separations

MDPI

Article

A Simple Stability-Indicating UPLC Method for the Concurrent Assessment of Paracetamol and Caffeine in Pharmaceutical Formulations

Wasim Ahmad [1,*], Yousif Amin Hassan [1], Ayaz Ahmad [1], Manal Suroor [1], Mohammad Sarafroz [2], Prawez Alam [3,*], Shadma Wahab [4] and Shahana Salam [5]

[1] Department of Pharmacy, Mohammed Al-Mana College for Medical Sciences, Dammam 34222, Saudi Arabia
[2] Department of Pharmaceutical Chemistry, College of Clinical Pharmacy, Imam Abdul Rahman Bin Faisal University, Dammam 34222, Saudi Arabia
[3] Department of Pharmacognosy, College of Pharmacy, Prince Sattam Bin Abdulaziz University, P.O. Box 173, Al-Kharj 11942, Saudi Arabia
[4] Department of Pharmacognosy, College of Pharmacy, King Khalid University, Abha 61421, Saudi Arabia
[5] Department of Pharmaceutical Chemistry, College of Pharmacy, Prince Sattam Bin Abdulaziz University, P.O. Box 173, Al-Kharj 11942, Saudi Arabia
* Correspondence: wasimahmadansari@yahoo.com (W.A.); p.alam@psau.edu.sa (P.A.)

Abstract: A fixed-dose combination of paracetamol (PCM) and caffeine (CAF) tablets/capsules is the most frequently used over-the-counter medicine for fever and headache. In this paper, a simple, reliable, sensitive, rapid, and stability-indicating ultra-performance liquid chromatography (UPLC) analytical method was proposed for simultaneously assessing PCM and CAF in pharmaceutical formulations. The UPLC method was developed on an Acquity UPLC® CSH™ C_{18} column, and the column oven temperature was maintained at 35 ± 5 °C with isocratic elution by using a solution of methanol and water (30:70, *v*/*v*). The maximum absorbance of PCM and CAF was observed at 272.5 nm. The flow rate was 0.2 mL/min, and the injection volume was 1 µL, with the total run time of 2 min for the separation of PCM and CAF. The proposed UPLC method was validated according to the ICH guidelines, and it demonstrated excellent linearity, with correlation coefficients of 0.9995 and 0.9999 over the concentration ranges of 40–400 and 7–70 ng/mL for PCM and CAF, respectively. The mean retention times of 0.82 ± 0.0 and 1.16 ± 0.02 were observed for PCM and CAF, respectively. The limits of detection and quantification were 16.62 and 3.86 for PCM, respectively, and 50.37 and 11.70 for CAF, respectively. PCM and CAF were subjected to acidic, alkali, oxidative, phytochemical, dry-heat, and wet-heat degradation. The method was found to well separate the analytes' peaks from degradation peaks, with no alterations in retention times. The proposed method is linear, precise, accurate, specific, and robust, and it can indicate stability and be used for the quantitative assessment of pharmaceutical formulations comprising PCM and CAF within a short period of time.

Keywords: paracetamol; caffeine; pharmaceutical formulation; stability-indicating UPLC method

Citation: Ahmad, W.; Hassan, Y.A.; Ahmad, A.; Suroor, M.; Sarafroz, M.; Alam, P.; Wahab, S.; Salam, S. A Simple Stability-Indicating UPLC Method for the Concurrent Assessment of Paracetamol and Caffeine in Pharmaceutical Formulations. *Separations* **2023**, *10*, 50. https://doi.org/10.3390/separations10010050

Academic Editor: Paraskevas D. Tzanavaras

Received: 10 December 2022
Revised: 6 January 2023
Accepted: 11 January 2023
Published: 12 January 2023

1. Introduction

Currently, drug analysis is one of the utmost concerns in the pharmaceutical industry. It can assist in selecting the dosage form by determining the strength of the active pharmaceutical ingredients, and it can detect scums in preparations [1]. Moreover, drug analysis is applicable not only to pharmaceutical industries but also to quantitative estimations of prohibited or abused substances in doping cases [2,3]. Therefore, the quantitative determination of these ingredients in formulations and biological fluids can help optimize their utilization and evade their adverse effects [4].

Paracetamol (acetamenophen, PCM) is a frequently used over-the-counter medicine for headache, body ache, arthritis, toothache, and fever, and it is commercially available

in different dosage forms [5–8] (Figure 1A). Caffeine (CAF), chemically known as 1,3,7-trimethyl xanthine, is a pseudo-alkaloid and is used as a psychoactive drug globally (Figure 1B) [9]. Other than this, CAF is used as a diuretic, CNS, and CVS stimulant [10], and it has potential antitumor activity [11]. The combination of PCM and CAF is most commonly used worldwide in clinical settings to treat conditions in humans, such as migraine headaches, a chronic and common disorder characterized by the recurrence of moderate-to-severe headaches, which mostly affect one side of the head; body aches; and fevers [12–15]. Thus, the qualitative and quantitative standardization of PCM and CAF in multicomponent drug formulations is required.

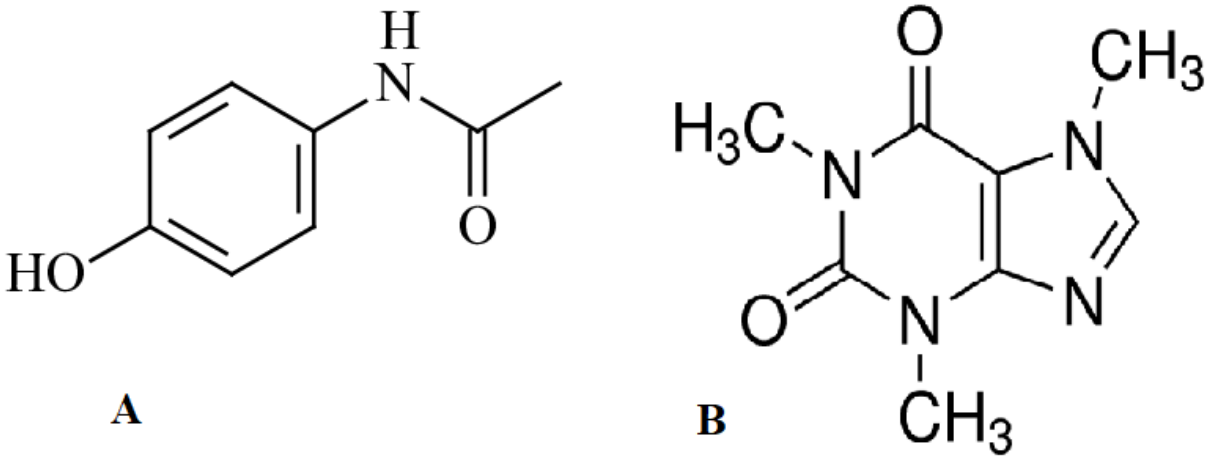

Figure 1. Chemical structures of (**A**) PCM and (**B**) CAF.

The literature has reported several analytical methods for concurrently estimating PCM and CFN in pharmaceutical formulations and body fluids. For simultaneously estimating PCM and CAF in several pharmaceutical formulations, many high-performance liquid chromatography (HPLC) methods are used [15–22]. Moreover, PCM and CAF are estimated in human body fluids by employing HPLC [23] and liquid chromatography–mass spectrometry [24] techniques. Several other techniques, including high-performance thin-layer chromatography [25–27], UPLC [6], several voltametric techniques [28–30], spectrophotometric methods [31,32], and FT-IR spectroscopy [33], are also used for the concurrent estimation of PCM and CAF. This study developed a new rapid, economical, specific, and stability-indicating UPLC method for the simultaneous assessment of PCM and CAF in formulations.

2. Results and Discussion

2.1. Analytical Method Optimization

Preliminary studies with various mobile phases were conducted to obtain the suitable eluent phase for the resolution and separation of PCM and CAF. The mobile phase was selected depending on the cost of the solvents, polarities, and the solubility of the standard. Several mobile phases in various quantities of solvents, such as isopropyl alcohol, acetonitrile, formic acid, methanol, and water, were studied. In the isocratic mode using a C18 column with an oven temperature of 35 °C maintained constant using a flow rate of 0.2 mL/min, methanol and water (30:70, v/v) provided high resolutions of PCM and CAF within the minimum retention time. Moreover, for PCM and CAF, the absorbance maximum was observed at 272.5 nm, when the spectrum indexes for PCM and CAF were recorded using the PDA mode (Figure 2). As a result, the assessment of PCM and CAF took place at 272.5 nm. The mean retention times of 0.82 ± 0.0 and 1.16 ± 0.02 were observed for PCM and CAF, respectively (Figure 3), within 2 min of the total runtime (Figures 4 and 5). The assessing factors used for selecting the optimum UPLC conditions were the solvent's

cost effectiveness; shorter analysis time; the reproducibility of the retention times; and the separation of peaks from mixtures.

