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Original Article

Simultaneous Assay of Teneligliptin and Pioglitazone in Bulk and Combined Formulations by a Validated RP-HPLC Method

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Abstract

A reverse-phase high-performance liquid chromatography (RP-HPLC) method has been developed to simultaneously determine Teneligliptin and Pioglitazone in their combined dosage form. Both the analytes were separated on a C18 column (4.6x250mm, 5µm) with a mobile phase comprising acetonitrile: 5 mM phosphate buffer (pH 4.8) [65 35 v/v] at a flow rate of 0.5ml/min and PDA detection at 254nm. The method was applied successfully to analyze stressed samples without any interference with the peaks of the primary analytes. The method was validated for various parameters to comply with the ICH Q2(R) requirements. The method is sensitive with a quantitation limit of 2.243 µg/ml and 4.757 µg/ml for Teneligliptin and Pioglitazone, respectively and is suitable for routine quality control of the drugs in pharmaceutical dosage forms.

Keywords: *HPLC; ICH Guidelines; Pioglitazone; Stress Study; Teneligliptin; Validation*

Introduction

The International Diabetes Federation (Atlas, 2015) estimates that a staggering 537 million people worldwide had diabetes mellitus (DM), among them 96% of cases classified as Type 2 diabetes (T2DM). People aged 65 to 95 years with a high BMI or obesity are significantly more susceptible to developing T2DM due to multiple risk factors, including changes in the food system, reduced physical activity, inadequate treatment, and socioeconomic challenges (IDF.DA, 2021). Effective management of hyperglycemia, hypertension, and lipid profiles is crucial in preventing the progression to advanced complications that augment morbidity and mortality in patients with T2DM (Association, 2021; Pradhan *et al*., 2025). In T2DM management, the primary objective of the therapy is to control glycemic levels to the normal range as early as possible via increasing insulin sensitivity. A holistic combination therapy approach becomes essential for effective management and better therapeutic benefit when the first-line monotherapy fails. It has been seen that there has been a failure in immunotherapy treatment for Type II DM patients with co-morbidities. In recent years, due to the use of multi-drug pharmaceutical formulations, there has been a tremendous transformation in the management of chronic disorders (Chhipa *et al*., 2024). Kadowaki & Kondo (2013) conducted a clinical trial (double-blind, placebo-controlled, parallel-group) to confirm the safety and efficacy of add-on TEN and PGL therapy in Japanese patients with T2DM inadequately controlled with PGL monotherapy. The study concluded that the addition of TEN to PEG monotherapy is well tolerated by T2DM patients and resulted in

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improved glycemic control with very low peripheral edema and hypoglycaemia incidences. When comparing the efficacy, Kumar *et al*. (2019) evaluated and reported that add-on therapy of Teneligliptin (TEN) 20 mg and Pioglitazone (PGL) 15 mg provided a significant reduction in HbA1c, FPG, 2-h PPG and significant changes in Lipid profile when compared with Metformin Plus Sulfonylurea. A study demonstrated that patients, after the treatment of Metformin plus Sulfonylurea combined therapy, had inadequately controlled T2DM (Kumar *et al*., 2019). DCGI approved the new fixed dosage combinations of TEN and PGL for the treatment of T2DM on February 25, 2022 (CDSCO Marketing approval, 2022). TEN is a newer oral dipeptidyl peptidase 4 inhibitor, which exhibits insulinotropic effects by promoting incretin actions (Sharma *et al*., 2016). PGL (peroxisome proliferator-activated receptor-γ agonist) is a second-line therapy with a hypoglycaemic effect that accelerates lipid metabolism and decreases insulin resistance in peripheral tissue (Marchand *et al*., 2020).

Chemically, TEN is {(2S, 4S)-4- [4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-1-piperazinyl]- pyrrolidinyl} (1, 3-thiazolidine-3-yl) methanone and PGL is (RS)-5-[(4-(2-(5-ethylpyridin-2-yl) ethoxy) phenyl) methyl] thiazolidine-2,4-dione hydrochloride (Figure 1). The literature study provides evidence that several HPLC methods are available for the determination of TEN (Nagarajan *et al*., 2024; Godase *et al*., 2024; Biswas *et al*., 2020; Maruthi *et al*., 2020) and PGL (Souri *et al*., 2008; Kumar *et al*., 2011; Reddy & Rao, 2012) in pharmaceutical dosage forms individually. However, for the simultaneous determination of TEN and PGL, only two HPLC methods are available in the literature (Prajapati *et al*., 2023; Sen *et al*., 2024).

