Research Article

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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF PARACETAMOL AND PAMABROM IN BULK DRUG AND MARKETED FORMULATION

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ABSTRACT:

Aim: A HPLC method has been described for simultaneous determination of Pamabrom and Paracetamol in marketed formulation. Methods: This method is based on HPLC separation of the two drugs on the Kromasil C18 column (250 mm × 4.6 mm, 5.0µ), with isocratic conditions and mobile phase containing water: methanol: acetonitrile in a ratio of 70:20:10, v/v/v at a flow rate of 1 ml/min, using UV detection at 279 nm. This method has been applied to formulation without any interference of excipients of formulation. Results: The linear regression analysis data for the calibration plots showed a good linear relationship over the concentration range of 100 – 500 µg/ml for Paracetamol and 5 – 25 µg/ml for Pamabrom respectively. The method was validated as per the ICH guidelines. The limit of detection (LOD) and limit of quantitation (LOQ) was 4.28 µg/ml and 12. 99 µg/ml for Paracetamol and 0.23 µg/ml and 0.95 µg/ml for Pamabrom, respectively. Result of assay and recovery study was statistically evaluated for its accuracy and precision. Conclusion: According to the validation results, the proposed method was found to be specific, accurate, precise and economic for the estimation of Paracetamol and Pamabrom in bulk and tablet dosage form.

KEYWORDS:

Paracetamol, Pamabrom, HPLC, Validation, ICH guidelines

INTRODUCTION

Pamabrom (PBM) is chemically, 1:1 mixture of 2-amino-2-methyl-1-propanol and 8-bromotheophyllinate (Figure 1), 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. It works, as all diuretics, by pulling excessive water from throughout the body. Literature review reveals plasma HPLC method for estimation of Pamabrom in pharmaceutical dosage form [4-5].

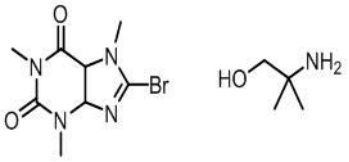

Figure1: Chemical structure of Pamabrom

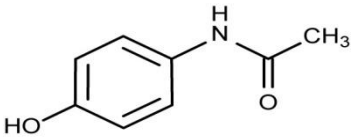

Figure 2: Chemical structure of Paracetamol

Paracetamol (PCM) chemically it is N-(4-hydroxyphenyl) acetamide (Figure 2). Paracetamol is official in IP, BP, USP and JP [6-8]. It is classified as a mild analgesic and antipyretic properties. It is much safer than aspirin in terms of gastric irritation, ulceration and bleeding. Literature survey revealed that number of methods has been reported in literature for the individual analysis of paracetamol and pamabrom by UV spectrophotometric, RP-HPLC and HPTLC method alone and in combination with other drugs. The suggested method was validated as per ICH guidelines. The validation parameters studied was accuracy, precision, linearity, LOD and LOQ, robustness[9-13]. However, none of the previous method reports was sensitive, selective and economic enough for the estimation of pamabrom and paracetamol. The aim of present work was to develop the comparatively simple, economical, accurate HPLC method for the estimation of paracetamol and pamabrom in bulk drug and tablet dosage form.

MATERIALS AND METHODS

Materials & Reagents

Working standards of pharmaceutical grade PCM, was received as gift sample from Emcure Pharmaceuticals Ltd, Pune and PBM was obtained as generous gift from Pan Drugs, Ahmadabad, India. Combination tablet BACKAID MAX containing 500 mg PCM and 25 mg PBM was used for study. All the chemicals used were of HPLC grade, purchased from Merck Chemicals, India.

Instrumentation

The instrument used was Waters 510 HPLC system equipped with a rheodyne injecting facility programmed at 20µl capacity per injection was used. The detector consisted of UV–Visible detector operated at wavelength 279 nm. Data acquisition was made with DataAce software. The column used was Kromasil C-18 (250mm x 4.6mm, 5µm). Analytical balance used for weighing was Schimadzu AUX-220. Ultrasonicator used was Sonarex Super RK 102 (Berlin, Germany) equipment with thermostatically controlled heating (30–80 ◦C).

Preparation of Mobile Phase

Mobile phase was prepared by mixing water: methanol: acetonitrile in a ratio of 70:20:10, v/v/v. The mobile phase was ultrasonicated for 20 minutes and then it was filtered through 0.45 µ membrane filter.

Chromatographic Conditions

The mobile phase consisted of water: methanol: acetonitrile was used as a mobile phase in a ratio water: methanol: acetonitrile in a ratio of 70:20:10, v/v/v. Detection was carried out at 279 nm. Study was carried out using Kromasil C18 (250 X 4.6 mm, 5µm) column at ambient temperature with a flow rate 1.0 ml/min. Mobile phase was filtered through a 0.45µm nylon membrane (Millipore Pvt. Ltd. Bangalore, India) and degassed in an ultrasonic bath.

