Rapid analytical method development and validation of Piroxicam by RP-HPLC

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ABSTRACT
This paper describes the analytical method suitable for validation of Piroxicam by reversed Phase High Performance liquid chromatography (RP-HPLC) method. The method utilized RP-HPLC (Youglin with UV-detector) model and a column, 150mm × 4.6 mm, 5m (Symmetry, ODS-3V, 150mm × 4.6mm, 5m). The mobile phases were comprised of Methanol and Water pH 3.2 (55:45v/v). Validation experiments were performed to demonstrate System suitability, precision, linearity and Range, Accuracy study, stability of analytical solution and robustness. The method was linear over the concentration range of 1-200 mg/ML-1. The method showed good recoveries (99.8 – 102.9%).

Keywords: RP-HPLC, Piroxicam, Analytical method, Quality control, validation.

INTRODUCTION
Piroxicam (PXM), (3E)-3-[hydroxy-(pyridine-2-yI amino) methylidene]-2-methyl-1, 1 dioxobenzo[e]thiazin-4-one (C15H13N3O4S). PXM is a class of drug called Nonsteroidal Anti-Inflammatory Drugs (NSAIDs). PXM works by reducing hormones that cause inflammation and pain in the body. PXM is used to reduce the pain, inflammation, and stiffness caused by rheumatoid arthritis and osteoarthritis. The anti-inflammatory effect of PXM may result from the reversible inhibition of cyclooxygenase, causing the peripheral inhibition of prostaglandin synthesis. The prostaglandins are produced by an enzyme called as Cox-1. PXM blocks the Cox-1 enzyme, resulting in to the disruption of production of prostaglandins. PXM also inhibits the migration of leucocytes in to sites of inflammation and prevents the formation of thromboxane
A2, an aggregating agent, by the platelets. PXM, 4-Hydroxy-2-methyl-N-(pyridin-2-yl)-2H-1, 2-benzothiazine-3-carboxamide 1, 1-dioxides is analgesic and anti-inflammatory agent [1-5]. PXM is official in Indian Pharmacopoeia [6], British Pharmacopoeia [7], European Pharmacopoeia [8] and United States Pharmacopeia [9].

![Fig. 1: Structure of Piroxicam](image)

PXM is Oxicam derivative having analgesic and anti-inflammatory action [10-12]. Various analytical methods had reported in literature for estimation of PXM individually and in combination with other drugs [13-18]. Pharmaceutical validations among these methods undergo the world ‘Validation’ means ‘Assessment’ of validity or action of providing effectiveness [19, 20], and validation as per ICH guidelines [21].

**EXPERIMENTAL SECTION**

**Apparatus**
The analysis was performed by using the analytical balance G285 (Mettler Toledo), pH meter 744 (metrohm), the HPLC used is of Younglin with UV-detector. Column used in HPLC is of 150mm ´ 4.6mm 5 µ (Symmetry, ODS-3V, 150mm ´4.6mm, 5 µ is suitable) with a flow rate of 1.2ml/min (Isocratic). The mobile phase consists of A & B with mixture of Methanol and the Water pH (3.2) at different proportions A & B which are degassed in a sonicator for about 10minutes the injection volume is 10ml and the ultra violet detection was at 240nm.

**Reagents and solutions**
Pure sample of Piroxicam (USP) and other ingredients such as Methanol and water used were of HPLC and milli-Q grade. All other chemicals like Potassium Di-hydrogen phosphate used were of AR grade. Optimized chromatographic conditions are listed in table no.1.

**Standard preparation**
Accurately weigh and transfer 10mg of PXM into a 10ml of volumetric flask, add about 10ml of diluent, sonicate to dissolve, make up to volume with diluent. Transfer 1 ml of the above solution into 10ml volumetric flask, dilute to the volume with mobile phase and mix well. Filter the solution through the 0.45µ filter.

**Accuracy**
Accuracy for the PXM is determined by applying the method in triplicate samples of mixture of placebo to which known amount of PXM standard is added at different levels (80%, 100% and
The sample were filtered through 0.45µ membrane filter and injected into the chromatographic system.

**Precision**
The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision expressed as %RSD. The %RSD was found to be 0.17% in the results of precision.