However, the HPTLC method for simultaneous estimation of teneligliptin hydrobromide hydrate and pioglitazone hydrochloride has several limitations in routine quality control compared to the claimed advantages. Being less precise and using toxic solvents like chloroform and ammonia does not confirm the analytical performance and environmental appeal. In contrast, the RP-HPLC method developed by Prajapati *et al*. (2023) claims to overcome these issues by integrating white analytical chemistry principles and using ethanol as the organic solvent. However, specific issues do not fulfill its claim of eco-friendliness and robust validation through designed experiments. The method is less sensitive (LODs in the µg/mL range) and is insufficient for trace-level detection of analytes. The absence of forced degradation studies weakens the method's applicability for stability-indicating purposes. Additionally, the method claim of generating only 100 mL of organic waste is not quantified accurately, influencing its eco-friendly claim. Based on the above observations, with an intent to overcome the operational, environmental, and analytical limitations of existing HPTLC and RP-HPLC methods, the authors felt it necessary to develop a new RP-HPLC approach. The proposed work aims to create and authenticate a simple and highly sensitive stability-indicating RPHPLC technique for the determination of both the analytes in bulk and pharmaceutical dosage forms.

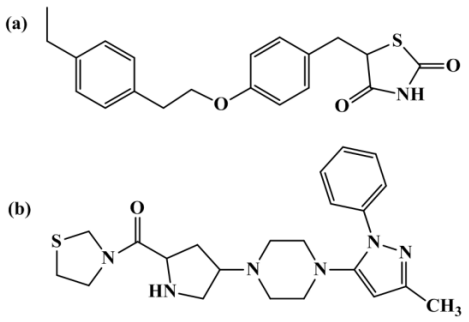


Figure 1: *Chemical Structure Of (A) PGL ((RS)-5-[(4-(2-(5-Ethylpyridin-2-Yl) Ethoxy) Phenyl) Methyl] Thiazolidine-2,4-Dione) And (B) TEN ({(2S, 4S)-4- [4-(3-Methyl-1-Phenyl-1H-Pyrazol-5-Yl)-1-Piperazinyl]- Pyrrolidinyl} (1, 3-Thiazolidine-3-Yl) Methanone).*

Experimental

Chemicals and Reagents

A Hyderabad-based pharmaceutical company in Telangana, India, has given a complimentary sample of pharmaceutical-grade CRB with a purity level of 99.98%. The solutions were made using ultra-pure

water obtained from the Millipore Milli-Q Plus water purification system located in Bedford, MA, USA. To prepare the solution HPLC-grade acetonitrile and phosphate buffer (Finar, India) were brought. All additional chemicals and solvents of analytical grade were acquired from commercial supply sources.

Apparatus and Equipment Used

The study was conducted using a binary gradient HPLC system manufactured by Shimadzu in Kyoto, Japan. The system consists of a diode array detector (SPD-M20A) and a dual pump (LC-20AD) that comprises a manual injector. Both drugs have been separated by a reverse-phase ShimPack GWS C18 column (4.6x250mm, 5µm). The LC-Solution (Shimadzu, Japan) software aids facilitate the analysis and integration of record chromatographic data. The hydrolytic study was conducted using a water bath having an MV controller from Thermostatic Classic Scientific India Ltd., a company from Mumbai, India. Labline Sun Scientific Ltd. humidity chamber was employed for stability research and a photo stability chamber (model 95 Th-400 G) from Thermo lab in Mumbai, India, for photolytic investigation. A hot air oven from Kumar Scientific Works in Pune, India, is used for the purpose of thermal stability research. We used an Elico pH meter to regulate the pH of the liquids.