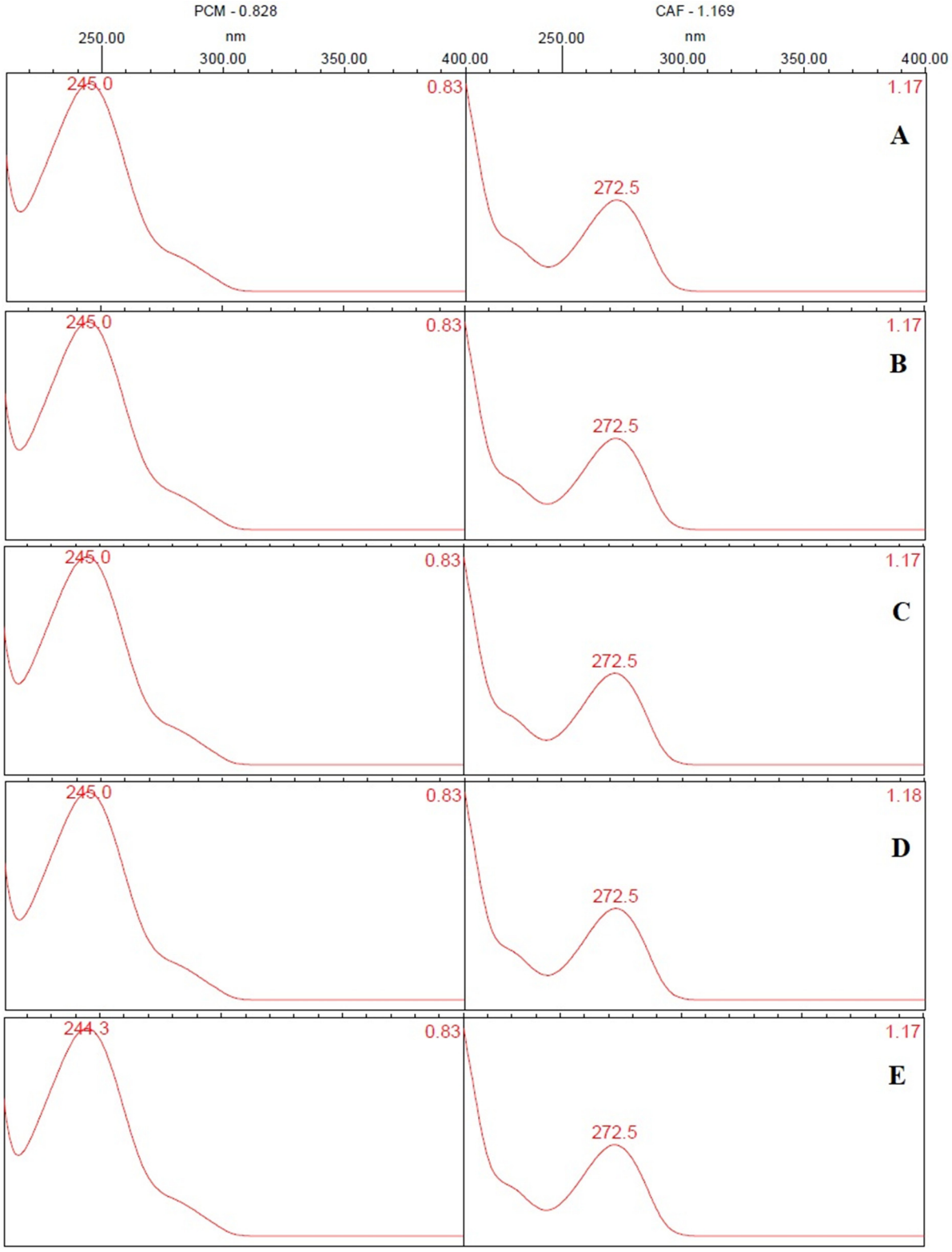

Figure 2. Spectra of PCM and CAF obtained via PDA detector in (**A**) standard, (**B**) Tab-1, (**C**) Tab-2, (**D**) Tab-3, (**E**) Cap-1.

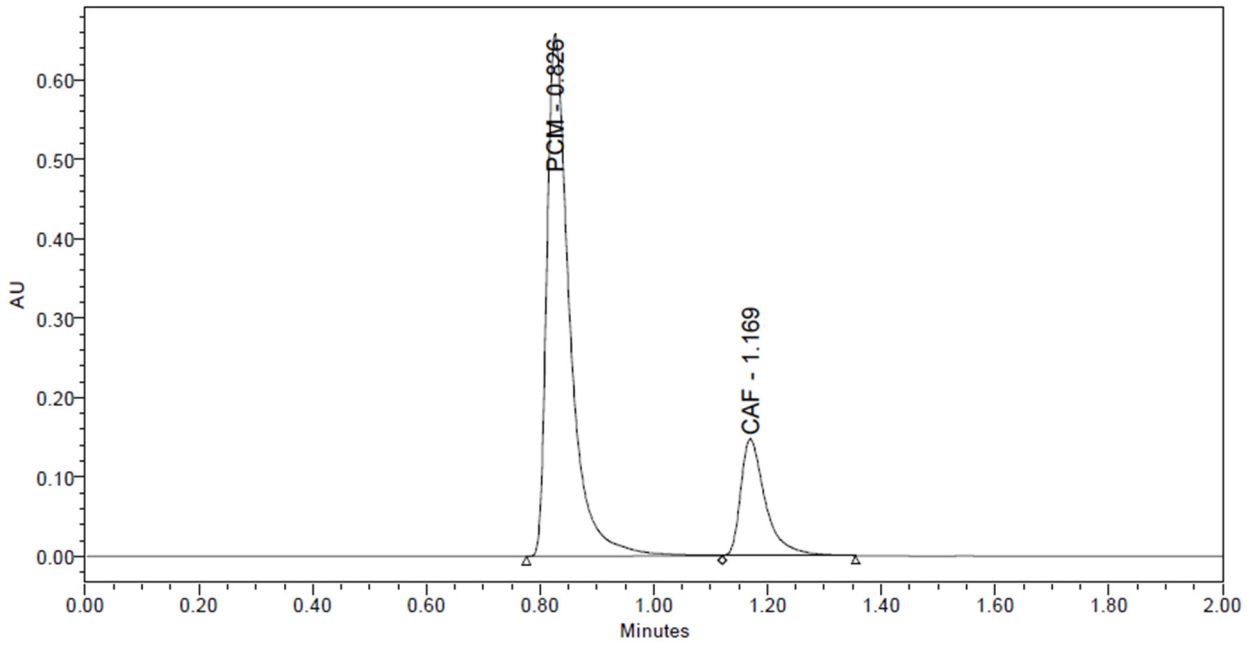

Figure 3. Chromatogram of PCM and CAF at 273 nm.

