Research Article

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Simultaneous quantification of mefenamic acid and paracetamol in fixed-dose combination tablet dosage forms using the green HPTLC method

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Abstract: In this study, a green reversed-phase "high-performance thin-layer chromatography (HPTLC)" technique is established and verified for the detection of mefenamic acid (MEF) and paracetamol (PCM) simultaneously in their fixed-dose combination tablets. MEF and PCM were measured simultaneously using a green developing system consisting of ethanol, water, and glacial acetic acid in a 75:23:2 (v/v/v) ternary ratio. Both MEF and PCM were concurrently identified at a wavelength of 235 nm. Three distinct greenness methodologies – analytical eco-scale (AES), chloroform toxicity (ChlorTox), and analytical GREEnness (AGREE) – were used to assess the method's greenness profile. For both medications, the devised technique was linear in the 25–800 ng·band^{-1} range. After validation, the devised approach was found to be accurate, precise, sensitive, robust, and environmentally friendly. Each of the greenness tools' results, including those from AGREE (0.82), ChlorTox (0.82 g), and AES (91), showed that the current approach had a noticeably greener profile. Using the current method, it was determined that the MEF and PCM levels in commercial fixed-dose combination tablet brands A and B were within the 100 ± 2% limit. The results of investigation showed that MEF and PCM in commercial combination tablets could be reliably analyzed using the recommended method.

Keywords: green HPTLC, mefenamic acid, paracetamol, simultaneous measurement, validation

1 Introduction

One popular nonsteroidal anti-inflammatory drug is mefenamic acid (MEF) (Figure 1a) [1]. Sports injuries, osteoarthritis, nonarticular rheumatism, and rheumatoid arthritis are among the conditions for which it is used to alleviate pain and inflammation [1,2]. Paracetamol (PCM) (Figure 1b) is the most widely used analgesic and antipyretic medicine, especially for elderly and pediatric patients [3,4]. A range of dosage forms are commercially marketed for it [4]. Numerous harmful outcomes may result from MEF and PCM overdoses [1,5]. MEF and PCM together are frequently utilized to treat a variety of inflammatory diseases and pains [5]. These medications, including PCM and MEF, are present in a number of multicomponent formulations that are sold commercially. Therefore, in commercially available multicomponent formulations, MEF and PCM must be standardized in both qualitative and quantitative parameters.

Different analytical approaches for simultaneously determining MEF and PCM in commercial dosage forms and biological materials have been found in the literature. MEF and PCM have been identified in their commercial products using a variety of ultraviolet (UV) spectroscopy and derivative UV spectrometry approaches [6–11]. An UV spectroscopy approach using an artificial neural network has also been described to determine MEF and PCM in their tablet dosage forms [12]. Numerous high-performance liquid chromatography (HPLC) approaches have also been utilized to detect MEF and PCM in pharmaceutical suspensions and

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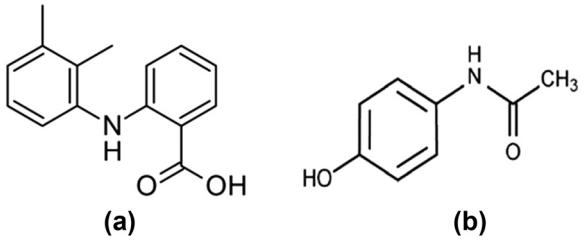

Figure 1: Chemical structures of (a) MEF and (b) PCM.

commercial tablet dosage forms [13–16]. To determine MEF and PCM in pharmaceutical suspension, an ultra-performance liquid chromatography technique has also been documented [17]. Additionally, MEF and PCM have been measured in their commercial tablets using a single high-performance thin-layer chromatography (HPTLC) approach [18]. Furthermore, MEF and PCM were detected in their commercial tablets using a proton nuclear magnetic resonance spectroscopy method [19]. In addition, MEF and PCM were analyzed in commercial tablets, human urine, and human serum samples using a copper-doped zeolite-modified carbon paste electrode sensor [20]. A second-order spectrofluorimetry approach was also used to determine MEF and PCM in urine samples [21]. The literature indicated no green HPTLC methods for the determination of MEF and PCM. However, some green HPTLC methods have been reported for the determination of PCM in combination with other drugs [22,23]. For example, both normal and reversed-phase green HPTLC approaches have been documented for the detection of PCM in combination with caffeine in commercial dosage forms [22]. Additionally, PCM was determined in combination with ibuprofen and caffeine in commercial dosage forms using a green HPTLC approach [23].

HPTLC is an extension of TLC, which is a simple, reliable, quick, and effective approach for pharmaceutical analysis [24]. A review of the literature found that MEF and PCM may be detected in their commercial tablets using a single HPTLC method [18]. However, many green HPTLC methods are not available to detect MEF and PCM. One of the 12 principles of green analytical chemistry (GAC) is the use of ecologically friendly solvent alternatives to reduce the adverse environmental consequences of toxic or hazardous solvents [25]. According to a review of the literature, the use of environmentally friendly solvents has grown significantly during the past few decades [26–30]. HPTLC approaches are currently used for the green analysis of pharmaceutical products [29–33]. A range of greenness approaches for assessing the greenness profiles of analytical methodologies are described in the literature [34–42]. White analytical chemistry approaches have also been established in the literature to develop eco-friendly analytical methods [43,44]. We employed three distinct methods to evaluate the greenness profile of the current HPTLC method: Analytical Eco-Scale (AES) [37], Chloroform Toxicity (ChlorTox) [41], and analytical GREEnness (AGREE) [42]. Penalty points were given for non-greenness features in the semi-quantitative AES approach [37]. The ChlorTox approach was used to assess the ChlorTox scale of the solvents in comparison to the standard chloroform. The low value of the ChlorTox scale of the green analytical methods indicates the environmental safety compared to the high value of ChlorTox scale for non-green methods [41]. All 12 GAC principles were applied in the AGREE methodology to evaluate the greenness [42]. In order to evaluate MEF and PCM in their fixed-dose combination tablets concurrently, the current method sought to design and validate a reversed-phase HPTLC technique that is fast, sensitive, and eco-friendly. It is based on the data and conclusions that were previously mentioned. The proposed HPTLC approach for concurrently determining MEF and PCM was confirmed utilizing the standards provided in "The International Council for Harmonization (ICH)-Q2-R2" [45].

