

Research Article

HPTLC-Densitometric Analysis of Eperisone Hydrochloride and Paracetamol in Their Combined Tablet Dosage Form

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A simple, precise, accurate, and reliable HPTLC method has been developed and validated for the analysis of EPE-Eperisone hydrochloride and PCM-Paracetamol in their combined dosage form. Identification and analysis were performed on 100 mm × 100 mm layer thickness 0.2 mm, precoated silica gel G₆₀-F₂₅₄, aluminum sheet, prewashed with methanol, and dried in an oven at 50°C for 5 min. Toluene : methanol : ethyl acetate : glacial acetic acid (4 : 3.5 : 2.5 : 0.05) (v/v/v/v) was used as mobile phase. Calibration plots were established showing the dependence of response (peak area) on the amount chromatographed. The validated calibration ranges were 200–700 ng/spot and 1300–4550 ng/spot for EPE and PCM with correlation coefficient (R^2) 0.994 and 0.996, respectively. Average % recovery was between 98.61–100.94% and 99.18–100.57% for EPE and PCM, respectively. The spots were scanned at 248 nm in a reflectance mode. The proposed method was validated as per ICH guidelines and successfully applied to the estimation of EPE and PCM in their combined tablet dosage form.

1. Introduction

EPE is chemically (2RS)-1-(4-ethylphenyl)-2-methyl-3-(1-piperidinyl)propan-1-one and hydrochloride (1:1) (Figure 1(a)) [1, 2]. Eperisone HCl is a centrally acting muscle relaxant; it acts at the level of spinal cord by blocking sodium channels and calcium channels. Eperisone HCl exerts its spinal reflex inhibitory action predominantly via a presynaptic inhibition of the transmitter release from the primary afferent endings via a combined action on voltage-gated sodium and calcium channels. Eperisone HCl increases the blood supply to skeletal muscles; this action is noteworthy since a muscle contracture may compress the small blood vessels and induce an ischemia leading to release of pain stimulating compounds [3]. EPE is official in Japanese pharmacopoeia [1]. Chemically PCM is N-(4-hydroxyphenyl)acetamide (Figure 1(b)). Paracetamol is a weak inhibitor of PG-Prostaglandin synthesis of COX-1 and COX-2. Cyclooxygenase serves as a pain activator, is responsible for the biosynthesis of prostaglandins, is used for the relief of headaches, fever, pains, and is a major

ingredient in numerous cold and flu remedies [4]. Paracetamol is official in Japanese Pharmacopoeia [1], British Pharmacopoeia [5], United States Pharmacopoeia [6], and Indian Pharmacopoeia [7].

The review of the literature revealed that analytical methods involving spectrophotometry [8], LC-ESI-MS [9], have been reported for EPE in single form and in combination with other drugs. Several analytical methods have been reported for PCM in single form and in combination with other drugs including spectrophotometry [10–14], HPLC [15–17], and HPTLC [18, 19].

To the best of our knowledge, there is no published HPTLC method for this combination. So, the present paper describes a simple, accurate, and precise method for simultaneous estimation of EPE and PCM in combined tablet dosage form by HPTLC method. The developed method was validated in accordance with ICH guidelines [20] and successfully employed for the assay of EPE and PCM in their combined dosage form.

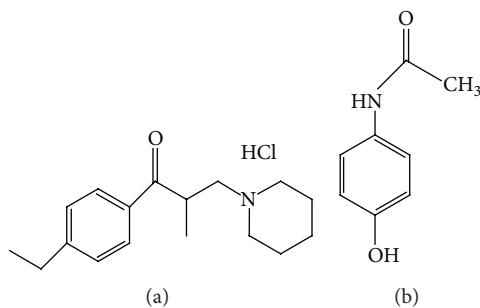


FIGURE 1: Chemical structure of (a) EPE and (b) PCM.

2. Materials and Methods

2.1. Reagents and Chemicals. Analytically pure EPE and PCM were kindly provided by Macleods Pharmaceuticals Ltd., Mumbai, Maharashtra, India, and Elysium Pharmaceutical Ltd., Vadodara, Gujarat, India, respectively, as gratis samples. Analytical grade methanol was purchased from SRL limited, Mumbai, India. Tablet of EPE and PCM in combined dosage form, MYOSONE PLUS, with a 50 mg EPE and 325 mg PCM label claim, manufactured by Macleods Pharmaceuticals Ltd., was procured from local market.

