Research Article

Development and Validation of HPTLC Method for Simultaneous Estimation of Paracetamol and Pamabrom in Bulk and Synthetic Mixture

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ABSTRACT

A simple, precise, accurate and specific high performance thin layer chromatographic method has been developed for the simultaneous estimation of pamabrom and paracetamol in tablet dosage form. The method employed silica gel $60F_{254}$ precoated plates as stationary phase and a mixture of Chloroform: ammonia: ethyl acetate (6:3.9: 0.1)as mobile phase.The developed method was validated for accuracy, linearity, precision, and specificity, limit of quantitation and limit of detection as per ICH guidelines. The $R_f$values for pamabrom and paracetamolwere found to be 0.39±0.1 and 0.78±0.2 respectively. The calibration curve was found to be linear between 100-600 ng/spot for pamabrom and 2000-4500 ng/spot for paracetamol. The limit of detection and limit of quantitation for pamabrom were found to be 8.77ng/spotand 7.73 ng/spotand for paracetamol 26.58ng/spot and 23.04ng/spot respectively. The proposed method can be successfully used to determine the drug content in synthetic mixture.

Keywords: Pamabrom, Paracetamol, HPTLC, Method Development, Validation.

INTRODUCTION

Pamabrom is chemically, 1:1 mixture of 2-amino-2-methyl-1-propanol and 8-bromotheophyllinate, it has a diuretic property[1]. It is official in US pharmacopoeia[2]. It is assayed by liquid chromatography as per USP[3]. Pamabrom, a xanthine derivative, is a safe and effective diuretic in relieving the water-accumulation symptoms of water-weight gain, bloating, swelling, and/or full feeling associated with the premenstrual and menstrual periods. As an over-the-counter diuretic, pamabrom is typically recommended to women to alleviate symptoms associated with menstrual cycles. Physicians also prescribe the use of such a water pill for other conditions involving water-weight gain[4]. The medication works, as all diuretics, by pulling excessive water from throughout the body and increasing how frequently patients need to urinate. By flushing excess water from the system through increased urination, patients gain relief from the uncomfortable bloating and swelling associated with water-weight gain. Literature review reveals plasma HPLC method for estimation of pamabrom in pharmaceutical dosage form[5]. Paracetamol is chemically N-(4hydroxy-phenyl) etanamidea compound with analgesic and antipyretic properties[6].It is much safer than aspirin in terms of gastricirritation, ulceration and bleeding. It is official in IP[7],JP[8], USP and BP[9] and is estimated by UV-visible spectrophotometric method as per IP, USP and JP. In BP a redox titration for paracetamol is given for drug substance,literature review also reveals HPLC,UV spectrophotometric and HPTLC method for estimation of paracetamol with other drugs. The suggested method is validated by using ICH validation parameters like accuracy, precision, linearity, LOD and LOQ respectively[10-16].However, there is no analytical method reported for the Estimation of pamabrom and paracetamol in combination. The aim of present work to develop the simple, economical, accurate, HPTLC method for the estimation of pamabrom and paracetamol in bulk and synthetic mixture. This method was validated as per ICH guideline.

MATERIALS AND METHODS

Pamabromreceived as gift samples from Pan drug Ltd, Ahmedabad, Indiaand paracetamol were received as gift samples from Emcure Pharmaceuticals Ltd, Pune. The solvent used

were ammonia(AR grade), ethyl acetate(AR grade), chloroform (AR grade) purchased from Merck Chemicals (Mumbai, India).

Equipment

Camag HPTLC system consisting Linomat 5 applicator, camag TLC scanner 3 and WinCATS software V-1.4.4 was used for chromatographic separation. Spotting of samples was done by using Hamilton microliter syringe.

Preparation of Standard stock solution of pamabrom

Standard stock solution of pamabrom was prepared by dissolving 10 mg of drug in 100 ml of methanol to get concentration 100µg/ml. 2 ml standard stock solution of pamabrom was then diluted up to 10 mlmethanol to get working standard solution 20 µg/ml.(100 ng/5µl).From the stock solution 2, 4, 6,8 and 10µl were applied on TLC plate, at a distance 10mm from both x-axis and y-axis.