2 Materials and methods

2.1 Materials

The reference standards of MEF and PCM and glacial acetic acid (GAA) were procured from Sigma Aldrich (St. Louis, MO, USA). Liquid chromatography-grade green organic eluents, such as ethanol (EtOH), acetone, and ethyl acetate, were obtained from E-Merck (Darmstadt, Germany). Liquid chromatography-grade water (H_2O) was purified using a Milli-Q (Lyon, France) device. Marketed fixed-dose combination tablets brand A (Pacimol® MF) and brand B (Paracim-M®) were obtained from an Indian pharmacy (New Delhi, India). Both the marketed fixed-dose combination tablet brands (A and B) contained 500 mg of MEF and 325 mg of PCM. The other chemicals and solvents that were employed were all of AR grade.

2.2 Chromatography and equipment

MEF and PCM in commercially available fixed-dose combination tablets were quantified using a HPTLC system (CAMAG, Muttenz, Switzerland). An Automatic TLC Sampler 4 (ATS4) Sample Applicator (CAMAG, Geneva, Switzerland) was used to apply the generated samples in the form of 6 mm bands. The microliter syringe (Hamilton, Bonaduz,

Switzerland) was attached to the sample applicator. Glass plates (plate size: 10 × 20 cm^2) pre-coated with reversed-phase silica gel (particle size: 5 µm), 60F254S plates (E-Merck, Darmstadt, Germany) were used as the stationary phase. The ternary form of EtOH–H_2O–GAA (75:23:2 v/v/v) was utilized as the environmentally favorable development system. In order to simultaneously measure MEF and PCM, the rate of application was set to 150 nL·s^{-1}. The plates were developed in an 8 cm spacing linear ascending mode in an automated developing chamber 2 (ADC2) (CAMAG, Muttenz, Switzerland). Vapors from the developing system were introduced into the development chamber, which was kept at 22°C for half an hour. At 235 nm, using a TLC scanner-III (CAMAG, Muttenz, Switzerland), both MEF and PCM were detected. The scanner speed (20 mm·s^{-1}) and slit dimensions (4 × 0.45 mm^2) were the settings that were used. For each measurement, either three or six replications were used. WinCAT's (version 1.4.3.6336, CAMAG, Muttenz, Switzerland) was the program that was utilized for data processing analysis.

2.3 Quality control (QC) samples and calibration curves for MEF and PCM

To create separate stock solutions for MEF and PCM, necessary amounts of each drug were dissolved in appropriate volumes of the green developing system, i.e., EtOH–H_2O–GAA. Each medication's final stock solution included 100 µg·mL^{-1}. The stock solutions were diluted in various ratios using the developing system, yielding the 25–800 ng·$band^{-1}$ levels of both drugs. In this experiment, the developing system was used as a diluent to ensure uniformity in HPTLC analysis and sample preparation. The peak area for each of the MEF and PCM concentrations was measured using the present approach, and 10 µL of each concentration was applied to TLC plates. The concentrations of the two medications were plotted against the peak areas that were obtained for six replicates (n = 6) to create MEF and PCM calibration curves. To assess a variety of validation variables, three different QC samples were created.

2.4 Sample preparation for commercial fixed-dose combination tablets for simultaneous detection of MEF and PCM

The developed method was used to determine MEF and PCM in commercial fixed-dose combination tablets and to concurrently quantify these medications in their bulk or pure forms. Twenty tablets of each brand were taken in order to determine the MEF and PCM in combination tablet brands A and B. Next, the average weight of each combination tablet brand was computed. The declared composition of each combination tablet was 500 mg of MEF and 325 mg of PCM. A glass pestle and mortar was used to crush and triturate each tablet brand into a fine powder. About 100 mL of the developing system was mixed with a finely powdered quantity equivalent to 500 mg of MEF and 325 mg of PCM. Approximately 1.0 mL of this stock solution was diluted with the developing system to produce a 100 mL solution. The solutions prepared for each brand of combination tablets were filtered using a 0.45 µm membrane filter to remove any insoluble materials after 10 min of sonication. Using the current technology, the collected samples were subjected to simultaneous analysis of MEF and PCM in both products.

2.5 Validation of green HPTLC method

The ICH-Q2-R2 criteria were used to validate the suggested approach for measuring MEF and PCM simultaneously for a number of parameters [45]. The linear ranges for MEF and PCM were evaluated by plotting the concentrations against the observed peak area. The linearity of MEF and PCM for the current approach was assessed for the range of 25–800 ng·$band^{-1}$ (n = 6).

The system suitability parameters for the suggested approach of evaluating MEF and PCM were obtained from the results of estimating the "retardation factor (R_f), peak tailing factor (As), and theoretical plates/meter (N/m)." For the suggested approach, the parameters "R_f, As, and N/m" were calculated using their standard equations [32].

