Research Article

Mohammed Hamed Alqarni, Faiyaz Shakeel, Sultan Alshehri*, Ahmed Ibrahim Foudah, Tariq Mohammed Aljarba, Fatma Mohamed Abdel Bar, and Prawez Alam*

Simultaneous estimation of ibuprofen, caffeine, and paracetamol in commercial products using a green reverse-phase HPTLC method

https://doi.org/10.1515/gps-2023-0220
received October 26, 2023; accepted January 02, 2024

Abstract: A fast, sensitive, and green reverse-phase "high-performance thin-layer chromatography" approach for the simultaneous estimation of ibuprofen (IBF), caffeine (CAF), and paracetamol (PCM) in marketed formulations was established and verified in this study. The binary combination of acetone and water (80:20 v/v) was used as the green eluent system. The current method's greenness was predicted using four different approaches, namely National Environmental Method Index, Analytical Eco-Score (89), ChlorTox (1.08 g), and the Analytical GREENness (83) approaches, which demonstrated an outstanding greener profile. The present approach was linear in the range of 25–800 ng·band^{-1} for the simultaneous estimation of IBF, CAF, and PCM. In addition, the current method was accurate (% recoveries = 100 ± 2), precise (%CV < 2%), robust (%CV < 2), sensitive (LOD = 1.13–2.71 ng·band^{-1} and LOQ = 3.39–8.10 ng·band^{-1}), and green. The amount of IBF, CAF, and PCM in commercial tablets was determined to be 99.51%, 98.25%, and 100.64%, respectively. The present method for the simultaneous determination of IBF, CAF, and PCM in marketed tablets is supported by these data. The findings of this study suggested that the current approach may be consistently applied to analyze IBF, CAF, and PCM in marketed tablets.

Keywords: caffeine, green reverse-phase HPTLC, greenness tools, ibuprofen, paracetamol

1 Introduction

Ibuprofen (IBF) is a commonly used anti-inflammatory medicine (Figure 1a) [1]. It is advised to use it to treat mild to moderate pain and inflammation, including that caused by dysmenorrhea, migraines, dental pain, post-operative pain, and muscle and joint condition [1,2]. It works by inhibiting the enzyme cyclooxygenase-2 (COX-2) [1]. Caffeine (CAF) is a pseudo-alkaloidal medicine, which is also used to treat various kind of pain (Figure 1b) [3,4]. The most popular anti-inflammatory and antipyretic medication, particularly for pediatric and geriatric patients, is paracetamol (PCM) (Figure 1c) [5,6]. It is marketed and offered in a variety of dosage forms [6]. The combination of IBF, CAF, and PCM is commonly used to treat several pains and inflammatory conditions [7]. These drugs such as IBF, CAF, and PCM are violable in various commercially available multicomponent formulations. It is therefore required to standardize IBF, CAF, and PCM in qualitative and quantitative terms in marketed multicomponent products.

There have been many published analysis techniques for the simultaneous determination of IBF, CAF, and PCM in marketed products. For the simultaneous estimation of CAF and PCM in standard drug and formulations, numerous analytical methodologies, such as derivative spectrometry [8–13], high-performance liquid chromatography (HPLC) [4,14–17], high-performance thin-layer chromatography (HPTLC) [18,19], voltammetry [20–22], electrospray laser desorption ionization mass spectrometry [23], electrochemical method with 3D-printed technology [24], near-infrared spectroscopy [25], flow-injection spectroscopy [26], and micellar liquid chromatographic methods [27,28] have been reported.

* **Corresponding author: Sultan Alshehri,** Department of Pharmaceutical Sciences, College of Pharmacy, AlMaarefa University, P.O. Box 71666, Diriyah 13713, Riyadh, Saudi Arabia, e-mail: sshehri@um.edu.sa
* **Corresponding author: Prawez Alam,** Department of Pharmacognosy, College of Pharmacy, Prince Sattam Bin Abdulaziz University, P.O. Box 173, Al-Kharj 11942, Saudi Arabia, e-mail: p.alam@psau.edu.sa
Mohammed Hamed Alqarni, Ahmed Ibrahim Foudah, Tariq Mohammed Aljarba, Fatma Mohamed Abdel Bar: Department of Pharmacognosy, College of Pharmacy, Prince Sattam Bin Abdulaziz University, P.O. Box 173, Al-Kharj 11942, Saudi Arabia
Faiyaz Shakeel: Department of Pharmaceutics, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

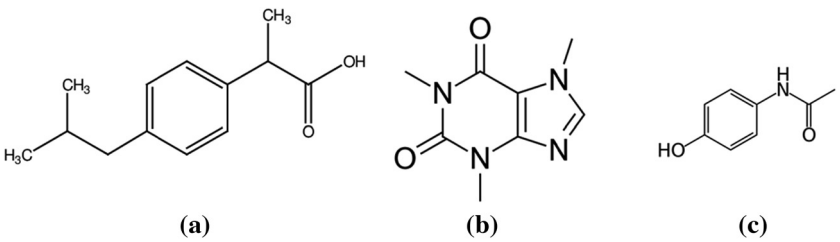

Figure 1: Molecular structures of (a) IBF, (b) CAF, and (c) PCM.

Various spectrometry [29–31], absorption-based spectrometry [7], and derivative spectrometry approaches [32,33] have been established for the simultaneous estimation of IBF, CAF, and PCM in bulk forms and multicomponent preparations. Numerous HPLC methods have also been developed and validated for the simultaneous estimation of IBF, CAF, and PCM in bulk forms and multicomponent pharmaceutical products [31,34,35]. Capillary electrophoresis [34] and chemometry [36] approaches have also been suggested for the simultaneous estimation of IBF, CAF, and PCM in bulk forms and multicomponent pharmaceutical preparations. The simultaneous quantification of IBF, CAF, and PCM in mixed dosage forms has also been done using an HPTLC approach [37]. Furthermore, our research group has produced an environmentally friendly HPTLC methodology for the quantification of CAF in commercially available energy drinks and dosage forms [38]. Green normal-phase and reverse-phase HPTLC methods have also been reported for the simultaneous estimation of CAF and PCM in commercial formulations by our research team [39]. The simultaneous estimation of IBF, CAF, and PCM in commercial formulations, however, has not been reported using the green HPTLC techniques.