2.2. Instrumentation and Conditions. Chromatography was performed on 100 mm × 100 mm on precoated silica gel G₆₀-F₂₅₄ aluminum sheet (E. Merck, Mumbai, India). Before use the plates were washed with methanol then dried at room temperature. Samples were applied as 6 mm bands by means of a Camag Linomat V (Muttenz, Switzerland) sample applicator equipped with 100 μL syringe; operated with settings of band length, 6 mm; distance between bands, 5 mm; distance from the plate edge, 10 mm; and distance from the bottom of the plate, 10 mm. The constant application rate was 15 s μL⁻¹, and a nitrogen aspirator was used. Ascending development of plate, migration distance 70 mm was performed at ambient temperature with Toluene : methanol : ethyl acetate : glacial acetic acid (4 : 3.5 : 2.5 : 0.05) (v/v/v/v), as mobile phase in a 10 cm × 10 cm Camag twin-trough chamber previously saturated for 15 min. After development the plates were dried with hot-hair dryer and viewed in a CAMAG UV cabinet. Densitometric scanning at 272 nm was then performed with a Camag TLC Scanner 3 equipped with winCATS 3.2.1 software. The scanning rate was 20 mm s⁻¹. The source of radiation used was the deuterium lamp.

2.3. Calibration

2.3.1. Eperisone Hydrochloride (50 μg/mL) and Paracetamol (325 μg/mL) Standard Stock Solution. Standard EPE 50 mg and PCM 325 mg were weighed and transferred to a 100 mL volumetric flask and dissolved in methanol. The flask was sonicated, and volume was made up to the mark with methanol to give a solution containing 500 μg/mL EPE and 3250 μg/mL PCM. One mL of this aliquot was added to 10 mL volumetric flask, and volume was made up to the mark with

TABLE 1: System suitability test parameters.

System suitability parameter	EPE	PCM
Peak purity	0.994	0.996
R _f value	0.26 ± 0.017224	0.79 ± 0.010328

methanol to give a solution containing 50 μg/mL EPE and 325 μg/mL PCM.

2.3.2. Calibration Curve for EPE and PCM. Semiautomatic spotter was used containing a syringe having capacity of 100 μL. Mixed stock solution having concentration of 50 μg/mL of EPE and 325 μg/mL PCM was filled in the syringe, and under nitrogen stream, it was applied in the form of band of desired concentration range for each drug on a single plate having concentration of 200 to 700 ng/spot for EPE and 1300 to 4550 ng/spot for PCM. Plate was developed using the above-mentioned conditions. Plots of peak area versus concentration for both drugs were obtained.

2.4. Analysis of Marketed Formulation. Twenty tablets were weighed accurately; average weight was found and finely powdered. A quantity equivalent to 50 mg EPE and 325 mg PCM was accurately weighed and transferred to volumetric flask of 100 mL capacity. 80 mL of methanol was transferred to this volumetric flask and sonicated for 20 min to dissolve the drug. Resulting solution was filtered through Whatman filter paper (0.45 μ) into a 100 mL volumetric flask. The flask was shaken, and volume was made up to the mark with methanol to give a solution containing 500 μg/mL of EPE and 3250 μg/mL of PCM. One mL of this aliquot was added to 10 mL volumetric flask, and volume was made up to the mark with methanol to give a solution containing 50 μg/mL of EPE and 325 μg/mL of PCM. Now this prepared sample solution was applied on TLC plate, developed, dried in air, and photometrically analyzed as described above. From the peak area obtained in the chromatogram, the amounts of both of the drugs were calculated.

2.5. Validation of the Method

2.5.1. Linearity and Range of the HPTLC Method. Calibration graphs were constructed by plotting peak areas versus concentrations of EPE and PCM, and the regression equations were calculated. The calibration graphs were plotted over 6 different concentrations in the range of 200–700 ng/spot for EPE and 1300–4550 ng/spot for PCM by applying different volumes stock solution containing EPE and PCM (50 μg/mL of EPE and 325 μg/mL PCM). The calibration graphs were developed by plotting peak area versus concentrations (n = 6) with the help of the winCATS software.

