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Stability-Indicating TLC-Densitometric and HPLC Methods for Simultaneous Determination of Teneligliptin and Pioglitazone in Pharmaceutical Dosage Forms with Eco-Friendly Assessment

Ashok H Akabari (ashokakabari@gmail.com)
Shree Naranjibhai Lalbhai Patel College of Pharmacy
Harsh Gajiwala
Shree Naranjibhai Lalbhai Patel College of Pharmacy
Sagarkumar K Patel
Labcorp Drug Development Inc
Jasmina Surati
Shree Naranjibhai Lalbhai Patel College of Pharmacy
Divya Solanki
Shree Naranjibhai Lalbhai Patel College of Pharmacy
Ketan V Shah
Shree Naranjibhai Lalbhai Patel College of Pharmacy
Tejas J Patel
Shree Naranjibhai Lalbhai Patel College of Pharmacy
Sagar P Patel
Vidhyadeep Institute of Pharmacy

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Abstract

The combination of multiple drugs in pharmaceutical formulations has greatly improved the management of complex medical conditions, particularly benefiting patients with type 2 diabetes mellitus. Two powerful antidiabetic agents, teneligliptin hydrobromide hydrate (TEN) and pioglitazone hydrochloride (PIO), play key roles in regulating blood glucose levels. In this study, we introduce innovative methods for the simultaneous quantification of TEN and PIO in pharmaceutical formulations, ensuring accuracy and stability assessment. Our TLC-densitometric approach employs a mobile phase consisting of Methanol, Toluene, Ethyl Acetate, and Triethylamine (1:7:2:0.1, v/v/v/v) on TLC silica gel plates, followed by densitometric scanning at 268 nm. Meanwhile, the RP-HPLC method utilizes an isocratic elution with acetonitrile and acetate buffer (pH 2.3, 60:40 v/v) on a C18 column, delivering diode-array detection at 235 nm. Both methods offer exceptional accuracy and reliability, serving as valuable tools for pharmaceutical quality control. Furthermore, our research incorporates an environmental impact assessment to align with global sustainability goals. We consider factors such as solvent consumption, waste generation, and energy usage, using assessment tools like the eco-scale assessment, AGREE, Green Analytical Procedure Index (GAPI), and the national environmental method index (NEMI) to gauge the environmental impact of our methods. By adopting these techniques, pharmaceutical companies can enhance their drug quality control processes and fulfill their environmental responsibilities. Comprehensive statistical comparisons, including t-tests and F-tests, validate the outcomes of the TLC-densitometric and RP-HPLC methods, ensuring their effectiveness in drug formulation analysis.

INTRODUCTION

The use of multiple drugs in pharmaceutical formulations has transformed the management of complex medical conditions, offering enhanced therapeutic outcomes. Among such therapeutic combinations, teneligliptin hydrobromide hydrate (TEN) and pioglitazone hydrochloride (PIO) have emerged as potent antidiabetic agents, demonstrating promising efficacy in the treatment of type 2 diabetes mellitus. TEN, chemically known as (2S,4S)-4-(4-(3-Methyl-1-phenyl-1H-pyrazol-5-yl)-1-piperazinyl)-2-(2,4,5-trifluorophenyl) butan-2-ol hydrobromide hydrate, is a dipeptidyl peptidase-4 inhibitor, while PIO, with the chemical name (RS)-5-[(4-(2-(5-ethylpyridin-2-yl) ethoxy) phenyl) methyl] thiazolidine-2,4-dione hydrochloride, belongs to the thiazolidinediones class, enhancing insulin sensitivity. These compounds play pivotal roles in regulating blood glucose levels and hold immense clinical significance. TEN enhances glycemic control by inhibiting the dipeptidyl peptidase-4 enzyme, preventing the degradation of incretin hormones, while PIO improves insulin sensitivity and reduces insulin resistance, collectively leading to effective glucose homeostasis. Exploration of literature reveals numerous analytical approaches for the determination of TEN, both as an individual compound and in combination with concurrently administered medications. These methods include UV spectrophotometry [1–3], high-performance liquid chromatography (HPLC) [4–9], and high-performance thin-layer chromatography (HPTLC)[10–12]. Conversely, PIO, whether as a single drug or in combination with other coadministered drugs, has been previously evaluated through diverse methodologies, including UV spectrophotometry [13, 14], HPLC [15–26] and HPTLC [27, 28] .

In this study, we present stability-indicating TLC-densitometric and high-performance liquid chromatography (RP-HPLC) methods for the simultaneous determination of TEN and PIO in pharmaceutical dosage forms. These stability-indicating methods are designed to accurately quantify these drugs and their potential degradation products, providing crucial information on drug stability during storage and use.

Furthermore, we have integrated an innovative Environmental Impact Assessment to evaluate the "greenness" of the developed analytical methods. By considering the environmental impact of the methods, we aim to contribute to sustainable and environmentally friendly analytical practices. The assessment takes into account factors such as solvent consumption, waste generation, and energy usage during the analytical process. It will evaluate the ecological footprint of the developed methods, allowing us to quantify their environmental impact in comparison to conventional analytical techniques [29]. The incorporation of "green" practices in analytical methods aligns with the growing global emphasis on sustainable development and environmentally responsible research [30].

Currently, there are several methods available to assess the environmental sustainability of an analytical technique. These methodologies include eco-scale assessment, the analytical greenness metric approach (AGREE), the Green Analytical Procedure Index (GAPI), and the national environmental method index (NEMI) [29–32]. These tools offer the capability to effectively assess the eco-friendliness attributes of a given analytical procedure. This study aims to advance pharmaceutical research and quality control practices by providing reliable stability-indicating methods for TEN and PIO determination, while also contributing to a more sustainable and eco-friendly analytical approach. The results obtained from the innovative "green" assessment will guide us in developing analytical methodologies that minimize their ecological impact, thus promoting greener and more sustainable practices in the pharmaceutical industry. To ensure the safety, efficacy, and environmental sustainability of these drug formulations, the development of stability-indicating methods for their simultaneous determination and a comprehensive environmental impact assessment is of paramount importance. As of now, the scientific realm remains uncharted with regards to the unveiling of TLC-densitometric and RP-HPLC methodologies devised for the concurrent quantification of TEN and PIO in pharmaceutical formulations, even when these compounds dance alongside their potential degradation products. Consequently, our paramount mission in this scholarly expedition was to craft with painstaking precision, sensitivity, and trustworthiness, TLC-densitometric and RP-HPLC techniques, tailored to the task of analyzing TEN and PIO in tandem, resilient even in the face of lurking degradation products.