Preparation of Standard stock solution of paracetamol

Standard stock solution of paracetamol was prepared by dissolving 100 mg of drug in 100 ml of methanol to get concentration 1000µg/ml. 2 ml standard stock solution of paracetamolwas then diluted up to 10 mlmethanol to get working standard solution 20 µg/ml(100 ng/5µl).From the stock solution 2, 4, 6,8 and 10µl were applied on TLC plate, at a distance 10mm from both x-axis and y-axis.

Preparation of synthetic mixture

The synthetic mixture of Pamabrom and paracetamol was prepared by accurately weighed 25 mg of Pamabrom and paracetamol 500 mg. then mixed with commonly used formulation excipients such as starch, magnesium stearate and lactose which were used in tablet formulation, were added in this mixture. The synthetic mixture was then transferred to 100 ml volumetric flask containing 70 ml of methanol and sonicatad for 20 minutes. This solution was filtered through the 0.45µm filter (Millifilter) and the volume was adjusted up to mark with methanol to get final concentration of paracetamol (500µg/ml) and pamabrom (25µg/ml).6µL of this solution applied on TLC plate followed by development and scanning and the analysis was repeated.

Method Validation

The method of analysis was validated as per the recommendations of ICH for the parameters like linearity, accuracy, limit of detection, limit of quantitation, specificity, intra-day and inter-day precision, repeatability of peak area measurement and repeatability of sample applicationand the observations are reported in Table 6.

A solvent system that would give dense and compact spots with appropriate and significantly different compact spots with appropriate and significantly different R_f values was desired for quantification of pamabrom and paracetamol in pharmaceutical formulations. The mobile phase consisting of Chloroform: ammonia: ethyl acetate (6:3.9: 0.1)gave R_f values of 0.39 and 0.78 for pamabromand paracetamolrespectively.

Linearity

A stock solution of pamabrom (100µg/ml) was prepared in methanol. Different volumes of stock solution as 1, 2,3,4,5, and 6µl were spotted on TLC plate to obtain concentration of 100, 200, 300,400,500 and 600ng/band of pamabrom, respectively. The data of peak area versus drug concentration were treated by linear least-square regression analysis. The response for the drug was found to be linear in the concentration range 100–600ng/band.A stock solution of paracetamol(1000µg/ml) was prepared in methanol. Different volumes of stock solution as 2, 2.5, 3, 3.5,4 and 4.5µl were spotted on TLC plate to obtain concentration of 2000, 2500,3000, 3500, 4000, and 4500ng/band of paracetamol, respectively. The data of peak area versus drug concentration were treated by linear least-square regression analysis. The response for the drug was found to be linear in the concentration range 2000–4500ng/band. The calibration curve is shown in fig.3 and fig.4.The observations are reported in Table 1 and 2.

Precision

The precision study of pamabrom and paracetamol the method was demonstrated by intra-day and inter-day variation studies. In the interday and intraday studies three different concentrations 250, 300 and 350ng/band and 2500, 3000 and 3500 ng/spot of standard stock solution were spotted in triplicate and were analyzed. The percentage RSD was calculated.The observations are reported in Table 3.

Limit of Detection (LOD)

LOD is calculated from the formula: -

$$DL = \frac{3.3\ \sigma}{S}$$

Where,

σ = the standard deviation of the response for the lowest conc. in the range

S = the slope of the calibration curve.

Limit of Quantification (LOQ)

The quantitation limit (QL) may be expressed as:

$$QL = \frac{10\ \sigma}{S}$$

Where,

σ = the standard deviation of the response for the lowest conc. in the range

S = the slope of the calibration curve.

Repeatability

Repeatability of sample application was assessed by spotting 200ng/spot of pamabrom and 2500 ng/spot of paracetamol solution six times on TLC plate, followed by development of plate and recording the peak area for six spots. Results were reported in terms relative standard deviation.Repeatability of measurement of peak area was determined by spotting 200ng/spot of pamabrom and 2500 ng/spot of paracetamol solution on TLC plate and developing the plate. The separated spot was scanned six times without changing the position of the plate and results were reported in terms relative standard deviation.The observations are reported in Table 4.

