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Determination of Pterostilbene in Pharmaceutical Products Using a New HPLC Method and Its Application to Solubility and Stability Samples

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Abstract: The quantification of a natural bioactive compound, pterostilbene (PTT), in commercial capsule dosage form, solubility, and stability samples was carried out using a rapid and sensitive high-performance liquid chromatography (HPLC) approach. PTT was quantified on a Nucleodur (150 mm × 4.6 mm) RP C_{18} column with a particle size of 5 µm. Acetonitrile and water (90:10 *v/v*) made up the mobile phase, which was pumped at a flow speed of 1.0 mL/min. At a wavelength of 254 nm, PTT was detected. The developed HPLC approach was linear in 1–75 µg/g range, with a determination coefficient of 0.9995. The developed HPLC approach for PTT estimation was also rapid (R_t = 2.54 min), accurate (%recoveries = 98.10–101.93), precise (%CV = 0.59–1.25), and sensitive (LOD = 2.65 ng/g and LOQ = 7.95 ng/g). The applicability of developed HPLC approach was revealed by determining PTT in commercial capsule dosage form, solubility, and stability samples. The % assay of PTT in marketed capsules was determined to be 99.31%. The solubility of PTT in five different green solvents, including water, propylene glycol, ethanol, polyethylene glycol-400, and Carbitol was found to be 0.0180 mg/g, 1127 mg/g, 710.0 mg/g, 340.0 mg/g, and 571.0 mg/g, respectively. In addition, the precision and accuracy of stability samples were within the acceptable limit, hence PTT was found to be stable in solution. These results suggested that PTT in commercial products, solubility, and stability samples may be routinely determined using the established HPLC method.

Keywords: dosage form; HPLC; pterostilbene; solubility; stability; validation

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1. Introduction

As strong antioxidants, natural polyphenols have a key role in regulating a variety of physiological diseases [1,2]. Pterostilbene (PTT) is one of the polyphenolic antioxidants with the chemical structure shown in Figure 1 [3]. Although it can be found in a wide range of plants and fruits, PTT is primarily derived from *Pterocarpus marsupium* [4–6]. It is used to manage diabetes and hypertension in conventional medical care [7]. In the literature, it has also demonstrated for a number of therapeutic efficacies, including antioxidant [8,9], anti-inflammatory [9], anticancer [9,10], antidiabetic [11], cardioprotective [12], and neuroprotective [13] effects, among others.

Due to its wide spectrum of medicinal efficacies, PTT's quality control and standardization in its commercial polyherbal products are crucial. For the qualitative and quantitative detection of PTT in plant extracts, commercial products, commercial polyherbal products, and biological samples, numerous analytical approaches have been reported. These

analytical approaches include ultraviolet (UV) spectrometry, high-performance liquid chromatography (HPLC), and ultra-high-performance liquid chromatography (UHPLC) for the determination of PTT [14–20]. PTT has also been identified in biological samples using several HPLC and UHPLC techniques, either alone or in conjunction with other bioactive chemicals [21–24]. To determine PTT in plant extracts, a high-performance thin-layer chromatography (HPTLC) method has also been used [25]. Recently, a greener and sustainable HPTLC approach has also been used by our research group to determine PTT in commercial capsule dosage forms [26].

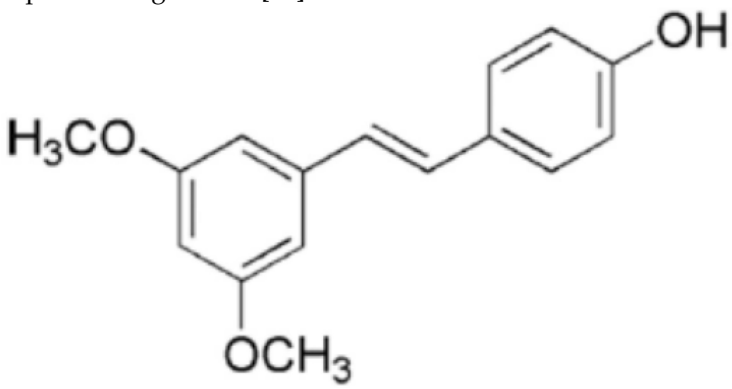

Figure 1. Chemical structure of pterostilbene (PTT).