Experimental

Apparatus and Analytical Conditions

An HPTLC system (CAMAG, Muttenz, Switzerland) was used for thin-layer densitometric analysis. It had a Linomat V sample applicator, a CAMAG twin-trough developing chamber (10 x 10 cm), a UV chamber with dual-wavelength UV lamps, a TLC Scanner IV densitometer (CAMAG), a Hamilton syringe with a 100 µL

capacity (Bonaduz, Switzerland), winCATS software (version 1.4.6, 2002; CAMAG), and aluminum-backed TLC plates measuring 1 A deuterium lamp was used as a radiation source for the TLC densitometric method, which was performed in absorption mode. The slit size was set to 6 × 0.45 mm, and the reading speed was set to 20 mm/s. To put the samples on the TLC plates (10 × 10 cm, 250 εm thick), 10 µL of the stock standard solution for each component was spread out in 6 mm wide bands using the Linomat V sample spreader. The bands were placed 10 mm from the plate's bottom edge, with 5 mm between each band. The separation process took place in a chromatographic TLC chamber that had been filled with a developing system of methanol, toluene, ethyl acetate, and triethylamine (in a ratio of 1:7:2:0.1, v/v/v/v) at room temperature for 30 minutes. Densitometric measurements were taken at 268 nm.

HPLC Method

The chromatographic method was developed using the Shimadzu P Series High-Performance Liquid Chromatographic System CTO-A10ASvp (SHIMADZU LAB, JAPAN), which includes a Shimpack ODS Column: C18 (250 × 4.6 mm id, 5 µm particle size), Thermostated Column Compartment (CTO-10AS vp), PDA detector, and LC 20 AD UFLC series quaternary Data were collected and processed using lab solution data system software. The mobile phase was 60:40 v/v acetonitrile and pH 2.3 acetate buffer at 1 mL/min. The injection volume was 10 µL, the column temperature was 30°C, and detection was at 235 nm. The best separation was achieved with a 10-minute isocratic run.

Reagents

Teneligliptin hydrobromide hydrate and pioglitazone hydrochloride were kindly supplied by Zota Healthcare, the pharmaceutical industry, Surat, Gujarat. Zita Plus-Pio Tablet was manufactured by Glenmark Pharmaceuticals Ltd. Each tablet claimed to contain 20 mg TEN and 15 mg PIO and was used to prepare the sample solution. Methanol, acetonitrile, water (HPLC grade, Rankem), potassium hydroxide, sodium acetate anhydrous, glacial acetic acid (ACS Chemicals), toluene (HPLC grade, Loba Chemie Pvt. Ltd.), ethyl acetate (AR grade, Allied Chemicals Corporation) was used for the development of methods. Analytical-grade reagents and solvents were used in this study.

(a) Stock Standard Solutions: Teneligliptin hydrobromide hydrate (TEN) (0.1g) and pioglitazone hydrochloride (PIO) (0.1g) were weighed and transferred into separate 10 mL volumetric flasks. They were dissolved in 5 ml of methanol and diluted to volume with methanol to achieve a 1000 µg/mL stock standard solution for each component.

(b) Working Standard Solutions: Accurately transfer 1 mL each of TEN and PIO from their respective stock standard solutions (1 mg/mL) into two separate 10 mL volumetric flasks, then dilute to 10 ml with methanol to achieve a 100 µg/mL working standard solution for each component.

(c) Sodium acetate buffer pH 3 Preparation for HPLC: A total of 0.6 gm of anhydrous sodium acetate was accurately weighed and dissolved in 100 ml of HPLC-grade water. Sonicate the solution for 10 minutes. Glacial acetic acid was added to bring the volume up to 150 ml to achieve a pH 2.3 buffer solution. The solution was filtered and sonicated for 15 minutes.

Validation of Methods:

Linearity:

The TLC-densitometric method involves transferring 10–50 g/mL aliquots of TEN and 7.5–45 g/mL aliquots of PIO from their respective 100 µg/mL working standard solutions. Each solution was applied in bands of 10 microliters and scanned at 268 nm. By plotting the integrated peak area against the corresponding concentrations of each component, calibration curves were constructed, and regression equations were computed.

For the RP-HPLC Method, 90–300 µg/mL and 120–400 µg/mL aliquots of TEN and PIO were taken from their respective 1000 µg/mL stock standard solutions and placed in two series of 10 mL volumetric flasks. The mobile phase was used to dilute these solutions to the desired concentration. At a rate of 1 mL/min, triplicate injections of each concentration (10 µL) were made. A diode-array detector at 235 nm was used to scan the effluent. The peak regions observed for TEN and PIO were correlated with their concentrations, and regression models were then used to establish calibration curves.

Accuracy

We spiked TEN and PIO at three concentrations (50, 100, and 150%) to determine their accuracy. Accurate sample preparation and analysis were performed under the method conditions determined to be optimal. The average % recovery was computed after determining the true concentration of each sample (n = 3).

Precision

Repeatability, intra-day, and inter-day variations over defined time periods (within a single day and across three consecutive days) were calculated as part of the precision evaluation. Both TEN and PIO samples were processed and analyzed under the idealized conditions of the procedure. The data is shown as the RSD, or the variation between the mean and the standard deviation.

Sensitivity

The developed method's Limit of Detection (LOD) and Limit of Quantification (LOQ) were used to evaluate its sensitivity for the simultaneous measurement of TEN and PIO. Values for LOD and LOQ were derived from the six calibration curves using the provided formulas

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma / S$

Where σ = standard deviation of the intercept of six calibration curves, and S = mean slope of six calibration curves.

Specificity

Standard and sample solutions containing TEN and PIO were analyzed under precise chromatographic conditions to assess the method's specificity. Tablet excipients did not affect TEN and PIO measurements. The TLC densitometric and RP-HPLC techniques were also tested for degradation product interference.

Robustness

To investigate resilience, we purposefully altered the chromatographic conditions in modest ways. Variations in flow rate, pH, and detection wavelength were used to evaluate RP-HPLC's stability. Modifications to the mobile phase composition, chamber saturation time, and wavelength were developed and tested for their impact on the peak area in TLC-densitometric analysis. Both proposed approaches were evaluated in terms of their robustness as measured by the percentage RSD (relative standard deviation). The % RSD was calculated after triplicate determination of robustness at 200 ng/band for TEN and 150 ng/band for PIO.

System Suitability

Parameters including the Rf value, tailing factor, and selectivity factor for both TEN and PIO were evaluated to guarantee system appropriateness for the TLC densitometric approach. Peak retention duration, theoretical plates, tailing factor, and resolution were evaluated for both TEN and PIO to verify HPLC's system appropriateness. The theoretical plates > 2000, tailing factor 2.0, and resolution > 2 thresholds were predetermined acceptance requirements for these characteristics. For these analyses, we employed 20 µg/mL TEN and 15 µg/mL PIO standard solutions, respectively. Six separate sample assays confirmed the analytical system's uniformity and dependability.

Force Degradation Study

In accordance with ICH guidelines [33, 34], forced degradation studies were performed.