Accuracy

The accuracy of the method was determined by calculating percentage recovery of Pamabrom and paracetamol for both the drugs, recovery studies were carried out by applying the method to drug sample to which known amount of pamabrom and paracetamol corresponding to 80, 100 and 120 % of label claim had been added (standard addition method). At each level of the amount six determinations were performed and the results obtained were compared. The observations are reported in Table 5

Specificity

To confirm the specificity of the proposed method, the solution of the synthetic mixture was spotted on the TLC plate, developed and scanned. It was observed that the excipients present in the synthetic mixture did not interfere with the peak of pamabrom and paracetamol.

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase ratio, dimensions of chamber were altered and the effects on the Rf values and area were noted. In case of mobile phase ratio, the percentage change in Rf was not more than 0.4 % & the percentage change in area was not more than 0.06 %. In case of Chamber change, percentage change in Rf value was not more than 0.01 % & the percentage change in area was not more than 0.06 %. The method was found to be robust since, the monitored parameters were not significantly affected.

RESULTS AND DISCUSSION

The mobile phase consisting ofChloroform: ammonia: ethyl acetate (6:3.9: 0.1)gave R_f values of 0.38 ±0.1and 0.78±0.2 for pamabrom and paracetamol respectively. The linearity of pamabromand paracetamol was found to be in the range of 100 ng/spot to 600 ng/spot and 2000 ng/spot to 4500 ng/spot respectively. The correlation coefficient of pamabromand paracetamol was 0.9999±0.0001 and 0.9912±0.00015 respectively. LOD and LOQ were found to be 8.77 ng/spotand 7.73ng/spot for pamabromand 26.58ng/spot and 23.04ng/spotfor paracetamol respectively.

The intra-day and inter-day %RSD were found to be less than 2 for both pamabromand paracetamol. These values indicate that the method is precise.The percentage recovery was found to be 100.11% and 99.79% for pamabromand paracetamol respectively, ensuring that the method is accurate.

For the repeatability of sample application %RSD of pamabrom and paracetamol was found to be 0.12 and 0.01 respectively. For the repeatability of measurement of peak area %RSD of pamabrom and paracetamol was found to be 0.0045 and 0.0653 respectively. Specificity of the method was well demonstrated by efficient separation of both drugs by the solvent system.

The developed HPTLC technique is simple, precise, specific and accurate, and statistical analysis proved that method is reproducible and selective for the analysis of pamabromand paracetamol simultaneously in bulk drug and synthetic mixture.

ACKNOWLEDGEMENT

Authors are grateful to theAyushakti Ayurved Pvt. Ltd., Palghar for providing instrumentation and necessary facilities to carry out the research work.Thanks are also extended to Emcure Pharmaceuticals Ltd, Pune, India andPan drug Ltd, Ahmedabad, India for providing gift samples of the pure drugs for research work.

Table 1: Result of calibration reading for pamabrom and paracetamol

Conc. (ng/spot)	Rf	Area mean ± SD*	% RSD	Conc. (ng/spot)	Rf	Area mean ± SD*	%RSD
100	0.39	639.65±7.01	0.15	2000	0.78	6893.66±2.69	0.06
200	0.38	1275.36±2.43	0.02	2500	0.79	8069.69±3.18	0.09
300	0.39	1919.62±3.20	0.10	3000	0.78	9689.39±2.43	0.05
400	0.38	2558.39±6.43	0.09	3500	0.78	9987.78±1.84	0.02
500	0.39	3199.68±4.56	0.13	4000	0.78	11269.36±2.39	0.08
600	0.39	3839.65±5.69	0.12	4500	0.78	12986.89±4.79	0.11

Table 2: Linear regression data for calibration curves

Parameters (units)	Pamabrom	Paracetamol
Linearity range (ng/spot)	100-600	2000-4500
r^2±SD	0.9999±0.0001	0.9912±0.00015
Slope ±SD	6.4123±0.008591	2.0855±0.001801
Intercept ±SD	0.3613±1.35778	2768.8±4.1146