Preparation of Analytical Solution

1 mg/ml stock solution of both the drugs, TEN and PGL, was prepared with acetonitrile. A 100 µg/ml solution was prepared by thoroughly mixing the stock solutions with a diluent of acetonitrile and phosphate buffer (65:35). The working standard solution was appropriately diluted to provide appropriate concentrations for conducting method validation activities (Mota-Lugo *et al*., 2021).

Specificity and Stress Degradation Studies

In order to carry out all of the stress degradation studies, a sample solution of 20µl of each was required. For the acid and alkaline hydrolysis studies, 200µl of acid (0.1N HCl) and alkali (0.1N NaOH) were added and allowed to stand for 30 min. Subsequently, the mixture was neutralized with equal volumes of the counterparts. In case of peroxide degradation, the drug aliquot was exposed to 200 µl of hydrogen peroxide and was diluted to the final mark by mobile phase. For thermolysis and photolysis studies, the drug aliquot was exposed to 80 °C temperature on a water bath and 365nm UV light, respectively, for 30 min. After the final volume, the solutions were injected onto the LC column and analyzed (ICH, 2003; Baertschi *et al*., 2011).

Condition for Chromatographic Separation

Chromatographic analysis has been done with ShimPack GWS C18 analytical column (4.6 × 250 mm, 5 µm). The mobile phase was the combination of acetonitrile and 5 mM phosphate buffer (pH 4.8) [65:35 v/v]. The flow rate of the mobile phase was 0.5 ml/min. For all of the trials, the sample injection volume was set at 10 µl, and the isobestic point of both samples was determined to be 254 nm (Dong, 2006; Tripathy *et al*., 2023).

Method Validation

According to ICH guidelines Q2 (R1), the analytical technique was effectively validated for the specified parameters (ICH, 2005).

Linearity And Range

The method's linearity was assessed within the concentration range of 14µg/ml to 26µg/ml for TEN and 10.5 µg/ml to 19.5 µg/ml for PGL. Every solution was administered three times. Least squares linear regression was used to analyse data on the drug's peak area vs concentration (ICH, 2005; Snyder *et al*., 2011).

Precision

The method's intra-day precision was assessed by analysing three quality control samples of each drug, 16, 20 and 24µg/ml for TEN and 12, 15 and 18µg/ml for PGL with three injections on a day. The percentage relative standard deviation (%RSD) was calculated. Additionally, inter-day precision was

assessed by repeating similar experiments on the three following days (ICH, 2005; Ermer & Nethercote, 2025).

Specificity

The method's specificity was determined according to ICH, 2005, by examining the resolution of the primary analyte peak from the closest degrading peaks.

LOD, LOQ and Other Parameters

The method's sensitivity was assessed by determining the limit of detection = 3.3 × standard deviation (σ) / slope (S) and the limit of quantitation = 10 × standard deviation (σ) / slope (S). Peak area, retention time (Rt), and tailing factor (T) are like chromatographic parameters to be evaluated. Additional verification was conducted on the responses obtained from the 13 trial runs to ensure their repeatability and compliance with the accepted criteria (ICH, 2005).

Results

Method Development and Optimization

An isocratic RP-HPLC method was developed for the quick quantitative analysis of TEN and PGL. Optimization of the process was achieved by selecting the optimal peak parameters and shape using a simple and cost-effective mobile phase that effectively separates the TEN and PGL from their primary degradants produced under different stress situations. A combination of acetonitrile and phosphate buffer (65:35) was used as the mobile phase, and degradation product retention and relative retention times under different stress conditions with acetonitrile and phosphate buffer (65:35) as mobile phase are shown in Table 1. Figure 2a shows that the unstressed peaks of TEN and PGL are unaffected by any interference, demonstrating that the approach has desired stability, indicating efficacy.