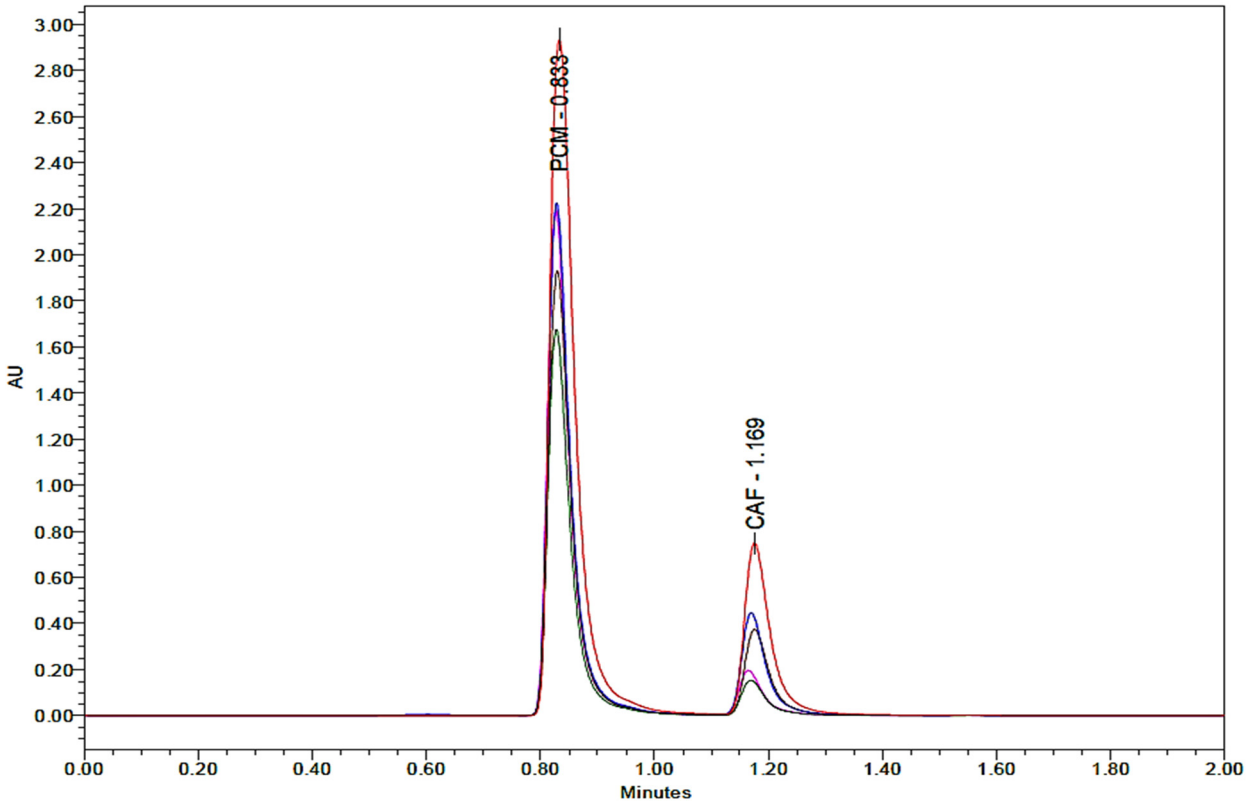

Figure 4. Overlay chromatogram of standard PCM and CAF of different levels.

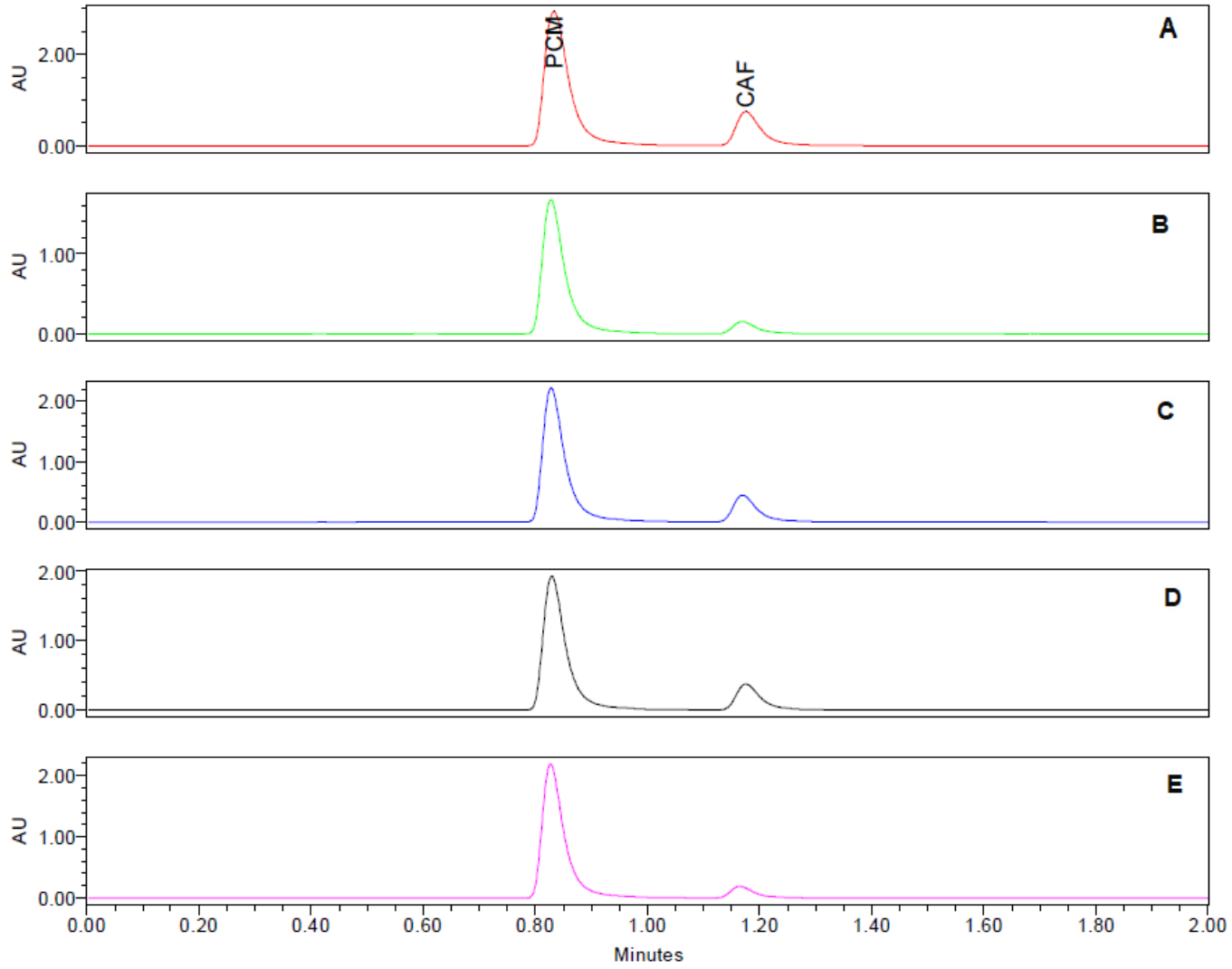

Figure 5. Stacked chromatograms of standard PCM and CAF and formulations: (**A**) standard, (**B**) Tab-1, (**C**) Tab-2, (**D**) Tab-3, (**E**) Cap-1.

2.2. Method Validation

To assess the various parameters for the concurrent assessment of PCM and CAF, the ICH guidelines were used. The linearity of the procedure was assessed by studying the regression of the standard calibration curve. The linear coefficient regression analysis was found to be r2 0.9995 and 0.9999 for PCM and CAF, respectively (Figures 6 and 7). The method showed the linearities of 40–200 and 7–70 ng/mL for PCM and CAF, respectively. These findings suggest the reliability of the UPLC method for the concurrent assessment of PCM and CAF. The LODs were 16.62 and 3.86 ng/mL for PCM and CAF, respectively, and the LOQs were 50.37 and 11.70 ng/mL for PCM and CAF, respectively (Table 1). These results indicate the method's sensitivity for the concurrent assessment of both PCM and CAF. The accuracy of the proposed procedure was assessed by investigating recovery by employing the standard inclusion technique at three concentrations of standard PCM and CAF. Moreover, the mean recovery results were within 98.80–101.14%, and the % RSD was below the value specified by the ICH guidelines (Table 2). The precision of the ultra-performance liquid chromatography was examined, and it is presented as percentage RSD. Table 3 presents the precision results for the concurrent quantification of these ingredients using UPLC. The % RSDs of PCM and CAF for intra-day precision were 0.32–1.01 and 0.68–1.03, respectively, and those for inter-day precision were 0.91–1.30 and 0.75–1.02, respectively. This procedure was precise because the % RSD was <2. The robustness of

the procedure was examined by slightly modifying the chromatographic settings. The small changes in the flow rate (±1) and wavelength (±2) did not adversely affect the proposed method. The robustness results showed no considerable differences after the modification of the chromatographic conditions. These results suggest that the proposed UPLC procedure exhibited a high robustness (Table 4).

