



SIMULTANEOUS ESTIMATION OF CETRIZINE HYDROCHLORIDE AND PHENYLPROPANOLAMINE HYDROCHLORIDE BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY METHOD

PINAK PATEL*, FALGUN MEHTA[§], SWAYAMPRAKASH PATEL[#]

*Department of pharmaceutical chemistry, Dharmaj degree Pharmacy College (Indubhai Patel college of Pharmacy and research center), Dharmaj, Gujarat-388430, India, SICART (Sophisticated Instrumentation center for Applied Science research and technology), Vallabh Vidyanagar, Gujarat-388120, India, [§]Department of pharmaceutical chemistry, Indukaka Ipcowala College of pharmacy, New Vallabh Vidyanagar, Gujarat-388120, India, [#]Department of Pharmaceutics & Pharmaceutical Technology, Dharmaj degree Pharmacy College (Indubhai Patel college of Pharmacy and research center), Dharmaj, Gujarat-388430, India. Email: pinakqa@gmail.com

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ABSTRACT

Simple, accurate, economic and less time consuming method for analysis of cetirizine hydrochloride (CET) and phenylpropanolamine hydrochloride (PPA) in their combined dosage forms was developed and validated using high-performance thin layer chromatography (HPTLC). HPTLC method analysis was performed on CAMAG, silica gel 60F254 HPTLC plates, in absorbance mode at 254nm, using ethyl acetate-methanol (5.5+4.5 v/v) as mobile phase. Both the drugs were well resolved and retention factor was found to be 0.28 and 0.78 for CET and PPA respectively. The calibration curves were linear in the range of 7.5-17.5 µg/spot for CET and 37.5-87.5µg/spot for PPA. The limit of detection for CET and PPA was found to be 740 ng/spot and 2710 ng/spot respectively. The method was found to be accurate with % recovery 98.6% - 103% for CET and 98.5% - 100.46% for PPA. Developed method was validated and found to be accurate, precise and specific. Developed method was successfully applied for the estimation of CET and PPA in their combined dosage forms.

Key words: Cetirizine Hydrochloride (CET), Phenylpropanolamine hydrochloride (PPA), High performance thin layer chromatography (HPTLC), Validation

INTRODUCTION

Cetirizine is (±) - [2-[4- [(4-chlorophenyl) phenyl methyl] -1-piperaziny] ethoxy] acetic acid, dihydrochloride¹. Cetirizine Hydrochloride antihistamine is a major metabolite of hydroxyzine, and a racemic selective H1 receptor inverse agonist used in the treatment of allergies, hay fever, angioedema, and urticaria. Histamines cause symptoms of allergy when released by allergic reactions in the body. Cetirizine competes with histamine for binding at H1 receptor sites on the effector cell surface (H1 receptor blocker) resulting in suppression of histaminic edema, flare, and purities². Phenylpropanolamine is chemically (1R, 2S)-2-amino-1-phenyl-propan-1-ol³. Phenylpropanolamine (PPA), a sympathomimetic agent structurally similar to pseudoephedrine, is used to treat nasal congestion. Phenylpropanolamine acts directly on alpha receptor and to a lesser degree, beta adrenergic receptor in the mucosa of respiratory tract. Stimulation of alpha-adrenergic receptors produces vasoconstriction, reduces tissue hyperemia, edema, and nasal congestion, and increases nasal airway potency. PPA indirectly stimulates beta-receptors and produce Tachycardia, a positive inotropic effect⁴. CET is combined with PPA to obtain additive effect in treatment of cough & cold that may be associated with hay fever or allergic rhinitis⁵. A literature survey revealed that methods have been reported for the estimation of CET using liquid chromatography⁶ & spectrophotometry alone & with combination of other drugs⁷. PPA has been determined by various pharmacopeial and non-pharmacopeial methods. The pharmacopeial methods include potentiometric titration and liquid chromatographic method. Many liquid chromatography methods have been reported for the estimation of PPA alone in biological fluids and in combination with other drugs. As far as we are aware, no HPTLC method available for the estimation of CET and PPA in their combined dosage forms.

MATERIAL AND METHODS

Drugs and chemicals

CET and PPA were kindly supplied as gift sample by vital formulations Limited, Vallabh Vidyanagar GIDC, Gujarat, India. It was certified to contain 99.81 % (w/w) of CET and 99.45 % (w/w) of PPA on dried basis. All other chemicals and reagents were

purchased from Merck chemicals limited, Mumbai, India and S.D. Fine chemicals limited, India.