Various analytical techniques were recommended in published articles on the simultaneous estimation of CAF and PCM or IBF, CAF, and PCM. However, none of the reported analytical methods approximated the greenness scale. Furthermore, the simultaneous estimation of IBF, CAF, and PCM has not employed green HPTLC techniques. One of the twelve guiding principles of "green analytical chemistry (GAC)" is the use of substitute environmentally safe solvents to decrease the detrimental effects of toxic/hazardous eluents on the environment [40]. The application of green solvents has increased exponentially during the past few decades, according to a literature search [41–43]. Several qualitative and quantitative techniques have been described in the literature to evaluate the greenness profiles of analytical methodologies. These include the Analytical GREENness (AGREE), Red, Green, and Blue, ChlorTox, Analytical Eco-Score (AES), Green Analytical Procedure Index, and the National Environmental Method Index (NEMI) [44–50]. In the present study, four different tools, namely, NEMI, AES, ChlorTox, and AGREE approaches, were used to gauge the greener profile of the present methodology [44,45,49,50]. Compared to traditional liquid chromatographic techniques, the green HPTLC method has a number of benefits, including low solvent consumption, short analysis times, nondestructive mode of detection, simplicity of use, minimal pretreatment, sensitivity, efficiency, simultaneous analysis of multiple samples, non-toxicity, and environmental friendliness [51–56]. Based on the aforementioned data and observations, the current method seeks to create and establish a fast, sensitive, and green reverse-phase HPTLC method for the simultaneous estimation of IBF, CAF, and PCM in commercially available tablets. The proposed method for the simultaneous estimation of IBF, CAF, and PCM was verified using "The International Council for Harmonization (ICH)-Q2-R1" guidelines [57].

2 Materials and methods

2.1 Materials

The reference standards of IBF, CAF, and PCM were obtained from "Sigma Aldrich (St. Louis, MO, USA)." Liquid chromatography-grade acetone was obtained from "E-Merck (Darmstadt, Germany)." The Milli-Q device was used to obtain the purified water. The marketed multicomponent tablets (containing 200 mg of IBF, 40 mg of CAF, and 325 mg of PCM) were bought at the neighborhood pharmacy in "Riyadh, Saudi Arabia." The other chemicals and solvents used were of the analytical variety.

2.2 Instrumentation and chromatographic conditions

The "HPTLC CAMAG TLC system (CAMAG, Muttenz, Switzerland)" was utilized for the simultaneous estimation of IBF, CAF, and PCM in the bulk forms and procured formulations.

The samples were applied as 6 mm bands using the "CAMAG Automatic TLC Sampler 4 (ATS4) Sample Applicator (CAMAG, Geneva, Switzerland)." The "CAMAG microliter Syringe (Hamilton, Bonaduz, Switzerland)" was loaded into the sample applicator. The TLC plates were "glass plates (plate size: 10 × 20 cm^2) pre-coated with reverse-phase silica gel (particle size: 5 µm) 60F254S plates," which were used as the stationary phase. The binary combination of acetone-water (80:20 v/v) was used as the green eluent system. For the concurrent measurement of IBF, CAF, and PCM, the application rates were set at 150 $nL \cdot s^{-1}$ each. The TLC plates were developed in a "CAMAG automated developing chamber 2 (ADC2) (CAMAG, Muttenz, Switzerland)" under a linear ascending mode at a distance of 8 cm. The development chamber was filled with vapors from a green eluent system for 30 minutes at 22°C. A wavelength of 260 nm was used to concurrently detect IBF, CAF, and PCM. Scanner speed and slit diameter were both adjusted to 20 $mm \cdot s^{-1}$ and 4 × 0.45 mm^2, respectively. For each measurement, there were three or six replications used. It was "WinCAT's (version 1.4.3.6336, CAMAG, Muttenz, Switzerland)" program that was used for data processing.

2.3 Calibration plots and quality control (QC) solutions for IBF, CAF, and PCM

Separate batches of IBF, CAF, and PCM stock solutions were made by dissolving the necessary quantities of each medication in the appropriate amount of the green eluent system. The resultant stock solution of each medication included 100 $\mu g \cdot mL^{-1}$ of active ingredient. IBF, CAF, and PCM concentrations in the 25–800 $ng \cdot band^{-1}$ range were created by diluting varying volumes of the stock solutions using the green eluent system. The peak area of each concentration of IBF, CAF, and PCM was recorded after 20 µL of each concentration of IBF, CAF, and PCM were placed on TLC plates for the current approach. IBF, CAF, and PCM calibration plots were produced by graphing the concentrations of IBF, CAF, and PCM versus the measured peak response in six replications (n = 6). In order to assess numerous validation criteria, three distinct QC solutions were produced.

2.4 Sample preparations for the simultaneous estimation of IBF, CAF, and PCM in procured tablets

Twenty commercial tablets were measured, and the mean weight for the concurrent determination of IBF, CAF, and PCM in procured tablets was computed. Each commercial tablet included 200 mg of IBF, 40 mg of CAF, and 325 mg of PCM. The commercial tablets were powdered after being roughly crushed. An amount of fine powder, equal to the mean weight of a tablet, was dispersed in 100 mL of the green eluent system. For the current procedure, 1 mL of this commercial tablet solution was diluted once more using 10 mL of the proposed solvent system. In order to remove any insoluble excipients, the produced solutions for procured formulations were sonicated for approximately 10 minutes and filtered. The obtained samples were used to assess IBF, CAF, and PCM concurrently in commercially available tablets by the existing methodology.

2.5 Validation studies

Using the ICH-Q2-R1 guidelines, the current method for the simultaneous estimation of IBF, CAF, and PCM was validated for numerous parameters [57]. Plotting the concentrations of IBF, CAF, and PCM against the measured peak area allowed for the establishment of their linear ranges. The current approach's IBF, CAF, and PCM linearity was evaluated between 25 and 800 $ng \cdot band^{-1}$ (n = 6).

To assess the parameters for the proposed approach's system suitability for the simultaneous estimation of IBF, CAF, and PCM, "retardation factor (R_f), asymmetry factor (As), theoretical plates number per meter ($N \cdot m^{-1}$), and resolution factor (Rs)" computation was used. For the current method, the "R_f, As, $N \cdot m^{-1}$, and Rs" values were derived by their reported equations [43,58].

Using the spiking/standard addition methodology, the accuracy of the present method for the concurrent determination of IBF, CAF, and PCM was assessed as % recoveries [57]. An additional 50%, 100%, and 150% of the IBF, CAF, and PCM solutions were spiked into the previously measured IBF, CAF, and PCM solutions (100 $ng \cdot band^{-1}$) in order to establish low-QC (LQC) solutions of IBF, CAF, and PCM of 150 $ng \cdot band^{-1}$, moderate-QC (MQC) levels of 200 $ng \cdot band^{-1}$, and high-QC (HQC) levels of 250 $ng \cdot band^{-1}$. The above IBF, CAF, and PCM QC solutions were reanalyzed to measure the accuracy. The % recovery was computed at each concentration level of IBF, CAF, and PCM. Six replicates (n = 6) were utilized to measure the accuracy.

Intra/inter-assay precision was evaluated for the existing method for the simultaneous estimation of IBF, CAF, and PCM. Examining intra-assay variation for these three substances was made possible by quantifying freshly generated IBF, CAF, and PCM solutions at previously described QC levels on the same day (n = 6). Examination of freshly prepared

solutions was done at previously described QC levels on three consecutive days (n = 6) as part of the evaluation of inter-assay variation for IBF, CAF, and PCM solutions for the current technique.

The robustness of IBF, CAF, and PCM was evaluated for the current method by introducing some, purposeful alterations to the composition of the green eluent system. After switching between acetone–water (82:18 v/v) and acetone–water (78:22 v/v) as the green eluent systems for IBF, CAF, and PCM, the alterations in peak area and R_f data were observed (n = 6).