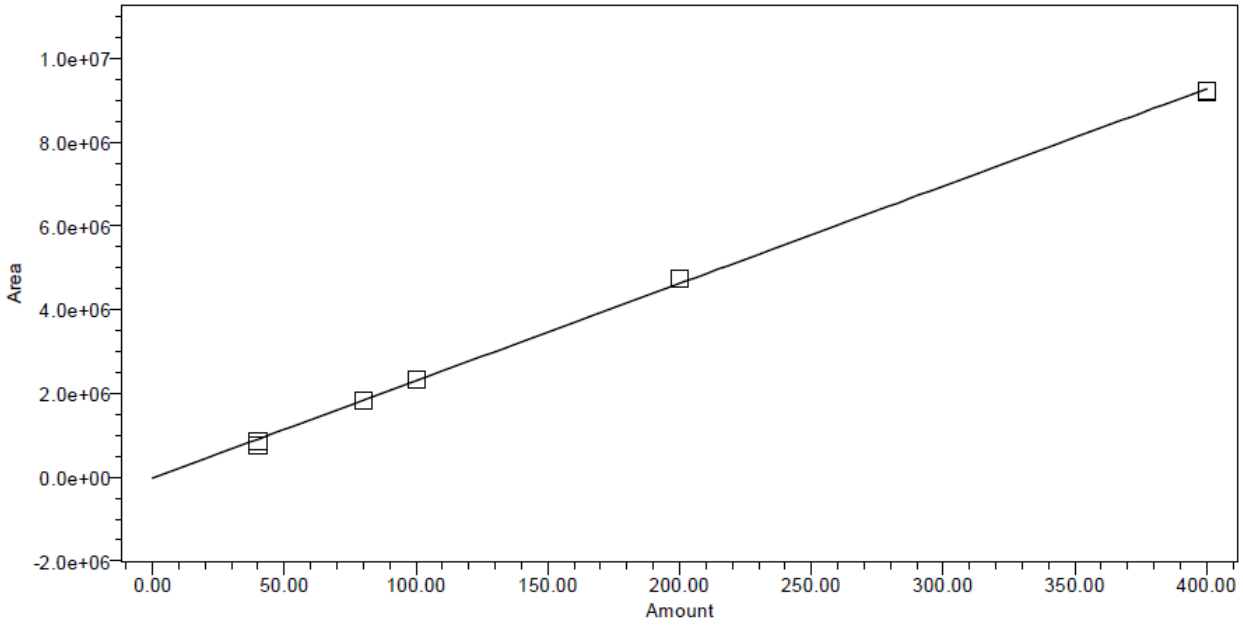

Figure 6. Calibration curve of PCM.

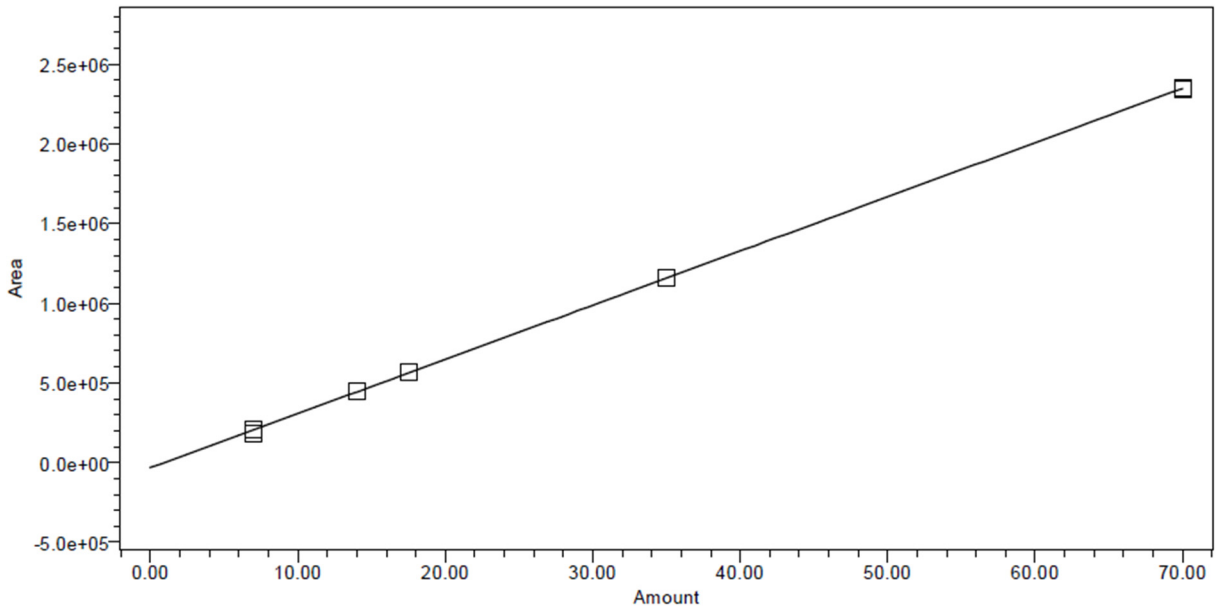

Figure 7. Calibration curve of CAF.

Table 1. Linear regression analysis for the concurrent quantification of PCM and CAF via UPLC.

Parameters	Paracetamol (PCM)	Caffeine (CAF)
Linearity range ng/mL	40–400	7–70
Correlation coefficient (R^2)	0.9995	0.9999
LOD	16.62	3.86
LOQ	50.37	11.70

Table 2. Accuracy of PCM and CAF contents.

Concentration ng/mL	Conc. Found (ng/mL) ± SD	% Recovery	% RSD
	Paracetamol		
40	40.33 ± 0.30	100.71	0.27
100	99.49 ± 1.02	99.49	0.48
200	197.61 ± 1.11	98.80	0.32
	Caffeine		
14	14.09 ± 0.15	100.64	0.44
35	35.40 ± 0.30	101.14	0.61
70	69.18 ± 0.87	98.82	0.29

Table 3. Precision of UPLC method for the concurrent quantification of PCM and CAF.

Amount	Intra-Day Precision		Inter-Day Precision	
ng/mL	Mean Peak Area ± SD	% RSD	Mean Peak Area ± SD	% RSD
		Paracetamol		
80	1,838,879.49 ± 12,548.57	0.68	1,782,189.92 ± 19,573.91	1.09
100	2,327,147.74 ± 7629.42	0.32	2,227,539.85 ± 28,977.18	1.30
200	4,766,558.97 ± 48,335.17	1.01	4,530,725.72 ± 41,486.14	0.91
		Caffeine		
17.5	565,071.77 ± 4217.98	0.74	524,396.78 ± 5356.17	1.02
35	1,160,103.46 ± 7986.68	0.68	1,106,601.09 ± 8302.87	0.75
70	2,308,756.33 ± 23,827.56	1.03	2,282,042.71 ± 20,951.76	0.91

Table 4. Results of robustness of PCM and CAF.

Compound Name			Mean Peak Area ± SD	Mean Rt Area ± SD	% RSD of Area	% RSD of Rt
Paracetamol	Flow rate mL/Min	0.1	2,303,872.31 ± 5875.08	0.83 ± 0.03	0.25	0.44
		0.2	2,325,199.84 ± 2500.15	0.82 ± 0.01	0.10	1.22
		0.3	2,311,573.43 ± 26,773.89	0.85 ± 0.00	1.15	0.58
	Change in wavelength (nm)	271	2,306,660.26 ± 12,685.84	0.83 ± 0.005	0.54	0.66
		273	2,325,754.95 ± 2387.25	0.82 ± 0.001	0.10	0.20
		275	2,312,139.36 ± 20,891.05	0.84 ± 0.02	0.90	0.29
	Column oven temperature	30 °C	2,301,993.59 ± 17,963.03	0.84 ± 0.009	0.78	1.11
		35 °C	2,325,088.28 ± 2125.32	0.82 ± 0.00	0.09	0.09
		40 °C	2,311,969.69 ± 5126.76	0.85 ± 0.01	0.22	1.17
Caffeine	Flow rate mL/Min	0.1	1,131,460.71 ± 9370.95	1.17 ± 0.00	0.82	0.56
		0.2	1,160,798.46 ± 1281.09	1.16 ± 0.08	0.11	0.68
		0.3	1,153,484.54 ± 11,663.54	1.18 ± 0.07	1.01	0.62
	Change in wavelength (nm)	271	1,137,662.35 ± 8282.21	1.15 ± 0.04	0.72	0.36
		273	1,159,837.97 ± 1511.62	1.16 ± 0.02	0.13	0.21
		275	1,147,068.33 ± 15,097.40	1.17 ± 0.004	1.31	0.38
	Column oven temperature	30 °C	1,131,329.02 ± 10,126.08	1.15 ± 0.02	0.89	0.17
		35 °C	1,159,771.30 ± 2830.96	1.16 ± 0.00	0.24	0.38
		40 °C	1,152,934.33 ± 12,675.51	1.17 ± 0.002	1.09	0.22

2.3. Analytical Assays

The proposed UPLC procedure was used for simultaneously assessing PCM and CAF in formulations (tablets and capsules). The chromatograms of PCM and CAF from marketed tablets and capsules were identified by comparing the retention times of 0.82 ± 0.0 for PCM and 1.16 ± 0.02 for CAF with those of standard PCM and CAF using the UPLC procedure.

