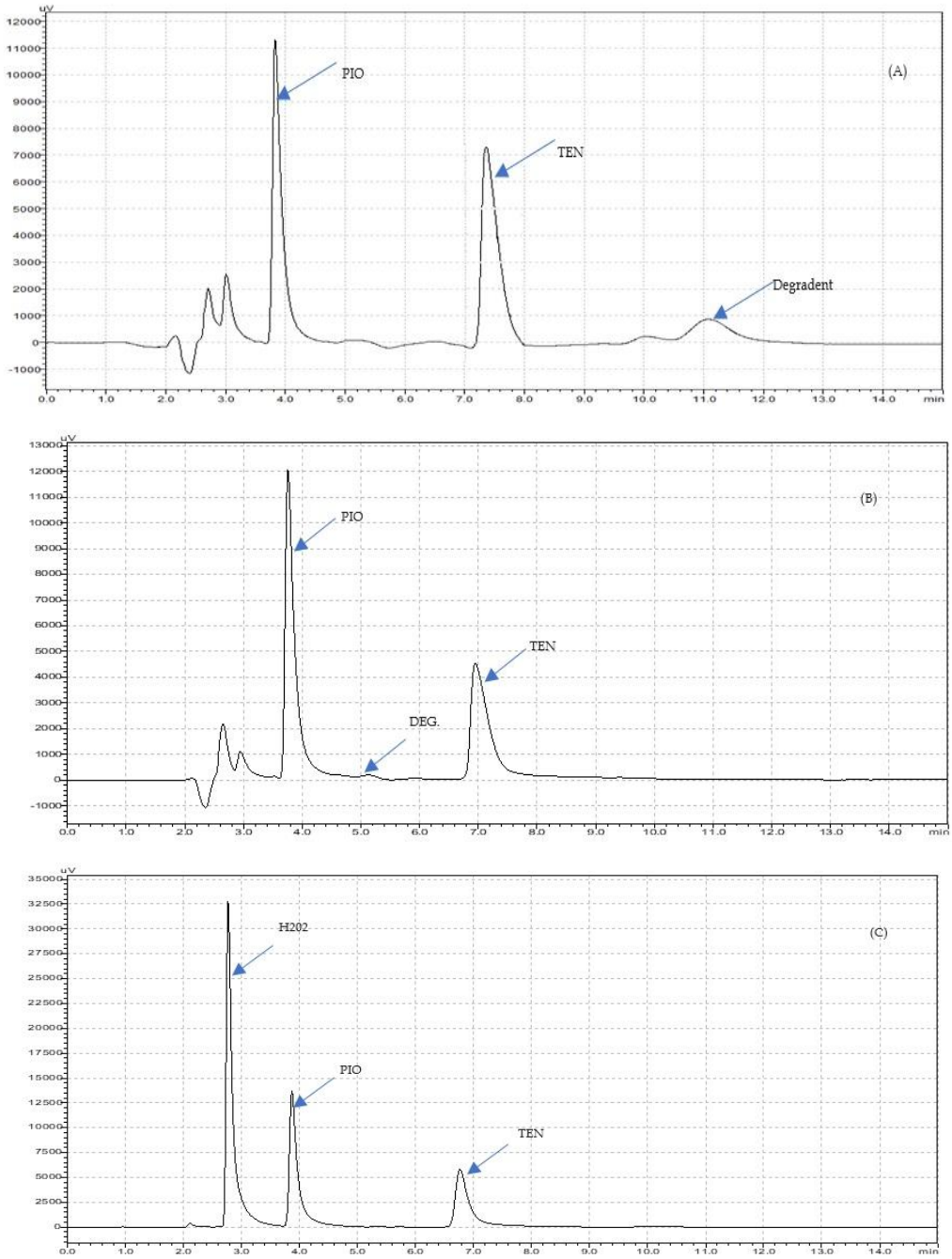
the peak areas of the two drugs to the standard chromatogram, it was discovered that the degradation of TEN and PIO was 14.62% and 16.62%, respectively. Fig. 6C displays the chromatogram.

**Photolytic Degradation**

Teneligliptin and pioglitazone solid API were exposed to sunshine for six hours in order to cause photolytic breakdown. The absence of notable degradation was noted in both medications, suggesting the stability of TEN and PIO against photolytic degradation. Fig. 6D displays the chromatogram.

**Thermal Degradation**

Teneligliptin and pioglitazone solid API were subjected to 60 °C for one hour in a hot air oven to cause thermal degradation. PIO is more susceptible to thermal degradation than TEN, as evidenced by the fact that both drugs peak areas decrease when compared to the standard chromatogram, with 1.75% and 12.65% degradation for TEN and PIO, respectively. In Fig. 6E, the chromatogram is displayed. Table 8 displays the findings of all the investigations on forced degradation.