2.5.2. Accuracy (Recovery). Known amounts of standard solution of EPE (300, 400, and 500 ng/spot) and PCM (2050, 2600, and 3250 ng/spot) for the HPTLC method were added

TABLE 2

Concentration (ng/spot)	Area mean \pm S.D. ($n = 6$)	CV
Result of calibration readings for EPE by HPTLC method		
200	1194.333 \pm 13.80821	1.15
300	1675.967 \pm 14.6988	0.87
400	2093.35 \pm 17.86894	0.85
500	2504.867 \pm 35.20316	1.4
600	3171.433 \pm 29.04952	0.91
700	3645.45 \pm 23.91458	0.66
Result of calibration readings for PCM by HPTLC method		
1300	9337.983 \pm 86.3672	0.92
1950	11511.37 \pm 122.5659	1.06
2600	13646.1 \pm 86.2213	0.63
3250	16556.83 \pm 104.0746	0.62
3900	18071.97 \pm 129.3408	0.71
4550	20566.1 \pm 110.7175	0.53

TABLE 3: Determination of accuracy.

% Level	Amount added (ng/spot)		Amount recovered (ng/spot) ($n = 3$)		% Recovered \pm S.D	
	EPE	PCM	EPE	PCM	EPE	PCM
50	300	2050	302.82	2057.17	100.94 \pm 0.89	100.35 \pm 0.41
100	400	2600	394.44	2578.68	98.61 \pm 0.74	99.18 \pm 0.50
150	500	3250	492.95	3268.52	98.59 \pm 0.57	100.57 \pm 0.93

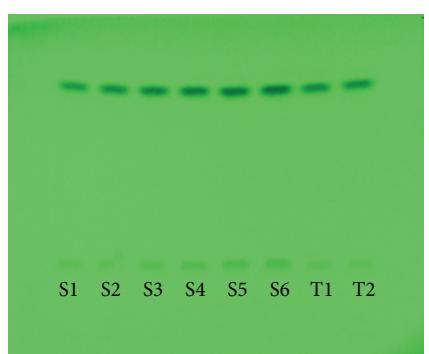


FIGURE 2: Photograph of developed HPTLC plate of EPE and PCM at 248 nm.

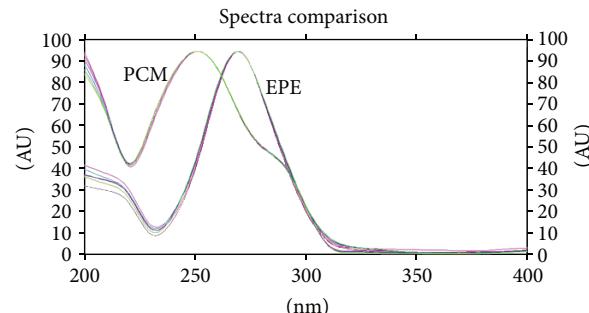


FIGURE 4: Overlaid spectrums of EPE and PCM at 248 nm by HPTLC method.

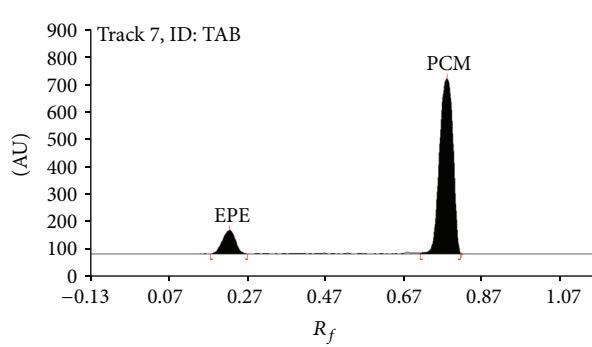


FIGURE 3: Densitogram of market formulation containing EPE and PCM 400 ng/spot and 2600 ng/spot, respectively.

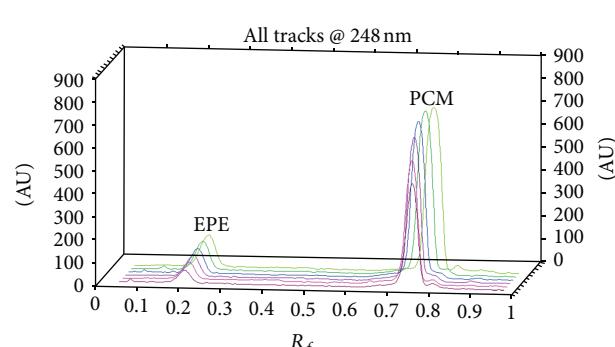


FIGURE 5: 3D overlain spectra of EPE and PCM by HPTLC method.