Preparation of Standard Stock Solution

An accurately weighed quantity of PCM (125.0 mg) and PBM (6.25 mg) was transferred to 50. 0 ml volumetric flask dissolved and diluted up to mark with mobile phase. From this solution, 1.0 ml was transferred to 10.0 ml volumetric flask and diluted to the mark with mobile phase. (Concentration of 250 µg/ml PCM and 12.5 µg/ml PBM, respectively). The solution was found to be stable during the period of study.

Analysis of Formulation

Accurately weighed 20 tablets, each containing 500 mg PCM and 25 mg PBM was crushed to fine powder and accurately weighed quantity equivalent to about 125.0 mg paracetamol and 6.25 mg pamabrom was transferred to 50.0 ml volumetric flasks, dissolved and diluted up to mark with mobile phase. From this solution, 1.0 ml was transferred to 10.0 ml volumetric flask and diluted

to the mark with mobile phase. The solution was filtered through 0.45 µ membrane filter. Equal volume of standard and sample solution (20 µL) were injected (in triplicate) into the column and chromatographed using optimized chromatographic conditions.

METHOD VALIDATION PARAMETERS

The method was validated for linearity, accuracy, precision, limit of detection, limit of quantitation and robustness.

Linearity

The standard solution was prepared by dilution of stock solution containing 1000µg/ml PCM and 500µg/ml PBM. Linearity test solutions were prepared at five different concentration levels ranging from 100 to 500 µg/ml PCM and 5 to 25 µg/ml PBM concentration. Three replicate of each concentration was injected. The peak area was plotted against the corresponding concentration to obtain the calibration graphs.

Precision

The intra-day precision study of PCM and PBM was carried out by estimating the correspondence responses six times on the same day and inter-day precision study was carried out by estimating the correspondence responses six times next day. The repeatability of sample application and measurement of peak area for active compound were expressed in terms of relative standard deviation (%R.S.D.) and standard error (S.E.). Method repeatability was obtained from R.S.D value by repeating the assay six times in same day for intra-day precision. The intraday and inter-day variation for determination of pamabrom and paracetamol was carried out at three different concentration levels 80, 100 & 120 %.

Recovery Study

The recovery study was carried out by applying the method to marketed formulation to which known amount of API of PCM and PBM corresponding to 80, 100 and 120% of label claim had been added (standard addition method). At each level of the amount three determinations were performed and the results obtained were compared with expected results.

Limit of Detection & Limit of Quantitation

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated for the proposed method which was based on the standard deviation of the y intercept and the slope of the calibration curves.

LOD is calculated from the formula:

$$\text{LOD} = \frac{3.3\,\sigma}{S}$$

Where,

σ = The standard deviation of the response for the lowest concentration in the range

S = The slope of the calibration curve.

LOQ is calculated from the formula:

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

Robustness

To evaluate HPLC method robustness, a few parameters were deliberately varied. The parameters included variation of flow rate, mobile phase composition and wavelength.

RESULTS AND DISCUSSION

The proposed method describes a new RP-HPLC method for the determination of PCM and PBM in tablet dosage form (Backaid Max) employing Waters 510 HPLC system, UV/VIS detector, Kromasil C-18 (4.6 × 250mm, 5µm) column and mobile phase comprising of water :methanol : acetonitrile (70:20:10 v/v/v). This method was found to be sensitive, accurate and economical.

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of water: methanol: acetonitrile (70:20:10 v/v/v) at 1.0 ml/min flow rate. The optimum wavelength for detection was set at 279 nm at which better detector responses of drugs were obtained. The retention time was found to be 3.38 and 8.55 min. The obtained chromatogram is shown in **Figure 3**.

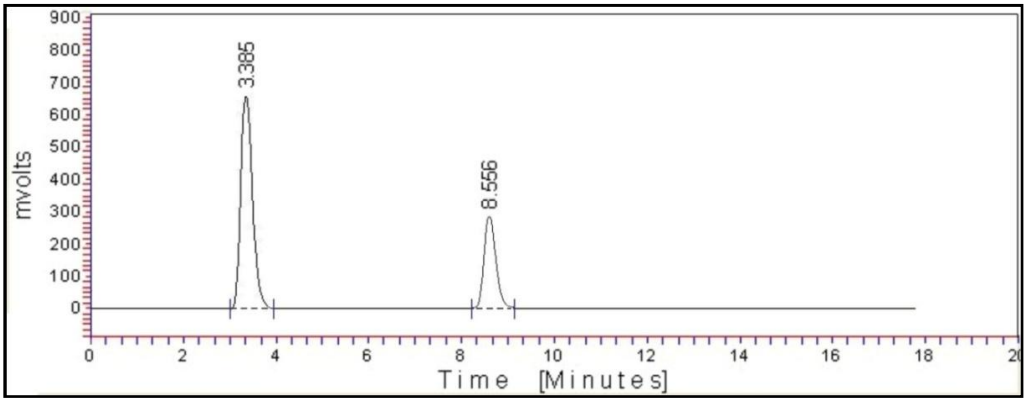

Figure 3: Typical Chromatogram of PCM & PBM (R_t 3.385 & 8.556) respectively.