Figure 5 summarizes the recorded chromatograms of PCM and CAF in the marketed tablets and capsules, which revealed that the chromatograms of PCM and CAF are similar to those of standard PCM and CAF in marketed tablets and capsules. To estimate the amounts of PCM and CAF in the tablets (Tab-1, Tab-2, and Tab-3) and the capsule (Cap-1), the samples were examined using the proposed procedure. The results are presented in Table 5. The obtained mean amounts of PCM and CAF are compared with their defined concentrations in Table 5. The amounts of PCM and CAF were within the recommended range of 90%–110% for the labeled quantity in the analyzed fixed-dose combination tablets and capsule [34].

Table 5. PCM and CAF contents in fixed-dose combinations.

Brand Name	Labeled Claim		Observed Content		% *w*/*w*	
	PCM (mg)	CAF (mg)	PCM (mg)	CAF (mg)	PCM	CAF
Tab 1	500	30	484.13	30.14	96.98 ± 1.12	100.46 ± 0.43
Tab 2	500	65	512.37	67.73	102.48 ± 0.73	104.20 ± 0.36
Tab 3	500	65	492.31	67.45	98.46 ± 0.52	103.69 ± 0.32
Cap 1	500	30	510.22	30.45	102.04 ± 0.61	101.50 ± 0.31

2.4. Forced Degradation of PCM and CAF

Stressed sample solutions were prepared and assessed as described previously. The extent of degradation was calculated as % recoveries of several stressed sample solutions. The results for various stressed samples are presented in Table 6. The chromatograms of the degraded samples showed satisfactory separation and resolutions. The retention times of PCM and CAF did not considerably shift in the presence of degradation peaks, indicating the stability of the proposed method.

Table 6. Results for stress degradation studies of PCM and CAF.

Compound Name	Degradation Condition	Recovery (%) (±SD, *n* = 3)
PCM	Acid	0
	Base	0
	H_2O_2	0
	Sunlight	97.87 ± 0.09
	Dry Heat	96.19 ± 0.02
	Wet Heat	96.78 ± 0.02
	Room Temp	99.89 ± 0.03
CAF	Acid	91.51 ± 0.06
	Base	0
	H_2O_2	12.49 ± 0.02
	Sunlight	98.35 ± 0.08
	Dry Heat	99.73 ± 0.04
	Wet Heat	93.66 ± 0.03
	Room Temp	102.21 ± 0.04

PCM showed complete loss upon exposure to 2 M HCl, 2 M NaOH, and 30% H2O2, and CAF exhibited considerable degradation in 2 M HCl and 30% H2O2 and complete loss in 2 M NaOH (Figure 8). Photolytic degradation was not substantial for PCM and CAF (Figure 9). The dry-heat samples did not show any additional peaks or substantial degradation. However, the wet-heat samples exhibited considerable degradation and two additional peaks, and the percentages of drug recovery were 96.78% and 93.66% for the stressed samples of PCM and CAF, respectively (Figure 10). The room-temperature sample did not show substantial degradation. Under all stress conditions, the retention times of PCM and CAF remained constant. Thus, the developed method is stable and can be employed to separate both PCM and CAF, even in the presence of degraded products.

The analytes were estimated quantitatively, and the degradation products were separated, demonstrating the specificity of the UPLC procedure and its stability-indicating power.

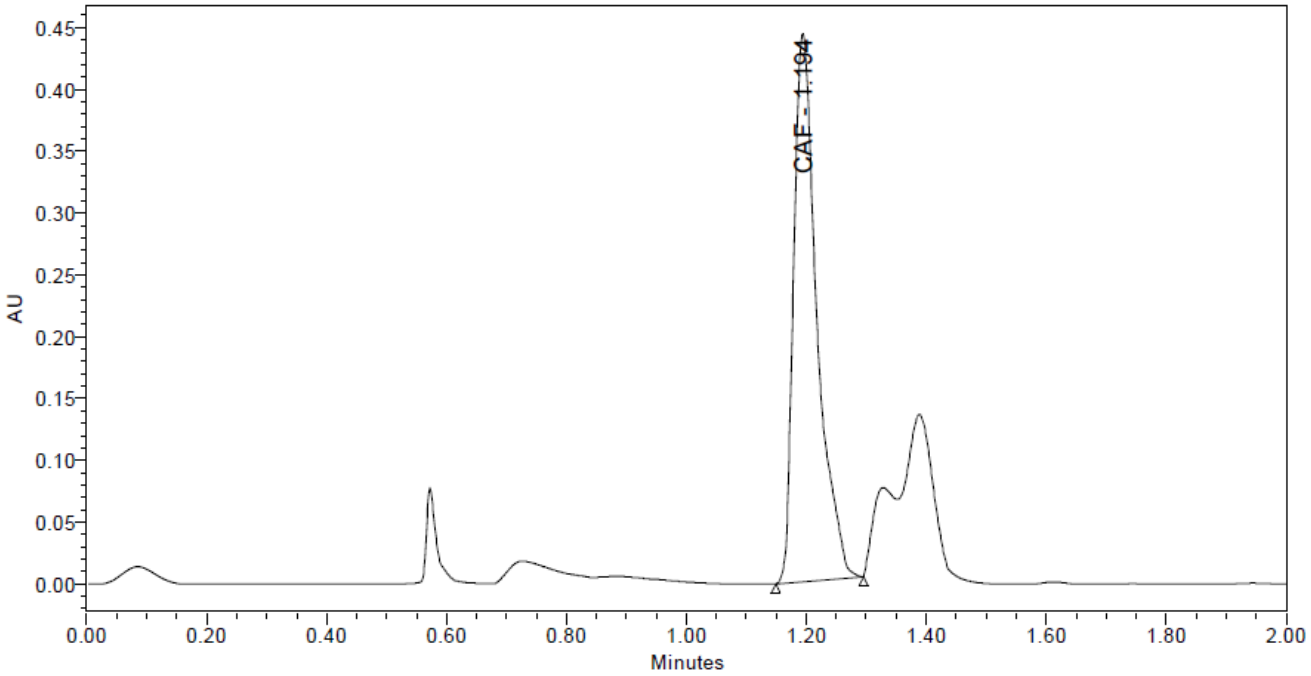

Figure 8. Chromatogram of PCM and CAF after forced degradation by 2 M HCl.

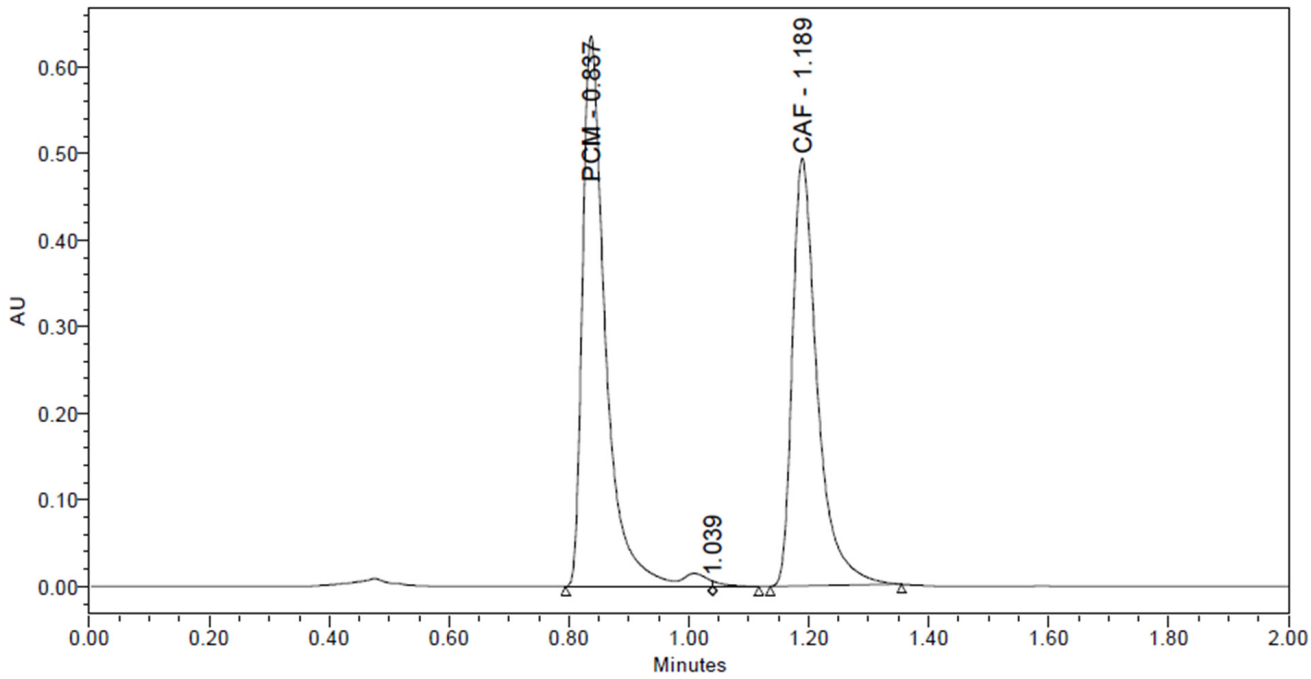

Figure 9. Chromatogram of PCM and CAF for photolytic degradation.