Instrumentation and conditions

Solutions of CET and PPA were applied to silica gel 60F254 HPTLC plates (10 × 10 cm) by means of a Linomat V automatic spotter equipped with a 100 µL syringe and 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 plate was developed in a twin-trough chamber previously saturated for 30 min with the mobile phase ethyl acetate: methanol (5.5:4.5, v/v), for a distance of 8 cm. For densitometric analysis, the spots on the air-dried plate were scanned with the Scanner III at 254 nm using the deuterium source.

Calibration

Cetirizine (CET) standard stock solution: (2500 µg/ml)

Standard CET 25.0 mg was weighed and transferred to a 10 ml volumetric flask and dissolved in methanol. The flask was shaken and volume was made up to the mark with methanol to give a solution containing 2500 µg/ml CET.

Phenylpropanolamine (PPA) standard stock solution: (5000 µg/ml)

Standard PPA 50.0 mg was weighed and transferred to a 10 ml volumetric flask and dissolved in methanol. The flask was shaken and volume was made up to the mark with methanol to give a solution containing 5000 µg/ml PPA.

Calibration curve for CET and PPA

Semi automatic spotter was used containing a syringe having capacity of 100 µl. Mixed stock solution having concentration of 5000 µg/ml of CET and 2500µg/ml PPA was filled in the syringe and under nitrogen stream, it was applied in form of band in desired concentration range. Each of drug on a single plate having concentration of 7.5 to 17.5 µg/spot for CET & 37.5 to 87.5 µg/spot for PPA. Plate was developed using above mentioned conditions. Plots of peak area vs. concentration for both drugs were obtained. Spectra of both drugs were recorded in the range of 200-800 nm and

purity of chromatographic peak was checked by scanning individual peak at 3 different positions (peak start, peak apex and peak end).

Validation⁸

Linearity and range of the HPTLC method:

Calibration graphs were constructed by plotting peak areas vs concentrations of CET and PPA, and the regression equations were calculated. The calibration graphs were plotted over 5 different concentrations in the range of 7.5-17.5 µg/ml for CET and 37.5-87.5µg/ml for PPA by applying different volumes stock solution containing CET and PPA (500 µg/ml of CET and 2500µg/ml PPA). The calibration graphs were developed by plotting peak area vs concentrations ($n = 5$) with the help of the winCATS software.

Accuracy (Recovery)

Known amounts of standard solution of CET (2, 4, 8 µg/spot) and PPA (10, 20, 40 µg/spot) for the HPTLC method were added to prequantitated sample solutions of tablet dosage forms. The amounts of CET and PPA were estimated by applying values of peak area to the regression equations of the calibration graph.

Method precision (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 CET and PPA (10 µg/spot of CET and 50 µg/spot of PPA) without changing the positions of the plate.

Intermediate precision (reproducibility)

Precisions of the proposed HPTLC methods were determined by analyzing mixed standard solution of CET and PPA at 3 different concentrations (7.5, 12.5, 17.5 µg/spot for CET and 37.5, 62.5, 87.5 µg/spot for PPA) 3 times on the same day and on 3 different days. The results are reported in terms of relative standard deviation (RSD).

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD with signal-to-noise (S/N) ratio of 3:1 and the LOQ with S/N ratio of 10:1 were calculated for both drugs using the following equations according to International Conference on Harmonization guidelines.

$$\text{LOD} = 3.3 \times \sigma / S, \text{LOQ} = 10 \times \sigma / S$$

Where, σ = the standard deviation (SD) of the response and

S = The SD of the y-intercept of the regression line.

Specificity

The excipients such as hydroxypropyl cellulose, polyethylene glycol 6000 and lactose monohydrate were spiked into pre-weighed quantity of drugs to assess the specificity of the methods. The peak area was measured to determine the quantity of the drugs.

Robustness

Robustness of the methods was studied by changing the composition and the pH of mobile phase and determining the stability of the drugs in methanol for 24 Hrs. at ambient temperature. Spot stability was observed by performing 2-dimensional HPTLC development using the same mobile phase.

Analysis of marketed formulation

Twenty tablets were weighed accurately average weight was found and finely powered. A quantity equivalent to 5 mg CET and 25 mg PPA was accurately weighed and transferred to volumetric flask of 10 ml capacity. 5 ml of methanol was transferred to this volumetric flask and sonicated for 5 min to dissolve the drug. Resulting solution was filtered through whattman filter paper (0.45µ) in to a 10ml 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 CET & 2500 µg/ml of PPA. Now this prepared sample solution was applied on HPTLC plate. Now this plate was developed, dried in air and analyzed photometrically as described above. From the peak area obtained in the chromatogram, the amounts of both the drugs were calculated.

