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Research Article

STABILITY INDICATING THIN LAYER CHROMATOGRAPHY METHOD FOR SIMULTANEOUS ESTIMATION OF PARACETAMOL AND PAMABROM IN BULK AND COMBINED PHARMACEUTICAL DOSAGE FORM

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ABSTRACT
A simple stability indicating thin layer chromatographic method was developed for simultaneous estimation of Paracetamol and Pamabrom in bulk and combined pharmaceutical dosage form. Thin layer chromatography was performed, using a mixture of Chloroform: Acetonitrile (5:5 v/v) as a mobile phase. The R_f value for Paracetamol and Pamabrom was found to be 0.66 and 0.65 respectively. Paracetamol and Pamabrom were subjected to stress conditions including acidic, alkaline, oxidation, thermal and sunlight degradation. The method was validated as per International conference harmonization (ICH) guidelines.
Keywords: Thin layer chromatography (TLC), Stability indicating method, Paracetamol (PCM), Pamabrom (PAM)

INTRODUCTION

PCM (N- 4-hydroxyphenyl acetamide) is an Analgesic-antipyretic drug with poor Anti-inflammatory action and belongs to para-aminophenol derivative categories of NSAIDs. The main mechanism of action of PCM is considered to be the inhibition of cyclooxygenase (COX). Recent findings suggest that it is highly selective for COX-2 because of its selectivity for COX-2 it does not significantly inhibit the production of the pro-clotting thromboxanes.[1] PAM is Diuretic drug that increases the rate of urine flow; however, clinically useful diuretics also increase the rate of excretion of Na^+ (natriuresis) and of an accompanying anion, usually Cl^-.[2] PCM and PAM tablet is used for premenstrual dysphoric disorder, back pain, premenstrual syndrome.[3] The purpose of stability indicating method is to provide evidence on how the quality of a drug substance or product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light and to establish retest period for the drug substance, or a shelf life for the drug product, and recommended storage condition. According to ICH, in forced degradation studies a variety of conditions like pH, light, oxidation, dry heat etc. and separation of drug from degradation product was done.[4-5] PCM is an official in Indian Pharmacopoeia, British Pharmacopoeia and United Pharmacopoeia. PAM is an official in United Pharmacopoeia. Very few analytical methods have been reported for estimation of PCM and PAM in combined pharmaceutical dosage form. There is no stability indicating method has been reported for combination of PCM and PAM till date. Combined formulation of PCM and PAM is approved by Central Drugs. Standard Control Organization (CDSCO) dated on 07/12/2011.[6]

MATERIALS AND METHODS

Chemicals and solvents

Ammonia, ethyal acetate, methanol, acetonitrile, chloroform of AR grade chemicals were used for preparing mobile phase. Pure sample of PCM and PAM were gifted by Astron chemicals, Ahmadabad, India and Ami Life sciences Pvt Ltd, Vadodara, India. MENSODOL™ TAB (PCM-325 mg, PAM-25 mg) was gifted from Anthus Pharmaceuticals, Chennai, India.

Chromatographic conditions

Stationary phase: Precoated Silica gel G60 F_{254} aluminium sheets
Mobile phase: Chloroform: Acetonitrile (5:5 v/v)
Chamber saturation time: 30 minutes
Temperature: ambient

Preparation of mobile phase

Mobile phase was prepared by mixing 5 ml of Chloroform and 5 ml of Acetonitrile, filtered through 0.45 µm whatman filters and sonicated for 10 minutes.

Preparation of standard stock solutions

PCM Standard stock solution (650 µg/ml)
65 mg of PCM was accurately weighed and transferred to a 10 ml volumetric flask and dissolved in methanol and sonicated for about 20 minutes. Volume was made up to the mark with methanol to give a solution containing 650 µg/ml PCM.