By utilizing a "standard deviation" approach, the sensitivity of the current method for the concurrent determination of IBF, CAF, and PCM was measured as "limit of detection (LOD) and limit of quantification (LOQ)." IBF, CAF, and PCM "LOD and LOQ" were computed using Eqs. 1 and 2 (n = 6) [57]:

$$\text{LOD} = \frac{3.3 \times \sigma}{S} \tag{1}$$

$$\text{LOQ} = \frac{10 \times \sigma}{S} \tag{2}$$

where S is the slope of the calibration curve for IBF, CAF, and PCM, and σ is the standard deviation of the intercept.

To assess the specificity of the current approach for the simultaneous estimation of IBF, CAF, and PCM, the R_f data and 3D spectrum of IBF, CAF, and PCM in marketed tablets were contrasted to that of standards IBF, CAF, and PCM.

2.6 Application of current approach in the concurrent determination of IBF, CAF, and PCM in procured formulations

Under the same experimental settings as the simultaneous estimation of standards IBF, CAF, and PCM, the chromatographic responses of the processed samples of procured formulations were determined on TLC plates for the current approach (n = 3). The calibration curves for IBF, CAF, and PCM were used to approximate the amounts of IBF, CAF, and PCM in procured formulations for the present methodology.

2.7 Greenness assessment

Four different approaches, namely, NEMI, AES, ChlorTox, and AGREE, were used to assess the greenness profile of the current method for the simultaneous estimation of IBF, CAF, and PCM [44,45,49,50]. NEMI is used to obtain preliminary judgment based on persistent, bioaccumulative, and toxic (PBT), hazardous, corrosive, and waste [44]. According to the NEMI method, four quarter circles are drawn and each quarter is colored green or left blank indicating the PBT, hazardous, corrosive, and waste [44].

AES is a semi-quantitative approach, which considers all steps of analytical procedures, instruments, and waste. Analysis of compounds with no or minimal use of reagents, low energy consumption, and no waste is expected to be an ideal analysis with 100 points. If any of these parameters are deviated, penalty points are assigned, and total penalty points are subtracted from 100 [45].

The ChlorTox technique states that the ChlorTox score is determined using Eq. 3 [49]:

$$\text{ChlorTox} = \frac{\text{CH}_{\text{sub}}}{\text{CH}_{\text{CHCl3}}} \times m_{\text{sub}} \tag{3}$$

where m_{sub} is the mass of the substance of interest (acetone in the present study) needed for a single analysis, CH_{sub} is the chemical risks of the acetone, and CH_{CHCl3} is the chemical hazard of standard chloroform. With the aid of the safety data sheet from "Sigma Aldrich (St. Louis, MO, USA)," the values of CH_{sub} and CH_{CHCl3} were determined using the weighted hazards number (WHN) model [49]. Using the WHN model and safety data sheet from "Sigma Aldrich (St. Louis, MO, USA)," CH_{sub} value was determined using Eq. 4:

$$\text{CH}_{\text{sub}} = (1 \times N_{\text{cat1}}) + (0.75 \times N_{\text{cat2}}) + (0.50 \times N_{\text{cat3}}) + (0.25 \times N_{\text{cat4}}), \tag{4}$$

where, N_{cat1}, N_{cat2}, N_{cat3}, and N_{cat4} are the number of toxicities under the categories 1, 2, 3, and 4, respectively. For substance (acetone), N_{cat1} = 0, N_{cat2} = 2, N_{cat3} = 1, and N_{cat4} = 0 were taken from the safety data sheet of "Sigma Aldrich (St. Louis, MO, USA)."

Hence, $\text{CH}_{\text{sub}} = (1 \times 0) + (0.75 \times 2) + (0.50 \times 1) + (0.25 \times 0) = 2.0$.

For chloroform, N_{cat1} = 1, N_{cat2} = 4, N_{cat3} = 3, and N_{cat4} = 1 were taken from the safety data sheet of "Sigma Aldrich (St. Louis, MO, USA)."

Hence, $\text{CH}_{\text{CHCl3}} = (1 \times 1) + (0.75 \times 4) + (0.50 \times 3) + (0.25 \times 1) = 5.75$.

Finally, CH_{sub} and CH_{CHCl3} values were determined to be 2.0 and 5.75, respectively. ChlorTox values were then calculated using Eq. 3.

The AGREE-metric technique was used to evaluate the AGREE score for the current method for the simultaneous estimation of IBF, CAF, and PCM [50]. This method assigns 0–1 scores to each of the twelve GAC components, after which the average score is determined. The "AGREE: The

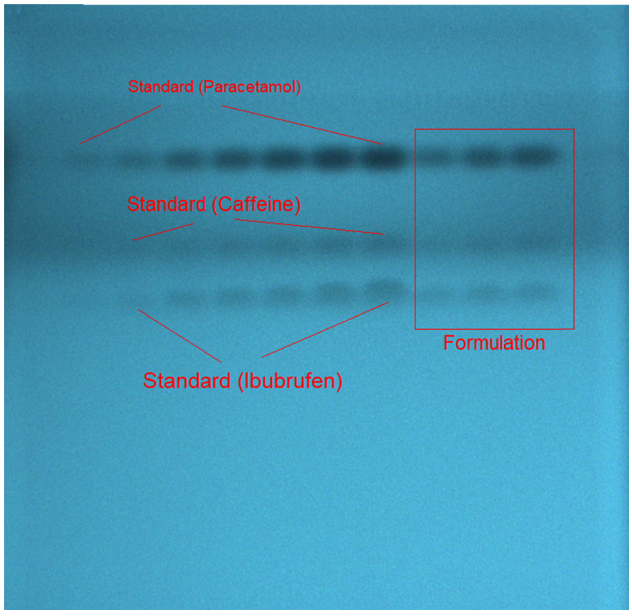

Figure 2: The representative TLC image for standard IBF, CAF, PCM, and marketed tablets established utilizing acetone-water (80:20 v/v) as the green eluent system for the current method.

Analytical Greenness Calculator (version 0.5, Gdansk University of Technology, Gdansk, Poland, 2020)" was used to gauge the AGREE scores in the range from 0.0 to 1.0 for the current procedure.

3 Results and discussions

3.1 Method development

A variety of acetone-to-water ratios were studied as green eluent systems, including acetone–water ratios of 50:50, 60:40, 70:30, 80:20, and 90:10 v/v. The development of every green eluent system put to the test took place in saturated chambers (Figure 2). The green eluent systems such as acetone–water (50:50 v/v), acetone–water (60:40 v/v), acetone–water (70:30 v/v), and acetone–water (90:10 v/v) displayed unsatisfactory chromatography signals of IBF, CAF, and PCM with unreliable As for IBF (As > 1.20), CAF (As > 1.25), and PCM (As > 1.30). It was observed when the green eluent system acetone–water (80:20 v/v) was examined that this green eluent system displayed well-separated and unbroken chromatography signals of IBF at R_f = 0.55 ± 0.01, CAF at R_f = 0.67 ± 0.02, and of PCM at R_f = 0.85 ± 0.02 (Figure 3a). Additionally, As values of 1.08, 1.12, and 1.05, respectively, were projected for IBF, CAF, and PCM, all of which are extremely reliable values. As a consequence, it was agreed that acetone–water (80:20 v/v) would be the final eluent system for the simultaneous estimation of IBF, CAF, and PCM in commercial tablets using the existing method. The densitometric recording of the IBF, CAF, and PCM spectral bands revealed that the strongest response was at 260 nm in wavelength. The entire simultaneous estimation of the IBF, CAF, and PCM therefore occurred at 260 nm.