A thorough literature evaluation suggested a variety of analytical approaches to determine PTT in different kinds of sample matrices. However, the methods in the literature have not been utilized for the measurement of PTT solubility. The solubility of bioactive compounds such as PTT is an important characteristic, and therefore its measurement in a variety of green solvents is important. As a result, simple and cost-effective analytical approaches are still required for its analysis and for further applications. Therefore, the aim of this research was to design and validate a simple, rapid, and cost-effective HPLC approach to determine PTT in commercial capsule dosage forms, solubility, and stability samples. The developed HPLC approach for determining PTT was validated according to the "International Council for Harmonization (ICH)-Q2-R1" protocols [27].

2. Materials and Methods

2.1. Materials

The working standard of PTT was provided by Sigma-Aldrich (St. Louis, MO, USA). Chromatography-grade solvents, including, methanol, acetonitrile, and ethanol were provided by E-Merck (Darmstadt, Germany). High pure water was obtained from Milli-Q® water purifier (Millipore, Lyon, France). All other materials used were of analytical grade. Commercial PTT capsules were bought from the neighborhood pharmacy in Riyadh, Saudi Arabia.

2.2. Instrumentation and Analytical Conditions

PTT was measured at 25 ± 1 °C using a Waters HPLC system 1515 isocratic pump, a 717 automatic sampler, a programmed UV-visible variable wavelength detector, a column oven, a SCL 10AVP system controller, and an inline vacuum degasser. The data were processed and analyzed using Millennium software (version 32, Waters, Milford, MA, USA). PTT was determined using a Nucleodur (150 mm × 4.6 mm) RP C_{18} column with 5 µm-sized particles. The mixture of acetonitrile and water (90:10% *v/v*) was used as the mobile phase. The mobile phase was flowed with a flow speed of 1.0 mL/min. At a wavelength of 254 nm, PTT was detected. The samples (20 µL) were injected into the system using a Waters autosampler.

2.3. PTT Calibration Curve

An appropriate quantity of PTT was dispensed in a mobile phase to create a PTT stock solution with a 100 µg/g concentration. The required aliquots from the stock solution of

PTT (100 μg/g) were diluted with the mobile phase to create the serial dilutions in the necessary range (1–75 μg/g). Instead of volume/volume, all of the dilutions were prepared on a mass/mass basis. Using the established HPLC method, the chromatographic area for each concentration of PTT was identified. To create the PTT calibration curve, eight different PTT concentrations (1, 5, 10, 15, 20, 25, 50, and 75 μg/g) were plotted against the measured chromatographic area. All the sample preparation and experiments were performed in three replicates (n = 3).

2.4. Analytical Method Development

As the eluent system/mobile phase, various combinations of organic/hydro-organic solvents were investigated for the development of a trustworthy HPLC approach for the detection of PTT in commercial capsules, solubility, and stability samples. The mixtures of methanol–water, ethanol–water, acetonitrile–water, methanol–ethanol, acetonitrile–methanol, acetonitrile–ethanol, methanol–formic acid, ethanol–formic acid, and acetonitrile–formic acid were among the numerous solvents that were investigated. Numerous aspects were taken into account when determining the best solvent or combination of solvents, including the solvents' affordability, the assay's sensitivity, the length of the analysis, the chromatographic parameters and the solvents' compatibility with one another. As a result, different solvents including methanol, ethanol, and acetonitrile were investigated as the mobile phase in both their individual and combined forms with water and formic acid.

2.5. Validation Parameters

Following ICH-Q2-R1 procedures, the developed analytical approach for the measurement of PTT was verified for several parameters [27]. By drawing the linearity plots, the linearity of the developed analytical approach could be investigated in the 1–75 μg/g range. PTT solutions that had just been produced were added to the HPLC apparatus in triplicates (n = 3), and the peak area was estimated. A PTT calibration curve was obtained by plotting PTT concentration vs. peak area.

The peak symmetry, tailing factor (As), capacity factor (k), and theoretical plates number (N) were obtained to examine the system suitability parameters for the developed analytical approach [28,29].

The developed analytical approach's intra-day and inter-day accuracy was estimated using the percent recovery technique. Three replicates (n = 3) were performed on the same day to test intra-day accuracy at three different quality control (QC) levels: low QC (LQC = 10 μg/g), middle QC (MQC = 50 μg/g), and high QC (HQC = 75 μg/g). On three separate days, three replicates (n = 3) of the PTT's LQC, MQC, and HQC levels were used to test inter-day accuracy. The percentage recovery, percentage coefficient of variance (%CV), and standard error were computed for each QC level.

