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Research Article

DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR SIMULTANEOUS DETERMINATION OF PAMABROM AND PARACETAMOL IN SYNTHETIC MIXTURE

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ABSTRACT

A new, simple, precise, accurate and selective high performance thin-layer chromatographic (HPTLC) method has been developed and validated for the simultaneous determination of pamabrom and paracetamol in a synthetic mixture. Chromatographic separation was carried out on Merck TLC aluminium sheets of silica gel $60F_{254}$ using Chloroform: Acetonitrile (5.0: 5.0 % v/v) as mobile phase followed by densitometric analysis at 277 nm. This system was found to give compact spots for pamabrom (Rf value of 0.34 ± 0.004) and paracetamol (Rf value of 0.56 ± 0.004). The method was validated in terms of linearity, accuracy, precision, limit of detection, limit of quantification and specificity in accordance with International Conference on Harmonization (ICH) guidelines. The calibration curve was found to be linear between 100 to 350 and 1300 to 4550 ng/spot for pamabrom and paracetamol, respectively with significantly high value of correlation coefficient ($r^2 > 0.99$). The limits of detection and quantitation were found to be 7.65 and 23.17 ng/spot, respectively for pamabrom and 52.63 and 159.48 ng/spot, respectively for paracetamol. The proposed method was found to be accurate, precise, reproducible, specific and sensitive and can be applicable for the simultaneous determination of pamabrom and paracetamol in Synthetic mixture.

KEY WORDS: Pamabrom, Paracetamol, HPTLC, simultaneous determination, validation.

INTRODUCTION

Pamabrom (PAM) is chemically 8-Bromo-3,7-dihydro-1,3-dimethyl-1-H-purine-2,6-dione compound with 2-amino-2-methyl-1-propanol (1:1) (Figure 1) is a diuretic drug[1,2]. It is official in US Pharmacopoiea[3]. It is assayed by Liquid chromatography as per USP. Literature review reveals HPLC method for estimation of PAM in pharmaceutical dosage form[4]. Paracetamol (PCM) is chemically N-(4-hydroxy-phenyl) ethanamide (Figure 2) used as analgesic and antipyretic.[2,5] It is official in IP[6], BP[7], USP[3] and JP[8] and is estimated by UV-Visible Spectrophotometric method as per IP, USP and JP. In BP a redox titration for PCM is given for drug substance. Literature review also reveals HPLC, UV spectrophotometric and HPTLC method for the estimation of PCM with other drugs and one HPLC method with PAM in human plasma.[9] Literature survey does not reveal any simple HPTLC method for simultaneous determination of PAM and PCM in Pharmaceutical dosage form/Synthetic mixture. The present developed method is new, simple, precise, accurate and selective for simultaneous determination of both drugs in their synthetic mixture as per International Conference on Harmonization (ICH) guidelines.[10]

MATERIALS AND METHODS

Material and reagents

Pamabrom was kindly supplied by Suven Life sciences, Hyderabad, India, as gratis sample and Paracetamol was obtained from A.R. College of Pharmacy & G.H. Patel Institute of Pharmacy, v.v. nagar, Gujarat, India. Chloroform and Acetonitrile were used as solvents to prepare the mobile phase. All reagents used were of analytical reagent grade (Allied Chemical Corporation, Vadodara).

Instrumentation and chromatographic conditions

The HPTLC system (Camag, Muttenz, Switzerland) consisted of Linomat V autosprayer connected to a nitrogen cylinder, a twin trough chamber (20 × 10 cm), a derivatization chamber, and a plate heater. Pre-coated silica gel 60 F254 TLC plates (10 × 10 cm, layer thickness 0.2 mm (E. Merck KGaA, Darmstadt, Germany) was used as stationary phase. TLC plates were pre-washed twice with 10 mL of methanol and activated at 80^0C for 5 min prior to sample application. The standard and formulation samples of PAM and PCM in mixture were spotted on Pre-coated TLC plates in the form of narrow bands of lengths 6 mm. Samples were applied under continuous drying stream of nitrogen gas at constant application rate of 150 nL/s. The mobile phase consists of Chloroform: Acetonitrile (5: 5, v/v). Linear ascending development was carried out in twin trough chamber (10 x 10 cm). The optimized chamber saturation time for mobile phase was 30 min, at 25^0C ± 2; the length of chromatogram run was 70 mm and TLC plates were air dried. Densitometric scanning was performed on CAMAG TLC scanner III in absorbance mode and operated by winCATS planar chromatography version 1.3.4. The source of radiation utilized was deuterium lamp. The spots were analyzed at a wavelength of 277 nm. The slit dimensions used in the analysis were length and width of 5 mm and 0.45 mm, respectively, with a scanning rate of 20 mm/s. The parameters were selected as recommended by the CAMAG TLC scanner III manual. Evaluation was performed using linear regression analysis via peak areas.