RESULTS AND DISCUSSION

Method optimization

Several mobile phases were tried to accomplish good separation of CET and PPA. Using the mobile phase ethyl acetate: methanol (5.5: 4.5v/v) and 10 × 10 cm HPTLC silica gel 60F254 aluminum-backed plates. Good separation was attained with retention factor (R_f) values of 0.28 for CET and 0.78 for PPA. A wavelength of 254 nm was used for the quantification of the drugs. Fig. 1 shows the detection of both the drug in their combined dosage form at 254nm by HPTLC method.

Resolution of the peaks with clear baseline separation was found. Fig. 2 shows the densitogram of mixture which has a clear baseline.

Fig. 3 showed a good linearity when overlapped and scanned between 200nm to 400nm. Fig. 4 shows a 3D overlapped spectrum of both the drugs which has good linearity.

The system suitability test parameters for the developed method are shown in Table 1.



Fig. 1: UV detection at 254 nm in HPTLC

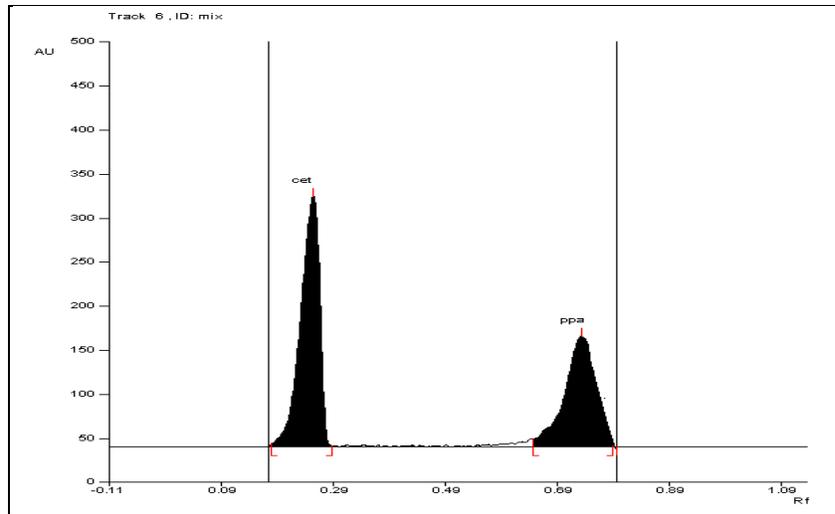


Fig. 2: Densitogram of mixture of CET (8 µg/spot) and PPA (16µg/spot)