The developed analytical approach's precision was evaluated using intra-day and inter-day variations. On the same day, the same QC levels of PTT (as those used for accuracy) were used to determine the intraday precision. At the same QC levels of the PTT on three consecutive days, inter-day precision was assessed. Both precisions were measured in three replicates (n = 3).

To investigate the impact of intentional chromatographic alterations on PTT analysis, the robustness of the developed analytical approach was evaluated. The PTT MQC (50 μg/g) was selected for the robustness analysis. By adjusting the mobile phase's composition, flow speed, and detecting wavelength, robustness was examined. The initial acetonitrile: water (90:10 *v/v*) mobile phase was adjusted to acetonitrile: water (92:8 *v/v*) and acetonitrile: water (88:12 *v/v*) for the robustness investigation, and the differences in chromatographic response were recorded for each combination of mobile phase. The original flow speed (1 mL/min) was changed to flow rates of 1.1 mL/min and 0.9 mL/min for robustness evaluation by adjusting flow speed, and the variations in chromatographic response were recorded for each set of flow rates. The initial detection wavelength (254 nm) was changed to detection wavelengths of 256 nm and 252 nm for the robustness evaluation

by altering the detection wavelength, and the variations in chromatographic response were recorded at each wavelength.

The developed analytical technique's sensitivity was evaluated in terms of the limit of detection (LOD) and limit of quantitation (LOQ), utilizing the standard deviation approach [27]. After the sample was injected into the HPLC system three times (n = 3), the standard deviation of the response was calculated. The LOD and LOQ for PTT were determined using the following equations [27,28]:

$$\text{LOD} = 3.3 \times \frac{\sigma}{S} \quad (1)$$

$$\text{LOQ} = 10 \times \frac{\sigma}{S} \quad (2)$$

where σ is the standard deviation of the response and S is the slope of the calibration curve of PTT.

2.6. Application of Developed HPLC Approach in the Assay of PTT in Commercial Capsules

Ten capsules (each containing an equivalent of 200 mg of PTT) were consumed at random for the test of PTT in commercial capsules, and the average weight was determined. The capsule contents were taken out from the capsule shell and mixed well to obtain the fine powder. The fine powder, with an equivalent to 200 mg of PTT, was dispersed in 100 g of methanol and sonicated for about 15 min. Then, 1 g of this solution was further diluted with methanol to obtain the stock of 100 g. The obtained mixtures of capsules were filtered [26]. The obtained solutions were used for the pharmaceutical assay of PTT in commercial capsules using the developed HPLC approach.

2.7. Application of the Developed HPLC Approach in the Determination of PTT in Solutions

The main purpose of measuring PTT solubility was to enhance the application of the developed method. The solubility of PTT in five different green solvents including water, propylene glycol (PG), ethanol, polyethylene glycol-400 (PEG-400), and Carbitol was determined at 25 °C using a previously reported shake flask method [30]. The excess of PTT was placed into known amounts (10 g) of each green solvent and examined in three replicates (n = 3). The obtained concentrated suspensions were vortexed for about 5 and transferred to a biological shaker for continuous shaking at 100 rpm speed for 72 h [31,32]. The samples were cautiously removed from the shaker once equilibrium had been reached. All the samples were centrifuged at 500 rpm for 30 min. The supernatants from each sample were taken, diluted with mobile phase (wherever required), and subjected to determination of PTT using the developed HPLC approach at a wavelength of 254 nm.

2.8. Application of the Developed HPLC Approach in the Determination of the Stability of PTT in Solutions

The main purpose of determining PTT stability was to enhance the application of developed method. The stability of PTT solution was performed at MQC level (50 µg/g) at two different temperatures, i.e., bench temperature (25 ± 1 °C) and refrigeration temperature (4 ± 0.5 °C). In this work, solution studies were performed; these studies were performed for a short period of time (72 h). The MQC of PTT solution was prepared in mobile phase and stored at 25 ± 1 °C and 4 ± 0.5 °C for about 72 h, and the decomposition of PTT was determined by measuring the rest of PTT after storage.