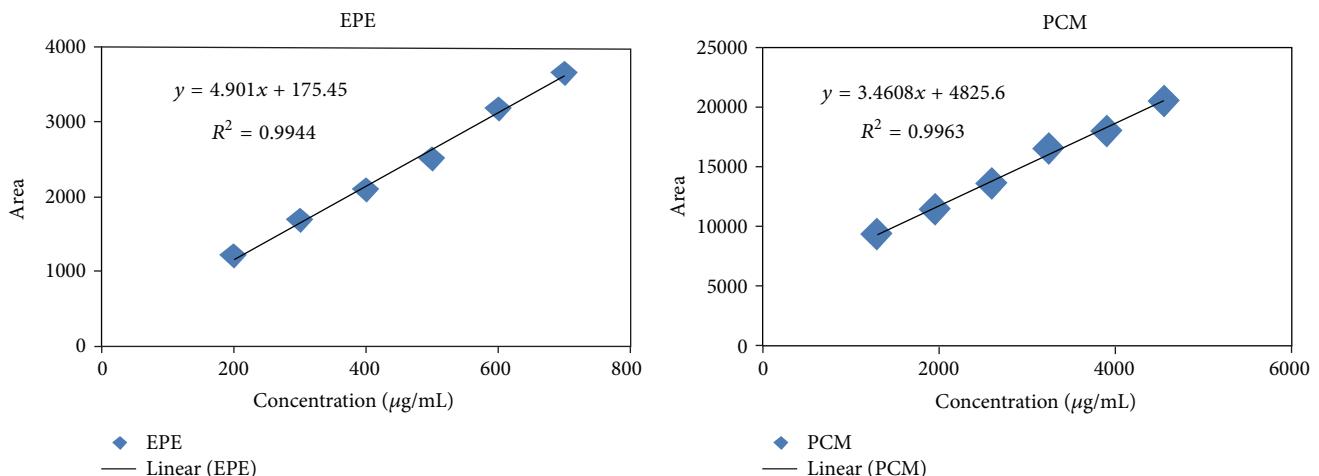


FIGURE 6: Calibration curve of EPE and PCM.

to prequantitated sample solutions of tablet dosage forms. The amounts of EPE and PCM were estimated by applying values of peak area to the regression equations of the calibration graph.

2.5.3. Precision. Precisions of the proposed HPTLC methods were determined by analyzing mixed standard solution of EPE and PCM at 3 different concentrations (300, 400, and 500 ng/spot for EPE and 1950, 2600, and 3250 ng/spot for PCM) 3 times in the same day and in 3 different days. The results are reported in terms of coefficient of variance (CV).

2.5.4. Repeatability. Repeatability of method was assessed by applying the same sample solution 6 times on a plate with the automatic spotter using the same syringe and by taking 6 scans of the sample spot for both EPE and PCM (400 ng/spot of EPE and 2600 ng/spot of PCM) without changing the positions of the plate.

2.5.5. Specificity. The specificity of the method was ascertained by analyzing standard drug and sample. The band of EPE and PCM in sample was confirmed by comparing the R_f and spectra of the band with that of standard. The peak purity of both drugs was assessed by comparing the spectra at 3 different levels, that is, peak start (S), peak apex (M), and peak end (E) position of the band.

2.5.6. Limit of Detection and Limit of Quantification. The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by using the following equations as per International Conference on Harmonization (ICH) guidelines which is based on the calibration curve:

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

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

where σ is the standard deviation of y -intercepts of regression lines and S is slope of calibration curve.

2.5.7. Robustness. Sample solution was prepared and then analyzed with change in the typical analytical conditions like amount of mobile phase, proportion of mobile phase, saturation time, plate pretreatment, and stability of analytical solution.

3. Results and Discussion

3.1. Method Optimization. Several mobile phases were tried to accomplish good separation of EPE and PCM. Using the mobile phase Toluene : methanol : ethyl acetate : glacial acetic acid (4:3.5:2.5:0.05) (v/v/v) and 10×10 cm HPTLC silica gel 60 F254 aluminum-backed plates, good separation was attained with retention factor (R_f) values of 0.26 for EPE and 0.79 for PCM. A wavelength of 248 nm was used for the quantification of the drugs. Figure 2 shows the detection of both of the drugs in their combined dosage form at 248 nm by HPTLC method. Resolution of the peaks with clear baseline separation was found. Figure 3 shows the densitogram of mixture which has a clear baseline. Figure 4 showed a good linearity when overlapped and scanned between 200 nm to 400 nm. Figure 5 shows a 3D overlapped spectrum of both drugs which has good linearity. The system suitability test parameters for the developed method are shown in Table 1.