System Suitability Testing

The system suitability test was applied to a representative chromatogram to check the various parameters such as column efficiency, resolution, precision and peak tailing. The result obtained is shown in **Table No. 01**.

Table No: 1 System Suitability Parameters

Sr. No.	Parameter	PCM	PBM
1.	Resolution (R)	7.12	
2.	Peak Asymmetry (As)	1.15	1.11
3.	No. of therotical plates (N)	11412	4583

Linearity

Calibration curve was obtained in a concentration range from 5 - 25 µg/ml for PBM. The response of the drug was found to be linear in the investigation. The linear regression equation was Y= 11839x + 14459 with correlation coefficient 0.999. The

standard calibration curve of Mean Peak Area vs. Concentration is depicted in **Figure 04**.

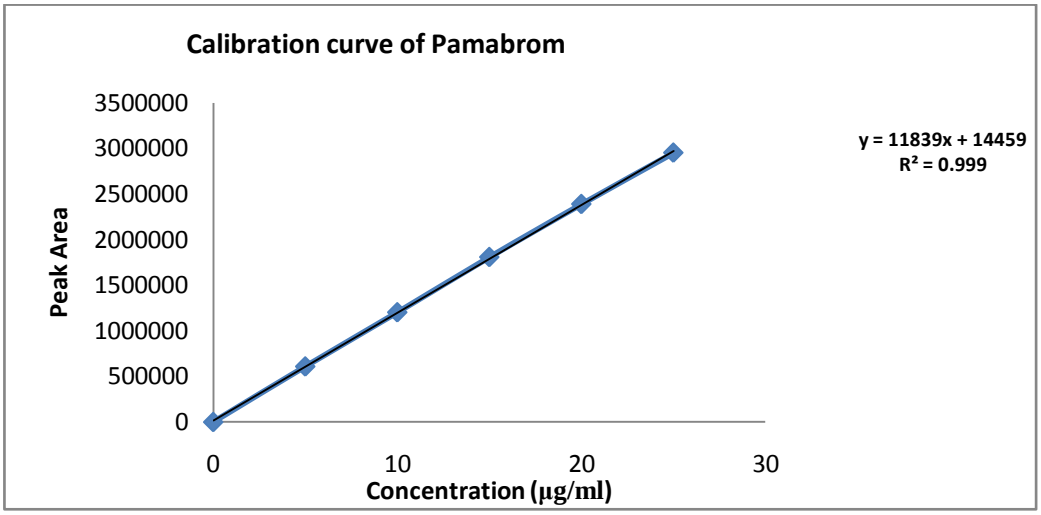

Figure 4: calibration curve of Pamabrom

Calibration curve was obtained in a concentration range from 100 - 500 µg/ml for PCM. The response of the drug was found to be linear in the investigation. The linear regression equation was Y= 20075x + 38698 with correlation coefficient 0.999. The standard calibration curve of Mean Peak Area vs. Concentration is depicted in **Figure 05**.

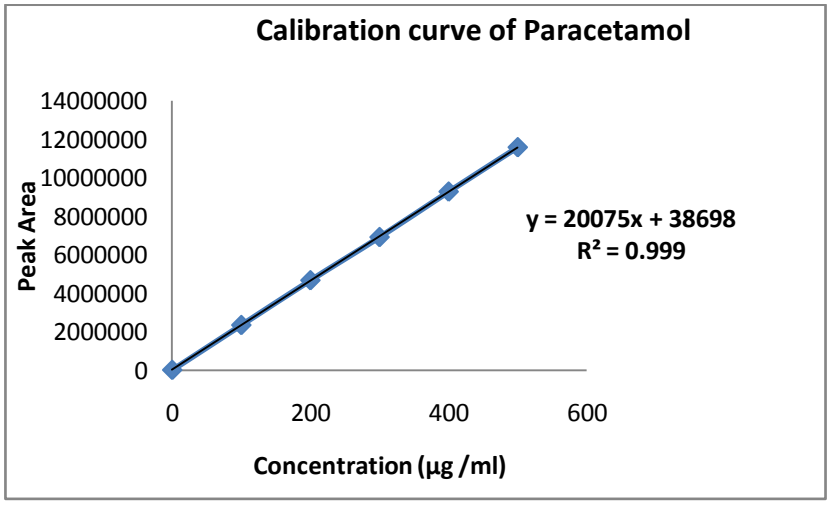

Figure 5: Standard Calibration Curve for Paracetamol

Precision

Three different concentrations of working standard solution of pamabrom and paracetamol were prepared. All the solutions were analyzed thrice, in order to record any intra-day variation in the result. The result obtained for intra-day variations are shown in the **Table No. 02.** For inter-day variation study, three different concentrations of the combined standards were analyzed for three days. The result obtained for inter-day variations are shown in the **Table No. 03**.