3. Results and Discussion

3.1. Analytical Method Development

Table 1 provides a summary of the measured chromatographic characteristics and the composition of various eluent systems. The application of methanol and water in various ratios during the analytical method development step led to a subpar chromatographic response of PTT, which exhibited higher As values (As > 1.30) with low N values (<3000).

Additionally, the use of ethanol and water in various ratios caused PTT to have a poor chromatographic response as well as increased As values (As > 1.45) and low N values (<2000). The combination of organic solvents, including acetonitrile and ethanol, acetonitrile and methanol, and methanol and ethanol, was also looked at as an eluent system. With high As values (As > 1.35) and low N values (<3000), the chromatographic response of PTT was once more subpar. We also looked at the binary combinations of organic solvents with formic acid, including methanol: formic acid and ethanol: formic acid. Additionally, the PTT chromatographic response of these binary combinations was subpar, with bigger As values (As > 1.35), and lower N values (<2000).

Table 1. Summary of the eluent systems and measured analytical responses for pterostilbene (PTT) (mean ± SD, n = 3).

Eluent System	As	N	R_t
Methanol: water (50:50 *v/v*)	1.34 ± 0.02	2478 ± 3.21	2.78 ± 0.04
Methanol: water (90:10 *v/v*)	1.30 ± 0.03	2771 ± 3.38	2.72 ± 0.05
Ethanol: water (50:50 *v/v*)	1.47 ± 0.07	1856 ± 2.63	2.68 ± 0.06
Ethanol: water (90:10 *v/v*)	1.59 ± 0.08	1715 ± 2.54	2.62 ± 0.07
Acetonitrile: water (50:50 *v/v*)	1.18 ± 0.03	4163 ± 4.22	2.59 ± 0.04
Acetonitrile: water (90:10 *v/v*)	1.07 ± 0.03	5125 ± 5.84	2.54 ± 0.02
Methanol: ethanol (50:50 *v/v*)	1.41 ± 0.08	2364 ± 3.11	2.81 ± 0.06
Acetonitrile: methanol (50:50 *v/v*)	1.37 ± 0.07	2814 ± 3.32	2.74 ± 0.07
Acetonitrile: ethanol (50:50 *v/v*)	1.44 ± 0.06	2932 ± 3.39	2.71 ± 0.04
Methanol: formic acid (90:10 *v/v*)	1.38 ± 0.05	1942 ± 2.26	2.86 ± 0.07
Ethanol: formic acid (90:10 *v/v*)	1.62 ± 0.10	1564 ± 1.97	2.91 ± 0.08
Acetonitrile: formic acid (90:10 *v/v*)	1.26 ± 0.05	3741 ± 4.51	2.61 ± 0.04

As: tailing factor; N: number of theoretical plates; R_t: retention time.

However, a well-resolved and intact PTT chromatographic peak with good As values and greater N values was shown by the binary mixture of acetonitrile and water in various ratios. The binary mixture of acetonitrile and water (90:10 *v/v*) gave the best chromatographic response (Figure 2). As a consequence, this mixture was chosen as the final eluent system for measuring PTT, with an acceptable As (1.07) and N (5125), rapid analysis (R_t = 2.54 ± 0.02 min), and a suitable analysis duration (5 min). Therefore, the most trustworthy eluent system for future investigation was a 90:10, volume-to-volume blend of acetonitrile and water.