Table 1: *Degradation Product Retention and Relative Retention Times Under Different Stress Conditions with Acetonitrile and Phosphate Buffer (65:35)*

Condition	Retention time ± SD; RSD%					
0.1N HCl	3.512± 0.02; 0.712	4.484 ±0.03; 0.623				
0.1N NaOH		4.166±0.008; 0.285				
3% H2O2		4.521 ±0.006; 0.127	4.554 ± 0.025; 0.587	5.249 ± 0.008; 0.155	5.973 ± 0.012; 0.202	7.484 ± 0.10; 0.138
80° C Thermal		4.413 ±0.010; 0.224				
UV light 365nm		4.402 ±0.016; 0.385				

Note: All the Values are Expressed in Retention time ± SD; RSD% (n=3)

Method Validation

Robustness Study

Consistently and systematically changing the parameters (% organic solvent, flow of mobile phase) within a suitable range and evaluating the effect on method performance, the robustness research guarantees the reliability and consistency of the RP-HPLC technique. An evaluation of the RP-HPLC method's capacity to maintain impartiality in the presence of minor fluctuations in the two crucial operating parameters is helpful. An analysis of the variance (Table 2) for flow rate, % acetonitrile, Rt, peak area, and telling factor showed that the regression models were statistically significant.

Table 2*: Operational Specifications and Thresholds for Robustness Analysis*

Operational Parameter	Low	Optimal	High
Flow rate	0.45ml/min	0.5 ml/min	0.55ml/min
% of Acetonitrile	63	65	67

Linearity and Sensitivity

The method was observed Linear from 14 to 26 µg/ml for TEN and 10.5 to 19.5 µg/ml for PGL. The calibration curve was established by plotting peak regions versus medication concentrations. Determination coefficient (R2) for TEN and PGL are 0.984 and 0.987, respectively, in the above concentration range. TEN and PGL had LODs of 0.740 µg/ml and 1.570 µg/ml, respectively, and LOQs of 2.243 µg/ml and 4.757 µg/ml, as measured.

Precision Study

All the precision study trials were done in triplicate and the data are shown in Table 3. In order to determine the intra-day and inter-day precision, experiments were conducted on quality control samples at three distinct concentrations: 16, 20, and 24µg/ml for TEN and 12, 15 and 18 µg/ml for PGL. The method was found to be precise, with an estimated relative standard deviation (RSD) of less than 1.573%.

Table 3: *Intra-Day and Inter-Day Precision Data in Triplicate*

	Precision				
	Teniligliptin		**Pioglitazone**		
Theoretical Conc.	Intra-day (Measured concentration µg/ml ± SD; RSD%)	Inter-day (Measured concentration µg/ml ± SD; RSD%)	Theoretical Conc.	Intra-day (Measured concentration µg/ml ± SD; RSD%)	Inter-day (Measured concentration µg/ml ± SD; RSD%)
16	16.039 ±0.067; 0.419	16.15 ±0.129; 0.67	12	12.114 ±0.168; 1.387	12.189 ±0.191; 1.573
20	20.127 ± 0.209; 1.035	20.134 ±0.225; 1.121	15	15.181 ± 0.143; 0.947	15.128 ± 0.206; 1.352
24	23.632 ± 0.305; 1.292	23.867 ± 0.153; 0.064	18	17.824 ± 0.142; 0.799	17.924 ± 0.123; 0.687

Note: All the values are expressed in concentration µg/ml ± SD; RSD% (n=3)

Degradation Behavior

The following degradation behavior has been speculated based on the results of stress testing of TEN and PGL under a broad range of situations with the assistance of RP-HPLC.

Acid Hydrolysis

The drug undergoes a gradual degradation process in highly acidic conditions over an extended period. This suggested that the TEN is hydrolysed under acidic conditions, potentially resulting in a decrease in the peak area of 55.068%. It shows two degradation peaks at Rt 3.521 min and 4.484 min, but PGL is found to be stable in the acid condition and no degradation occurs. (Figure 2b)

Base Hydrolysis

The drugs TEN and PGL exhibit high stability in base hydrolysis, with retention times (Rt) of 4.693 min and 6.78 min, respectively. A single and very small degradation peak was observed at a retention time (Rt) of 4.166 min, with a peak area of 2640, when the drug solution was heated with 0.1 N NaOH at 60 °C for 30 min. (Figure 2c)