3.2 Validation studies

The ICH-Q2-R1 criteria were utilized to measure a range of factors for the simultaneous estimation of IBF, CAF, and PCM [57]. Table 1 shows the outcomes of the linearity evaluation of the IBF, CAF, and PCM calibration plots performed using the current method. The 25–800 ng·band^{-1} range of the IBF, CAF, and PCM calibration curves were linear. All drugs showed linearity over the 25–800 ng·band^{-1} range. The linearity was not maintained beyond 25–800 ng·band^{-1} range. As a result, it was the same for all drugs. IBF, CAF, and PCM's correlation coefficient (R^2) were predicted to be 0.9982, 0.9950, and 0.9949, respectively. Regression coefficients (R) for IBF, CAF, and PCM were calculated as 0.9991, 0.9974, and 0.9974,

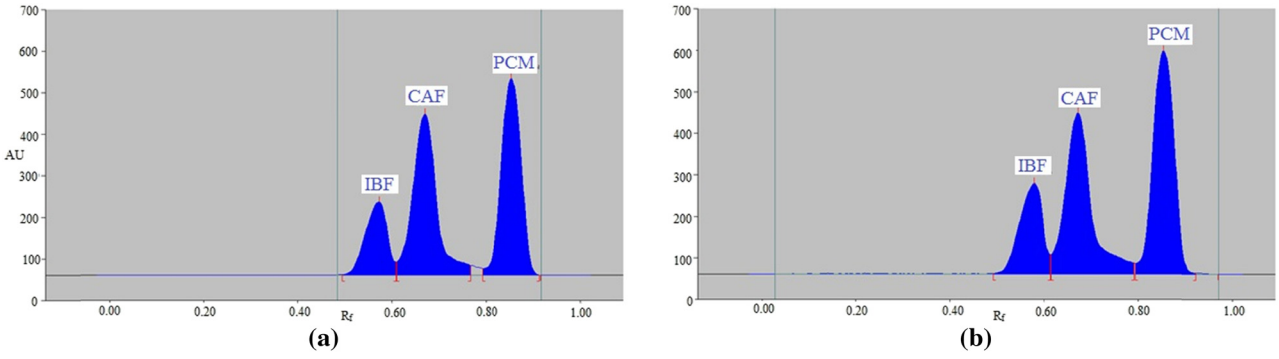

Figure 3: Reversed-phase HPTLC chromatogram of (a) standard IBF, CAF, and PCM and (b) IBF, CAF, and PCM in commercial tablets.

Table 1: Results of linearity assessment for the simultaneous estimation of IBF, CAF, and PCM utilizing the proposed methodology (mean ± SD; $n = 6$)

Parameters	IBF	CAF	PCM
Linear range (ng·band^{-1})	25–800	25–800	25–800
Regression equation	$y = 15.812x + 1{,}210.9$	$y = 30.867x + 2{,}677.6$	$y = 30.844x + 3{,}241.9$
R^2	0.9982	0.9950	0.9949
R	0.9991	0.9974	0.9974
Standard error of slope	0.30	0.66	0.70
Standard error of intercept	2.21	8.74	10.35
95% confidence interval of slope	14.51–17.11	27.98–33.74	27.80–33.88
95% confidence interval of intercept	1,201.37–1,220.42	2,639.96–2,715.23	3,196.931–3,286.04
LOD ± SD (ng·band^{-1})	1.13 ± 0.03	2.29 ± 0.05	2.71 ± 0.06
LOQ ± SD (ng·band^{-1})	3.39 ± 0.09	6.87 ± 0.15	8.10 ± 0.18

respectively. For IBF, CAF, and PCM, the R^2 and R data were considerably significant ($p < 0.05$). These outcomes suggested a significant correlation between IBF, CAF, and PCM concentration and measured responses. The linear range for IBF, CAF, and PCM for a reported HPTLC method was found to be 300–1,100 ng·band^{-1} [37]. The reported linear range for IBF, CAF, and PCM was much inferior to the current method [37]. All these results showed the reliability of the current method for the simultaneous estimation of IBF, CAF, and PCM.

Table 2 mentions the system appropriateness criteria for the current technique. The "R_f, As, N·m^{-1}, and Rs" for the current approach were computed to be adequate for the concurrent determination of IBF, CAF, and PCM.

The accuracy of the current method was assessed as the % recovery for the simultaneous estimation of the IBF, CAF, and PCM. Table 3 contains the accuracy measurement outcomes for the current methodology. The % recoveries of IBF, CAF, and PCM at three distinct QC solutions were determined to be 98.90–101.24, 99.12–100.82, and 98.24–101.99, respectively, using the current approach. The % recovery for IBF, CAF, and PCM for a reported HPTLC method was found to be 99.31–99.99, 99.14–99.96, and 99.66–101.25, respectively [37]. The reported % recovery for IBF, CAF, and PCM was identical to the current method [37]. All these outcomes showed that the current method was accurate for the concurrent determination of IBF, CAF, and PCM.

Table 2: The system suitability criteria for IBF, CAF, and PCM for the proposed methodology (mean ± SD; $n = 3$)

Parameters	IBF	CAF	PCM
R_f	0.55 ± 0.01	0.67 ± 0.02	0.85 ± 0.02
As	1.08 ± 0.03	1.12 ± 0.04	1.05 ± 0.02
N·m^{-1}	4,782 ± 5.24	4,612 ± 4.41	5,182 ± 5.87
Rs	0.15 ± 0.00	0.30 ± 0.01	0.38 ± 0.01

The precision of the current method was evaluated as intra/inter-assay precision and reported as a percentage of coefficient of variation (%CV) for the simultaneous determination of IBF, CAF, and PCM. The results of the simultaneous estimation of IBF, CAF, and PCM utilizing the current technique are shown in Table 4 for intra- and inter-day precisions. IBF, CAF, and PCM %CVs were calculated to be 0.88–0.92%, 0.92–1.08%, and 0.91–1.08%, respectively, for the intra-assay precision. The %CVs of IBF, CAF, and PCM for inter-day variation were measured to be 0.95–1.03%, 0.95–1.13%, and 0.98–1.13%, respectively. The %CVs for IBF, CAF, and PCM for a reported HPTLC method were found to be 1.16–1.50, 1.29–1.64, and 1.21–1.62, respectively [37]. The reported %CVs for IBF, CAF, and PCM were identical to the current method [37]. All of these outcomes showed that the current method was precise for the simultaneous estimation of IBF, CAF, and PCM.