Acid Degradation Study:

To study acid degradation, 2 mL solutions were prepared from TEN (2000 µg/mL), PIO (1500 µg/mL), and a mixture of both (2000 µg/mL TEN and 1500 µg/mL PIO) in three 10 mL volumetric flasks. Add 2 mL of 0.1 N HCl and reflux at 70°C for 2 hours. After neutralization with 0.1 N NaOH, the volume was adjusted to 10 mL with methanol, resulting in a final concentration of 200 µg/mL TEN and 150 µg/mL PIO. For the acid degradation investigation, TLC-densitometric and RP-HPLC were used to examine the produced solutions.

Alkali Degradation Study:

To study alkali degradation, 2 mL solutions were prepared from TEN (2000 µg/mL), PIO (1500 µg/mL), or a mixture of both (2000 µg/mL TEN and 1500 µg/mL PIO) in three 10 mL volumetric flasks. The solutions were refluxed with 2 mL 0.1 N NaOH at 70°C for 2 hours. After neutralizing with 0.1 N HCl, the volume was

adjusted to 10 mL with methanol, resulting in a final concentration of 200 µg/mL TEN and 150 µg/mL PIO. Alkali degradation was studied using TLC-densitometric and RP-HPLC on the produced solutions.

Oxidative Degradation Study:

To evaluate oxidative degradation, 2 mL solutions were prepared from TEN (2000 µg/mL), PIO (1500 µg/mL), and a mixture of both (2000 µg/mL TEN and 1500 µg/mL PIO) in three 10 mL volumetric flasks. Each flask received 2 mL of 3% hydrogen peroxide and was heated to 70°C for 2 hours. After cooling, methanol was added to reach a final concentration of 200 µg/mL TEN and 150 µg/mL PIO. The proposed TLC-densitometric and RP-HPLC procedures examined the solutions for oxidative deterioration.

Thermal Degradation Study:

Pure TEN and PIO samples, along with tablet powder, were baked in an oven at 70 ^{0}C for six hours to investigate thermal degradation. Following this incubation time, methanol solutions containing 200 µg/mL TEN and 150 µg/mL PIO were produced from the samples. The proposed TLC-densitometric and RP-HPLC methods were then used to these solutions.

Photolytic Degradation Study:

Pure TEN, PIO, and tablet powder were exposed to UV light for 24 hours for the light degradation investigation. Following this, methanol solutions were produced from samples, resulting in 200 µg/mL TEN and 150 µg/mL PIO concentrations. The proposed TLC-densitometric and RP-HPLC procedures examined these solutions.

Results and Discussion

Method Development and Optimization

TLC-Densitometric Method: In order to improve chromatographic separation, it is crucial to investigate the impact of various factors. The pursuit of the optimal parameters that govern maximum separation involves considering factors such as solvent migration distance, chamber saturation time, detection wavelength, and mobile phase composition. When developing the densitometric method, we evaluated different organic compositions and ratios to determine their suitability as mobile phases. This included combinations such as acetonitrile-methanol, chloroform-methanol, and methanol-ethyl acetate-toluene. Notably, the presence of ethyl acetate in the developing system proved to be crucial for effectively distinguishing between TEN, PIO, and their degradation products. Consequently, we varied the ratio of methanol: ethyl acetate: toluene: triethylamine, and the inclusion of triethylamine notably improved separation and peak symmetry between TEN and PIO. The most optimal mobile phase composition was determined to be methanol: toluene: ethyl acetate: triethylamine (1:7:2:0.1, v/v/v/v). This selection resulted in robust separation between the analyzed components, producing desirable RF-values and minimal tailing of separated bands.

The proposed TLC-densitometric method offers high selectivity and sensitivity for TEN and PIO analysis, using methanol: toluene: ethyl acetate: triethylamine (1:7:2:0.1, v/v/v/v) as the developing system. Extensive evaluation of scanning wavelengths indicated that 268 nm provided maximum sensitivity, generating sharp, symmetrical peaks with minimal noise. This configuration demonstrated excellent sensitivity for the simultaneous determination of both drugs in the presence of each other. For solvent migration distance and chamber saturation time, values of 80 mm and 30 minutes, respectively, were found to be optimal for effective separation. The distinct RF-values of 0.21 and 0.45 for TEN and PIO, respectively, underscored the efficacy of the separation, as depicted in Fig. 1.

RP-HPLC Method: Developing the proposed RP-HPLC method required systematic consideration of various parameters affecting sensitivity, selectivity, and chromatographic efficiency. Initial experiments with different mobile phase compositions, such as acetonitrile, methanol, and water, resulted in peak splitting for TEN and broad peaks for PIO. To address these issues, we replaced water with phosphate and acetate buffer to reduce peak broadening. We conducted several trials with varying ratios of acetonitrile, methanol, and phosphate buffer pH 3 to enhance chromatographic resolution. Although peak broadening decreased, peak shape remained suboptimal, and analysis time increased. The next step involved replacing phosphate buffer pH 3 with acetate buffer pH 2.3, adjusted with glacial acetic acid. This change significantly affected the retention time, resolution, and peak shape of TEN and PIO. After extensive experimentation, we determined that the most effective separation and resolution were achieved using acetonitrile and sodium acetate buffer (pH 2.3, adjusted with glacial acetic acid) at a ratio of 60:40 (v/v) as the mobile phase. This mobile phase configuration provided efficient separation with shorter retention times and minimal peak tailing, as illustrated in Fig. 2. For detection, a diode-array detector set at 235 nm yielded optimal results in terms of sensitivity and peak shape. Furthermore, we identified an optimum flow rate of 1 mL/min, which delivered satisfactory separation under the defined experimental conditions. Using these conditions, we determined retention times of 4.274 minutes for PIO and 6.117 minutes for TEN, as shown in Fig. 2.

Method Validation

International Conference on Harmonization (ICH) standards were followed throughout the validation of the suggested procedures [26].

(a) Linearity: The proposed methods were found to be linear within the evaluated ranges. The linearity for TEN using the TLC-densitometric technique was 100–500 ng/band, and that for PIO was 75–450 ng/band. However, the linearity range for the RP-HPLC technique was wider, covering concentrations of TEN from 90 to 300 ng/mL and PIO from 120 to 400 ng/mL. Table 1 displays the results of calculating regression equations for these approaches.
(b) Accuracy: Accuracy was determined by calculating pure TEN and PIO samples using the corresponding regression equations. Tablet dose forms of TEN and PIO were also validated for accuracy using the standard addition method. Positive results from this analysis confirmed the efficacy of the established procedures (Table 1), suggesting that there was no interference from excipients in the pharmaceutical formulation.