The spiking/standard addition technique was used to evaluate the accuracy of the suggested methodology for analyzing MEF and PCM as percentage recoveries [45]. Additionally, 50%, 100%, and 150% MEF and PCM solutions were added to the previously analyzed MEF and PCM solutions of 200 ng·$band^{-1}$ in order to produce MEF and PCM low-QC (LQC) solutions of 300 ng·$band^{-1}$, middle-QC (MQC) solutions of 400 ng·$band^{-1}$, and high-QC (HQC) solutions of 500 ng·$band^{-1}$. To determine the accuracy, the aforementioned MEF and PCM QC solutions were reassessed. Each of MEF and PCM concentrations' percentage recovery was determined. Six replicates (n = 6) were used for accuracy measurements.

For the analysis of MEF and PCM, the intra- and inter-batch precisions of the current procedure were assessed. By measuring freshly prepared QC solutions at the previously mentioned QC levels, it was feasible to evaluate

the intra-batch precision for both drugs on the same day (n = 6). In order to examine the inter-batch variance for MEF and PCM for the proposed approach, freshly prepared solutions were assessed over a period of 3 days (n = 6) at the previously defined QC levels.

The robustness of the proposed methodology for both MEF and PCM was determined by intentionally changing the green developing system's composition. In the green development system for MEF and PCM, the peak area (a quantitative parameter) and R_f (a separation parameter) were assessed after the transfer from EtOH–H_2O–GAA (77:21:2 v/v/v) to EtOH–H_2O–GAA (73:25:2 v/v/v) (n = 6).

Using a standard deviation methodology, the "limit of detection (LOD) and limit of quantification (LOQ)" were used to quantify the sensitivity of the suggested approach. Six replicates (n = 6) of the blank samples – which lacked MEF or PCM – were injected, and the standard deviation of the response from blank sample was calculated. To obtain MEF and PCM "LOD and LOQ" (n = 6) [45], Eqs. 1 and 2 were used.

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

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

where σ is the blank sample's standard deviation and S is the slope of the MEF and PCM calibration curves.

The R_f values and UV absorption spectra of the standards were used to evaluate the peak purity and specificity of the current method for simultaneously analyzing MEF and PCM. These were compared with the corresponding values obtained from the commercial combination tablets.

2.6 Commercial combination tablets' simultaneous measurement of MEF and PCM using the current technique

The marketed combination tablet preparation samples were placed on TLC plates for this approach using the same experimental setup as the pure MEF and PCM measurements (n = 3). The peak area was then measured. Utilizing the MEF and PCM calibration curves, the amounts of these compounds in commercial combination tablets were estimated using the current approach.

2.7 Greenness evaluation

The greenness parameters of the suggested method for concurrently measuring MEF and PCM were assessed utilizing three different approaches: AES [37], ChlorTox [41], and AGREE [42]. AES is a semi-quantitative approach which takes into account waste, tools, and each stage of analysis. The solvents/reagents with an ideal analysis of 100 points are expected to have minimal or no waste, energy consumption, and reagent usage. If any of these conditions are not met, penalty points are awarded, and they were deducted from the final score of 100 [37].

The ChlorTox scale was determined utilizing Eq. 3 [41] according to the ChlorTox methodology.

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

where CH_{sub} stands for the substance of interest's chemical risks, m_{sub} for the mass of the substance of interest required for a single analysis, and CH_{CHCl3} for the chemical hazards of standard chloroform. The weighted hazard number (WHN) method and the safety data sheet from Sigma Aldrich (St. Louis, MO, USA) were used to get the values of CH_{sub} and CH_{CHCl3} [41]. The AGREE approach was used to assess the AGREE scale for the suggested methodology for concurrently measuring MEF and PCM [42]. The AGREE scales for the proposed method were created using AGREE: The Analytical Greenness Calculator (version 0.5, Gdansk University of Technology, Gdansk, Poland, 2020). The AGREE scale, which varied from 0.0 to 1.0, was based on 12 different GAC criteria. The AGREE considers 12 GAC criteria for the calculation of the AGREE scale. Each GAC principle could obtain the value of 0.0–1.0. The overall AGREE scale for the analytical method is considered to be the average of 12 GAC components.

3 Results and discussion

3.1 Development and optimization of chromatographic conditions and green HPTLC method

Using HPTLC plates precoated with silica gel 60F254 on an aluminum substrate, a green HPTLC method was created and optimized based on previous studies for the evaluation of MEF and PCM in their combination tablet dosage forms [46–48]. A range of pure green solvents, such as EtOH, acetone, ethyl acetate, GAA, and H_2O, were examined in order to optimize the R_f value and peak response of MEF and PCM. Based on the initial findings, a range of binary combinations were then assessed using pure green solvents, including EtOH/H_2O, acetone/H_2O, EtOH/ethyl acetate, and

acetone/ethyl acetate in different proportions. When compared to various binary combinations, it was found that EtOH/H_2O generated the best chromatographic performance. Then, the binary combinations of EtOH/H_2O in different proportions were investigated. Among the binary mixtures of EtOH and H_2O, the proportion of EtOH in the 45–85% (v/v) range was investigated to be the green developing system. Chromatographic responses of MEF and PCM that are not acceptable regarding MEF (As > 1.35) and PCM (As > 1.40), they were found in every binary combination involving EtOH and H_2O. Subsequently, the ternary combination of EtOH, H_2O, and GAA was investigated in different proportions. The purpose of adding GAA in the developing system was to improve the separation efficiency and chromatographic performance. GAA can interact with the polar functional groups of the analytes and the stationary phase, resulting in the improvement of separation efficiency and resolution of compounds. Therefore, GAA was added into the developing system in this study. The following ternary combinations were investigated as the green developing systems: EtOH–H_2O–GAA (45:53:2 v/v/v), EtOH–H_2O–GAA (55:43:2 v/v/v), EtOH–H_2O–GAA (65:33:2 v/v/v), EtOH–H_2O–GAA (75:23:2 v/v/v), and EtOH–H_2O–GAA (85:13:2 v/v). Acetonitrile, methanol, and chloroform are examples of high volatile organic solvents that are typically suggested as the developing system for HPTLC analysis. Nevertheless, the identification and quantification of drugs and pharmaceuticals was accomplished with the help of these volatile solvents. These solvents are highly toxic and present significant environmental danger [48]. One of the 12 GAC principles stresses the use of green solvents, including H_2O, to lessen the negative environmental consequences of hazardous eluents [25]. The solvents studied herein are ethyl acetate, acetone, H_2O, and EtOH; these are considered green solvents since they do not harm the environment [49,50]. Since H_2O is the greenest solvent, it was combined with other green organic solvents to reduce the environmental toxicity of the existing analytical process [48]. The saturated chambers utilized for the preparation of each of the developing systems under evaluation are depicted in Figure 2.