By making minor, deliberate changes to the suggested solvent system, the robustness of the current method for

Table 3: Accuracy results of IBF, CAF, and PCM for the proposed technology (mean ± SD; $n = 6$)

Conc. (ng·band^{-1})	Conc. found (ng·band^{-1}) ± SD	Recovery (%)	CV (%)
IBF			
150	148.64 ± 1.81	99.09	1.21
200	197.81 ± 2.24	98.90	1.13
250	253.10 ± 2.64	101.24	1.04
CAF			
150	151.23 ± 1.92	100.82	1.26
200	201.41 ± 2.31	100.70	1.14
250	247.81 ± 2.71	99.12	1.09
PCM			
150	152.81 ± 1.96	101.87	1.28
200	203.98 ± 2.44	101.99	1.19
250	245.61 ± 2.87	98.24	1.16

Table 4: Assessment of intra/inter-day precision of IBF, CAF, and PCM for the current method (mean ± SD; n = 6)

Conc. (ng·band^{-1})	Intraday precision			Interday precision		
	Conc. (ng·band^{-1}) ± SD	Standard error	CV (%)	Conc. (ng·band^{-1}) ± SD	Standard error	CV (%)
IBF						
150	152.21 ± 1.41	0.57	0.92	149.12 ± 1.54	0.62	1.03
200	203.14 ± 1.84	0.75	0.90	198.71 ± 1.95	0.79	0.98
250	247.34 ± 2.18	0.89	0.88	251.41 ± 2.39	0.97	0.95
CAF						
150	148.49 ± 1.61	0.65	1.08	147.89 ± 1.68	0.68	1.13
200	202.35 ± 1.91	0.77	0.94	201.61 ± 2.01	0.82	0.99
250	253.61 ± 2.35	0.95	0.92	253.44 ± 2.42	0.98	0.95
PCM						
150	147.91 ± 1.61	0.65	1.08	146.84 ± 1.66	0.67	1.13
200	196.31 ± 1.91	0.77	0.97	195.41 ± 1.98	0.80	1.01
250	256.21 ± 2.35	0.95	0.91	254.61 ± 2.51	1.02	0.98

the simultaneous determination of IBF, CAF, and PCM was evaluated. The results of the robustness research carried out with the current methodology are shown in Table 5. It was established that the %CVs for IBF, CAF, and PCM were 0.87–0.97, 0.93–0.99, and 0.95–1.02, respectively. It was discovered that the IBF, CAF, and PCM R_f values were 0.54–0.56, 0.66–0.68, and 0.84–0.86, respectively.

The sensitivity of the current approach for the simultaneous determination of IBF, CAF, and PCM was assessed as "LOD and LOQ." The computed values of "LOD and LOQ" for IBF, CAF, and PCM are included in Table 1. For the present methodology, the "LOD and LOQ" for IBF were calculated to be 1.13 ± 0.03 and 3.39 ± 0.09 ng·band^{-1}, respectively. The "LOD and LOQ" for CAF were determined using the current method to be 2.29 ± 0.05 and 6.87 ± 0.15 ng·band^{-1}, respectively. The "LOD and LOQ" for PCM were determined using the current method to be 2.71 ± 0.06 and 8.10 ± 0.18 ng·band^{-1}, respectively. Using a reported HPTLC method, the "LOD and LOQ" for IBF were found to be 430 and 1,304 ng·mL^{-1}, respectively [37]. Using a reported HPTLC method, the "LOD and LOQ" for CAF were found to be 36 and 109 ng·mL^{-1}, respectively [37]. Using a reported HPTLC method, the "LOD and LOQ" for PCM were found to be 3 and 10 ng·mL^{-1}, respectively [37]. The reported "LOD and LOQ" for IBF, CAF, and PCM were much inferior to those of the current method [37]. Thus, compared to the previously disclosed HPTLC method, the proposed approach was significantly more sensitive for the simultaneous estimation of IBF, CAF, and PCM [37]. All of these findings indicated that the current method was highly sensitive for the simultaneous estimation of IBF, CAF, and PCM.

Table 5: Robustness assessment results of IBF, CAF, and PCM for the current method (mean ± SD; n = 6)

Conc. (ng·band^{-1})	Green eluent system composition (acetone–water)			Results		
	Original	Used		(ng·band^{-1}) ± SD	%CV	R_f
IFB						
		82:18	+2.0	195.41 ± 1.71	0.87	0.54
200	80:20	80:20	0.0	198.92 ± 1.95	0.91	0.55
		78:22	−2.0	203.32 ± 1.99	0.97	0.56
CAF						
		82:18	+2.0	194.41 ± 1.81	0.93	0.66
200	80:20	80:20	0.0	199.81 ± 1.93	0.96	0.67
		78:22	−2.0	204.35 ± 2.04	0.99	0.68
PCM						
200	80:20	82:18	+2.0	193.64 ± 1.84	0.95	0.84
		80:20	0.0	197.65 ± 1.97	0.99	0.85
		78:22	−2.0	203.29 ± 2.08	1.02	0.86

R_f: retardation factor.

By contrasting the R_f data and 3D spectrum of IBF, CAF, and PCM in marketed tablets with that of standards IBF, CAF, and PCM, the specificity of the current method for the simultaneous determination of IBF, CAF, and PCM was evaluated. The results are shown in Figure 4.

At a wavelength of 260 nm, IBF, CAF, and PCM in standards and marketed tablets showed the greatest chromatographic response. The specificity of the present methodology for the concurrent determination of IBF, CAF, and PCM was revealed by the similar 3D spectrum, R_f values, and wavelengths found in standards and marketed tablets. Overall, all validation parameters, including system suitability parameters, were acceptable for the concurrent determination of IBF, CAF, and PCM.

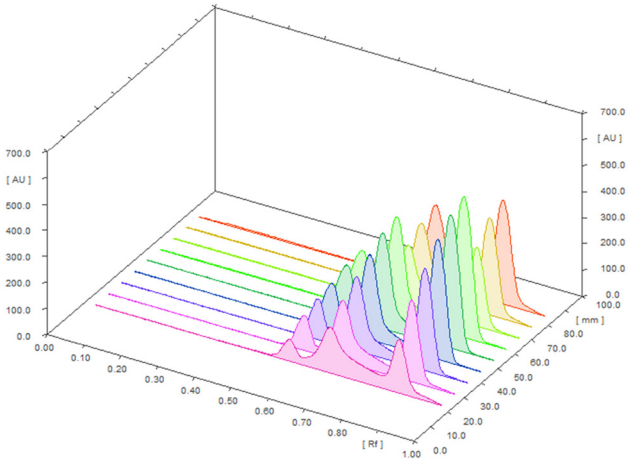

Figure 4: 3D Chromatograms of standard IBF, CAF, and PCM and formulation.

Figure 5: NEMI Evaluation of greenness for the current methodology.