(c) Precision: Suitable for use in quality control analysis of TEN and PIO in their pharmaceutical formulation, the methodologies showed intra- and inter-day variance that was acceptable. Table 1 shows that the RSD for each concentration should be kept below 2% in accordance with the ICH recommendations [41]. The % RSD values of all the samples examined fell within the specified tolerances, demonstrating the accuracy of the suggested procedures.
(d) Sensitivity: Lower LOD and LOQ values for both TEN and PIO demonstrate the sensitivity of the new approaches (Table 1). The determination of LOD and LOQ was based on SD and slope, with the formulas: LOD = 3.3 × SD/slope and LOQ = 10 × SD/slope.
(e) Specificity: TLC densitogram and HPLC chromatograms, as shown in Fig. 4, provide evidence of specificity. Parameters such as selectivity and resolution factor that were determined also under the acceptable criteria (Table 2).
(f) Robustness: During the robustness analysis, the experimental circumstances were purposefully changed to see how it affected the peak area. TLC-densitometric analysis allowed for a wide range of customizations, such as a ± 0.1% shift in developing system composition, as well as shifts in chamber saturation time, mobile phase composition, and wavelength. The total peak area was unaffected by these alterations. Variations in the HPLC procedure included shifting the flow rate (± 0.1 mL/min), shifting the wavelength at which the sample was detected, and shifting the percentage of acetonitrile in the mobile phase composition (± 5 v/v). Table 3 displays RSD values that are very small, demonstrating the reliability of the new techniques.
(g) System suitability testing: Computing characteristics such tailing factor (T), selectivity factor ( ), and resolution (RS), as described in Table 2, allowed us to assess the suitability of both the TLC-densitometric and RP-HPLC systems. There was no outlier among the estimated values, which is indicative of the approaches' strong selectivity and confirms the system's performance as a whole.

Table 1
Regression data obtained for TEN and PIO using the proposed TLC-densitometric and HPLC methods

Parameters	TLC-densitometric method		HPLC method	
	TEN	PIO	TEN	PIO
Linearity and Range	100–600 ng/band	75–450 ng/band	120–400 µg/ml	90–300 µg/ml
Regression Equation	y = 4.3467x + 467.19	y = 10.157x + 916.09	y = 944.13x + 46625	y = 1319.3x + 17932
Regression Coefficient	0.9983	0.9986	0.9975	0.9989
Accuracy (% Recovery)	100.10–101.90	99.61-100.43	99.42–100.20	99.73–101.90
Precision (% RSD) Repeatability	1.02	1.44	1.50	1.16
Intraday Precision (% RSD)	0.52–0.92	0.64–1.10	0.72–0.88	0.85–1.36
Interday Precision (% RSD)	0.83–1.05	0.99–1.24	0.84–1.19	0.66–1.60
LOD	9.94 ng/band	6.76 ng/band	12.18 µg/ml	11.51 µg/ml
LOQ	30.12 ng/band	20.49 ng/band	38.84 µg/ml	34.87 µg/ml

Table 2
System Suitability parameters of TEN and PIO

Drugs	TLC-densitometric method		HPLC method	
	Parameters	Mean ± SD (n = 6)	Parameters	Mean ± SD (n = 6)
TEN	R_f Value	0.19 ± 0.02	Retention Time	6.226 ± 0.124
	Tailing Factor	1.10	Theoretical Plates	19181 ± 383.62
	Selectivity (α)	-	Tailing Factor	1.074 ± 0.022
PIO	R_f Value	0.41 ± 0.03	Retention Time	4.355 ± 0.087
	Tailing Factor	0.99	Theoretical Plates	15455 ± 309.1
	Selectivity (α)	2.21	Tailing Factor	1.009 ± 0.020
	Resolution	4.11 ± 0.075	Resolution	4.577 ± 0.092

Table 3
Robustness data for TEN and PIO as determined by both the TLC-densitometric method and the HPLC method

Method	***TLC-densitometric method***			***HPLC method***		
Parameter	**Change in condition**	**%RSD**		**Change in condition**	**%RSD**	
		TEN	**PIO**		**TEN**	**PIO**
Detection Wavelength	266	0.76	1.48	280nm	1.16	1.34
	268	0.69	0.93	283nm	1.01	0.77
	270	1.70	1.40	286nm	1.04	1.44
Chamber Saturation Time / Flow Rate	25 min	1.43	1.20	0.9 ml/min.	1.02	0.52
	30 min	0.69	0.93	1 ml/min.	1.01	0.77
	35 min	0.64	1.54	1.1 ml/min.	1.05	0.97
Mobile Phase Composition	Methanol:Toluene: Ethyl Acetate :Triethylamine (1.1:7:1.9:0.1)	1.37	1.71	ACN : Buffer (65 : 35)	1.44	1.05
	Methanol:Toluene:Ethyl Acetate :Triethylamine (1:7:2:0.1)	0.69	0.93	ACN : Buffer (60 : 40)	1.01	0.77
	Methanol:Toluene:Ethyl Acetate :Triethylamine (1:7.7:1.2:0.1)	1.08	1.48	ACN : Buffer (55 : 45)	0.80	1.51

Force degradation study

The stability-indicating properties of PIO and TEN were investigated by subjecting them to a variety of degrading treatments, including TLC-densitometric and HPLC techniques. Drug degradation was measured as a decrease in the peak area of PIO and TEN under different circumstances, with the greatest degradation happening under alkaline conditions and the least under acidic ones. Table 4 provides a summary of the percentages of degradation under various situations. The standard drug's degradation products can be resolved using the stability-indicating assay method, proving its usefulness. The peaks of the analytes were clearly separated from those of the degraded chemical entities.

Table 4
Stability study results for TEN and PIO

Degradation Condition	% Degradation of TEN		% Degradation of PIO	
	TLC-densitometric method	HPLC	TLC-densitometric method	HPLC
Acid Hydrolysis 0.1 M HCl 70°C for 2 hours	5.82	5.74	11.58	12.63
Base Hydrolysis 0.1 N NaOH 70°C for 2 hours	7.11	7.00	14.41	15.44
Oxidative Degradation 3% H_2O_2 70°C for 2 hours	8.16	8.81	13.01	13.43
Thermal degradation (Hot air oven at 70°C for 8 hours)	4.17	4.47	4.70	5.83
Photolytic degradation (UV Light for 24 hours)	5.38	5.85	7.76	8.42

In the HPTLC method, exposure to acidic stress induced a degradation of 5.82% for TEN, with one unidentified degradation peak at 0.27 (Fig. 3 (a)). Similarly, PIO experienced an 11.58% degradation with one unidentified degradation peak at 0.73 (Fig. 3 (b)), while the determination of TEN and PIO remained unaffected by degradants, as depicted in Fig. 3 (c). Alkaline stress resulted in a 7.11% degradation for TEN, accompanied by two unknown degradation peaks at 0.24 and 0.32 (Fig. 3 (d)). PIO exhibited a 14.41% degradation with one unidentified degradation peak at 0.27 (Fig. 3 (e)), with no interference from degradants in the determination of TEN and PIO, as illustrated in Fig. 3 (f). Peroxide stress caused an 8.16% degradation, and PIO experienced a 13.01% degradation without detectable degradation products (Fig. 4 (a)). Thermal degradation studies of TEN and PIO indicated a reduction in peak area by 4.17% and 4.70%, respectively, without detectable degradation products (Fig. 4 (b)). Photolytic conditions resulted in a 5.38% decrease in peak area for TEN and a 7.76% decrease for PIO (Fig. 4 (c)).