Table 3: Intraday and Interday study of pamabrom and paracetamol

Drug	Amount applied (ng/spot)	Intraday precision± SD*	%RSD	Interday precision± SD*	%RSD
Pamabrom	250	1597.63±15.84	0.15	1586.29±13.01	0.09
	300	1912.39±22.20	0.13	1915.38±12.69	0.11
	350	2234.69±13.01	0.11	2239.46±13.56	0.13
Paracetamol	2500	8072.38±06.08	0.06	8077.99±04.20	0.21
	3000	9695.69±05.10	0.03	9696.78±09.01	0.26
	3500	9985.45±03.02	0.10	9989.10±06.84	0.11

Table 4: Repeatability study of pamabrom and paracetamol

Concentration	Pamabrom (200ng\spot)	Paracetamol (2500ng\spot)
Area	1275.69	8070.10
	1280.12	8071.69
	1278.36	8073.22
	1276.96	8069.99
	1278.45	8072.86
	1277.36	8069.97
Mean	1277.82	8071.30
SD	1.51	1.49
%RSD	0.12	0.01

Table 5: Recovery study of pamabrom and paracetamol

Drug	Conc.(ng/spot) Spiked of synthetic mixture	Conc.of Pure drug (ng/spot)	Mean Area	Recovered conc.	% Recovery	%RSD
Pamabrom	200	160	2235.36	359.60	99.79%	0.15
	200	200	2559.45	401.03	100.02%	0.11
	200	240	2876.31	449.79	100.11%	0.09
Paracetamol	1500	1200	8879.54	2792.99	99.97%	0.11
	1500	1500	9684.26	2997.39	100.02%	0.07
	1500	1800	10627.36	3295.85	99.97%	0.09

Table 6: Summary of validation parameters

Parameter		Pamabrom	Paracetamol
Linearity range(ng/spot)		100-600	2000-4500
Regression coefficients (r^2)		0.9999±0.0001	0.9912±0.0015
Limit of detection (ng/spot)		8.77	7.73
Limit of quantitation (ng/spot)		26.58	23.04
Precision	Intra-day (%RSD)	0.31	0.44
	Inter-day (%RSD)	0.66	0.31
Rf value		0.39±0.1	0.78±0.2

