Development and Validation of RP-HPLC Method For Estimation of Pamabrom in Bulk And Marketed Formulation

Minal T. Harde*1,3, Sagar B. Wankhede2 and Pravin D. Chaudhari3
1Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University, Hyderbad- 500085, A.P., India.
2Pad. Dr. D.Y. Patil Institute of pharmaceutical Science and Research, Sant Tukaram Nagar, Pimpri, Pune - 411018, Maharashtra, India.
3P. E Society’s Modern College of Pharmacy, Sector no. 21, Yamunanagar, Nigdi, Pune - 411044, Maharashtra, India.

Received on: 21-12-2013; Revised on: 08-05-2014; Accepted on: 20-10-2014

ABSTRACT

Background: A rapid, sensitive and specific RP-HPLC method involving PDA detection was developed and validated for estimation of pamabrom in bulk drug and tablet dosage form. Method: The method was developed in terms of accuracy, precision, specificity, and robustness, limit of detection and limit of quantitation. The mobile phase used was water: methanol: acetonitrile in a ratio of 70:20:10, v/v/v. The detection of tablet dosage form was carried out at 279 nm at a constant flow rate of 1.0 ml/min. Result and discussion: The method was found linear over the range 10 – 50 µg/ml for pamabrom. Correlation coefficient (r²) of the regression equation was found to be 0.999. Detection limit and quantitation limit was found to be 0.45 & 1.86 respectively. Conclusion: Result of assay and recovery study was statistically evaluated for its accuracy and precision. According to the validation results, the proposed method was found to be specific, accurate, precise and economic for the estimation of Pamabrom in bulk and tablet dosage form.


INTRODUCTION

Pamabrom is chemically, 1:1 mixture of 2-amino-2-methyl-1-propanol and 8-bromotheophyllinate (Fig.1), it has a diuretic property¹. It is official in US pharmacopoeia². It is assayed by liquid chromatography as per USP³. 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 periods⁴⁻⁶. Literature review reveals the plasma HPLC method for estimation of Pamabrom in pharmaceutical dosage form. The suggested method was validated by using ICH validation parameters like accuracy, precision, linearity, LOD and LOQ respectively. However, there is no analytical method reported for the estimation of Pamabrom by HPLC using marketed formulation⁷⁻¹². Hence, the purpose of the current study was to develop a sensitive, accurate and comparatively simple method for quantification of Pamabrom in bulk and tablet dosage form.

MATERIALS AND METHODS

Chemicals and reagents
Reference standard Pamabrom was obtained as gift sample from Pan drug Ltd, Ahmadabad, India. Tablet (Diurex Max) used was purchased from local pharmacy shop. HPLC grade water, methanol and acetonitrile were obtained from Merck specialities Pvt. Ltd., Mumbai (India).

Instruments
The instrument used was Waters 510 HPLC system equipped with a rheodyne injecting facility programmed at 20 µl capacity per injection was used. The detector consisted of PDA detector operated at wave-length 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 70ml water, 20ml methanol and...
10ml acetonitrile. This 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 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, 5 µm ) 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
12.5 mg Pamabrom was weighed and transferred to 50.0 ml volumetric flask, dissolved and diluted up to the 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 final concentration prepared was 25 µg/ml. The solution was found to be stable during the period of study.

Analysis of Formulation
Accurately weighed 20 tablets, each containing 50 mg Pamabrom was crushed to fine powder and quantity of powder equivalent to 12.5 mg was weighed and transferred to 50.0 ml volumetric flask, dissolved and diluted up to the 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 1000 µg/ml. Linearity test solutions for method were prepared at five different concentration levels ranging from 10 to 50 µg/ml of analyte 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 pamabrom 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 was carried out at three different concentration levels 80, 100 & 120 %.

Recovery Study
The recovery study was carried out by applying the method to drug sample to which known amount of Pamabrom 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 with expected results.