Standard solutions and calibration curves

Standard stock solution of combined drugs was prepared containing 0.025 g/L of PAM and 0.325 g/L of PCM in methanol. Which were further diluted with methanol to obtain 25 µg/mL of PAM and 325 µg/mL of PCM. Calibration was done by applying mixture of standard solutions ranging from 4.0 – 14.0 µL by Hamilton syringe with the help of Linomat V autosprayer on TLC plate that gave concentration 100-350 ng/spot for PAM and 1950-4550 ng/spot for PCM, respectively. Each concentration was spotted six times on TLC plates. From the developed plates calibration curve was plotted as peak areas versus corresponding concentrations (Figure 5 and 6).

Method Validation

HPTLC method development

In trial phase chloroform and methanol in ratio of 9:1 (v/v) was used and separation and peak shape is good but it shows

solvent fronting due to high content of chloroform. Then acetonitrile was incorporated to the mobile phase composition and tried with different ratios. By decreasing the chloroform in mobile phase composition solvent fronting was resolved and ultimately, mobile phase consisting chloroform and acetonitrile (5: 5 v/v) showed good resolution without solvent fronting. Both the peaks were symmetrical in nature and no tailing was observed when plate was scanned at 277 nm. The chamber was saturated with the mobile phase for 30 min at room temperature.

Linearity

Linearity responses for PAM and PCM were assessed in the concentration range 100-350 ng/spot and 1300-4550 ng/spot of standard solutions, respectively.

Precision

Precision of the method was determined in the terms of intra-day and inter-day variation (%RSD). Intra-day precision (%RSD) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Inter-day precision (%RSD) was assessed by analyzing drug solutions within the calibration range on three different days over a period of 7 days.

Accuracy

To the pre-analyzed sample a known amount of standard solution of pure drug (PAM and PCM) was spiked at three different levels. These solutions were subjected to re-analysis by the proposed method.

Sensitivity

The sensitivity of measurement of PAM and PCM by the use of proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ were calculated by equation.

Specificity

Specificity of the method was ascertained by analyzing standard drug and sample. The mobile phase resolved both the drugs very efficiently as shown in Figure 7. The spot for PAM and PCM was confirmed by comparing the R_f and spectra of the spot with that of standard. The wavelength 277 nm for detecting peak purity of PAM and PCM was assessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) positions of the spot.

Repeatability

Repeatability of sample application was assessed by spotting 8µL (200 ng/spot of PAM and 2600 ng/spot of PCM) of drug solution six times on a TLC, followed by development of plate and recording the peak area for six spots.

Analysis of PAM and PCM in synthetic mixture

Pamabrom (25 mg) and paracetamol (325 mg) standard drug powders were accurately weighed and then mixed with commonly used formulation excipients (113.35 mg avicel PH-101, 1.65 mg stearic acid[11]). The synthetic mixture was then transferred to 100 mL volumetric flask containing 50 mL methanol and sonicated for 20 min. The solution was filtered through 0.45 µm filter (Millifilter, MA) and the volume was adjusted up to mark with methanol. From the above solution 1 mL was taken into a 10 mL volumetric flask and the volume was adjusted up to mark with methanol to get a final concentration of PAM (25 µg/mL) and PCM (325 µg/mL). 6µL of this solution applied on TLC plate followed by development and scanning & the analysis was repeated for three times.

Table 1: Result of Calibration reading for PAM and PCM

Conc. (ng/spot)	R_f	Area Mean (n=6) ± SD	%RSD	Conc. (ng/spot)	R_f	Area Mean (n=6) ± SD	%RSD
100	0.33	1950.03± 14.596	0.748	1300	0.55	4952.4± 26.47	0.5346
150	0.34	2750.88± 20.430	0.723	1950	0.56	6740±17.01	0.2524
200	0.34	3617.42± 28.83	0.787	2600	0.56	8078.533±54.31	0.6722
250	0.34	4422.5± 24.494	0.561	3250	0.56	9414.2±43.70	0.4642
300	0.34	5111.83± 56.738	1.11	3900	0.56	10681.22±24.68	0.2311
350	0.34	5763.9± 29.896	0.518	4550	0.56	11887±47.01	0.3955