In the HPLC method, exposure to acidic stress led to a 5.74% degradation for TEN (Fig. 5 (a)) and a 12.63% degradation for PIO (Fig. 5 (b)), both without detectable degradation products. Figure 5 (c) illustrates no interference from degradants in the determination of TEN and PIO. Alkaline stress induced a 7.00% degradation for TEN with one unidentified degradation peak at 5.080 (Fig. 5 (d)), and a 15.44% degradation for PIO with one unidentified degradation peak at 6.668 (Fig. 5 (e)), while Fig. 5 (f) demonstrates no interference from degradants. Peroxide stress resulted in an 8.81% degradation, and PIO experienced a 13.43% degradation without detectable degradation products (Fig. 6 (a)). Thermal degradation studies showed a reduction in peak area by 4.47% for TEN and 5.83% for PIO, without detectable degradation products (Fig. 6 (b)). Photolytic conditions led to a decrease in peak area by 5.85% for TEN and 8.42% for PIO (Fig. 6 (c)).

Statistical Evaluation of the Developed TLC Densitometric and HPLC Methods

TLC-densitometric and HPLC-based TEN and PIO values were compared statistically in this study. The analysis was conducted using the student's t-test and the F-test for variance ratio at a 95% level of confidence. However, Table 5 shows that there is no appreciable difference in accuracy and precision between the proposed methods and the reported ones, as the computed t and F values are lower than their respective tabular values.

Table 5
Statistical comparison of proposed methods for the determination of TEN and PIO

Parameters	TLC-densitometric method		HPLC method	
	TEN	PIO	TEN	PIO
Mean (n = 6)	104.97	100.03	105.86	99.78
SD	0.938	1.001	1.103	1.046
Variance	1.28	1.002	1.84	1.095
N	5	5	5	5
df	4		4	
t-Critical two tail (2.776)[a]	1.11	-0.43	1.11	-0.43
F-Critical value (6.388)[a]	1.38	1.092	1.38	1.092

[a] Values in parentheses correspond to the theoretical values of t and F (P = 0.05).

Assessment of the Environmental Friendliness of the Developed Methods:

Four different ecological metrics were used to evaluate the environmental effects of the standard TLC densitometric and HPLC methods for TEN and PIO determination: eco-scale assessment [30], the analytical greenness metric approach (AGREE) [31], the Green Analytical Procedure Index (GAPI) [32], and the national environmental method index (NEMI) [28]. The recommended HPLC procedure scored an 86 on the analytical eco-scale [31, 35], while the TLC-densitometric approach scored a 77. Table 6 displays the results of an ecological score comparison between the proposed method and the documented method. The proposed method has a much higher ecological profile.

In terms of GAPI assessment [30, 36], the developed methodology underwent evaluation, resulting in a visual representation as illustrated in Table 6. In this representation, the proposed approach's pictograms, categorizing sample preparation, reagents and solvents, and instrumentation assessment, show a combination of yellow and green colors. This contrasts with the pictograms of established methods,

which tend to display 4 red indications for the HPLC method and 3 red indications for the TLC-densitometric method, signifying the higher environmental compatibility of the proposed method. Regarding the AGREE metric [30,38], which provides a novel software-driven means of quantifying environmental compatibility, the assessment tool is particularly noteworthy when compared to GAPI and the analytical eco-scale. The AGREE software-generated scores, as presented in Table 6, confirm that the proposed HPLC and TLC-densitometric methods are significantly more environmentally friendly, with scores for both methods exceeding 0.65.

The NEMI serves as an additional instrument for assessing the ecological viability of the formulated RP-HPLC and TLC-densitometric methods, considering four distinct criteria: waste generation, corrosiveness, deposition of harmful chemicals, and exposure to hazardous substances [30, 37]. In the context of the devised RP-HPLC and TLC-densitometric methodologies, the use of solvents such as Acetonitrile, Ammonium acetate, methanol, ethyl acetate, and toluene are noteworthy, as these solvents do not fall within the category of PBT (Persistent, Bioaccumulative, and Toxic) or hazardous agents, as assessed by the EPA's Toxic Release Inventory. The pH range of the mobile phase, spanning from 2 to 12, falls below the corrosive threshold (< 12). Waste production per individual run is calculated at 10.4 mL/sample for the RP-HPLC approach, contrasting with 5 mL/sample for the TLC-densitometric method. These findings collectively confirm that the newly developed methodologies adhere to the four parameters indicative of environmental compatibility, justifying their classification as eco-friendly techniques, as shown in Table 6.

Conclusions

Without the need for pre-separation processes, TLC-densitometric and RP-HPLC methods were developed and validated to separate and quantify TEN and PIO in pharmaceutical formulations containing their degradation products. The stability-indicating RP-HPLC method ensures the quantification of both intact drugs and their degradation products under a wide range of stress conditions, due to its superior separation and sensitivity. The HPTLC technology offers an efficient and economical alternative for the simultaneous investigation of various diabetic drugs. Visual inspection of the separated analytes improves technique specificity, while high-performance thin-layer chromatography (HPTLC) enables for the separation and quantification of numerous components in a single run using a small amount of the developing system. Both TEN and PIO in tablet form were successfully quantified using the methods developed. These assays represent the first of its category to be created, and they show promise as a means of determining TEN and PIO stability. The quantitative results obtained from TLC densitometric and HPLC methods did not show any statistically significant differences between them. The meticulous development of these methods not only produces robust analytical results but also underscores their exceptional environmental conscientiousness, as evidenced by their minimal waste generation, non-corrosive nature, and utilization of non-hazardous solvents.

Declarations

Author Contribution

Author Contribution in Development of Analytical MethodsAshok H Akabari- Conceptualization, Methodology, SoftwareSagarkumar K Patel- Writing - Review & EditingHarsh Gajiwala– Resources, InvestigationDivya Solanki – Resources, InvestigationJasmina Surati- Software, ValidationSagar P Patel - Software, Validation, InvestigationKetan V Shah, Tejas Patel- Formal analysis

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Table 6

Table 6 is available in the Supplementary Files section.