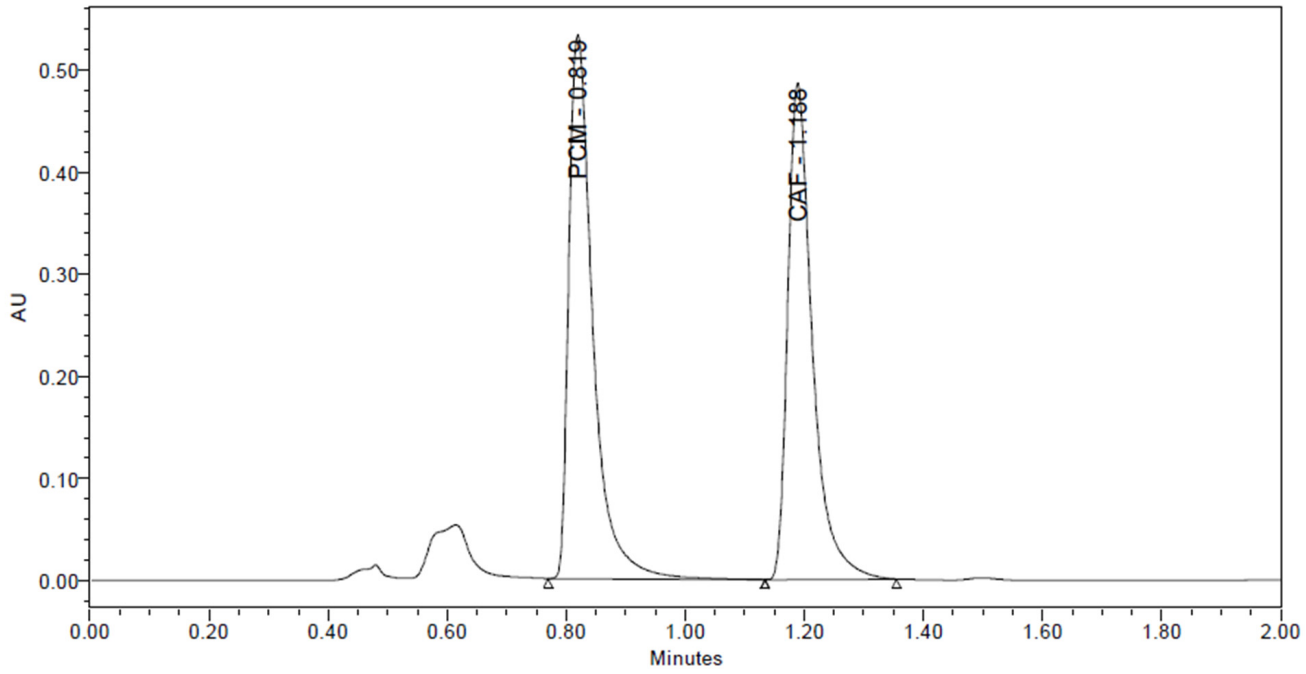

Figure 10. Chromatogram of PCM and CAF for wet heat degradation.

2.5. Comparison with Reported Analytical Methods

To study performance, few chromatographic characteristics of the proposed UPLC method were compared with those of existing methods; the comparison is presented in Table 7. Several chromatographic characteristics, including run time, linearity range, and retention time, of the proposed procedure were in contrast with those of some published studies. However, the linearity range (PCM 40–400 ng/mL and CAF 7–70 ng/mL) of the proposed procedure is lower than that of the reported methods. The linearity ranges of the UPLC-MS method presented in the literature have been reported to be 0.05–250 for PCM and 0.01–5 µg/mL for CAF, which were also inferior to those of the UPLC method [24]. The run time of the proposed procedure is considerably short and, thus, highly satisfactory for the separation of PCM and CAF. For PCM and CAF, the retention times of 0.82 and 1.16 min, respectively, obtained using the proposed method are substantially less than those acquired using other methods, except for those reported by Jena et al. (2017). Jena et al. reported the retention times of 0.68 and 1.78 min for PCM and CAF, respectively; this retention time for CAF is higher than that obtained with the proposed UPLC method. Compared with that of the reported methods, the mobile phase composition of the current method is simple and does not use any buffer for separating PCM and CAF. The stability-indicating method proposed for concurrently assessing PCM and CAF is better than other published methods in terms of simplicity, precision, spontaneity, and robustness.

Table 7. Comparison of some chromatographic characteristics of the current UPLC procedure with previously published methods for concurrent quantification of PCM and CAF.

S.N	Technique	Column	Run Time	Linearity (µg/mL)	Rt	Ref
1	HPLC	C18	9	PCM: 0.409–400 µg CAF: 0.151–200 µg	PCM: 4.88 CAF: 5.84	[12]
2	HPLC	C18	10	PCM: 15–300 µg CAF: 2.5–50 µg	PCM: 2.6 CAF: 3.5	[13]
3	HPLC	C18	10	PCM: 0.5–25 µg CAF: 0.1–30 µg	PCM: 3.4 CAF: 5.3	[14]

Table 7. *Cont.*

S.N	Technique	Column	Run Time	Linearity (µg/mL)	Rt	Ref
4	HPLC	C18	20	PCM: 42.8–127.6 µg CAF: 9.4–25 µg	PCM: 6.14 CAF: 14.44	[15]
5	HPLC	C18	17	PCM: 0.8–270 µg CAF: 0.4–250 µg	PCM: 3.8 CAF: 5.3	[16]
6	HPLC	C18	15	PCM: 1–500 µg CAF: 1–150 µg	PCM: 4.2 CAF: 7.2	[17]
7	HPLC	C18	17	PCM: 30–1100 ng CAF: 50–400 ng	PCM: 6.5 CAF: 12.1	[18]
8	HPLC	C18	10	PCM: 15–300 µg CAF: 0.01–5 µg	NR	[19]
9	UPLC	C18	7	P PCM: 325–2600 PPM CAF: 30–240 PPM	PCM: 0.68 CAF: 1.78	[6]
10	UPLC-MS	C18	4.5	PCM: 0.05–25 µg CAF: 0.01–5 µg	NR	[21]
11	HPLC	C18	24	PCM: 250–750 µg CAF: 15–45 µg	PCM: 11.03 CAF: 15.36	[35]
12	UPLC	C18	2.0	PCM: 40–400 ng/mL CAF: 7–70 ng/mL	PCM: 0.82 CAF: 1.16	CI

NR: not reported, CI: current investigation.

3. Materials and Methods

3.1. Materials

PCM and CAF (purity $\geq$ 99%) were purchased from Sigma Aldrich. Other HPLC-grade solvents used were procured from Chroma solve (Germany). Tablet and capsule formulations were obtained from a pharmacy in Rakkah, Dammam, Saudi Arabia.

3.2. Chromatographic Conditions

The analytical procedure was developed on a Waters UPLC by using a photodiode array (PDA) detector with a column oven. PCM and CAF were separated on a C18 column (1.7 µm, 2.1 $\times$ 50 mm) maintained at 35 $\pm$ 5 °C by using Empower software. A mixture of water and methanol (70:30, v/v) was used as the mobile phase, with an injection volume of 1 µL for isocratic elution at a flow rate of 0.2 mL/min and a detection wavelength of 273 nm.

3.3. Stock Solutions

Standard stock solutions of PCM and CAF (400 and 140 µg/mL, respectively) were prepared in a solution of water and methanol (70:30, v/v). Then, 1 mL of each standard of PCM and CAF was mixed to obtain the concentrations of 200 and 70 µg/mL, respectively. All the samples were filtered using 0.22 µm membrane filters.