Oxidative Hydrolysis

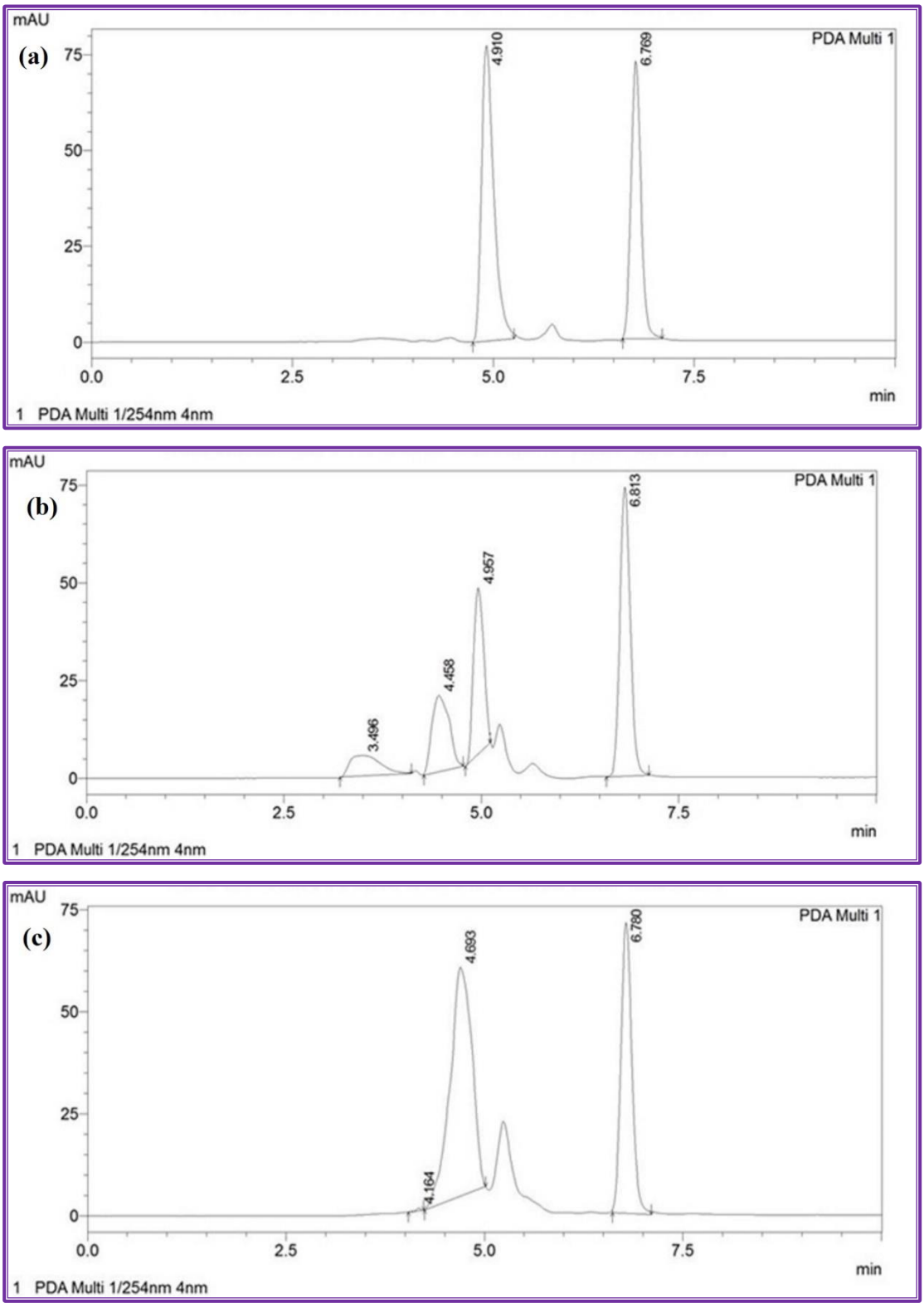
Both the drugs TEN and PGL showed a significant fall of 91.796% and 89.112% in peak area when treated with 3% hydrogen peroxide, respectively. Figure 2d presents data linking the degradation process to an increase in five degradation peaks, with Rt values of 4.521, 4.554, 5.249, 5.973 and 7.484 min, respectively.