Figures

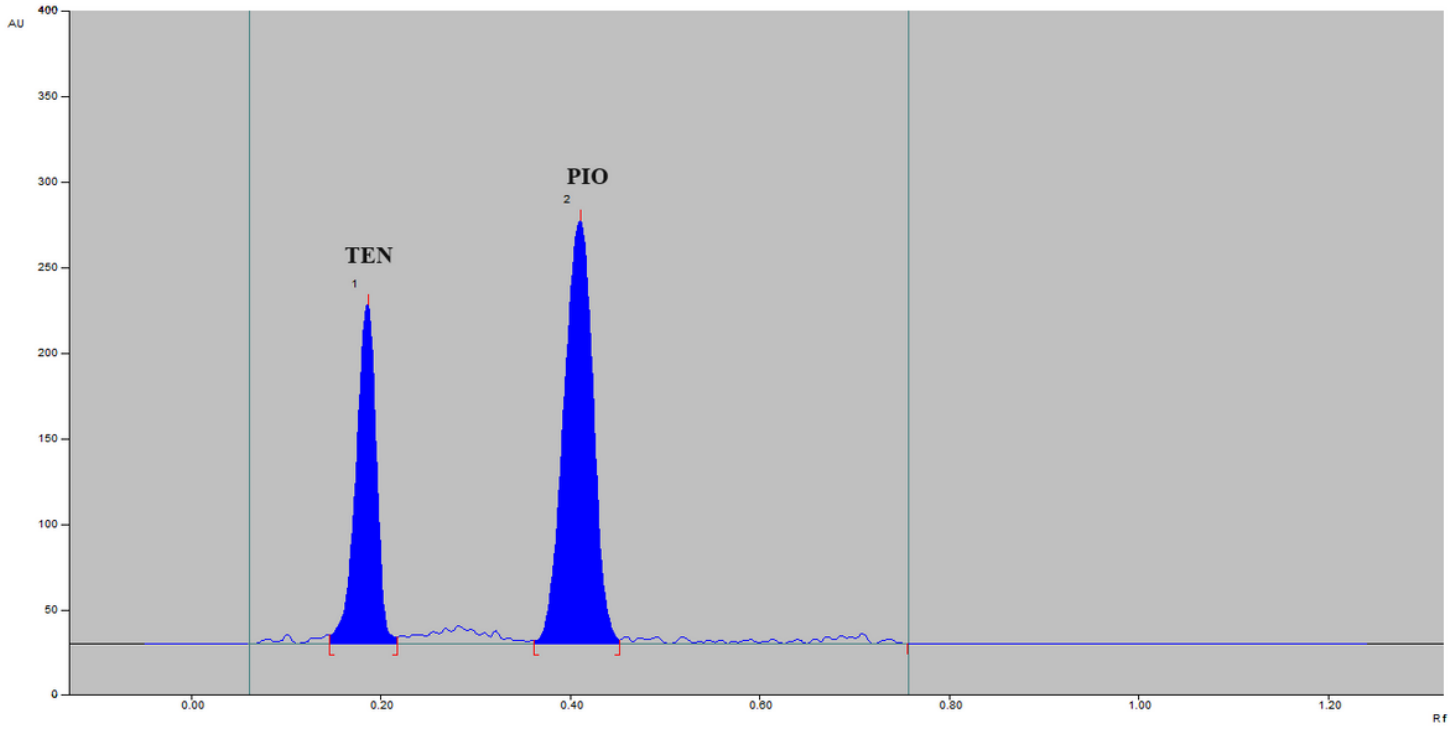

Figure 1

TLC densitogram of TEN and PIO using optimized densitometric condition

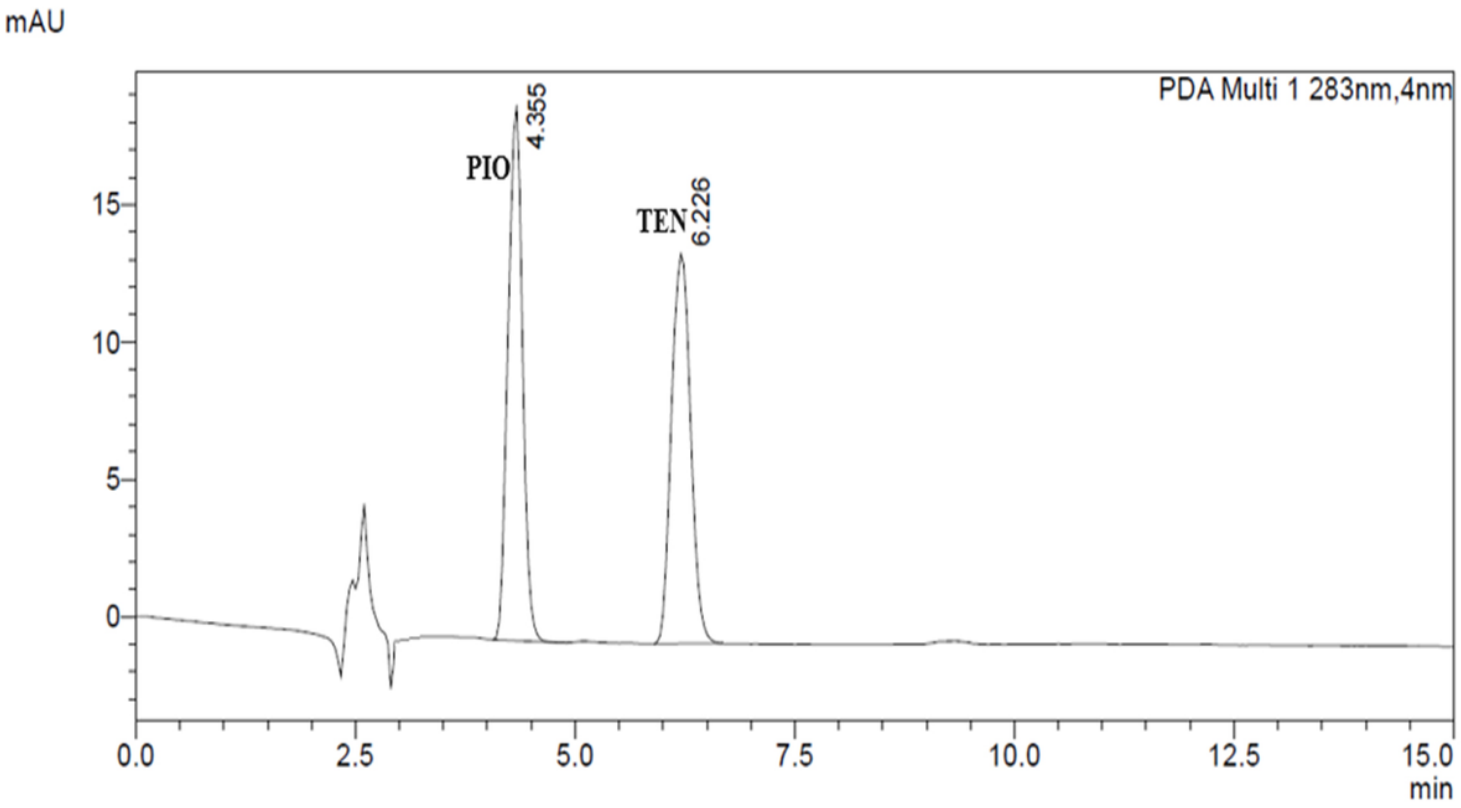

Figure 2

HPLC chromatogram of TEN and PIO using optimized chromatographic condition

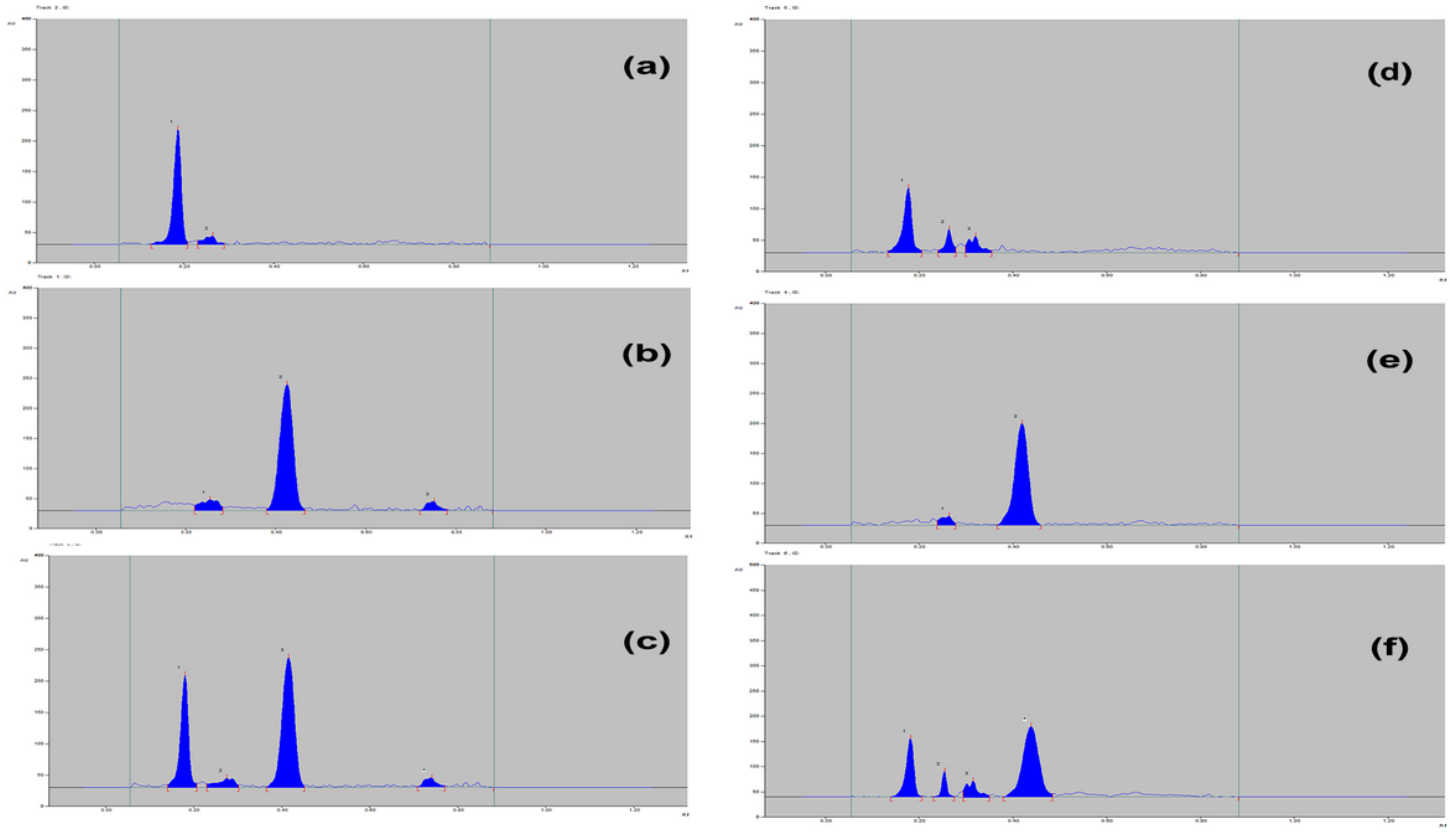

Figure 3

TLC densitogram of TEN and PIO under varied Conditions, including Acid Hydrolysis, Alkaline Hydrolysis a) TLC densitogram of TEN by acid hydrolysis