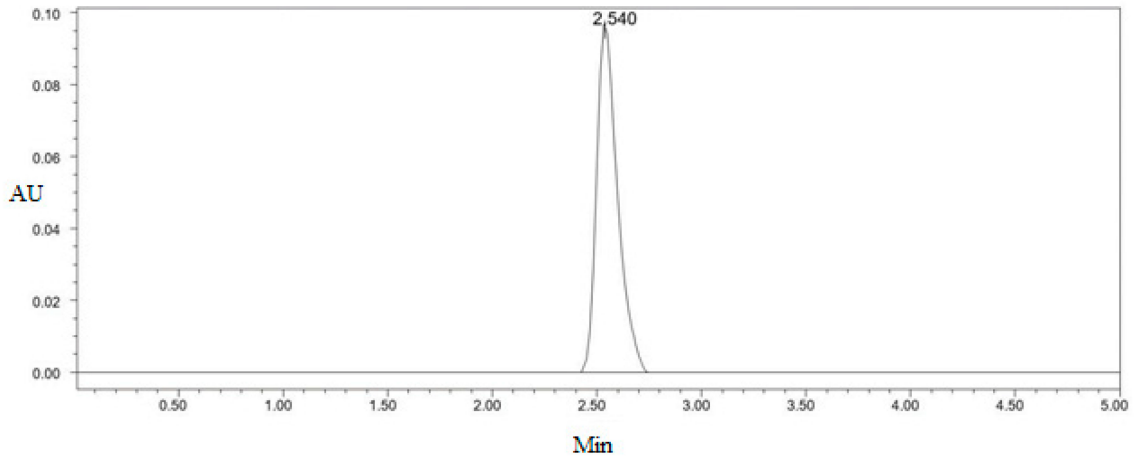

Figure 2. High-performance liquid chromatography (HPLC) chromatogram of PTT (10 µg/g concentration) in solution, produced using a binary eluent system that consisted of acetonitrile and water (90:10 *v/v*).

3.2. Validation Studies

Several validation parameters for the developed HPLC approach were determined following ICH-Q2-R1 protocols [27]. The linearity graphs were constructed using freshly produced PTT samples (1–75 µg/g). The outcomes of a linear regression analysis of the PTT calibration curve are shown in Table 2. The linear calibration curve for PTT was between 1 and 75 µg/g. According to estimates, the calibration curve's determination coefficient (R^2) and regression coefficient (R) values are 0.9995 and 0.9997, respectively. These outcomes revealed the efficiency of the developed analytical approach for determining PTT.

Table 2. Linear regression analysis for the calibration curve of PTT for the "high-performance liquid chromatography (HPLC)" approach (mean ± SD, n = 3).

Parameters	Values
Linearity range (µg/g)	1–75
Regression equation	y = 9207.2x–4565.5
R^2	0.9995
R	0.9997
Slope ± SD	9207.2 ± 12.13
Intercept ± SD	4565.5 ± 7.41
SE of slope	7.00
SE of intercept	4.27
95% CI of slope	9177.0–9237.3
95% CI of intercept	4547.0–4583.9
LOD (ng/g)	2.65 ± 0.09
LOQ (ng/g)	7.95 ± 0.27

R^2: determination coefficient; R: regression coefficient; SD: standard deviation; SE: standard error; CI: confidence interval; LOD: limit of detection; LOQ: limit of quantification.

The system's appropriateness parameters for the developed analytical approach were determined using the peak symmetry, As, k, and N. The results are shown in Table 3. The developed analytical approach's values for peak symmetry, As, k, and N were found to be satisfactory and acceptable for determining PTT.

Table 3. Optimized chromatographic peak parameters for the resolution of PTT for HPLC approach (mean ± SD, n = 3).

Drug	Peak Symmetry	As	K	N
PTT	1.684 ± 0.11	1.07 ± 0.03	2.78 ± 0.16	5125 ± 5.84

As: tailing factor; k: capacity factor; N: number of theoretical plates.

The percent recovery at LQC, MQC, and HQC was used to determine the intra-day and inter-day accuracy of the established HPLC technique. The results are shown in Table 4. At three different QC levels, the intra-day and inter-day percent recoveries of PTT were found to be 98–102 and 98–101 percent, respectively. According to ICH guidelines, the percent recoveries of analytical method should be within the limit of 100 ± 2% [27]. The percent recoveries of two literature HPLC methods have been reported as 98–99 and 96–100 percent, respectively [17,18]. The percent recoveries of current HPLC method were similar to first reported method [17] and superior to second reported method [18], as per ICH guidelines. High percent recoveries for the established HPLC method for determining PTT point to its accuracy.

The results of the intra-day and inter-day precisions are summarized in Table 5 and are indicated in %CV. For PTT, the intraday variation percent CVs were observed to range from 0.59 to 1.15%. On the contrary, the %CVs for inter-day precision ranged between 0.60 and 1.25 percent. The %CVs of current HPLC method were similar to reported methods [17,18]. Low %CVs in the devised HPLC method for calculating PTT indicated its precision.

Table 4. Intra-day and inter-day accuracy results of PTT for HPLC approach (mean ± SD; n = 3).

