Estimation of Piroxicam in Proliposomal Formulation Using RP HPLC Method

Mallesh Kurakula, Abdul Bari Mohd, Padmanabh Rao A, Prakash V Diwan
Department of Pharmaceutics, School of pharmacy (Formerly Lalitha college of Pharmacy), Venkatapur (V), Ghatkesar (M), R.R district, Andhra Pradesh, INDIA

INTRODUCTION

Piroxicam [(8E)-8-[hydroxy-(pyridin-2-ylamino)methylidene]-9-methyl-10-dioxo-10$\lambda_6$-thia-9-azabicyclo[4.4.0]deca-1,3,5-trien-7-one] [fig.1] is an Oxicam derivative belongs to Non-steroidal anti-inflammatory class of drugs (NSAIDs). Piroxicam is used to reduce the pain, inflammation, and stiffness caused by osteoarthritis and rheumatoid arthritis [1]. The anti-inflammatory activity of piroxicam is due to the reversible inhibition of Cyclooxygenase COX-1 resulting in disruption and production of prostaglandins [2].

Figure 1: Structure of piroxicam

Piroxicam also inhibits the migration of leucocytes in to sites of inflammation and prevents the formation of thromboxane A2, an aggregating agent, by the platelets. It is well absorbed following oral administration however its use has been associated with a number of undesirable side effects on the stomach and kidneys in addition to gastric mucosal damage. These side effects can be avoided by topical administration of the drug [3]. Piroxicam is commercially available in gel form drug delivery has release of the drug from the topical formulation, and its absorption into and through the skin, at the application site [4, 5]. Detailed literature survey revealed that the available analytical methods for piroxicam alone or in combination with other drugs [6, 7] or in combination with other drugs [8, 9], RP-HPLC [10]. And are no reports for the determination of piroxicam in proliposome formulation.

The main objective of the study is to develop and validate HPLC method so as to obtain an accurate, sensitive and precise for quantitative determination of piroxicam in proliposomal formulation.

MATERIALS AND METHODS

Piroxicam (API) was a gift sample from AKUMS Laboratories Ltd., Haridwar. Acetonitrile, potassium dihydrogen ortho phosphate and ortho phosphoric acid used in the study were of analytical grade and obtained from Qualigens Ltd., Mumbai. HPLC grade water was obtained from SD-Labostar (3 TWF-UV) water purification system. The chromatographic condition for method was optimized and depicted in Table 1.

Table 1: Optimized chromatographic conditions

<table>
<thead>
<tr>
<th>Chromatograph</th>
<th>Preeminence SCL-20AD Shimadzu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Columns:</td>
<td>Phenomenex Luna C18 5µm 4.6x250mm (i.d) column</td>
</tr>
<tr>
<td>Mobile phase:</td>
<td>A: 10mM potassium Dihydrogen orthophosphate Ph-3</td>
</tr>
<tr>
<td>Isocratic</td>
<td>50:50</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.0ml/min</td>
</tr>
<tr>
<td>Detector</td>
<td>UV, D2 lamp, 240 nm</td>
</tr>
<tr>
<td>Column temperature</td>
<td>Controlled Room Temperature (25°C)</td>
</tr>
<tr>
<td>Injection</td>
<td>20µL sample loop</td>
</tr>
</tbody>
</table>

Mobile Phase:
Preparation of 10mM potassium dihydrogen ortho phosphate pH 3.0 buffer: 6.8g of potassium dihydrogen ortho phosphate was dissolved in 400ml of HPLC-grade water and adjusted to pH 3.0 with diluted ortho phosphoric acid; the final volume was made up to 500ml using HPLC grade water.

Preparation of pre-mixed mobile phase: The mobile phase was prepared by using 10mM potassium dihydrogen ortho
phosphate buffer pH 3.0 combined with acetonitrile in the ratio of 50:50 v/v. Filtered through a membrane filter unit and degassed by sonication under vacuum for 5min and transferred to solvent reservoir.

The permutation and combination of the mobile phase were used and finally 10mM potassium dihydrogen ortho phosphate buffer pH 3.0: Acetonitrile (50:50) v/v was selected as an appropriate mobile phase which gave good resolution and acceptable system suitability parameters [Table: 2].

Procedure:

Preparation of standard solution: A standard stock solution was prepared by weighing 100 mg of piroxicam standard and transferred into 100ml volumetric flask. To the contents 25ml of mobile phase was added and sonicated for about 5 minutes for complete solubility and was made up to required volume with mobile phase to obtain a final concentration of 1mg/ml of piroxicam.

Calibration curve: From the stock solution, measured volumes of working standards were prepared in the concentration range of 1- 5µg/ml. 20μL injections were made in the range of 1-5µg/ml. The steady base line was recorded by using the optimized chromatographic conditions. The assay was subjected for calculating regression equation. The procedure was repeated for 6 times and the percentage of drug in the formulation was calculated.

Method Validation:

Linearity: The method was linear in the concentration of 1 to 5µg ml for piroxicam standard and results of linear regression data were depicted in table 4.

Precision: The precision of the method was demonstrated by inter day and intraday variation studies. In the intraday studies the solutions of standard and sample were repeated thrice in a day and percent relative standard deviation (%RSD) for response factor was calculated. The intraday %RSD of piroxicam was 0.3746 Where as in the inter day variation studies, injections of standard and sample solutions were made on three consecutive days and % RSD was calculated.