3.4. Sample Preparation

Four pharmaceutical formulations were used as samples. Among these, two commercial tablets comprised PCM (500 mg) and CAF (65 mg), one tablet comprised PCM (500 mg) and CAF (30 mg), and one capsule comprised PCM (500 mg) and CAF (30 mg). These samples are denoted as Tab-1 (PCM: 500 mg and CAF: 30 mg), Tab-2 and Tab-3 (PCM: 500 mg and CAF: 65 mg), and Cap-1 (PCM: 500 mg and CAF: 30 mg). A total of 10 samples of each of the aforementioned commercial tablets and the capsule were weighed accurately. An amount of powdered Tab-1, Tab-2, Tab-3, and Cap-1 was separately dissolved in 100 mL of a water and methanol (70:30, v/v) solution and sonicated for 10 min to dissolve the

powders completely. Then, 1 mL of this solution was diluted ten times by using the same solvent for analyses. All the samples were filtered using a 0.22 µm filter before analyses.

3.5. Method Validation

The UPLC method was validated according to the ICH guidelines [36–38] for the estimation of PCM and CAF; this included the following validation characteristics: precision, specificity, accuracy, robustness, LOD, and LOQ.

Specificity is the capability of an analytical procedure to detect analytes in the presence of other components and existing excipients. The specificity of the UPLC procedure was determined by comparing the retention time and the peak apex acquired during the sample tests for PCM and CAF with the retention time and the peak apex of standard PCM and CAF.

The linearity of the proposed method was assessed by plotting the peak areas obtained using the injection of PCM and CAF against the concentration employed for the calibration graph. The calibration curves were analyzed for regression analyses.

The accuracy of the procedure was estimated by studying recovery by employing the standard accumulation technique at three concentrations of PCM and CAF. A known quantity of PCM and CAF was examined, and the amounts were calculated. This experiment was performed in triplicate.

The inter-day and intra-day precisions of the developed procedure were measured. Intra-day precision was examined at three concentrations of 80, 100, and 200 ng/mL for PCM and at three concentrations of 17.5, 35, and 70 ng/mL for CAF, and the actual concentrations of PCM and CAF were estimated in triplicate within a day. The same procedure was used for the determination of intra-day precision. The concentrations of PCM and CAF were estimated, and the relative standard deviation (RSD) was calculated.

The robustness of the procedure was determined by analyzing the effects of slight variations in the experimental settings. Robustness was assessed by changing the flow rate, wavelength, and column oven temperature.

The LOD and LOQ of the developed procedure were assessed using a signal to noise ratio based method.

3.6. Forced Degradation of PCM and CAF

A stock solution comprising PCM (170 µg/mL) and CAF (90 µg/mL) was prepared and used for further studies. The forced degradation of PCM and CAF was performed to study the stability-indicating property and specificity of the proposed method.

This study was conducted by following the ICH guidelines [15,36,39]. The standard samples of PCM and CAF were degraded under different stress conditions, namely, acidic, alkali, oxidative, phytochemical, dry-heat, wet-heat, and normal conditions. For acidic and alkaline degradation, the samples were refluxed for 2 h at 80 °C with 2 M HCl and 2 M NaOH, respectively. Similarly, oxidative degradation was performed using 30% H_2O_2, and the sample was heated for 30 min at 60 °C. Photochemical-induced degradation was performed using methanol in the sample, and the sample was exposed to sunlight for 1 day (8:00 to 16:00 at 40–44 °C). Wet-heat degradation was performed using methanol in the sample, and the mixture was refluxed for 2 h, whereas dry-heat degradation was conducted by heating the sample in an oven at 100 °C for 2 h.

All the samples were diluted to obtain PCM (85 µg/mL) and CAF (45 µg/mL), except for the dry-heat sample. The dry-heat samples were diluted to 75 µg/mL for both PCM and CAF. Then, 1 µL was injected into the system, and a chromatogram was recorded to measure sample stability.

4. Conclusions

This study presented a simple, rapid, precise, accurate, and stability-indicating UPLC-PDA procedure for concurrently determining PCM and CAF in pharmaceutical formulations. The method was demonstrated to be superior compared with previous analytical

reports in terms of its simplicity, fast speed, time efficiency, and cost effectiveness, with a short run time of 2 min, which reduces solvent utilization. Furthermore, the mobile phase comprising methanol and water (30:70, *v*/*v*), used for sample preparation and washing the column, extended the method's considerable cost effectiveness compared to that of other methods. The proposed method also provided a detailed account of the quantification of PCM and CAF under stress conditions, indicating the excellent specificity of the UPLC procedure and its stability-indicating power. This is advantageous from economic and environmental perspectives. Therefore, the proposed procedure is suitable for quality control analysis and stability studies of pharmaceutical formulations comprising PCM and CAF as ingredients.

Author Contributions: Conceptualization, W.A. and P.A.; methodology, W.A., Y.A.H., A.A. and S.S.; software, W.A. and P.A.; validation, W.A., S.W. and P.A.; formal analysis, A.A., P.A. and M.S. (Manal Suroor); investigation, W.A., M.S.(Mohammad Sarafroz) and M.S. (Manal Suroor); data curation, W.A., A.A. and S.W.; writing—original draft preparation, W.A., P.A. and S.S.; writing—review and editing, S.W., A.A. and Y.A.H.; supervision, W.A., A.A. and Y.A.H.; project administration, W.A., S.W. and P.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Not Applicable.

Acknowledgments: All authors are thankful to Mohammed Al-Mana College for Medical Sciences for providing instrumentation facility.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

PCM	paracetamol
CAF	caffeine
UPLC	ultra-performance liquid chromatography
ICH	International Council for Harmonization
LOD	limit of detection
LOQ	limit of quantification
H_2O_2	hydrogen peroxide

References

1. Siddiqui, M.R.; AlOthman, Z.A.; Rahman, N. Analytical techniques in pharmaceutical analysis: A review. *Arab. J. Chem.* **2017**, *10*, S1409–S1421. [CrossRef]
2. Khorshed, A.A.; Khairy, M.; Banks, C.E. Electrochemical determination of antihypertensive drugs by employing costless and portable unmodified screen-printed electrodes. *Talanta* **2019**, *198*, 447–456. [CrossRef]
3. Duan, C.; Wu, Y.; Yang, J.; Chen, S.; Pu, Y.; Deng, H. Simultaneous Determination of Cortisol, Cortisone, and Multiple Illicit Drugs in Hair among Female Drug Addicts with LC-MS/MS. *Molecules* **2021**, *26*, 516. [CrossRef] [PubMed]
4. Terzopoulou, Z.; Papageorgiou, M.; Kyzas, G.Z.; Bikiaris, D.N.; Lambropoulou, D.A. Preparation of molecularly imprinted solid-phase microextraction fiber for the selective removal and extraction of the antiviral drug abacavir in environmental and biological matrices. *Anal. Chim. Acta.* **2016**, *913*, 63–75. [CrossRef] [PubMed]
5. Tunca, K. Quantitative Analysis of Paracetamol, Acetyl cysteine and Guaifenesin in Commercial Cold Medicines by UV-Vis Spectroscopy. *Sop. Trans. Anal. Chem.* **2014**, *1*, 40–47. [CrossRef]
6. Jena, B.R.; Babu, S.M.; Pradhan, D.P.; Swain, S. UPLC Analytical Method Development and Validation for the Simultaneous Estimation of Paracetamol and Caffeine Capsules Dosages Form. *Pharm. Regul. Aff.* **2017**, *6*, 186. [CrossRef]
7. Foudah, A.I.; Shakeel, F.; Alqarni, M.H.; Aljarba, T.M.; Alshehri, S.; Alam, P. Simultaneous Detection of Chlorzoxazone and Paracetamol Using a Greener Reverse-Phase HPTLC-UV Method. *Separations* **2022**, *9*, 300. [CrossRef]
8. Ilango, K.B.; Gowthaman, S.; Seramaan, K.I.; Chidambaram, K.; Bayan, M.F.; Rahamathulla, M.; Balakumar, C. Mucilage of Coccinia grandis as an Efficient Natural Polymer-Based Pharmaceutical Excipient. *Polymers* **2022**, *14*, 215. [CrossRef]
9. Alvi, S.N.; Hammami, M.M. Validated HPLC Method for Determination of caffeine Level in Human Plasma using Synthetic Plasma: Application to Bioavailability Studies. *J. Chromatogr. Sci.* **2011**, *49*, 292–296. [CrossRef] [PubMed]
10. Nehlig, A.; Daval, J.L.; Debry, G. Caffeine and the central nervous system: Mechanisms of action, biochemical, metabolic and psychostimulant effects. *Brain Res. Rev.* **1992**, *17*, 139–170.