b) TLC densitogram of PIO by acid hydrolysis c) TLC densitogram of TEN and PIO by acid hydrolysis d) TLC densitogram of TEN by alkaline hydrolysis e) TLC densitogram of PIO by alkaline hydrolysis f) TLC densitogram of TEN and PIO by alkaline hydrolysis

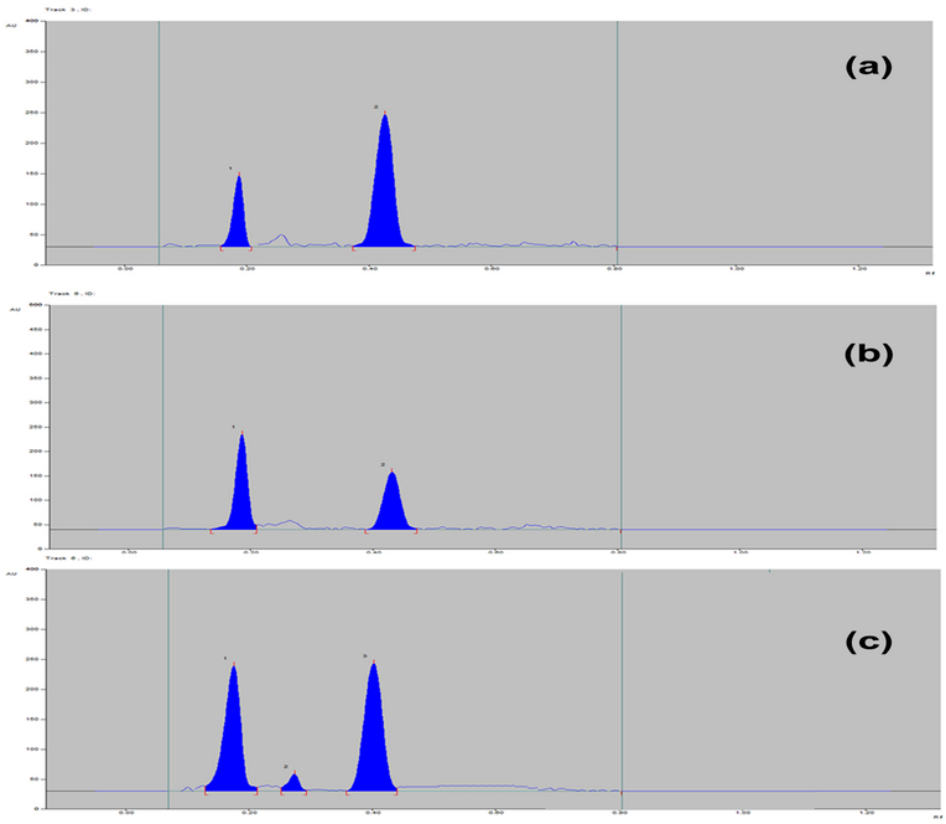

Figure 4

TLC densitogram of TEN and PIO under varied Conditions, including a) Oxidative Degradation, b) Thermal Degradation, and c) Photolytic Conditions