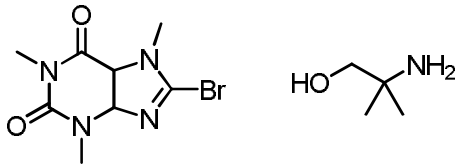

Fig. 1: Chemical structure of pamabrom

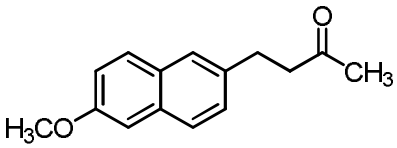

Fig. 2: chemical structure of paracetamol

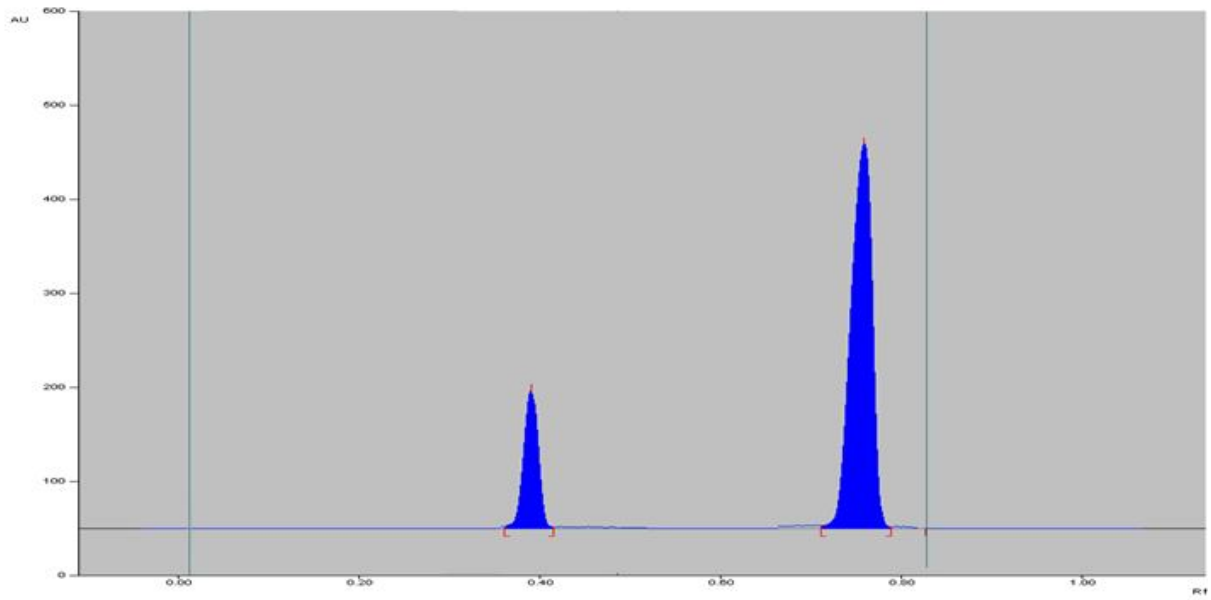

Fig. 3: HPTLC chromatogram of pamabrom (400ng/spot) of and paracetamol (3500ng/spot)

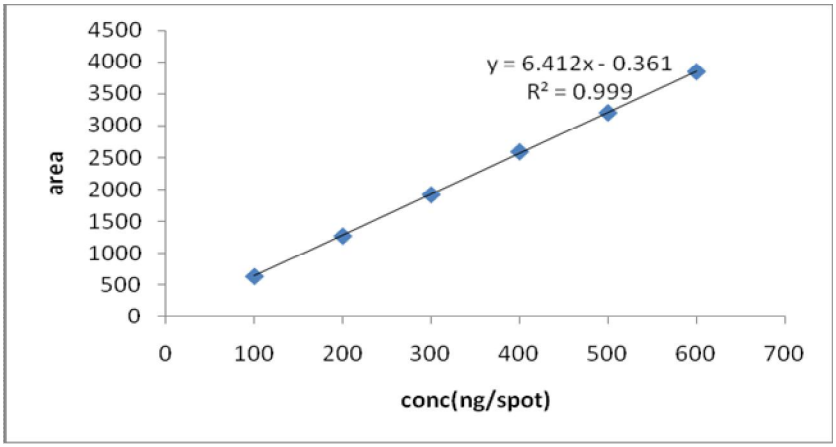

Fig. 4: calibration curve for pamabrom

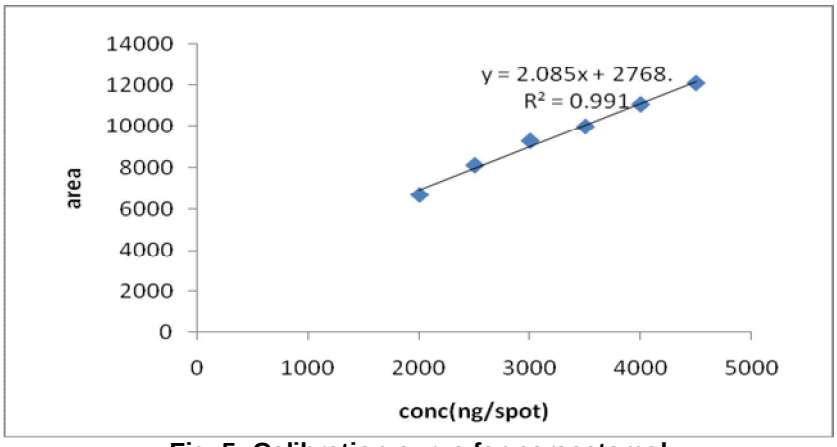

Fig. 5: Calibration curve for paracetamol

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