LOD and LOQ
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:

\[
LOD = \frac{3.3 \sigma}{S}
\]

Where,
\[
\sigma = \text{the standard deviation of the response for the lowest conc. in the range}
\]
\[
S = \text{the slope of the calibration curve.}
\]

The quantitation limit (QL) may be expressed as:

\[
QL = \frac{10 \sigma}{S}
\]

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 Pamabrom in tablet dosage form (Diurex Max) employing Waters 510 HPLC system equipped with PDA 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. Satisfactory separation and good peak symmetry was found in a mixture of water: methanol: acetonitrile in the ratio of 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 8.59 min.

The obtained chromatogram is shown in Fig. 2.

System suitability testing

The system suitability test was applied to a representative chromatogram to check the various parameters such as column efficiency, asymmetry factor, number of theoretical plates and retention time. The result obtained is shown in Table 1.

Table 1: System Suitability Parameters

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameter</th>
<th>PBM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Resolution</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Asymmetry factor (As)</td>
<td>1.1</td>
</tr>
<tr>
<td>3</td>
<td>No. of theoretical plates (N)</td>
<td>6754</td>
</tr>
<tr>
<td>4</td>
<td>Retention Time</td>
<td>8.59</td>
</tr>
</tbody>
</table>

Linearity

Calibration curve was obtained in a concentration range from 10 - 50 µg/ml for Pamabrom. The response of the drug was found to be linear in the investigation. The linear regression equation was Y = 50979x + 92845 with correlation coefficient 0.999. The linearity graph is shown in Fig. 3.

Precision

Three different concentrations of working standard solution of Pamabrom 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 2. 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 3.

Table 2: Intra-day Precision Data

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (µg/ml)</th>
<th>Mean Peak Area*</th>
<th>S. D.</th>
<th>% R.S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBM</td>
<td>20</td>
<td>1511205</td>
<td>± 1510</td>
<td>0.099</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>1891246</td>
<td>± 4964</td>
<td>0.262</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2265204</td>
<td>± 5878</td>
<td>0.259</td>
</tr>
</tbody>
</table>

* Mean of six determinations, SD: Standard Deviation, RSD: Relative Standard Deviation
Table 4: LOD and LOQ of Pamabrom

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PBM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limit of Detection (µg/ml)</td>
<td>0.45</td>
</tr>
<tr>
<td>Limit of Quantification (µg/ml)</td>
<td>1.86</td>
</tr>
</tbody>
</table>

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

Limit of Detection (LOD) and Limit of Quantitation (LOQ)
The sensitivity of method is described in terms of Limit of Detection and Limit of Quantitation. LOD and LOQ values for Pamabrom were found to be 0.45µg/ml and 1.86µg/ml. The results of LOD and LOQ studies are shown in Table 4.

Table 5: Statistical Validation for Recovery Study

<table>
<thead>
<tr>
<th>Level of recovery</th>
<th>% Mean Recovery</th>
<th>Standard Deviation</th>
<th>% R.S.D.</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 %</td>
<td>99.33</td>
<td>1.016</td>
<td>1.022</td>
<td>0.586</td>
</tr>
<tr>
<td>100 %</td>
<td>100.28</td>
<td>0.6729</td>
<td>0.671</td>
<td>0.388</td>
</tr>
<tr>
<td>120 %</td>
<td>99.66</td>
<td>1.271</td>
<td>1.275</td>
<td>0.733</td>
</tr>
</tbody>
</table>

*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 are indicated in Table 6. 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
In this work, a simple, efficient, economic, precise and reproducible RP-HPLC method has been developed for determination of pamabrom in bulk and tablet dosage form. The method was validated as per International Conference on Harmonization (ICH) guidelines and validation criteria met in all cases. The method was successfully developed and quantitized for bulk and tablet dosage form. Statistical analysis proves that the method is suitable for the analysis of Pamabrom.

ACKNOWLEDGEMENTS
The authors express their gratitude to the Principal, Modern college of pharmacy, Pune, India for providing necessary infrastructural facilities. Thanks are also extended to Pan drug Ltd, Ahmadabad, India for providing gift samples of the pure drugs for research work.

REFERENCES
9. ICH; Q2A: Text on Validation of Analytical Procedures; International Conference on Harmonization; Geneva; 1994; 1-5.

Source of support: Nil, Conflict of interest: None Declared