Table 2: Statistical Data of PAM and PCM

Parameters	Results	
	PAM	PCM
Linear Range(ng/spot)	100-350	1300-4550
Slope	15.329	2.119
Intercept	490.71	2414.8
Std. Deviation of Slope	0.145	0.0121
Std. Deviation of Intercept	35.692	33.656
Limit of Detection(ng/spot)	7.65	52.63
Limit of Quantification(ng/spot)	23.17	159.48
Regression Equation	y = 15.329x + 490.71	y = 2.1193x + 2414.8
Co-Relation Co-Efficient (r)	0.9985	0.9977
Co-Efficient of Determination (r^2)	0.9972	0.9955

Table 3: Intra Day and Inter Day study of PAM

Concentration (ng/spot)	Intra Day Area Mean (n=3) ± SD	%RSD	Inter Day Area Mean (n=3) ± SD	%RSD
150	2727.07±23.18	0.85	2708.47±27.59	1.019
200	3615.3±28.39	0.785	3593.43±36.12	1.005
250	4430.7±38.21	0.862	4422.8±34.89	0.789

Table 4: Intra Day and Inter Day study of PCM

Concentration (ng/spot)	Intra Day Area Mean (n=3) ± SD	%RSD	Inter Day Area Mean (n=3) ± SD	%RSD
1950	6737.53±16.84	0.25	6697.13±41.79	0.624
2300	8094.57±29.23	0.361	8062.13±54.70	0.679
3250	9412.03±43.18	0.459	9375.77±40.29	0.43

Table 5: Determination of Accuracy for PAM and PCM

Concentration of Sample Taken (ng/spot)	Concentration of Pure API spiked (ng/spot)	Total Concentration (ng/spot)	Mean Total Concentration Found (n=3) (ng/spot)	%Recovery Mean (n=3)	%RSD
PAM 100	50	150	148.53	99.02	0.273
	100	200	200.01	100.01	0.241
	150	250	252.21	100.88	0.161
PCM 1300	650	1950	1969.55	101.00	0.274
	1300	2600	2622.25	100.86	0.363
	1950	3250	3270.69	100.64	0.334

Table 6: Repeatability study of PAM and PCM

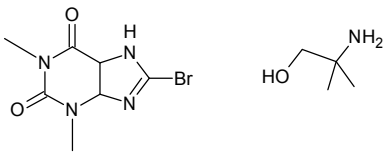
Concentration	PAM (200 ng/spot)	PCM (2600 ng/spot)
Area	3599	8100.1
	3623	8167.4
	3611.8	8045
	3579.6	8122
	3567	8099.8
	3601.1	8178
Mean	3596.92	8118.72
± SD	20.584	49.059
%RSD	0.572	0.604

Table 7: Assay Result of Synthetic mixture

Formulation	Synthetic mixture	
	PAM	**PCM**
Actual Concentration (ng/spot)	150	1950
Concentration Obtained (ng/spot)	149.03± 2.628	1964.10± 0.749
%Purity	99.35	100.72
%RSD	0.669	0.518
Limit[3, 6]	72.2%-76.6% for theophylline and 24.6%-26.6% for 2-amino-2-methyl-1-propanol	98.5% -101.0%

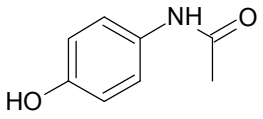
Table 8: Validation Parameters

Summary of Validation Parameters		
	PAM	**PCM**
Recovery (%)	99.02-100.88	100.64-101.0
Repeatability (%RSD)	0.572	0.604
Precision (CV)		
Intra-day (n=3)	0.00833	0.0077
Inter-day (n=3)	0.00938	0.00577
Specificity	Specific	Specific
Selectivity	Selective	Selective