PAM Standard stock solution (50 µg/ml)
50 mg of PAM was accurately weighed and transferred to a 10 ml volumetric flask and dissolved in methanol and sonicated for about 20 minutes. Volume was made up to the mark with methanol to give a solution containing 500 µg/ml PAM. 1 ml PAM solution was withdrawn to 10 ml volumetric flask and diluted up to mark with methanolto give a solution containing 50 µg/ml PAM.

Mixed working standard solution (PCM 32.5 µg/ml and PAM 2.5 µg/ml)
0.5 ml of PCM and 0.5 ml PAM standard stock solutions were transferred to a 10 ml of volumetric flask and volume was made up to the mark with methanolto give a solution containing 32.5 µg/ml PCM and 2.5 µg/ml PAM.

Preparation of sample solutions
Twenty tablets were weighed; their average weight was determined and finally powdered. An accurately weighed tablet powder equivalent to 650 mg of PCM and 50 mg PAM was then transferred to 10 ml volumetric flask containing 5 ml methanol and sonicated for 20 minutes. The solution was filtered through whatman filter paper and the volume was adjusted up to the mark with methanol. This solution is expected to contain 650 µg/ml PCM and 50 µg/ml PAM. This solution (0.5 ml) was transferred in to a 10 ml volumetric flask and the volume was adjusted up to mark with methanol to get a concentration of PCM (32.5 µg/ml) and PAM (2.5 µg/ml).

Mobile phase optimization
Forthe estimation of PCM and PAM, different mobile phases in varying composition were tried and tested, which are summarized in following Table 1.

Forced degradation studies
The study was intended to ensure the effective separation of PCM, PAM and its degradation spots of formulation ingredients of PCM and PAM. Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the methods. The proposed stability-indicating TLC method was validated as per ICH guidelines.[5]

Procedure for PCM, PAM and combined standard mixture solution
1 ml standard stock solution of PCM, PAM and mixture of standard solution were taken in 10 ml volumetric flask. These solutions were diluted to up 10 ml with methanol. The resulting solutions (6 µl, 65 µg/ml of PCM, 5 µg/ml of PAM and combined standard mixture of 65 µg/ml for PCM and 5 µg/ml of PAM) were applied on TLC. Chromatogram is shown in Figure 2. Result is shown in Table 2.

Procedure for PCM, PAM and combined standard mixture solution of acid degradation
1 ml standard stock solution of PCM, PAM and mixture of standard solution were taken. 2 ml of 0.1N HCl was added and kept for 1 hour at room temperature in 10 ml volumetric flask. After 1 hour the solutions were neutralized with 2 ml of 0.1N NaOH and volume was made up with methanol. The forced degradation was performed in the dark to exclude the possible degradation effect of light and controls of the respective solution was made at each stage of degradation study to eliminate possible changes due to heat and light. The resulting solutions (6 µl, 65 µg/ml of PCM, 5 µg/ml of PAM and combined standard mixture of 65 µg/ml for PCM and 5 µg/ml of PAM) were applied on TLC. Chromatogram is shown in Figure 3. Result is shown in Table 2.

Procedure for PCM, PAM and combined standard mixture solution of basic degradation
1 ml standard stock solutions of PCM, PAM and mixture of standard solution were taken. 2 ml of 0.1NNaOH was added and kept for 1 hour at room temperature in 10 ml volumetric flask. After 1 hour the solutions were neutralized with 2 ml of 0.1NHCl and make up the volume with methanol. The forced degradation was performed in the dark to exclude the possible degradation effect of light and controls of the respective solutions were made at each stage of degradation study to eliminate possible changes due to heat and light. The resulting solutions (6 µl, 65 µg/ml of PCM, 5 µg/ml of PAM and combined standard mixture of 65 µg/ml for PCM and 5 µg/ml of PAM) were applied on TLC. Chromatogram is shown in Figure 4. Result is shown in Table 2.