Conc. (µg/g)	Intra-Day Accuracy			Inter-Day Accuracy		
	Conc. Found (µg/g) ± SD	Recovery (%)	CV (%)	Conc. Found (µg/g) ± SD	Recovery (%)	CV (%)
10	9.84 ± 0.11	98	1.11	10.13 ± 0.12	101	1.18
50	50.56 ± 0.44	101	0.87	49.05 ± 0.43	98	0.87
75	76.45 ± 0.51	102	0.66	74.44 ± 0.55	99	0.73

Table 5. Intra-day and inter-day precision of PTT for HPLC approach (mean ± SD; n = 3).

Conc. (µg/g)	Intra-Day Precision			Inter-Day Precision		
	Conc. Found (µg/g) ± SD	SE	CV (%)	Conc. Found (µg/g) ± SD	SE	CV (%)
10	10.41 ± 0.12	0.06	1.15	10.32 ± 0.13	0.07	1.25
50	48.96 ± 0.38	0.21	0.77	49.65 ± 0.41	0.23	0.82
75	74.54 ± 0.44	0.25	0.59	76.24 ± 0.46	0.26	0.60

Table 6 contains the results of the robustness assessment for the MQC level of PTT. When evaluating robustness by altering the composition of the mobile phase, the %CV and R_t were discovered to be 0.78–1.18% and 2.53–2.55 min, respectively. The %CV and R_t were found to be 0.43–1.45% and 2.28–2.75 min, respectively, in the scenario of a robustness assessment when the flow speed was changed. The %CV and R_t were calculated to be 1.17–1.55% and 2.55–2.57 min, respectively, in the scenario of a robustness assessment by shifting detecting wavelength. Low CVs and minimal R_t value swings in the devised HPLC method for detecting PTT indicate its robustness.

Table 6. Robustness results of PTT at MQC (50 µg/g) for the HPLC approach (mean ± SD; n = 3).

Parameters	Conc. Found (µg/g) ± SD	CV (%)	R_t ± SD	CV (%)
Mobile phase composition				
(92:8 % *v/v*)	48.71 ± 0.51	1.04	2.53 ± 0.03	1.18
(88:12 % *v/v*)	50.68 ± 0.55	1.08	2.55 ± 0.02	0.78
Mobile phase flow rate				
(1.10 mL/min)	50.81 ± 0.61	1.20	2.28 ± 0.01	0.43
(0.90 mL/min)	48.42 ± 0.58	1.19	2.75 ± 0.04	1.45
Detection wavelength (nm)				
252	48.71 ± 0.57	1.17	2.55 ± 0.03	1.17
256	51.11 ± 0.64	1.25	2.57 ± 0.04	1.55

Table 2 lists the findings from evaluating the developed analytical approach's sensitivity in terms of LOD and LOQ. The LOD and LOQ for the developed analytical approach were discovered to be 2.65 ± 0.09 ng/g and 7.95 ± 0.27 ng/g, respectively. These results suggested that the developed analytical approach would have sufficient sensitivity to determine PTT.

The developed HPLC approach for the determination of PTT was compared with reported analytical assays used to determine PTT in solution form. The validation parameters of present HPLC approach compared with reported analytical methods are listed in Table 7. Most of the validation parameters of reported HPLC assays were within the limits of ICH protocol, and hence were similar to the present HPLC approach [17,18]. However, the linearity range, accuracy, precision, LOD, and LOQ values of the HPTLC approaches of PTT analysis in the literature were also found to be inferior to the present HPLC approach [25,26]. Furthermore, the LOD and LOQ values of PTT for the present method were lower than the reported HPLC and HPTLC methods, and were hence found to be more sensitive than the reported HPLC and HPTLC methods. Overall, the newly developed and validated HPTLC approach has been found to be reliable for the determination of PTT.

Table 7. Comparative summary of validation parameters of the present HPLC method with reported methods for the determination of PTT.