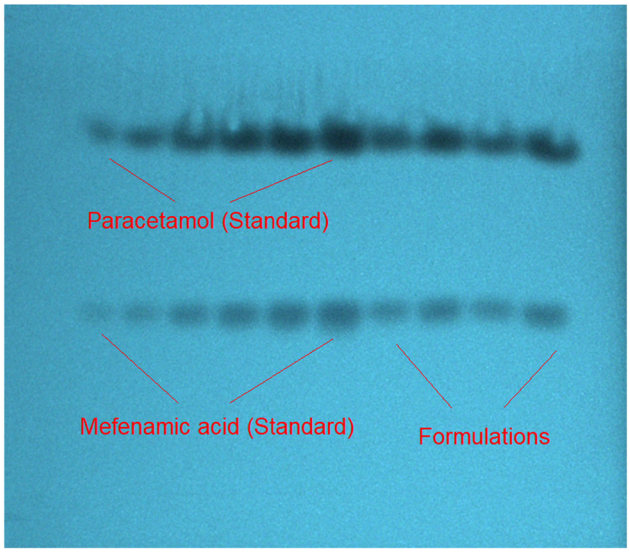

Figure 2: A typical TLC picture demonstrating the spots for commercial tablets, standard MEF, and PCM. EtOH–H_2O–GAA (75:23:2 v/v/v) was used as an environmentally friendly developing system for the current methodology.

The ternary combinations EtOH–H_2O–GAA (45:53:2 v/v/v), EtOH–H_2O–GAA (55:43:2 v/v/v), EtOH–H_2O–GAA (65:33:2 v/v/v), and EtOH–H_2O–GAA (85:13:2 v/v) also displayed poor chromatographic signals for MEF and PCM. As for MEF (As > 1.20) and PCM (As > 1.25), they were unreliable. Figure 3a displays the blank sample's chromatogram, which did not display any MEF or PCM peaks. Using the green development system EtOH–H_2O–GAA (75:23:2 v/v/v), it was discovered that the chromatographic responses of MEF at R_f = 0.39 ± 0.01 and PCM at R_f = 0.81 ± 0.01 were both intact and well separated (Figure 3b). Additionally, the As values of 1.07 and 1.05 for MEF and PCM, respectively, were projected; all of these are very trustworthy numbers. Therefore, it was determined that EtOH–H_2O–GAA (75:23:2 v/v/v) would be the final

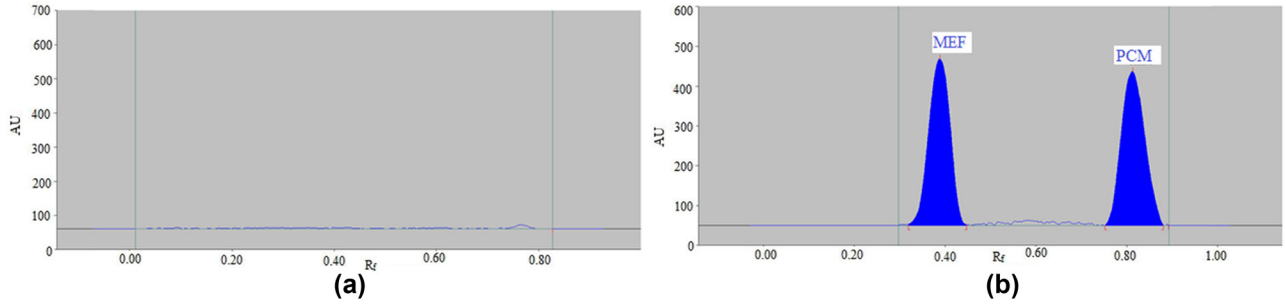

Figure 3: Typical chromatograms for the (a) blank sample and (b) standards MEF (R_f = 0.39) and PCM (R_f = 0.81).

developing system to assess MEF and PCM in marketed combination tablet dosage forms utilizing the proposed technique. The densitometry mode recording of the MEF and PCM spectral bands indicated the greatest response at 235 nm. Thus, at 235 nm, a thorough analysis of MEF and PCM was performed.

3.2 Validation of green HPTLC method

Using the ICH-Q2-R2 protocol, several parameters were developed to estimate MEF and PCM [45]. Table 1 lists the outcomes of the linearity assessment of the MEF and PCM calibration curves. In the 25–800 ng·band^{-1} range, the MEF and PCM calibration curves were both linear. MEF and PCM have calculated coefficients of determination (R^2) of 0.9985 and 0.9992, respectively. The correlation coefficients (R) for MEF and PCM were calculated to be 0.9992 and 0.9996, respectively. The R^2 and R values for MEF and PCM were both statistically significant ($p < 0.05$) based on Student's t-test. These outcomes suggest an excellent relationship between the levels of MEF and PCM and the measured peak areas. Finally, these outcomes suggest that the current methodology is linear enough to assess MEF and PCM.