Procedure for PCM, PAM and combined standard mixture solution of oxidative degradation
1 ml standard stock solution of PCM, PAM and mixture standard solution were taken in 10 ml volumetric flask. 2 ml of Hydrogen peroxide (3 %) was added 10 and kept for 1 hour at room temperature. After 1 hour solutions were made up with methanol. The forced degradation was performed in the dark to exclude the possible degradation effect of light and controls of the respective solutions were made at each stage of degradation study to eliminate possible changes due to heat and light. The resulting solutions (6 µl, 65 µg/ml of PCM, 5 µg/ml of PAM and combined standard mixture of 65 µg/ml for PCM and 5 µg/ml of PAM) were applied on TLC. Chromatogram is shown in Figure 5. Result is shown in Table 2.

Procedure for PCM, PAM and sample mixture solution in thermal condition
1 ml standard stock solution of PCM, PAM and mixture sample solution were taken solutions were exposed to temperature of 105°c for half an hour in an oven. After half an hour, solutions were diluted with methanol up to 10 ml. The resulting solutions (6 µl, 65 µg/ml of PCM, 5 µg/ml of PAM and combined standard mixture of 65 µg/ml for PCM and 5 µg/ml of PAM) were applied on TLC. Chromatogram is shown in Figure 6. Result is shown in Table 2.

Procedure for stress degradation in sunlight condition
1 ml standard stock solution of PCM, PAM and mixture sample solution were taken and exposed in the sunlight for 2 hours. After 2 hours solutions were diluted with methanol up to 10 ml. The resulting solutions (6 µl, 65 µg/ml of PCM, 5 µg/ml of PAM and combined standard mixture of 65 µg/ml for PCM and 5 µg/ml of PAM) were applied on TLC. Chromatogram is shown in Figure 7. Result is shown in Table 2.

RESULTS AND DISCUSSION

Forced Degradation study
Summary of the results for stress degradation studies of PCM and PAM are shown in the Table 2. The results of the methods lie within the prescribed limit, showing that method is free from interference from excipients. PCM was undergoing acid, basic, oxidation, thermal degradation very rapidly. The reaction showed extensive degradation for PCM with additional spots. R_f value for degradation of PCM in acid, basic, oxidation, thermal degradation were found to be about 0.35, 0.26, 0.12, 0.18 respectively.

CONCLUSION

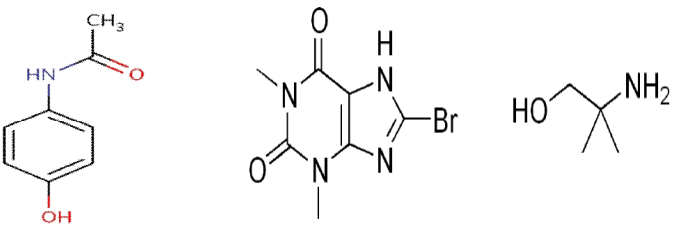
The proposed stability-indicating TLC method was validated as per ICH guidelines and applied for the determination of Paracetamol and Pamabrom in bulk and combined pharmaceutical dosage forms. It can also be successfully applied to perform long-term and accelerated stability studies of combined dosage formulations of Paracetamol and Pamabrom.

Table 1: Mobile Phase Optimization

Trial No.	Mobile phase	R_f of PCM	R_f of PAM	Comment
1.	Chloroform: ammonia: ethyl acetate (6:3:9 v/v)	-	-	No spot is found
2.	Chloroform: Methanol(9:1)	-	-	No spot is found
3.	**Chloroform: Acetonitrile(5.0:5.0 v/v)**	**0.66**	**0.65**	**Spots are fond and separated**

Table 2: Results of Forced Degradation Study of PCM, PAM and Mixture

Stress conditions	Drugs	Time (min)	R_f
Standard	PCM	-	0.66
	PAM		0.65
	Mixture of PCM and PAM		0.66
			0.65
0.1 N HCl	PCM	60	0.66
	PAM		0.64
	Mixture of PCM and PAM		0.66
			0.64
	Degradant of PCM		0.35
0.1 N NaOH	PCM	60	0.58
	PAM		0.56
	Mixture of PCM and PAM		0.58
			0.56
	Degradant of PCM		0.26
3 % H_2O_2	PCM	60	0.91
	PAM		0.89
	Mixture of PCM and PAM		0.91
			0.89
	Degradant of PCM		0.12
Heat exposure	PCM	30	0.74
	PAM		0.72
	Mixture of PCM and PAM		0.74
			0.72
	Degradant of PCM		0.18
Sunlight exposure	PCM	120	-
	PAM		-
	Mixture of PCM and PAM		-
	Degradant of PCM		-