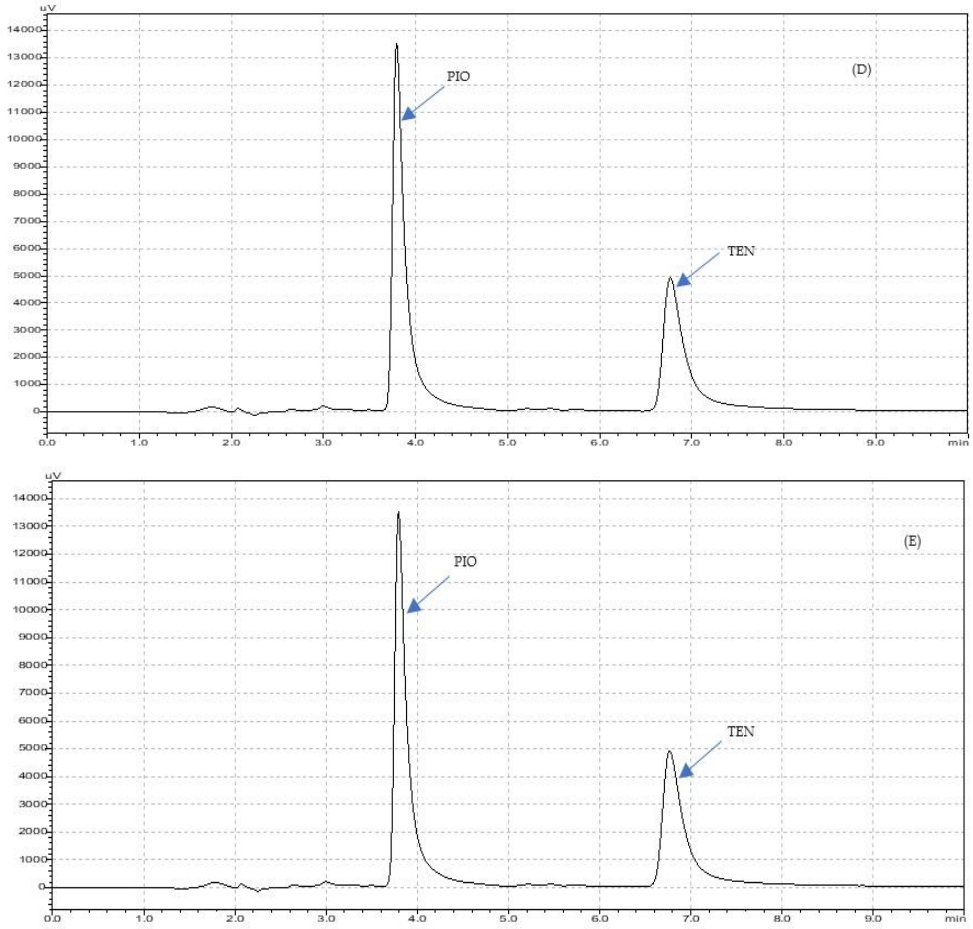



**Figure 6: (A) RP-HPLC chromatogram of acid hydrolysis (B) RP-HPLC chromatogram of base hydrolysis (C) RP-HPLC chromatogram of oxidative degradation (D) RP-HPLC chromatogram of photolytic degradation (E) RP-HPLC chromatogram of thermal degradation.**

**Table 8: Results of forced degradation studies of TEN and PIO.**

| Sr. No | Stress Study | Stress condition | % degradation | |
|---|---|---|---|---|
| | | | TEN | PIO |
| 1 | Acid hydrolysis | 0.1 N HCl 40°C for 30 min | 9.75% | 15% |
| 2 | Base hydrolysis | 0.1 N NaOH 40°C for 30 min | 5% | 13% |
| 3 | Oxidation | 3% v/v H2O2 at 40°C for 30 min | 14.62% | 16.46% |
| 4 | Photolytic | Direct sunlight for 6 h | - | 0.49% |
| 5 | Thermal | 60℃ in hot air oven for 1h | 1.75% | 12.65% |

## CONCLUSIONS

The developed RP-HPLC method can be utilised for routine analysis of TEN and PIO in bulk as well as their pharmaceutical formulations. It was proven to be accurate, precise, sensitive, and specific. The investigation on forced degradation came to the conclusion that the suggested method successfully separated medicinal compounds from degradation products produced under different stress conditions. It was determined that PIO was more susceptible to acidic, alkaline, oxidative, and thermal degradation, whereas TEN was more susceptible to oxidative degradation. Both medications were shown to be stable in photolytic degradation.

**CONFLICT OF INTEREST:** No conflicts of interest were disclosed by the authors.

## ACKNOWLEDGEMENT

The authors express their gratitude to Torrent Pharmaceuticals, located in Ahmedabad, India, and Live-More Pharmaceuticals Ltd., located in Vadodara, India, for providing gift samples of reference standards. The K. B. Institute of Pharmaceutical Education and research, Gandhinagar, Gujarat, is acknowledged by the authors for allowing them to use its facilities for study.

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