11. Ali, A.A.; Raouf, H.H.A. Synthesis and Antitumor Activity of New Pyrimidine and Caffeine Derivatives. *Lett. Drug Des. Discov.* **2015**, *12*, 471–478. [CrossRef]
12. Graham, G.G.; Davies, M.J.; Day, R.O.; Mohamudally, A.; Scott, K.F. The modern pharmacology of paracetamol: Therapeutic actions, mechanism of action, metabolism, toxicity and recent pharmacological findings. *Inflammopharmacology* **2013**, *21*, 201–232. [CrossRef]
13. Schug, S.A.; Goddard, C. Recent advances in the pharmacological management of acute and chronic pain. *Ann. Palliat. Med.* **2014**, *3*, 263–275.
14. Jendrzejewska, I.; Goryczka, T.; Pietrasik, E.; Klimontko, J.; Jampilek, J. X-ray and Thermal Analysis of Selected Drugs Containing Acetaminophen. *Molecules* **2020**, *25*, 5909. [CrossRef] [PubMed]
15. Altun, M.L. HPLC Method for the Analysis of Paracetamol, Caffeine and Dipyrone. *Turk. J. Chem.* **2002**, *26*, 521–528.
16. Aminu, N.; Chan, S.Y.; Khan, N.H.; Farhan, A.B.; Umar, M.N.; Toh, S.M. A Simple Stability-Indicating HPLC Method for Simultaneous Analysis of Paracetamol and Caffeine and Its Application to Determinations in Fixed-Dose Combination Tablet Dosage Form. *Acta. Chromatogr.* **2019**, *31*, 85–91. [CrossRef]
17. Mohammed, O.J.; Hamzah, M.J.; Saeed, A.M. RP–HPLC Method Validation for Simultaneous Estimation of Paracetamol and Caffeine in Formulating Pharmaceutical Form. *Res. J. Phar. Technol.* **2021**, *14*, 4743–4748. [CrossRef]
18. Soponar, F.; Staniloae, D.; Moise, G.; Szaniszlo, B.; David, V. Simultaneous determination of paracetamol, propyphenazone and caffeine from pharmaceutical preparations in the presence of related substances using a validated hplc-dad method. *Rev. Roum. Chim.* **2013**, *58*, 433–440.
19. Pereira, F.J.; Rodríguez-Cordero, A.; López, R.; Robles, L.C.; Aller, A.J. Development and Validation of an RP-HPLC-PDA Method for Determination of Paracetamol, Caffeine and Tramadol Hydrochloride in Pharmaceutical Formulations. *Pharmaceuticals* **2021**, *14*, 466. [CrossRef]
20. Acheampong, A.; Gyasi, W.O.; Darko, G.; Apau, J.; Addai-Arhin, S. Validated RP-HPLC method for simultaneous determination and quantification of chlorpheniramine maleate, paracetamol and caffeine in tablet formulation. *SpringerPlus* **2016**, *5*, 625. [CrossRef]
21. Rahimi, M.; Khorshidi, N.; Rouhollah, H. Simultaneous determination of paracetamol and caffeine in aqueous samples by ultrasound-assisted emulsification microextraction coupled with high-performance liquid chromatography. *Sep. Sci. Plus.* **2020**, *3*, 561–570. [CrossRef]
22. Cunha, R.R.; Chaves, S.C.; Ribeiro, M.M.; Torres, L.M.; Muñoz, R.A. Simultaneous determination of caffeine, paracetamol, and ibuprofen in pharmaceutical formulations by high-performance liquid chromatography with UV detection and by capillary electrophoresis with conductivity detection. *J. Sep. Sci.* **2015**, *38*, 1657–1662. [CrossRef] [PubMed]
23. Belal, F.; Omar, M.A.; Derayea, S.; Zayed, S.; Hammad, M.A.; Saleh, S.F. Simultaneous determination of paracetamol, caffeine and codeine in tablets and human plasma by micellar liquid chromatography. *Eur. J. Chem.* **2015**, *4*, 468–474. [CrossRef]
24. Wang, A.; Sun, J.; Feng, H.; Gao, S.; He, Z. Simultaneous Determination of Paracetamol and Caffeine in Human Plasma by LC–ESI–MS. *Chromatographia* **2008**, *67*, 281–285. [CrossRef]
25. Tavallali, H.; Zareiyan, S.F.; Naghian, M. An efficient and simultaneous analysis of caffeine and paracetamol in pharmaceutical formulations using TLC with a fluorescence plate reader. *J. AOAC Int.* **2011**, *94*, 1094–1099. [CrossRef]
26. Chabukswar, A.R.; Thakur, V.G.; Dharam, D.L.; Shah, M.H.; Kuchekar, B.S.; Sharma, S.N. Development and validation of HPTLC method for simultaneous estimation of paracetamol, ibuprofen and caffeine in bulk and pharmaceutical dosage form. *Res. J. Pharm. Technol.* **2012**, *5*, 1218–1222.
27. Halka-Grysinska, A.; Slazak, P.; Zareba, G.; Markowski, W.; Klimek-Turek, A.; Dzido, T.H. Simultaneous determination of acetaminophen, propyphenazone and caffeine in cefalgin preparation by pressurized planar electrochromatography and highperformance thin-layer chromatography. *Anal. Methods* **2012**, *4*, 973–982. [CrossRef]
28. Minh, T.T.; Phong, N.H.; Duc, H.V.; Khieu, D.Q. Microwave synthesis and voltametric simultaneous determination of paracetamol and caffeine using an MOF-199-based electrode. *J. Mat. Sci.* **2018**, *53*, 2453–2471. [CrossRef]
29. Silva, T.A.; Zanin, H.; Jos-Corat, E.; Fatibello-Filho, O. Simultaneous Voltammetric Determination of Paracetamol, Codeine and Caffeine on Diamond-like Carbon Porous Electrodes. *Electroanalysis* **2016**, *29*, 907–916. [CrossRef]
30. Feyisa, T.Y.; Kitte, S.A.; Yenealem, D.; Gebretsadik, G. Simultaneous Electrochemical Determination of Paracetamol and Caffeine Using Activated Glassy Carbon Electrode. *Anal. Bioanal. Electrochem.* **2020**, *12*, 93–106.
31. Boltia, S.A.; Soudi, A.T.; Elzanfaly, E.S.; Zaazaa, H.E. Simultaneous determination of paracetamol, orphenadrine citrate, and caffeine ternary mixture by different spectrophotometric methods. *J. Appl. Spectrosc.* **2019**, *6*, 731–739. [CrossRef]
32. Moţ, A.C.; Soponar, F.; Medvedovici, A.; Sârbu, C. Simultaneous Spectrophotometric Determination of Aspirin, Paracetamol, Caffeine, and Chlorphenamine from Pharmaceutical Formulations Using Multivariate Regression Methods. *Anal. Lett.* **2010**, *43*, 804–813. [CrossRef]
33. Nugrahani, I.; Manosa, E.Y.; Chintya, L. FTIR-derivative as a green method for simultaneous content determination of caffeine, paracetamol, and acetosal in a tablet compared to HPLC. *Vib. Spectrosc.* **2019**, *104*, 102941. [CrossRef]
34. British Pharmacopoeia Commission. *British Pharmacopoeia*; British Pharmacopoeia Commission: London, UK, 2009.
35. Dewani, A.P.; Patra, S. A single HPLC-DAD method for simultaneous analysis of paracetamol, phenylephrine, caffeine, and levocetirizine in bulk powder and tablet formulation: Application to in-vitro dissolution studies. *J. Chil. Chem. Soc.* **2015**, *60*, 2734. [CrossRef]

36. Ahmad, W.; Yusuf, M.; Ahmad, A.; Hassan, Y.A.; Amir, M.; Wahab, S. Development and Validation of Ultra Performance Liquid Chromatography (UPLC) Method for the Quantitative Estimation of Caffeine in Non-Alcoholic Soft and Energy Drinks. *J. AOAC Int.* **2022**, *105*, 1146–1152. [CrossRef] [PubMed]
37. ICH. *Validation of Analytical Procedures: Text and Methodology Q2 (R1)*; ICH: Geneva, Switzerland, 2005; Volume 2005.
38. Ahmad, W.; Zaidi, S.M.A.; Mujeeb, M.; Ansari, S.H.; Ahmad, S. HPLC and HPTLC methods by design for quantitative characterization and in vitro anti-oxidant activity of polyherbal formulation containing *Rheum emodi*. *J. Chromatogr. Sci.* **2014**, *2*, 911–918. [CrossRef]
39. ICH Harmonised Tripartite Guidelines. "Stability testing of new drug substances and products, Q1A (R2)", In international Conference on Hormonization of Technical Requirements for Registration of Pharmaceutical for Human Use. 2003. Available online: https://database.ich.org/sites/default/files/Q1A%28R2%29%20Guideline.pdf (accessed on 20 November 2022).