3.2. Validation of the Proposed Methods

3.2.1. Linearity. Figure 6 shows that linear correlation was obtained between peak areas and concentrations of EPE and PCM in the range of 200–700 ng/spot for EPE with $R^2 = 0.994$ and 1300–4550 ng/spot for PCM with $R^2 = 0.996$, respectively, and data are shown in Table 2.

3.2.2. Accuracy. The recovery experiments were performed by the standard addition method. The HPTLC method was found to be accurate with % recovery of 98.61–100.94% for EPE and 99.18–100.57% for PCM, respectively, (Table 3). The high values indicate that the method is accurate.

TABLE 4: Summary of validation parameters of HPTLC.

Parameters	EPE	PCM
Recovery %	99.59–100.94	99.18–100.57
Repeatability (CV, $n = 6$)	0.69	0.35
Precision (CV)		
Intraday ($n = 3$)	0.69–0.76	0.35–0.59
Interday ($n = 3$)	0.77–1.34	0.72–1.65
Limit of detection (ng/spot)	8.05	87.64
Limit of quantitation (ng/spot)	24.41	265.57
Robustness	Robust	Robust
Solvent suitability	Suitable for 24 hr	Suitable for 24 hr

TABLE 5: Assay result of marketed formulation.

Formulation	Drug	Amount taken (ng/spot)	Amount found (ng/spot) ($n = 3$)	Labeled claim (mg)	Amount found per tablet (mg)	% Label claim \pm SD
MYOSONE PLUS (tablet)	EPE	400	395.17	50	49.39	98.79 \pm 1.26
	PCM	2600	2544.47	325	318.06	97.86 \pm 0.84

3.2.3. Repeatability. The CV values for EPE and PCM were found to be 0.69 and 0.35, respectively. The CV values were found to be <1%, which indicates that the proposed methods are repeatable.

3.2.4. Precision. The CV values were found to be <2%, which indicates that the proposed method is precise.

3.2.5. Limit of Detection (LOD) and Limit of Quantification (LOQ). LOD values for EPE and PCM were found to be 8.05 ng/spot and 87.64 ng/spot, respectively. LOQ values for EPE and PCM were found to be 24.41 ng/spot and 265.57 ng/spot, respectively. These data show that nanogram quantity of both drugs can be accurately determined (Table 4).

3.2.6. Specificity. Excipients (Starch) used in the specificity studies did not interfere with the estimation of either of the drugs by the proposed methods. Hence, the methods were found to be specific for estimation of EPE and PCM.

3.2.7. Robustness. Peak area and retention time variation were found to be <1%. Also, no significant change in peak area was observed during 24 hr. No decomposition was observed in either the first or second direction of the 2-dimensional analysis for both drugs on the HPTLC plate. Hence, the method was found to be robust for estimation of EPE and PCM.

3.2.8. Assay of the Tablet Dosage Form (EPE 50 mg and PCM 325 mg per Tablet). The proposed validated method was successfully applied to determine EPE and PCM in their tablet dosage form (MYOSONE PLUS). The results obtained for EPE and PCM was comparable with the corresponding labelled amounts (Table 5).

4. Conclusion

Thus, the objective of project work was development and comparison of analytical method of EPE and PCM in their combined dosage form. The developed and validated HPTLC method for EPE and PCM was found to be simple, specific, and cost effective and can be routinely applied for analysis of EPE and PCM in their combined dosage form. We can say that HPTLC method is more sensitive giving precise results (interday, intraday) for both drugs, and also HPTLC method is more sensitive in terms of LOD and LOQ. It also requires least solvents for analysis. The proposed method has the advantages of simplicity and convenience for the separation and quantitation of EPE and PCM in combination and can be used for the assay of their dosage form. Also, the low solvent consumption and short analytical run time lead to environmentally friendly chromatographic procedures. The additives usually present in the pharmaceutical formulations of the assayed analytes did not interfere with determination of EPE and PCM. The method can be used for the routine simultaneous analysis of EPE and PCM in pharmaceutical preparations.

Disclosure

The usage of this trade mark symbol or company name is for proving the genuinity of the work and not for any another purpose. The authors of the paper, do not have any financial relation with the commercial identity mentioned in the paper.

Conflict of Interests

The authors have no conflict of interests or no financial gains in mentioning the company names or trade marks.

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