Thermal Hydrolysis

The Rt values for TEN and PGL were 4.906 and 6.786 min, respectively. When TEN and PGL were heated to 80 °C, they both showed a small degradation of 6.384% and 7.082%, respectively. This was shown by the appearance of a single deteriorated peak at Rt 4.413 min. (Figure 2e)

Photolysis Hydrolysis

PGL exhibits less deterioration of 11.052% under the conditions of photolytic stress, where the peak area of TEN immensely falls by 31.239%, as evidenced by the development of a degradation peak measured at Rt 4.402 min. (Figure 2f)



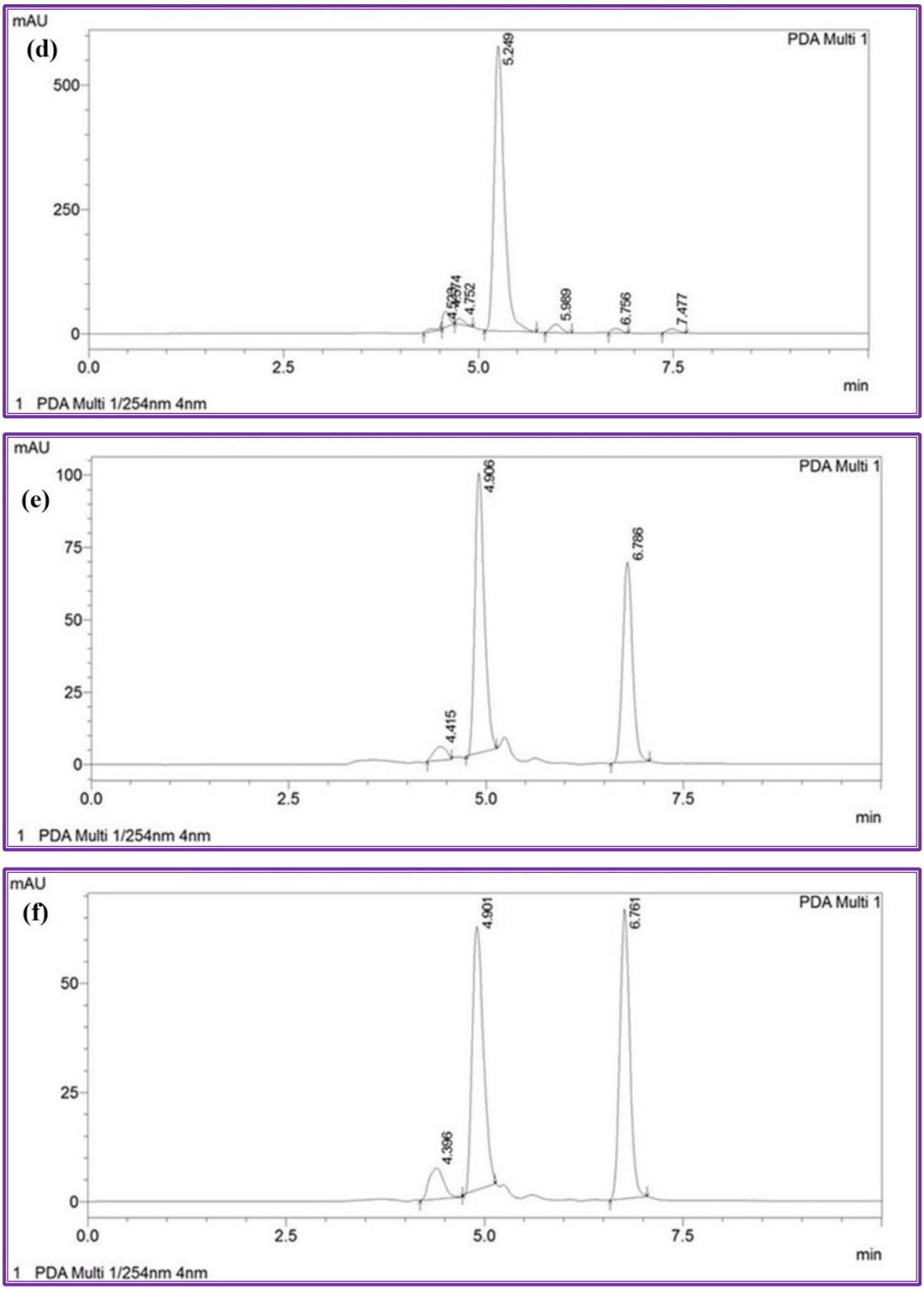


Figure 2: *Chromatograms of the Drug Solutions Under Stress Conditions Acquired During the Forced Degradation Experiments. Where, (A) Presents Chromatogram of Unstressed Sample, (B) Acid Hydrolysis, (C) Alkaline Hydrolysis, (D) Oxidative Hydrolysis, (E) Thermal Hydrolysis, (F) Photolysis Hydrolysis*