The system suitability parameters for the current methodology are shown in Table 2. The results show that the parameters R_f, As, and N/m are reliable for the simultaneous determination of MEF and PCM using the proposed approach.

By calculating the % recovery for the simultaneous analysis of MEF and PCM, the accuracy of the existing approach was assessed. The accuracy evaluation results for the current technique are shown in Table 3. With the aid of the current technique, the percentage recoveries of MEF and PCM at three distinct QC solutions were determined to be 99.74–100.57% and 99.66–101.42%, respectively. These outcomes demonstrate that the suggested methodology could accurately assess MEF and PCM simultaneously.

The suggested protocol's intra- and inter-day precisions were assessed for the simultaneous detection of MEF and PCM. The findings are expressed as a percentage of coefficient of variation (% CV). The precision findings for both MEF and PCM measured simultaneously using the suggested method are shown in Table 4. It shows that the percentage CVs of MEF and PCM during the intra-day fluctuation were 0.77 to 0.87% and 0.80 to 0.98%, respectively. The results show that the inter-day precision percentage CVs for MEF and PCM were 0.81–0.95% and 0.91–1.01%, respectively. All of these results show how precise the suggested technique is for simultaneously analyzing MEF and PCM.

By carefully modifying the composition of the proposed developing system, it is possible to concurrently perform a robust assessment of the current approach for both MEF and PCM. Table 5 displays the outcomes of robustness evaluation conducted with the present methodology. The % CVs for MEF and PCM were found to be 0.93–0.96% and 0.91–0.97%, respectively. It was found that the values of MEF and PCM R_f were 0.38–0.40 and 0.80–0.82,

Table 1: Evaluation of linearity for the current method's measurement of MEF and PCM (mean ± SD; n = 6)

Parameters	MEF	PCM
Linear range (ng·band^{-1})	25–800	25–800
Regression equation	$y = 29.624x + 42.974$	$y = 34.142x - 270.08$
R^2	0.9985	0.9992
R	0.9992	0.9996
SE of slope	0.39	0.42
SE of intercept	0.68	1.07
95% CI of slope	27.93–31.34	32.33–35.95
95% CI of intercept	40.03–45.90	265.44–274.71
LOD ± SD (ng·band^{-1})	8.41 ± 0.22	8.36 ± 0.20
LOQ ± SD (ng·band^{-1})	25.23 ± 0.66	25.08 ± 0.60

R^2: coefficient of determination; R: correlation coefficient; x: MEF or PCM concentration; y: MEF or PCM peak area; SE: standard error; CI: confidence interval; LOD: limit of detection; LOQ: limit of quantitation.

Table 2: System suitability factors for MEF and PCM for the present method (mean ± SD; n = 3)

Parameters	MEF	PCM
R_f	0.39 ± 0.01	0.81 ± 0.01
As	1.07 ± 0.04	1.05 ± 0.03
N/m	4,855 ± 4.92	5,022 ± 5.27

Table 3: MEF and PCM accuracy assessment for the current method (mean ± SD; n = 6)

Conc. (ng·band^{-1})	Conc. found (ng·band^{-1}) ± SD	Recovery (%)	CV (%)
	MEF		
300	300.54 ± 3.50	100.18	1.16
400	402.31 ± 4.10	100.57	1.01
500	498.71 ± 4.87	99.74	0.97
	PCM		
300	299.00 ± 3.29	99.66	1.10
400	405.05 ± 4.14	101.26	1.02
500	507.10 ± 4.98	101.42	0.98

CV: coefficient of variance.

Table 4: Results of the current method's intra/inter-day precision of MEF and PCM during simultaneous detection (mean ± SD; n = 6)

Conc. (ng·band^{-1})	Intra-day precision			Inter-day precision		
	Conc. (ng·band^{-1}) ± SD	Standard error	CV (%)	Conc. (ng·band^{-1}) ± SD	Standard error	CV (%)
			MEF			
300	297.81 ± 2.62	1.06	0.87	302.82 ± 2.89	1.18	0.95
400	395.61 ± 3.31	1.35	0.83	406.17 ± 3.56	1.45	0.87
500	503.86 ± 3.92	1.60	0.77	495.52 ± 4.02	1.64	0.81
			PCM			
300	302.33 ± 2.98	1.21	0.98	303.12 ± 3.09	1.26	1.01
400	407.12 ± 3.63	1.48	0.89	396.71 ± 3.80	1.55	0.95
500	511.23 ± 4.13	1.68	0.80	508.71 ± 4.66	1.90	0.91

respectively. The robustness of the recommended approach was suggested by the R_f data of MEF and PCM, together with the modest changes in peak response.

"LOD and LOQ" were utilized to evaluate the sensitivity of the present protocol for simultaneously measuring MEF and PCM. Table 1 includes the "LOD and LOQ" data which were generated using the present approach for MEF and PCM. Using the current approach, the "LOD and LOQ" for MEF were found to be 8.41 ± 0.22 and 25.23 ± 0.66 ng·band^{-1}, respectively. Utilizing the present procedure, the "LOD and LOQ" for PCM were found to be 8.36 ± 0.20 and 25.08 ± 0.60 ng·band^{-1}, respectively. The high sensitivity was demonstrated by the LOD values for both medications, which are much below the lower linear limit. Nonetheless, both medications' LOQ values fell below the lower linear limit, suggesting that they were appropriately quantified. These findings demonstrate the sensitivity of the existing technique for measuring MEF and PCM simultaneously.