Accuracy: The accuracy of the proposed method was determined by using recovery studies. It was determined by adding the known amounts of the piroxicam reference standard to the test sample at the beginning of the process and all solutions were prepared in triplicate. For recovery studies, proportions of piroxicam in proliposome formulation were made 1:1 by adding reference standard piroxicam into proliposome formulation.

Limit of Detection and Limit of Quantification: The Limit of detection and quantification were calculated using standard deviation of the response and slope of calibration curve. The LOD and LOQ for Piroxicam were observed as 36 and 64.3ng/ml.

Robustness: Robustness of the method was checked by making slight changes in chromatographic conditions such as mobile phase ratio and pH of buffer.

Stability of solutions: In order to obtain reliable experimental results, it is essential to evaluate the stability of standard

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mobile phase - A</th>
<th>Mobile phase - B</th>
<th>PH of Mobile phase</th>
<th>Ratio of A/B</th>
<th>Piroxicam Retention time</th>
<th>Tailing factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10mM</td>
<td>Acetonitrile</td>
<td>3.0</td>
<td>80/20</td>
<td>12.3 min</td>
<td>1.188</td>
</tr>
<tr>
<td>2</td>
<td>10mM</td>
<td>Acetonitrile</td>
<td>3.0</td>
<td>70/30</td>
<td>10.2 min</td>
<td>1.162</td>
</tr>
<tr>
<td>3</td>
<td>10mM</td>
<td>Acetonitrile</td>
<td>3.0</td>
<td>60/40</td>
<td>8.4 min</td>
<td>1.076</td>
</tr>
<tr>
<td>4</td>
<td>10mM</td>
<td>Acetonitrile</td>
<td>3.0</td>
<td>50/50</td>
<td>7.1 min</td>
<td>1.046</td>
</tr>
</tbody>
</table>

*The selected mobile phase was highlighted.
solution. The stability of the solution was validated as per ICH guidelines [12]. Standard solution was stored at room and refrigerated temperatures during three consecutive days (intermediate precision), and injections were made at every 3hrs interval.

RESULTS AND DISCUSSIONS

Proliposome formulation are newer generation of career based drug delivery which exhibit greater stability and show more easy of sterilization when compared to traditional liposomes. The present developed method is novel for the determination of piroxicam in proliposomes formulation. The retention time of standard piroxicam drug was 7.1minutes under optimized chromatographic conditions (fig-2). In our study the retention time of piroxicam in proliposome formulation was 7.1minutes. The method was found to be specific as excipients in the formulation did not interfere in the estimation of piroxicam in proliposome formulation (fig-3). The developed method was linear in the concentration range of 1-5µg/ml (fig-4). Accuracy of the method was indicated by recovery studies and it was in agreement with the standard piroxicam (100.14%). The LOD and LOQ for Piroxicam were found to be 36 and 64.3ng/ml respectively. The LOQ for piroxicam (64.3ng/ml) was 10times higher than in other described method [8-10]. The precision and accuracy at the LOQ were lower than 20%, as recommended in the literature [9]. The results of analysis showed that the amount of drug was in good agreement with label claim of developed proliposome formulation and tabulated in Table-3. It was observed that there were no marked changes in chromatogram, which demonstrated that the method developed was robust (Table-4). The stability study was performed under stress degradation conditions as per ICH guidelines [12]. The results indicated that the developed formulation was stable up to 12-14 hours which was sufficient for completing the analytical procedures. The developed method was specific and reproducible for the quantitative determination of piroxicam in proliposome formulation with a good resolution and high sensitivity.

| Table 3: Analysis of proliposome formulation |
|-----------------|--------|----------|-----------------|
| Formulation     | Analyte| Label claim (mg) | % Label claim estimated |
| Proliposome     | Piroxicam | 10        | 97.54           |

| Table 4: Robustness |
|---------------------|--------|
| Condition           | % RSD  |
| pH of the buffer (+ 0.2) | 0.4507 |
| pH of the buffer (~ 0.2)  | 0.5321 |
| Organic phase ratio (+ 2%)  | 0.4985 |
| Organic phase ratio (~ 2%)   | 0.7351 |

CONCLUSION

Proliposome formulation prepared shows sustain release intended for treatment of rheumatoid arthritis. There are no methods developed for quantitative determination of Piroxicam in proliposome formulation. Precision and accuracy for piroxicam were comparable with other HPLC methods previously described in the literature. The standard deviation and %RSD calculated for the proposed method are low, indicating high degree of precision of the method. Hence, it can be concluded that the proposed chromatographic method was accurate, precise, and selective can be employed successfully for the determination of piroxicam in bulk and proliposome formulation.
Figure 5: Typical chromatogram showing stability at room temperature for 12 hrs.

Figure 6: Typical chromatogram showing stability at refrigerated temperature for 14hrs.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. Rajeshwar Reddy chairman, Anurag group of institutions for providing facilities, Infrastructure for carrying out research work. The author is also grateful to Managing director of Akums Labs, Haridwar for providing standard piroxicam as gift sample.

REFERENCES


7. Y Kumar, SK Talwar, YKS Rathore, PD Sethi and CL Jain. Spectrophotometric estimation of piroxicam in pharmaceutical formulations through cobalt (II) and copper(II) chelation. Indian Drugs, 1990, 28(3), 139-141.


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