3.3 Application of current method in the concurrent determination of IBF, CAF, and PCM in marketed formulations

For the simultaneous estimation of IBF, CAF, and PCM in their commercially available tablets, the proposed technique was used as an alternative to traditional liquid chromatography procedures. By contrasting the TLC signals at R_f = 0.55 ± 0.01 for IBF, R_f = 0.67 ± 0.02 for CAF, and R_f = 0.85 ± 0.02 for PCM to those of standards IBF, CAF, and PCM utilizing the current methodology, the chromatograms of IBF, CAF, and PCM from procured tablets were recognized. Figure 3b shows the chromatograms of IBF, CAF, and PCM found in marketed tablets. These peaks were identical to those of the standards for IBF, CAF, and PCM. Using the current method, the amount of IBF, CAF, and PCM in commercial tablets was determined to be 99.51 ± 1.38%, 98.25 ± 1.32%, and 100.64 ± 1.46%, respectively. These results suggested the applicability of the current methodology for the simultaneous estimation of IBF, CAF, and PCM in procured tablets.

Table 6: Analytical eco-score (AES) and penalty point assessment for the greenness of the current method and comparison with reported HPTLC method

Reagents/instruments/waste	Penalty points	
	HPTLC [37]	Present HPTLC
Acetone		8
Water		0
Ethyl acetate	4	
Glacial acetic acid	8	
Methanol	18	
Instruments	0	0
Waste	3	3
Total penalty points	33	11
AES score	67	89

3.4 Greenness assessment

Numerous quantitative and qualitative approaches are established for the greenness assessment of pharmaceutical assays [44–50]. In the present work, four different approaches, namely NEMI, AES, ChlorTox, and AGREE approaches were used to assess the greenness of the

Table 7: Results of ChlorTox scores for the proposed method in comparison with the reported HPTLC method in terms of the relative hazards with respect to chloroform (CH_{sub}/CH_{CHCl3}) and the mass of individual reagents used for single analysis (m_{sub})

Stage	Solvent/reagent	Relative hazard (CH_{sub}/CH_{CHCl3})	m_{sub} (mg)	ChlorTox (g)	Total ChlorTox (g)	Ref.
Sample preparation	Acetone	0.34	1,600	0.54	1.08	Present method
HPTLC analysis	Acetone	0.34	1,600	0.54		
Sample preparation	Methanol	0.56	2,000	1.12	1.81	[37]
HPTLC analysis	Ethyl acetate	0.34	1,900	0.65		
	Glacial acetic acid	0.43	100	0.04		

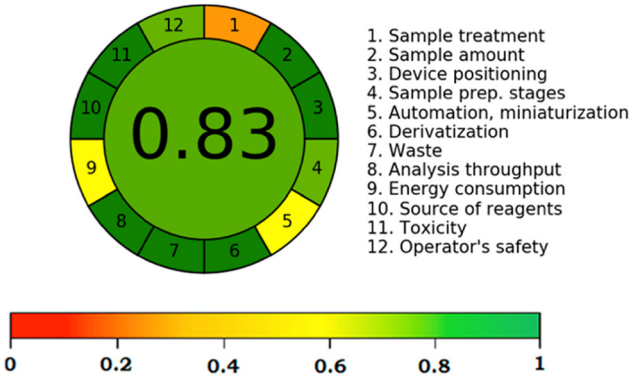

Figure 6: AGREE scale for the present methodology.

current approach [44,45,49,50]. NEMI is used to obtain the preliminary assessment. According to the NEMI method, four quarter circles are drawn and each quarter is colored green or left blank indicating the following criteria [44]: PBT, corrosive, hazardous, and waste. Figure 5 shows the representative diagram for the NEMI of the current method. As all of the reagents employed are neither toxic, PBT, or corrosive and produce minimal waste, the current procedure produced four circles that were green.

AES is a good semi-quantitative approach, which considers all the steps of the analytical procedures, instruments, and waste. The results of AES scores with penalty points for the current approach in comparison with the reported HPTLC approach are included in Table 6. The AES value of greater than 75 indicated an excellent greenness, the value of less than 75 but greater than 50 indicated adequate greenness, and the value of less than 50 indicated inadequate greenness [45]. The AES score of the present method was derived to be 89, indicating an excellent greenness profile. The AES score for the reported HPTLC approach was derived to be 67 [37]. The AES score of the present approach was much superior to the reported HPTLC method, indicating the excellent greenness profile of the present approach compared to the reported HPTLC method [37].

Table 7 includes the findings of the individual greener solvent ChlorTox scores and the overall ChlorTox for the suggested technique in comparison with the reported HPTLC approach. The total ChlorTox value for the suggested approach was anticipated to be 1.08 g, indicating that it was both relatively safe and environmentally friendly [49]. The total ChlorTox value for the reported HPTLC approach was calculated to be 1.81 g [37]. The total ChlorTox value of the present approach was much superior to the reported HPTLC method, indicating the excellent greenness and safety profile of the present approach compared to the reported HPTLC method [37].

The AGREE approach is the most widely used quantitative approach for greenness assessment as it consumes all 12 GAC principles [50]. Figure 6 displays the overall AGREE score for the current methodology. The AGREE score of greater than 0.75 indicated excellent greenness, the AGREE score of less than 0.75 but greater than 0.50 indicated adequate greenness, and AGREE score of less than 0.50 indicated inadequate greenness [50]. The current methodology projected that the overall AGREE score would be 0.83. The AGREE results again demonstrated the current method's excellent green features. Overall, the results of all greenness approaches indicated the excellent greener profile of the current method for the simultaneous estimation of IBF, CAF, and PCM in commercial products.

4 Conclusions

There are no green analytical methods in the literature for the simultaneous determination of IBF, CAF, and PCM. In this study, a fast, sensitive, and green HPTLC methodology was designed and validated for the simultaneous determination of IBF, CAF, and PCM in their commercially available products. For the simultaneous determination of IBF, CAF, and PCM, the current method is linear, accurate, precise, robust, highly sensitive, and green. The current method was successfully utilized to determine IBF, CAF, and PCM contents in their commercial tablets. The results of NEMI, AES, ChlorTox, and AGREE assessment showed an excellent greenness characteristic of the current method for the simultaneous determination of IBF, CAF, and PCM. The current method for the simultaneous determination of IBF, CAF, and PCM has been found more linear and highly sensitive than the previously reported HPTLC method. All of these findings suggested that the current method can be

regularly used for the simultaneous determination of IBF, CAF, and PCM in their commercial products.

Acknowledgments: The authors are thankful to the Researchers Supporting Project number (RSPD2024R1040), King Saud University, Riyadh, Saudi Arabia for supporting this work. The authors also thank Prince Sattam bin Abdulaziz University for supporting this work via project number (PSAU/2023/R/1444). Sultan Alshehri would like to express sincere gratitude to AlMaarefa University, Riyadh, Saudi Arabia, for providing funding to conduct this research.

Funding information: The article was supported by Researchers Supporting Project number (RSPD2024R1040), King Saud University, Riyadh, Saudi Arabia.

Author contributions: All authors contributed equally to this article.