(A) (B)

Figure 1: Chemical structure of (A) Paracetamol (B) Pamabrom

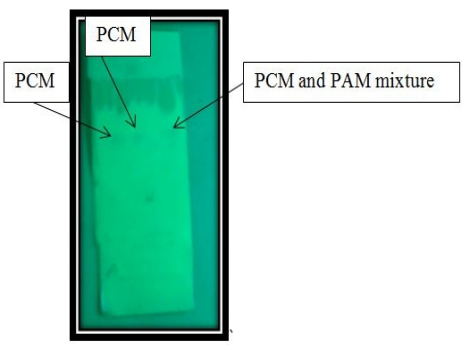


Figure 2 TLC of PCM, PAM and combined standard mixture

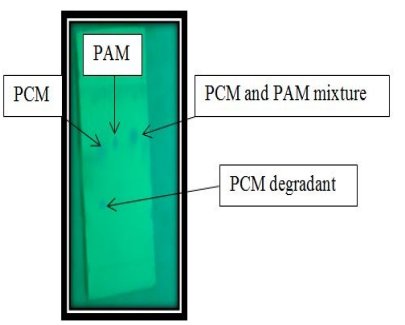


Figure 3 TLC of PCM, PAM and combined standard mixture in acid degradation (0.1 N HCl, 1 hour)

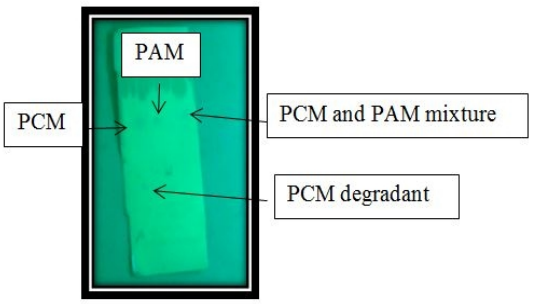


Figure 4 TLC of PCM, PAM and combined standard mixture in basic degradation (0.1 N NaOH, 1 hour)

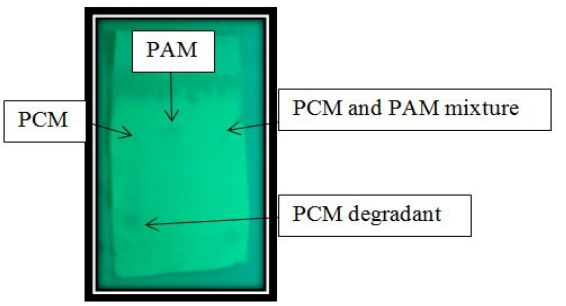


Figure 5 TLC of PCM, PAM and combined standard mixture in oxidation degradation (3% H_2O_2, 1 hour)

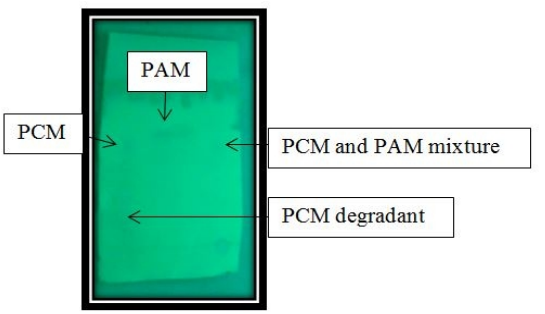


Figure 6 TLC of PCM, PAM and combined tablet mixture in thermal degradation (105°c, 30 min)

Figure 7 TLC of PCM, PAM and combined tablet mixture in sunlight degradation (2 hours)

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