The specificity and peak purity of the suggested approach for evaluating MEF and PCM simultaneously were evaluated by contrasting the UV-absorption spectra and R_f values of MEF and PCM in commercial combination tablets with those of standard MEF and PCM. Together with the standards MEF and PCM, Figure 4 displays the overlapping UV-absorption spectra of the commercial combination tablet brands A and B.

By contrasting the spectra at the peak start (S), peak apex (M), and peak end (E) positions of the spot, the peak purities of standard MEF and PCM and MEF and PCM in commercial combination tablets were determined [51,52]. For commercial combination tablet brands A and B, standard MEF, and standard PCM, estimated values of r(S,M) and r(M,E) more than 0.99 demonstrate the homogeneity of the peaks [53,54]. The wavelength at which the highest chromatographic response for MEF and PCM was seen in standards and commercial combination tablets was 235 nm. How specific the proposed method was for simultaneously evaluating MEF and PCM was demonstrated by the identical R_f values, wavelengths, and UV-absorption spectra of standards and commercial combination tablets.

Table 5: MEF and PCM robustness evaluation results for the suggested approach (mean ± SD; n = 6)

Conc. (ng·band^{-1})	Mobile phase composition (EtOH–H_2O–GAA)			Results		
	Original	Used		(ng·band^{-1}) ± SD	CV (%)	R_f
			MEF			
		77:21:2	+2.0	392.22 ± 3.66	0.93	0.38
400	75:23:2	75:23:2	0.0	397.11 ± 3.80	0.95	0.39
		73:25:2	−2.0	404.08 ± 3.90	0.96	0.40
			PCM			
		77:21:2	+2.0	393.41 ± 3.60	0.91	0.8 0
400	75:23:2	75:23:2	0.0	397.71 ± 3.72	0.93	0.81
		73:25:2	−2.0	405.66 ± 3.96	0.97	0.82

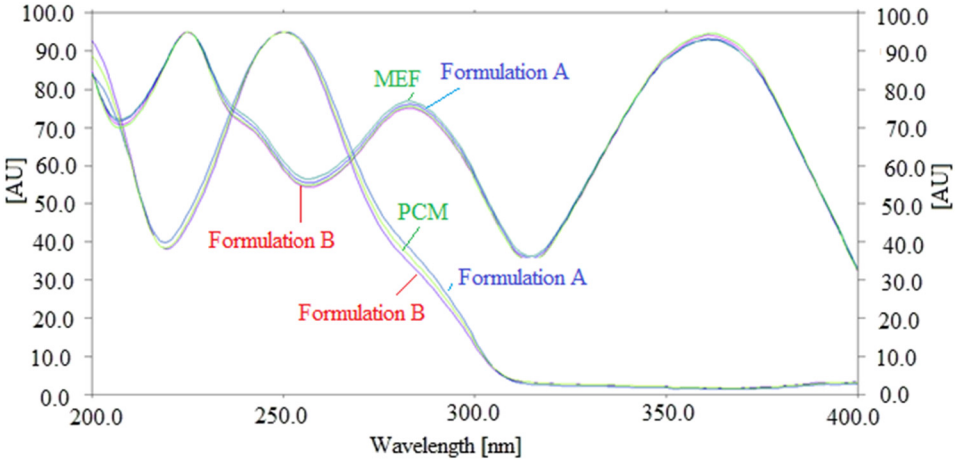

Figure 4: UV-absorption spectra of standard MEF and PCM and MEF and PCM in marketed combination tablets.

3.3 Comparative analysis of present HPTLC approach's validation variables with literature reported HPTLC and HPLC approaches

The current HPTLC method's validation variables were compared to reported HPTLC and HPLC approaches for the concurrent analysis of MEF and PCM. The findings are shown in Table 6. It has been discovered that the proposed HPTLC approach for the assessment of MEF and PCM has a better linear range for MEF and PCM than a previously published HPTLC approach [18]. Furthermore, all reported HPLC techniques have been found to have a narrow linear range compared to the broad linear range of the present HPTLC technique for the analysis of MEF and PCM [13–16]. The HPTLC approach used for the analysis of MEF and PCM seems to be similar to the published HPTLC [18] and the majority of HPLC techniques [13–16], which were reported to have accuracy (100 ± 2%) and precision (<2%) values within the range of the ICH-Q2-R2 criteria [45]. However, in terms of MEF accuracy [13] and PCM accuracy [16], the current HPTLC technique has been proven to be better than one of the published HPLC techniques. For the simultaneous detection of MEF and PCM using the HPTLC technique, the LOD and LOQ have not been published [18]. The LOD and LOQ for the majority of HPLC techniques, however, have been published and were determined to be sufficiently sensitive, such as the proposed HPTLC methodology for the detection of MEF and PCM [13–16]. Overall, the present HPTLC technique has been demonstrated to be superior in terms of linear range to the reported HPTLC and HPLC techniques for the

Table 6: Comparison of the current approach with previously published HPLC and HPTLC techniques for simultaneous MEF and PCM analysis