Conflict of interest: Authors state no conflict of interest.

Data availability statement: The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

[1] Adams SS. The propionic acids: A personal perspective. J Clin Pharmacol. 1992;32:317–23.
[2] Grisales JO, Arancibia JA, Olivieri AC. Determination of enantiomeric composition of ibuprofen in pharmaceutical formulations by partial least-squares regression of strongly overlapped chromatographic profiles. J Chromatogr B. 2012;910:78–83.
[3] Shakeel F, Ramadan W. Transdermal delivery of anticancer drug caffeine from water-in-oil nanoemulsions. Colloids Surf B. 2010;75:356–62.
[4] Rahimi M, Khorshidi N, Heydari R. Simultaneous determination of paracetamol and caffeine in aqueous samples by ultrasound-assisted emulsification microextraction coupled with high-performance liquid chromatography. Sep Sci Plus. 2020;3:561–70.
[5] Jimenez JA, Martinez F. Thermodynamic study of the solubility of acetaminophen in propylene glycol + water cosolvent mixtures. J Braz Chem Soc. 2006;17:125–34.
[6] Shakeel F, Alanazi FK, Alsarra IA, Haq N. Solubilization behavior of paracetamol in Transcutol-water mixtures at T = (298.15 to 333.15) K. J Chem Eng Data. 2012;58:3551–6.
[7] Tran BT, Tran TN, Tran AMT, Nguyen GCD, Nguyen QTT. Simultaneous determination of paracetamol, ibuprofen, and caffeine in tablets by molecular absorption spectroscopy combined with classical least square method. Molecules. 2022;27:E2657.
[8] Medina AR, de Cordova MLF, Molina-Diaz A. Simultaneous determination of paracetamol, caffeine and acetylsalicylic acid by means of FI ultraviolet plus multioptosensing device. J Pharm Biomed Anal. 1999;21:983–92.
[9] Tavallali H, Salami M. Simultaneous determination of caffeine and paracetamol by zero-crossing second derivative spectrophotometry in pharmaceutical preparations. Asian J Chem. 2009;21:1949–56.
[10] Tavallali H, Sheikhaei M. Simultaneous kinetic determination of paracetamol and caffeine by H-point standard addition method. Afr J Pure Appl Chem. 2009;3:11–9.
[11] Aktas AH, Kitis F. Spectrophotometric simultaneous determination of caffeine and paracetamol in commercial pharmaceutical by principal component regression, least squares and artificial neural networks chemometric methods. Croat Chem Acta. 2014;87:69–74.
[12] Uddin MN, Mondol A, Karim MM, Jahan RA, Rana AA. Chemometrics assisted spectrophotometric method for simultaneous determination of paracetamol and caffeine in pharmaceutical formulations. Bangladesh J Sci Ind Res. 2019;54:215–22.
[13] Sebaiy MM, Mattar AA. H-point assay method for simultaneous determination of paracetamol and caffeine in panadol extra dosage forms. Can J Biomed Res Tech. 2020;3:1–6.
[14] Acheampong A, Gyasi WO, Darko G, Apau J, Addai-Arhin S. Validated RP-HPLC method for simultaneous determination and quantification of chlorphenaramine maleate, paracetamol and caffeine in tablet formulation. Springer Plus. 2016;5:E625.
[15] Narayanan VL, Austin A. Determination of acetaminophen and caffeine using reverse phase liquid (RP-LC) chromatographic technique. Quest J Res Pharm Sci. 2016;3:5–10.
[16] Aminu N, Chan SY, Khan NH, Farhan AB, Umar MN, Toh SM. 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.
[17] Ali JG, Muhammad I, Hamid S, Muhammad AA, Shoaib H, Tasleem S. Simultaneous determination and quantification of paracetamol, caffeine and orphenadrine citrate using stability-indicating HPLC method in a fixed dose combination tablet dosage form. Ann Pharmacol Pharm. 2020;5:E118.
[18] Tavallali H, Zareiyan SF, 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;94:1094–9.
[19] Halka-Grysinska A, Slazak P, Zareba G, Markowski W, Klimek-Turek A, Dzido TH. Simultaneous determination of acetaminophen, propyphenazone and caffeine in cefalgin preparation by pressurized planar electrochromatography and high-performance thin-layer chromatography. Anal Methods. 2012;4:973–82.
[20] Yigit A, Yardrm Y, Senturk Z. Voltametric sensor based on boron-doped diamond electrode for simultaneous determination of paracetamol, caffeine and aspirin in pharmaceutical formulations. IEEE Sens. 2016;16:1674–80.
[21] Minh TT, Phong NH, Duc HV, Khieu DQ. Microwave synthesis and voltametric simultaneous determination of paracetamol and caffeine using an MOF-199-based electrode. J Mater Sci. 2018;53:2453–71.
[22] Hung NX, Quang DA, Toan TTT, Dung NN. The simultaneous determination of ascorbic acid, paracetamol, and caffeine by voltammetry method using cobalt Schiff base complex/SBA-15 modified electrode. ECS J Solid State Sci Technol. 2020;9:E101004.
[23] Meter MIV, Khan SM, Taulbee-Cotton BV, Dimmitt NH, Hubbard ND, Green AM, et al. Diagnosis of agglomeration and