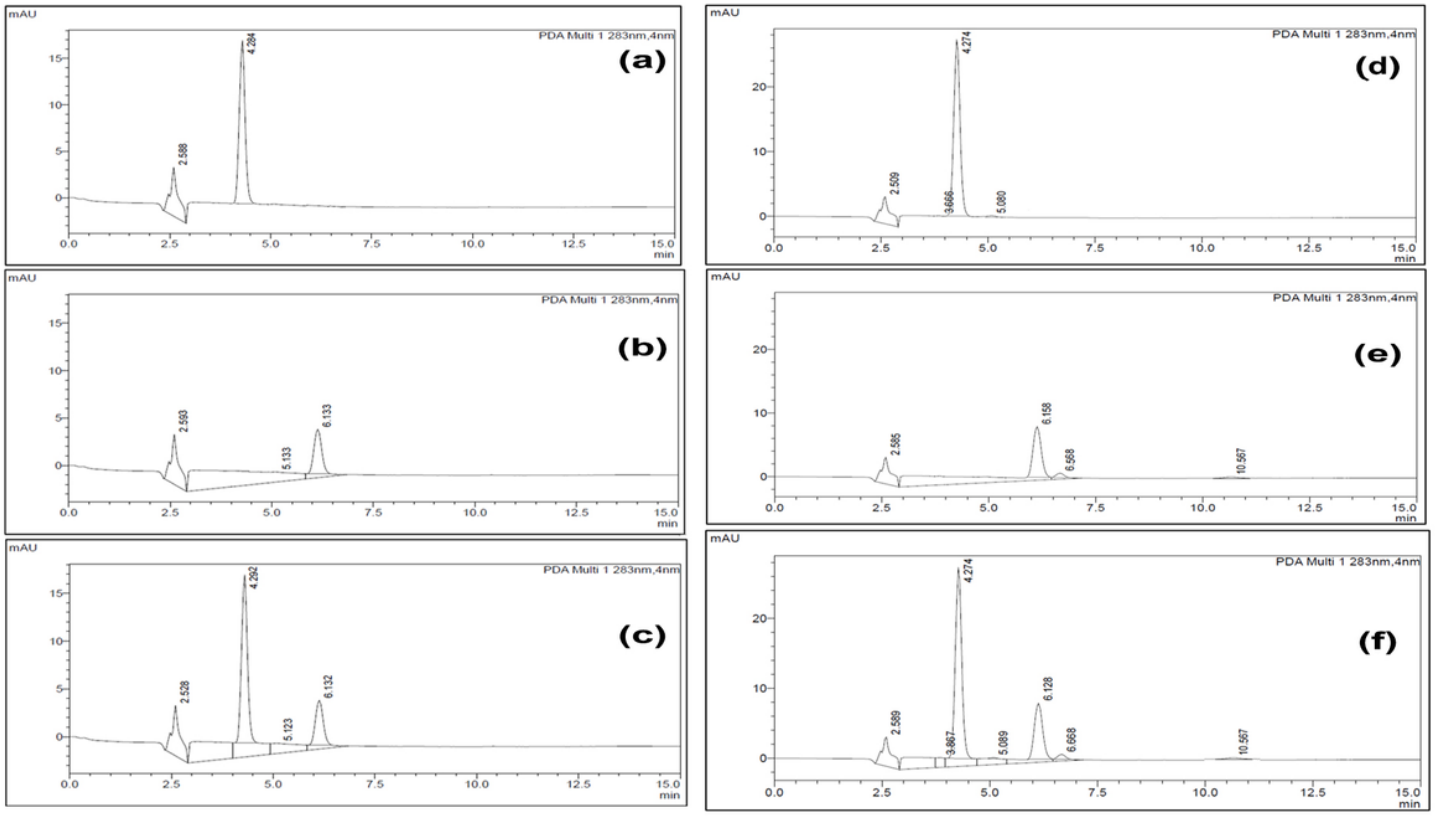

Figure 5

HPLC chromatogram of TEN and PIO under varied Conditions, including Acid Hydrolysis, Alkaline Hydrolysis a) HPLC chromatogram of TEN by acid hydrolysis b) HPLC chromatogram of PIO by acid hydrolysis c) HPLC chromatogram of TEN and PIO by acid hydrolysis d) HPLC chromatogram of TEN by alkaline hydrolysis e) HPLC chromatogram of PIO by alkaline hydrolysis f) HPLC chromatogram of TEN and PIO by alkaline hydrolysis

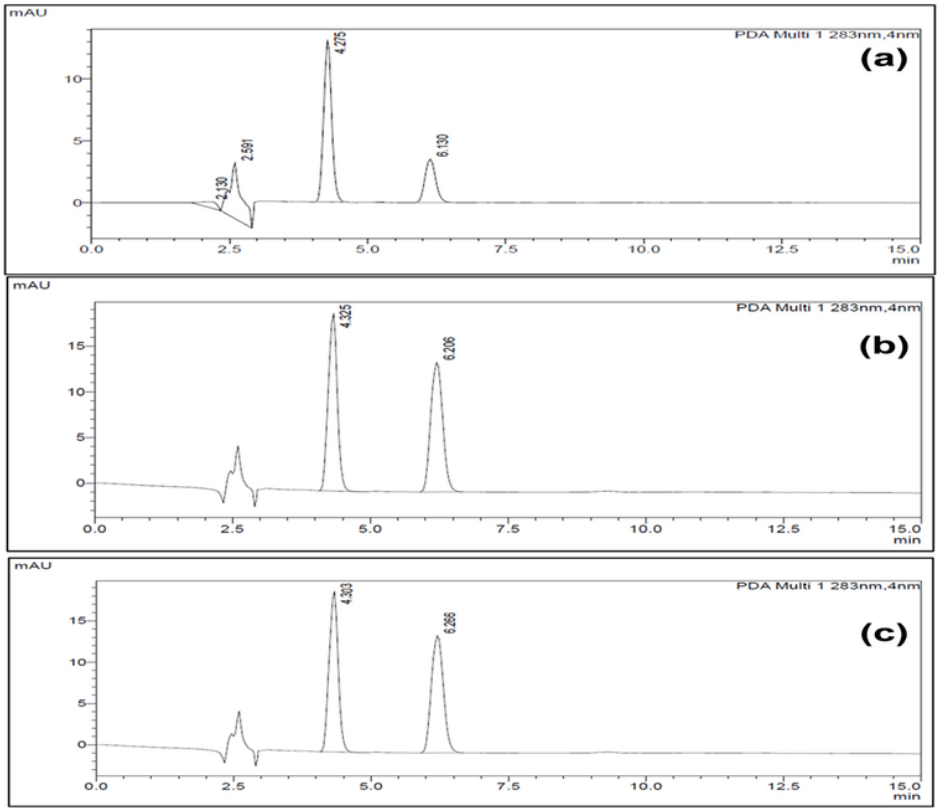

Figure 6

HPLC chromatogram of TEN and PIO under varied Conditions, including a) Oxidative Degradation, b) Thermal Degradation, and c) Photolytic Conditions

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table6.docx