1:1 Mixture of 2-amino-2-methyl-1-propanol and 8-bromotheophyllinate

Figure 1: Structure of Pamabrom



N-(4-hydroxy phenyl)-ethanamide

Figure 2: Structure of Paracetamol

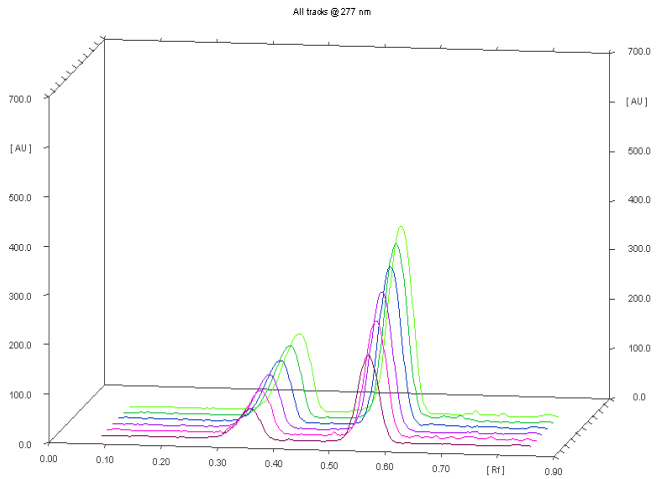


Figure 3: 3D Representation of Densitogram for Calibration curve of PAM and PCM

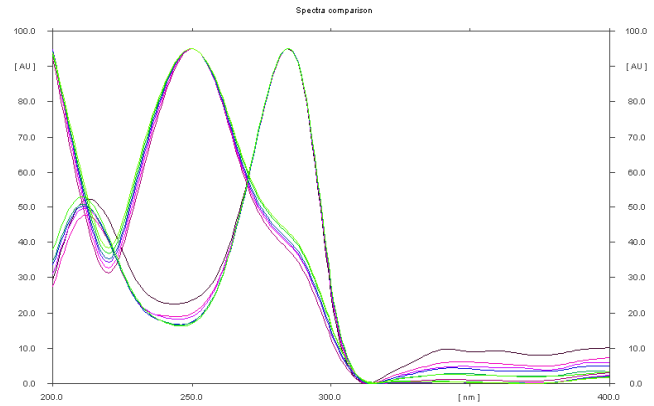


Figure 4: Overlay UV Absorption (Reflectance Mode) of the corresponding spots for PAM and PCM

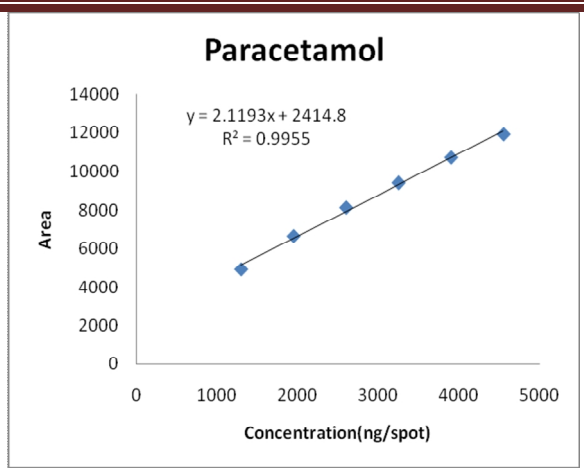


Figure 6: Calibration curve of PCM in Methanol at 277 nm

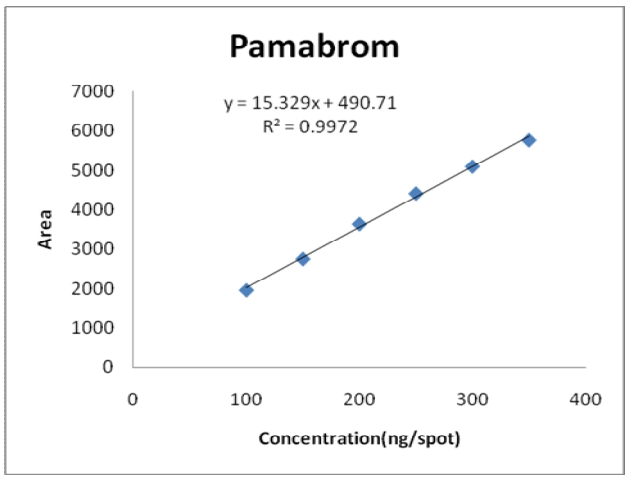


Figure 5: Calibration curve of PAM in Methanol at 277 nm

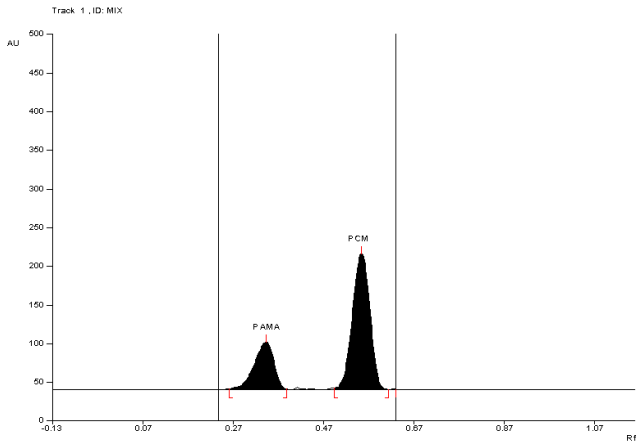


Figure 7: HPTLC Chromatogram of Standard PAM and PCM in mixture

RESULT AND DISCUSSION

Method development

The TLC procedure was optimized for simultaneous determination of PAM and PCM. The mobile phase Chloroform: Acetonitrile (5: 5 v/v) resulted in good resolution with sharp and symmetrical peaks of R_f 0.34 ± 0.004 for PAM and 0.56 ± 0.004 for PCM. It was observed that pre-washing of TLC plates with methanol (followed by drying and activation) and pre-saturation of TLC chamber with mobile phase for 30 min (optimum chamber saturation time) ensured reproducibility and proper peak shape of both drugs (Figure 3 and 7).