Method	Linear range	Accuracy (% recovery)	Precision (% CV)	LOD (ng·band^{-1})	LOQ (ng·band^{-1})	Ref.
		MEF				
HPTLC	40–200 µg·mL^{-1}	99.49–100.15	1.31	—	—	[18]
HPLC	100–300 µg·mL^{-1}	99.70–102.20	—	0.39 µg·mL^{-1}	1.20 µg·mL^{-1}	[13]
HPLC	40–200 µg·mL^{-1}	98.15–99.02	0.52	—	—	[14]
HPLC	0.50–10 µg·mL^{-1}	99.63–99.98	0.62–1.28	0.01 µg·mL^{-1}	0.05 µg·mL^{-1}	[15]
HPLC	6–14 µg·mL^{-1}	99.26–99.63	0.00	—	—	[16]
HPTLC	25–800 ng·band^{-1}	99.74–100.57	0.77–0.95	8.41 ng·band^{-1}	25.23 ng·band^{-1}	Present work
		PCM				
HPTLC	36–180 µg·mL^{-1}	99.10–99.97	0.89	—	—	[18]
HPLC	100–300 µg·mL^{-1}	99.30–99.50	—	0.39 µg·mL^{-1}	1.20 µg·mL^{-1}	[13]
HPLC	36–180 µg·mL^{-1}	98.32–100.78	0.48	—	—	[14]
HPLC	0.10–20 µg·mL^{-1}	99.79–99.99	0.75–1.60	0.03 µg·mL^{-1}	0.10 µg·mL^{-1}	[15]
HPLC	15–35 µg·mL^{-1}	100.94–103.27	0.00	—	—	[16]
HPTLC	25–800 ng·band^{-1}	99.66–101.42	0.80–1.01	8.36 ng·band^{-1}	25.08 ng·band^{-1}	Present work

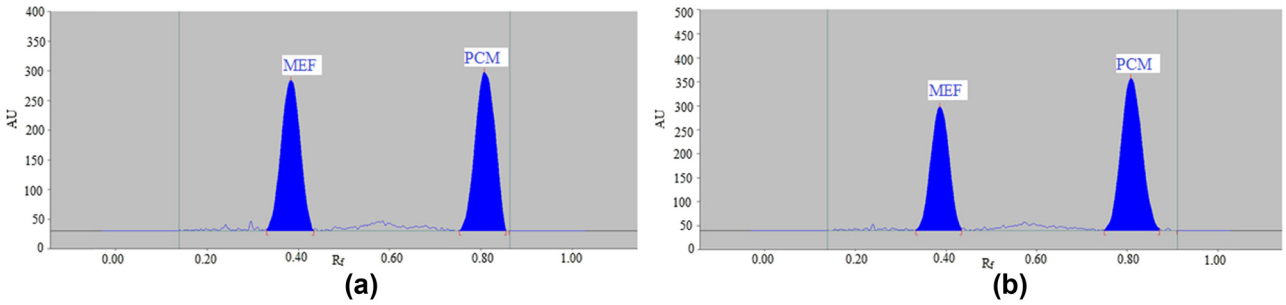

Figure 5: Representative chromatograms of MEF and PCM in (a) marketed combination tablet brand A and (b) marketed combination tablet brand B.

analysis of MEF and PCM when all factors are considered [13–16,18].

3.4 Commercial combination tablets' simultaneous measurement of MEF and PCM using the current technique

Liquid chromatography approaches, such as HPLC methods, have been successfully utilized in the concurrent analysis of multiple drugs [55]. However, the suggested approach was applied in place of conventional liquid chromatography methods to measure MEF and PCM concurrently in marketed combination tablets. Utilizing the suggested methodology, the chromatographic peaks of marketed combination tablet brand A and B were identified by contrasting them to those of standard MEF and PCM, at R_f = 0.39 ± 0.01 and R_f = 0.81 ± 0.01, respectively. Figure 5 displays the chromatographic peaks for MEF and PCM from commercial combination tablet brands A (Figure 5a) and B (Figure 5b). These peaks exactly matched those found in the MEF and PCM standards.

It was determined how much MEF was present in marketed combination tablets using the present technology. The results showed 99.27 ± 1.32% and 101.01 ± 1.36% in marketed combination tablet brands A and B, respectively. With the present methodology, 98.72 ± 1.26 and 100.12 ± 1.38% of PCM were found in marketed combination tablet brands A and B, respectively. The average % of MEF and PCM in marketed combination tablets was reported to be 100.66% and 99.40%, respectively [13]. The average % of MEF and PCM in commercial combination tablets was recorded to be 98.93% and 98.87%, respectively, by another report [14]. Student's *t*-test and the variance ratio F-test were used to evaluate the outcomes of the suggested method for the simultaneous analysis of MEF and PCM in commercial combination tablets with those of reported HPLC techniques [13,14]. There were no appreciable variations in the precision and accuracy of the performance of the compared techniques, as indicated by the recorded t and F values, which did not surpass their theoretical values. These findings demonstrated that the existing technique is appropriate for concurrently detecting MEF and PCM in commercial combination tablets.

Table 7: Current HPTLC method's greenness evaluation using the AES and penalty points, and its comparison to previously published HPTLC and HPLC methods for simultaneous determination of MEF and PCM

Reagents/instruments/waste	Penalty points					
	HPTLC [18]	HPLC [13]	HPLC [14]	HPLC [15]	HPLC [16]	Present HPTLC
EtOH	—	—	—	—	—	4
H_2O	—	0	—	—	—	0
GAA	—	—	—	—	—	2
Acetonitrile	8	12	—	12	—	
Toluene	12	—	—	—	—	—
Methanol	18	—	18	18	18	—
KH_2PO_4	—	0	0	0	0	—
Instruments	0	0	0	0	0	0
Waste	3	5	5	5	5	3
Total penalty points	41	17	23	35	23	9
AES scale	59	83	77	65	77	91

Table 8: ChlorTox scale results for the current HPTLC approach compared to previously published HPTLC and HPLC methods for the relative hazards of chloroform (CH_{sub}/CH_{CHCl3}), which were calculated using the WHN model