crystallinity of active pharmaceutical ingredients in over the counter headache medication by electrospray desorption ionization mass spectrometry imaging. Molecules. 2021;26:E610.
[24] Katseli V, Economou A, Kokkinos C. A novel all-3D-printed cell-on-a-chip device as a useful electroanalytical tool: application to the simultaneous voltametric determination of caffeine and paracetamol. Talanta. 2020;208:E12388.
[25] Muntean DM, Alecu C, Tomuta I. Simultaneous quantification of paracetamol and caffeine in powder blends for tableting by NIR-chemometry. J Spectrosc. 2017;2017:E7160675.
[26] Ortega-Barrales P, Padilla-Weigand R, Molina-Diaz A. Simultaneous determination of paracetamol and caffeine by flow injection-solid phase spectrometry using C_{18} silica gel as a sensing support. Anal Sci. 2002;18:1241–6.
[27] Kulikov AU, Verushkin AG. Simultaneous determination of paracetamol, caffeine, guaifenesin and preservatives in syrups by micellar LC. Chromatographia. 2008;67:347–55.
[28] Emre D, Ozaltrn N. Simultaneous determination of paracetamol, caffeine and propyphenazone in ternary mixtures by micellar electrokinetic capillary chromatography. J Chromatogr B. 2007;847:126–32.
[29] Harde M, Wankhede S, Chaudhari P. Development of validated UV spectrophotometric method for simultaneous estimation of ibuprofen, paracetamol and caffeine in bulk drug and marketed formulation. World J Pharm Res. 2015;4:1428–36.
[30] Marvaniya V, Patani P. A review on analytical method for paracetamol, caffeine, ibuprofen in marketed formulation. J Emerg Technol Innov Res. 2019;6:212–33.
[31] Dang HV, Thu HTT, Ha LDT, Mai HN. RP-HPLC and UV spectrophotometric analysis of paracetamol, ibuprofen, and caffeine in solid pharmaceutical dosage forms by derivative, fourier, and wavelet transforms: A comparison study. J Anal Methods Chem. 2020;2020:E8107571.
[32] Alkhafaji SL, Mahood AM. First-order derivative and UV-spectrophotometric methods for simultaneous determination of paracetamol, ibuprofen and caffeine in bulk drug and pharmaceutical formulation. J Pharm Res Int. 2018;25:1–14.
[33] Alsamarrai KF, Ameen ST. Simultaneous ratio derivative spectrophotometric determination of paracetamol, caffeine and ibuprofen in their ternary form. Baghdad Sci J. 2022;19:1276–85.
[34] Cunha RR, Chaves SC. Ribeiro MMAC, Torres LMFC, Munoz RAA, Santos WTPD, et al. 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;38:1657–62.
[35] Palled PJ, Dushyanth Reddy V, Mannor VS, Chowdary B. Validated isocratic/gradient RP-HPLC for simultaneous estimation of paracetamol, ibuprofen and caffeine in marketed formulations using diclofenac as internal standard. Anal Chem Indian J. 2017;17:E116.
[36] Khoshayand MR, Abdollahi H, Shariatpanahi M, Saadatfard A, Mohammadi A. Simultaneous spectrophotometric determination of paracetamol, ibuprofen and caffeine in pharmaceuticals by chemometric methods. Spectrochim Acta Part A. 2008;70:491–9.
[37] Chabukswar AR, Thakur VG, Dharam DL, Shah MH, Kuchekar BS, Sharma SN. Development and validation of HPTLC method for simultaneous estimation of paracetamol, ibuprofen and caffeine in bulk and pharmaceutical dosage form. Res J Pharm Tech. 2012;5:1218–22.
[38] Foudah AI, Shakeel F, Salkini MA, Alshehri S, Ghoneim MM, Alam P. A green high-performance thin-layer chromatography method for the determination of caffeine in commercial energy drinks and formulations. Materials. 2022;15:E2965.
[39] Alam P, Shakeel F, Ali A, Alqarni MH, Foudah AI, Aljarba TM, et al. Simultaneous determination of caffeine and paracetamol in commercial formulations using greener normal-phase and reversed-phase HPTLC methods: A contrast of validation parameters. Molecules. 2022;27:E405.
[40] Galuszka A, Migaszewski Z, Namiesnik J. The 12 principles of green analytical chemistry and the significance mnemonic of green analytical practices. Trends Anal Chem. 2013;50:78–84.
[41] Abdelrahman MM, Abdelwahab NS, Hegazy MA, Fares MY, El-Sayed GM. Determination of the abused intravenously administered madness drops (tropicamide) by liquid chromatography in rat plasma; an application to pharmacokinetic study and greenness profile assessment. Microchem J. 2020;159:E105582.
[42] Alam P, Salem-Bekhit MM, Al-Joufi FA, Alqarni MH, Shakeel F. Quantitative analysis of cabozantinib in pharmaceutical dosage forms using green RP-HPTLC and green NP-HPTLC methods: A comparative evaluation. Sus Chem Pharm. 2021;21:E100413.
[43] Foudah AI, Shakeel F, Alqarni MH, Alam P. A rapid and sensitive stability-indicating green RP-HPTLC method for the quantitation of flibanserin compared to green NP-HPTLC method: Validation studies and greenness assessment. Microchem J. 2021;164:E105960.
[44] Keith LH, Brass HJ, Sullivan DJ, Boiani JA, Alben KT. An introduction to the national environmental methods index. Environ Sci Technol. 2005;39:173A–6A.
[45] Duan X, Liu X, Dong Y, Yang J, Zhang J, He S, et al. A green HPLC method for determination of nine sulfonamides in milk and beef, and its greenness assessment with analytical eco-scale and greenness profile. J AOAC Int. 2020;103:1181–9.
[46] Plotka-Wasylka J. A new tool for the evaluation of the analytical procedure: Green analytical procedure index. Talanta. 2018;181:204–9.
[47] Nowak PM, Koscielnaik P. What color is your method? Adaptation of the RGB additive color model to analytical method evaluation. Anal Chem. 2019;91:10343–52.
[48] Nowak PM, Wietecha-Posluszny R, Pawliszyn J. White analytical chemistry: An approach to reconcile the principles of green analytical chemistry and functionality. Trends Anal Chem. 2021;138:E116223.
[49] Nowak PM, Wietecha-Posluszny R, Plotka-Wasylka J, Tobiszewski M. How to evaluate methods used in chemical laboratories in terms of the total chemical risk? a ChlorTox Scale. Green. Anal Chem 2023;5:E100056.
[50] Pena-Pereira F, Wojnowski W, Tobiszewski M. AGREE-Analytical GREEnness metric approach and software. Anal Chem. 2020;92:10076–82.
[51] Rezk MR, Monir HH, Marzouk HM. Novel determination of a new antiviral combination; sofosbuvir and velpatasvir by high performance thin layer chromatographic method; application to real human samples. Microchem J. 2019;146:828–34.
[52] Tantawy MA, Weshahy SA, Wadie M, Rezk MR. Novel HPTLC densitometric methods for determination of tamsulosin HCl and tadalafil in their newly formulated dosage form: Comparative study and green profile assessment. Biomed Chromatogr. 2020;34:E4850.
[53] Tantawy MA, Weshahy SA, Wadie M, Rezk MR. Stability-indicating HPTLC method for the simultaneous detection and quantification

of alfuzosin hydrochloride, solifenacin succinate along with four of their official impurities. Microchem J. 2020;157:E104905.
[54] Wadie M, Abdel-Moety EM, Rezk MR, Marzouk HM. A novel smartphone HPTLC assaying platform versus traditional densitometric method for simultaneous quantification of alfuzosin and solifenacin in their dosage forms as well as monitoring content uniformity and drug residues on the manufacturing equipment. RSC Adv. 2023;13:11642–51.
[55] Wadie M, Abdel-Moety EM, Rezk MR, Marzouk HM. Sustainable and smart HPTLC determination of silodosin and solifenacin using a constructed two illumination source chamber with a smartphone camera as a detector: Comparative study with conventional densitometric scanner. Sus Chem Pharm. 2023;33:E101095.
[56] Wadie M, Abdel-Moety EM, Rezk MR, Marzouk HM. Smartphone-based high-performance thin Layer chromatographic method along with benchtop densitometry for simultaneous quantification of co-formulated dutasteride with silodosin and their residuals on manufacturing equipment's surfaces. Bull Fac Pharm Cairo Univ. 2023;61:E4.
[57] International conference on harmonization (ICH), Q2 (R1): validation of analytical procedures–text and methodology, Geneva, Switzerland; 2005.
[58] Liu Y, Brettell TA, Victoria J, Wood MR, Staretz ME. High performance thin-layer chromatography (HPTLC) analysis of cannabinoids in cannabis extracts. Forensic Chem. 2020;19:E100249.