Validation

Linearity

Linear regression data for the calibration plots revealed linear relationships between area and concentration over the ranges 100-350 ng/spot for PAM and 1300-4550 ng/spot for PCM. The linear equations for the calibration plots were y = 15.329x + 490.71 and y = 2.1193x + 2414.8 with correlation coefficient (r) being 0.9973 and 0.9984 for PAM and PCM, respectively (Table 1 and 2).

Precision

The precision of the method was expressed as relative standard deviation (RSD, %). The results listed in Table 3 and 4 reveal the high precision of the method.

Accuracy

When the method was used for accuracy and subsequent analysis of both drugs from the synthetic mix, and spiked with 50, 100, and 150% of additional drug, the recovery was 99.02- 100.88 % for PAM and 100.64- 101.0 % for PCM (Table 5).

Sensitivity

The LOD and LOQ were calculated by equation. The LOD and LOQ values were 7.65 and 52.63 ng/spot for PAM and 23.17 and 159.48 ng/spot for PCM.

Specificity

The peak purity of PAM and PCM was assessed by comparing their respective spectra at peak start, apex and peak end positions of the spot i.e., r (S, M) = 0.9997 and r (M, E) =0.9993 for PAM and r (S, M) = 0.9991 and r (M, E) = 0.9995 for PCM. Good match was obtained between standard and sample spectra of PAM and PCM respectively. (Figure 4)

Repeatability

The % RSD for peak area values of PAM and PCM was found to be 0.572 and 0.604, respectively as given in Table 6.

Analysis of PAM and PCM in synthetic mixture

When synthetic mixture was analyzed, PAM and PCM gave sharp and well defined peaks at R_f 0.34± 0.004 and 0.56± 0.004, respectively, when scanned at 277 nm. The results in Table 7 indicate that there was no interference from the excipients commonly present in the tablets. The % purity was 99.35% for PAM and 100.72% for PCM.

CONCLUSION

The developed HPTLC method is simple, precise, accurate and reproducible and can be used in future for simultaneous determination of PAM and PCM in tablets as method gave results within range for synthetic mixture. The method was validated as per International Conference on Harmonization (ICH) guidelines.

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REFERENCES

1. Pamabrom drug profile, [cited 2012 July 7], Available from: http://en.wikipedia.org/wiki/Pamabrom
2. Drug information of Paracetamol and Pamabrom, [2012 September 30], Available from: http://druginformation-directory.blogspot.in/2010/05/acetaminophen-and-pamabrom.html
3. United State Pharmacopoeia- 34 and National Formulary-29, Asian edition, United State Pharmacopoeia Convention, Rockville, MD, USA, 2011; Vol II: 1720,3800.
4. Chen Z, Wen J, Li J, "Determination of pamabrom in pamabrom and pamabrom paracetamol tablets using RP-HPLC", Journal of Zhengzhou University(Medical Sciences),2008;1.
5. Paracetamol drug profile,[Cited 2012 July 7], Available from: http://en.wikipedia.org/wiki/Paracetamol
6. Indian pharmacopoeia, Ministry of health and family welfare, 6th edition, Indian Pharmacopoeial Commission, Ghaziabad, India, 2010; 2: 1859-61.
7. British pharmacopoeia, 6th edition, The Stationary Office, London Medicines and Healthcare Product Regulatory Agency, 2010;Vol II: 1612.
8. Japanese Pharmacopoeia, Society of Japanese Pharmacopoeia, 15th edition, Shibuya Tokyo, Japan, 2006: 267-68.
9. Zhou L, Gu L, Wang Y, "Determination of two constituents in compound acetaminophen and pamabrom tablets in human plasma by high performance liquid chromatography", Ch J of new drugs and Cli Rem, 2007;26:187-190.
10. ICH, Q2B (R1) Validation of Analytical Procedure: Methodology, International Conference on Harmonization, IFPMA, Geneva, Switzerland.
11. Lachman L, Lieberman HA, "The Theory and practice of industrial pharmacy", Special Indian dition, CBS publishers and distributors, 2009, 344.

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