Table No: 2 Intra-day Precision Data

Parameters	PCM			PBM		
	Concentration (µg/ml)			Concentration (µg/ml)		
	200	250	300	10	12.5	15
% Estimated	99.72	99.12	99.57	99.51	99.27	99.35
S. D.	± 0.6090	± 0.4050	± 0.4750	± 0.8280	± 0.4807	± 0.5901
C. V.	0.6107	0.4085	0.4771	0.8321	0.4842	0.5940

*** Mean of six determinations, SD: Standard Deviation, RSD: Relative Standard Deviation**

Table No: 3 Inter-Day Precision Data

Parameters	PCM			PBM		
	Concentration (µg/ml)			Concentration (µg/ml)		
	200	250	300	10	12.5	15
% Estimated	98.99	99.6	99.68	99.25	99.44	98.95
S. D.	± 0.3594	± 0.5682	± 0.7562	± 0.4932	± 0.6030	± 0.3881
C. V.	0.3631	0.5705	0.7586	0.4969	0.6064	0.3922

*** Mean of six determinations, SD: Standard Deviation, RSD: Relative Standard Deviation**

Limit of Detection & Limit of Quantitation

The sensitivity of method is described in terms of Limit of Detection and Limit of Quantitation. LOD and LOQ values for PCM and PBM were found to be 4.28 µg/ml, 12.99 µg/ml and 0.23µg/ml, 0.95 µg/ml, respectively. The results of LOD and LOQ studies are shown in **Table No. 04**.

Table No: 4 LOD & LOQ of PCM & PBM

Parameter	PCM	PBM
Limit of Detection (µg/ml)	4.28	0.23
Limit of Quantification (µg/ml)	12.99	0.95

Accuracy

The HPLC area responses for accuracy determination are depicted in **Table No. 05.** The results show that best recoveries (98.50 - 99.20 %) of the spiked drug were obtained at each added concentration; it indicates accuracy of the method.

Table No: 5 Statistical Validation for Recovery Study

Level of recovery	% Mean Recovery		Standard Deviation		% R.S.D.		S.E	
	PCM	PBM	PCM	PBM	PCM	PBM	PCM	PBM
80 %	98.86	99.53	± 0.268	± 0.832	0.271	0.835	0.155	0.480
100 %	99.52	98.56	± 0.413	± 0.697	0.414	0.707	0.238	0.402
120 %	99.44	98.75	± 0.232	± 0.276	0.233	0.279	0.134	0.159

***Average of three determinations, S.D. is Standard deviation; RSD is the Relative Standard deviation**

Table No: 6 Result of Robustness Studies

Chromatographic Changes					
Factor	**Level**	**Retention time**		**Tailing factor**	
Flow Rate (ml/min)		**PCM**	**PBM**	**PCM**	**PBM**
0.9	– 0.1	3.30	8.52	1.17	1.12
1.0	0	3.38	8.55	1.16	1.11
1.1	+ 0.1	3.33	8.59	1.16	1.13
	Mean	3.37	8.55	1.16	1.12
	S.D.	± 0.0360	± 0.0351	± 0.0057	± 0.0100
Mobile Phase (v/v/v)	**Level**	**PCM**	**PBM**	**PCM**	**PBM**
70:19:11	– 1.0	3.37	8.56	1.15	1.12
70:20:10	0	3.38	8.55	1.16	1.11
70:21:09	+1.0	3.35	8.56	1.20	1.18
	Mean	3.36	8.55	1.17	1.13
	S.D.	± 0.0152	± 0.0057	± 0.0264	± 0.0378
Change in Wavelength (nm)	**Level**	**PCM**	**PBM**	**PCM**	**PBM**
278	– 1.0	3.38	8.56	1.15	1.11
279	0	3.38	8.55	1.16	1.11
280	+1.0	3.39	8.56	1.16	1.10
	Mean	3.38	8.55	1.15	1.10
	S.D.	± 0.0057	± 0.0057	± 0.0057	± 0.0057

*** Average of three determinations, S.D. is Standard deviation; RSD is the Relative Standard Deviation**

Robustness

The result of robustness study of the developed assay method was established in **Table No. 06**. The result indicates during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

CONCLUSION

All these factors lead to the conclusion that the proposed method is simple, specific, accurate, precise and reproducible. Statistical analysis proves that the method is suitable for the analysis of paracetamol and pamabrom.

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