Discussion

TEN and pioglitazone had distinct peaks in the HPLC technique, demonstrating high specificity. Excipients and degradation products did not interfere under stress. This method is better than prior TLC or UV methods (Akabari *et al*., 2025), which had overlapping peaks and interference from other constituents in complicated mixes. This approach performed better because of the improved mobile phase composition.

The validation demonstrated that the method is robust, precise, sensitive, and linear over the studied concentration range, with correlation coefficients exceeding 0.999 for both analytes. This methodology showed a shorter runtime and superior baseline separation compared to Vijayasri (2025) technique, which demonstrated remarkable linearity and precision. The technique demonstrated superior efficacy under varying pH, flow rate, and temperature conditions, indicating its enhanced robustness.

In forced degradation trials, this technique was able to identify the difference between degradation products in all ICH-recommended stress conditions. The order of degradation was: oxidation > acid > heat > photolysis > base. This pattern resembles the findings of Akabari *et al*. (2025). However, our technique identified minor degradation peaks under oxidative and acidic stress, indicating greater sensitivity. For instance, Akabari *et al*. (2025) used eco-friendly TLC and HPLC procedures, but their system couldn't resolve low-level oxidative degradation impurities, but here. On the other hand, we clearly recognized these impurities with superior peak purity and symmetry.

Our results also revealed that both drugs are more stable when alkaline hydrolysis is used, which agrees with what Godase *et al*. (2024) ; Pandya and Vekaria (2025) found. But our method showed faster elution and more consistent peak profiles, with retention periods of less than 5 minutes for both analytes. This technique is far superior to the UHPLC methods described by Godase *et al*., (2024) which had larger retention windows and less effective degradation separation.

The simultaneous breakdown of TEN and PGL we saw here supports findings from recent reviews (Pandya & Vekaria, 2025), indicating that they are both easily affected by oxidative and acidic conditions. However, our method was better at separating the breakdown products from the original compounds, something that many earlier methods struggled with, especially when both were breaking down at the same time. In alignment with our findings, Shaikh and Singla (2025) successfully developed a stability-indicating HPLC method for TEN, PGL, and metformin in commercial formulations. The study effectively addressed stress-induced breakdown products, confirming the robustness and sensitivity of our methodology in acidic and oxidative conditions. Vinay *et al*. (2025) established an RP-HPLC protocol utilizing acetonitrile–KH_2PO_4 (60:40, pH 3.6), achieving high linearity ($R^2 \geq 0.999$), low LOD/LOQ, and demonstrating the efficiency of our methodology.

This technique is more useful and practical since it takes less time to analyze, requires less sample preparation, and has superior system appropriateness criteria, such as theoretical plates and tailing factor. It is also cost-effective, doesn't need specialist detection systems (like LC-MS/MS), and is easy to adapt to normal pharmaceutical QC labs.

Limitations

The existing approach was refined and proven, although not implemented for complex pharmaceutical formulations. It also did not assess drug long-term stability under real-time storage conditions.

Future Studies

Future research may involve the structural characterization of degradation products employing methodologies such as LC-MS/MS or NMR. Using the method on tablet or capsule formulations can help prove that it is good for regular quality control in the industry.

Conclusion

To conclude, the RP-HPLC approach for quantifying the combined dosage of TEN and PGL is reliable and effective. The technique's simplicity, speed, and sensitivity make it ideal for quality control in the industry. Extended validation studies validated the method's reliability and accuracy across varied analytical conditions, proving its sustainability. The method's specificity is shown by its ability to separate APIs from excipients and degradation products. This ensures that the current method can be used efficiently for the purpose of stability investigations. The method's ICH-compliant validation suggests it could be used for routine TEN and PGL analysis in combined pharmaceutical formulations.

Conflict of Interest

The authors report no conflicts of interest in this work.

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