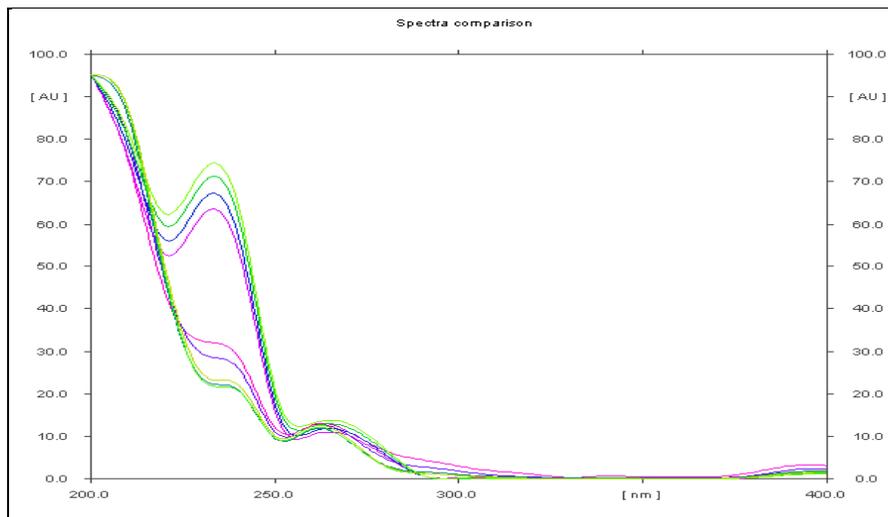


Fig. 3: Overlain spectra of CET & PPA at 254nm by HPTLC method

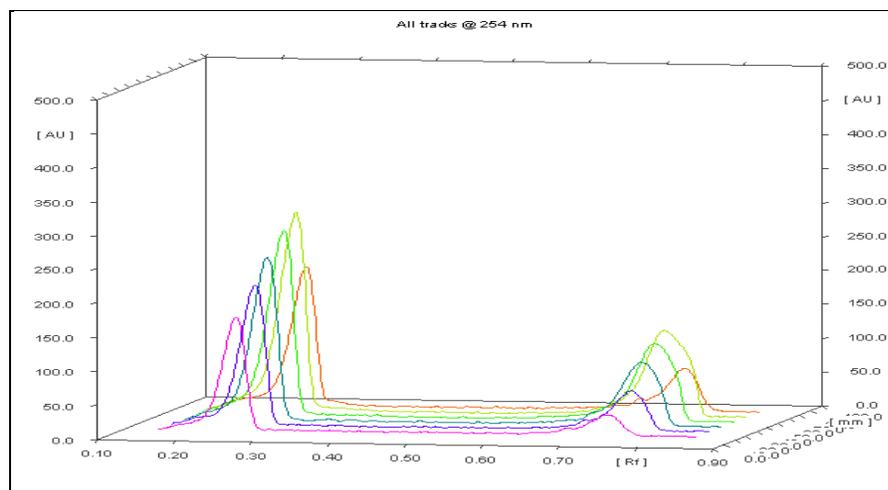


Fig. 4: 3D overlain spectra of CET & PPA by HPTLC method

Table 1: System suitability parameters for developed HPTLC method

System Suitability Parameters	By HPTLC method	
	CET	PPA
Peak Purity	0.9999	0.9998
R _f	0.28	0.78

Validation of the proposed methods**Linearity**

Linear correlation was obtained between peak areas and concentrations of CET and PPA in the range of 7.5–17.5 µg/mL with r²=0.997 and 37.5–87.5µg/ml for PPA with r²=0.998, respectively and data are shown in table 2.

Accuracy

The recovery experiments were performed by the standard addition method. The HPTLC method was found to be accurate with % recovery of 98.6-103 % for CET and 100.30-100.46% for PPA respectively (Table 3). The high values indicate that the method is accurate.

Table 2: Result of calibration readings for CET and PPA by HPTLC method

Concentration of drug (µg/spot)	Area		C.V.
	Mean ± S.D		
FOR CETRIZINE HYDROCHLORIDE			
7.5	4764.15 ± 74.65		1.56
10	6113.73 ± 111.95		1.83
12.5	7125.36 ± 48.14		0.67
15	8452.15 ± 55.83		0.66
17.5	9460.7 ± 50.73		0.62
FOR PHENYLPROPANOLAMINE HYDROCHLORIDE			
37.5	1057.06 ± 19.85		1.87
50	2430.28 ± 46.20		1.90
62.5	3853.56 ± 70.98		1.84
75	5234.56 ± 26.02		0.49
87.5	6392.26 ± 59.85		0.93

Table 3: Determination of accuracy by HPTLC method

Amount of drug		Amount of drug added		Amount of drug recovered		% Recovery	
CET (µg/spot)	PPA (µg/spot)	CET (µg/spot)	PPA (µg/spot)	CET (µg/spot)	PPA (µg/spot)	CET	PPA
8	40	-	-	-	-	-	-
8	40	2	10	10.3	50.16	103	100.32
8	40	4	20	12.16	60.02	101.3	100.30
8	40	8	40	15.78	80.37	98.6	100.46

Method precision

The RSD values for CET and PPA were found to be 0.007 and 0.013%, respectively. The RSD values were found to be <1%, which indicates that the proposed methods is repeatable.

Intermediate precision

The RSD values were found to be <2%, which indicates that the proposed method is reproducible.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD values for CET and PPA were found to be 740ng/mL and 2710ng/mL respectively. LOQ values for CET and PPA were found to be 2.24µg/mL and 8.2µg/mL respectively. These data show that microgram quantity of both drugs can be accurately determined.

Specificity

Excipients 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 CET and PPA.

Robustness

Peak area and retention time variation were found to be <1%. Also, no significant change in peak area was observed during 24 Hrs and 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 CET and PPA.

Assay of the tablet dosage form (CET 5mg and PPA 25 mg per tablet)

The proposed validated method was successfully applied to determine CET and PPA in their tablet dosage form (ALERID-D). The results obtained for CET and PPA was comparable with the corresponding labeled amounts (Table 4).

Table 4: Assay of marketed formulation

Formulation	Actual Prepared concentration ($\mu\text{g/ml}$)		% CET \pm S.D.	% PPA \pm S.D.
	CET	PPA		
Tablet (Alerid-D)*	500	2500	99.03 \pm 1.07	99.81 \pm 0.915

*Marketed formulation: Alerid-D (Label claim: 5mg Cetizinehydrochloride & 25mg Phenylpropanolamine hydrochloride).

CONCLUSION

Thus, the objective of project work was development and comparison of analytical method of CET and PPA in their combined dosage form. The developed and validated HPTLC method for CET and PPA was found to be simple specific and cost effective and can be routinely applied for analysis of CET and PPA in their combined dosage form. We can say that HPTLC method is more sensitive giving precise results (interday, intraday) for both the drugs & 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 CET and PPA 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 CET and PPA. The method can be used for the routine simultaneous analysis of PPA and CET in pharmaceutical preparations.

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