Stage	Solvent/reagent	Relative hazard (CH_{sub}/CH_{CHCl3})	m_{sub} (mg)	ChlorTox (g)	Total ChlorTox (g)	Ref.
Sample preparation	EtOH	0.26	1500	0.39	0.82	Present HPTLC
	GAA	0.43	40	0.02		
HPTLC analysis	EtOH	0.26	1500	0.39		
	GAA	0.43	40	0.02		
Sample preparation	Methanol	0.56	200	0.11	2.14	[18]
	Acetonitrile	0.39	1260	0.49		
	Toluene	0.87	540	0.47		
HPTLC analysis	Acetonitrile	0.39	1400	0.55		
	Toluene	0.87	600	0.52		
Sample preparation	Acetonitrile	0.39	393	0.15	1.99	[13]
HPLC analysis	Acetonitrile	0.39	4716	1.84		
Sample preparation	Methanol	0.56	792	0.44	3.10	[14]
HPLC analysis	Methanol	0.56	4752	2.66		
Sample preparation	Methanol	0.56	792	0.44	3.01	[15]
HPLC analysis	Acetonitrile	0.39	6602	2.57		
Sample preparation	Methanol	0.56	554	0.31	3.41	[16]
HPLC analysis	Methanol	0.56	5544	3.10		

3.5 Greenness assessment

As stated in Section 1, the greenness parameters of pharmaceutical analysis procedures can be evaluated utilizing a variety of greenness metric tools [34–42]. Three distinct methodologies were employed in the current method to evaluate the greenness of the suggested method: AES [37], ChlorTox [41], and AGREE [42]. Table 7 displays the outcomes of the AES scales with penalty points. According to Galuszka et al. (2012), an AES rating of 75 or higher indicated excellent greenness, a score of 75 or higher but less than 50 indicated good greenness, and a score of less than 50 indicated inadequate greenness [37]. The AES score for the suggested method was 91, indicating a very high degree of greenness. Additionally, the AES of one HPTLC and four different HPLC techniques was computed and contrasted with the current HPTLC technique. The reported HPTLC technique was found to have an AES scale of 59 [18]. However, the AES scale for four literature HPLC methods ranged from 65 to 83 [13–16]. The results show that previously reported HPTLC and HPLC methods to measure MEF and PCM simultaneously were subpar with respect to AES scales [13–16,18].

The outcomes of the individual green solvent ChlorTox scores and the overall ChlorTox scores for the present HPTLC method are shown in Table 8. The suggested method was shown to be reasonably safe and ecologically benign by the computed total ChlorTox scale of 0.82 g. The lower ChlorTox scale indicates the lower environmental and safety hazards [41]. Additionally, the ChlorTox of a previously published HPTLC and HPLC techniques was computed and contrasted with the current HPTLC technique. The documented HPTLC method was found to have a ChlorTox of 2.14 g [18]. However, the ChlorTox of the four published HPLC methods ranged from 1.99 to 3.41 g [13–16]. It was found that previously reported HPTLC and HPLC methods to assess MEF and PCM simultaneously were subpar with respect to ChlorTox scales [13–16,18].

The AGREE process, which takes into account all 12 GAC criteria, is the most widely used greenness metric approach for evaluating greenness [42]. Figure 6 displays the overall AGREE scale for the HPTLC method as it is currently used. A score of less than 0.75 is considered appropriate, a score of less than 0.50 is considered

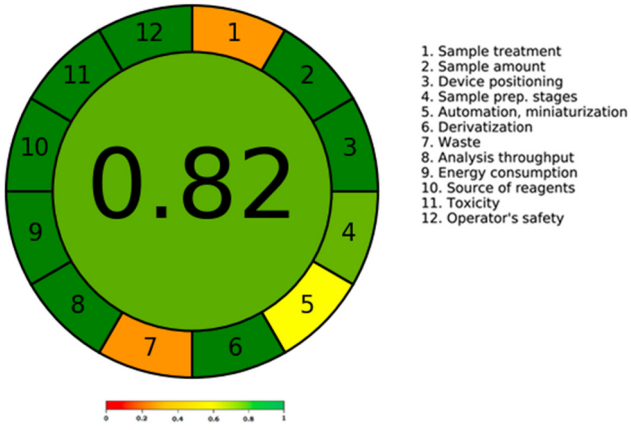

Figure 6: Overall AGREE score for the proposed HPTLC method.

insufficient, and a score of more than 0.75 is considered remarkable on the AGREE scale [42]. With the current method, the total AGREE scale was 0.82. The outstanding green qualities of the present HPTLC technology were once again shown by the AGREE results. The current HPTLC method to analyze MEF and PCM concurrently in commercial combination tablets has an excellent greener profile, according to the combined results of all greenness approaches.

4 Conclusions

For the concurrent measurement of MEF and PCM in physiological samples and commercial formulations, there are not many HPTLC methods available. Through this work, a fast, accurate, and eco-friendly HPTLC method for quantifying MEF and PCM in commercial combination tablets simultaneously was developed and validated. The suggested approach is linear (25–800 ng·band^{-1} range for both drugs), sensitive (LOD = 8.41 ng·band^{-1} for MEF, LOD = 8.36 ng·band^{-1} for PCM, LOQ = 25.23 ng·band^{-1} for MEF, and LOQ = 25.08 ng·band^{-1} for PCM), accurate (% recovery = 99.26–101.42 for both drugs), precise (% CV = 0.77–1.01 for both drugs), robust, and eco-friendly for measuring MEF and PCM simultaneously. With the current method, the MEF and PCM contents of commercial combination tablets were determined effectively. The AES and ChlorTox evaluation findings demonstrate how remarkably environmentally friendly the present technology is for concurrently assessing MEF and PCM when compared to the established HPTLC and HPLC methods. All of these findings demonstrate that the current method can be used to analyze MEF and PCM in marketed combination products.

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