Analytical Method	Nature of Sample	Linearity Range	Accuracy (% Recovery)	Precision (% CV)	LOD	LOQ	Ref.
HPLC	Solution	0.02–250 (µg/mL)	98.91–99.59	0.02–0.67	0.006 (µg/mL)	0.019 (µg/mL)	[17]
HPLC	Solution	1–20 (µg/mL)	96.88–100.77	0.20–1.65	0.290 (µg/mL)	0.090 (µg/mL)	[18]
HPTLC	Solution	200–500 (ng/band)	96.67–98.13	0.82–2.12	140 (ng/band)	200 (ng/band)	[25]
Routine HPTLC	Solution	30–400 (ng/band)	90.42–108.82	3.32–3.48	11.1 (ng/band)	33.3 (ng/band)	[26]
Sustainable HPTLC	Solution	10–1600 (ng/band)	98.79–100.94	0.18–0.64	3.51 (ng/band)	10.5 (ng/band)	[26]
HPLC	Solution	1–75 (µg/g)	98.10–101.93	0.59–1.25	2.65 (ng/g)	7.95 (ng/g)	Present work

3.3. Assay of PTT in Marketed Capsules

The developed analytical approach for the PTT assay was shown to be efficient, quick, and sensitive. This approach was therefore used to ascertain PTT in its commercial capsule dosage form. The PTT percentage assay was 99.31% in the commercial capsule dosage form. The PTT percentage in different brands of commercially available capsule dosage forms has been reported as 98.75–98.94% using an HPLC method from the literature [17]. The PTT percentage in the marketed capsule dosage form has been reported as 92.59 and 100.84%, respectively, using routine and sustainable HPTLC methods [26]. The current HPLC method in terms of PTT assay was identical to the reported HPLC and sustainable HPTLC methods [17,26]. However, it was much superior to the reported routine HPTLC method [26]. These findings suggest that the HPLC method would work well for determining PTT in commercially available dosage forms.

3.4. Determination of PTT in Solubility Samples

The potential of the developed HPLC approach was demonstrated by determining the solubility of PTT in five different green solvents, including water, PG, ethanol, PEG-400, and Carbitol, at 25 °C. At 25 °C, the solubility of PTT in water, PG, ethanol, PEG-400, and Carbitol was found to be 0.0180 mg/g, 1127 mg/g, 710.0 mg/g, 340.0 mg/g, and 571.0 mg/g, respectively. Based on these results, PTT was found to be poorly soluble in water, freely soluble in ethanol, PEG-400, and Carbitol, and very soluble in PG [33,34]. Similar solubility characteristics of PTT in water, PG, ethanol, PEG-400, and Carbitol at 25 °C have also been reported in the literature [31]. Hence, the obtained solubility results of PTT were in accordance with those reported in the literature [31]. These results suggested that the developed HPLC approach would be suitable for determining PTT in solubility samples.

3.5. Stability Studies of PTT in Solution

The potential of the developed HPLC approach was also demonstrated by determining the stability of PTT in solution at two different temperatures. The solution of PTT was prepared in mobile phase (acetonitrile: water, 90:10 *v/v*). The findings of stability evaluations at two different temperatures are included in Table 8. The PTT degradation was measured by determining the rest of PTT concentration after storage. The PTT degradation was very low when held for 72 h at 25 ± 1 °C, and at 4 ± 0.5 °C, when the peak areas of the stored PTT solution were compared to those obtained from a freshly made PTT solution. The precision of PTT in terms of %CV was found to be 1.04–1.07% at two different temperatures. Furthermore, the percent recovery of PTT was found to be 99.84–100.42 percent at two different temperatures. PTT was discovered to be sufficiently stable in solution form

at 25 and 4 °C as a result. These findings indicated that PTT stability in solution could be determined using the HPLC method that was established.

Table 8. Stability data of PTT at MCQ level at two different temperatures (mean ± SD; n = 3).

Stability	Nominal Conc. (µg/g)	Conc. Found (µg/g) ± SD	Precision (% CV)	Recovery (%)
Refrigeration (4 °C)	50	49.92 ± 0.52	1.04	99.84
Bench top (25 °C)	50	50.21± 0.54 h	1.07	100.42

4. Conclusions

A rapid, sensitive, and economical HPLC approach has been designed and validated for the quantification of PTT in its marketed products, solubility, and stability samples. The developed HPLC approach was validated per ICH-Q2-R1 protocols. The developed analytical approach is rapid, accurate, precise, robust, sensitive, and economical for estimating PTT. The developed HPLC approach was found to be reliable for the determination of PTT in commercial capsule dosage forms, solubility, and stability samples. Based on these findings, it is possible to effectively estimate PTT in a variety of sample matrices using the established HPLC approach. In future, further studies can be carried out to determine PTT in the complex matrices of biological samples, and to accomplish pharmacokinetic assessment of PTT.

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