**Linearity and Range**
The Linearity of detector response is established by plotting a graph to concentration versus area of PXM standard and determining the correlation coefficient. A series of solution of PXM standard solution in the concentration ranging from about 1-200ppm levels of the target concentration (100µg/ml of PXM) were prepared and injected into the HPLC system.

**RESULTS AND DISCUSSION**

PXM standard having concentration 100µg/ml was scanned in UV- region between 200- 400 nm. $\lambda_{\text{max}}$ of PXM was found to be at 240 nm. The PXM peak in the sample was identified by comparing with the PXM standard and the Retention time was found to be around 5.183 minutes. The estimation PXM was carried out by RP-HPLC using Mobile phase having a composition volumes of 55 volumes of Methanol and 45 volumes of Water (55:45 v/v). The ratio pH was found to be 3.2. Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10minutes. The column used was C18 Symmetry ODS 3V (150mm x 4.6mm; 5µ particle size). Flow rate of Mobile phase was 1.2ml/min, theoretical plates – 6013.72, and tailing factor – 1.32.

**Table – 1: Optimized chromatographic conditions**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Optimized condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromatograph</td>
<td>HPLC (Younglin HPLC with UV-detector)</td>
</tr>
<tr>
<td>Column</td>
<td>Symmetry-Extend C₁₈ (150 × 4.6mm, packed with 5µm) is suitable</td>
</tr>
<tr>
<td>Mobile Phase*</td>
<td>Water: Methanol (45:55)</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.2ml/min</td>
</tr>
<tr>
<td>Detection</td>
<td>UV at 240nm</td>
</tr>
<tr>
<td>Injection volume</td>
<td>20µl</td>
</tr>
<tr>
<td>Temperature column</td>
<td>Ambient</td>
</tr>
</tbody>
</table>

**Table – 2: System suitability parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Glimepiride Hydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration range (µg/ml)</td>
<td>1-200</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>6013.72</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.32</td>
</tr>
<tr>
<td>Correlation Coefficient($r^2$)</td>
<td>0.999</td>
</tr>
<tr>
<td>% Recovery</td>
<td>99.8% - 102.9%</td>
</tr>
<tr>
<td>System Suitability %RSD</td>
<td>0.04%</td>
</tr>
<tr>
<td>Method Precision %RSD</td>
<td>0.17%</td>
</tr>
<tr>
<td>Injection Precision %RSD</td>
<td>0.04%</td>
</tr>
</tbody>
</table>
The System Suitability parameters show the results very satisfactory. The acceptance criteria of System Suitability is RSD should be not more than 2.0% and the method show System Suitability 0.04% which shows that the method is repeatable. The acceptance criteria of Method Repeatability is RSD should be not more than 2.0% and the method show Method Repeatability 0.17% which shows that the method is precise.

Fig. 2: Chromatogram of standard for Piroxicam

![Chromatogram of standard for Piroxicam](image)

Fig. 3: Linearity of Piroxicam

![Linearity of Piroxicam](image)

The validation of developed method shows that the drug stability is well within the limits. The linearity of the detector response was found to be linear from 1-200µg/ml of target concentration for PXM standard with a correlation coefficient value is greater than 0.999. The correlation coefficient of \( r^2 \) = 0.999, which shows that the method is capable of producing good response
in UV-detector. The Accuracy limit is the % recovery should be in the range of 99.8-102.9%. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy.

Regression analysis of the calibration curve for PXM showed a linear relationship between the concentration and peak area with correlation coefficients higher than 0.999 in all the curves assayed.

**CONCLUSION**

HPLC is at present one of the most sophisticated tools of analysis. The estimation of PXM is done by RP-HPLC. The mobile phase consists of 55 volumes of Methanol and 45 volumes of Water (55:45). The ratio pH was found to be 3.2. Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10 minutes. The detection is carried out using UV-detector set at 240nm. The solutions are chromatographer at the constant flow rate of 1.2ml/min. The Retention time for PXM was around 5.183minutes. Linearity range for PXM is 1-200µg/ml. The quantitative estimation was carried out on the tablet by RP-HPLC taking a concentration of 100µg/ml. The % recovery 99.8% to 102.9% for PXM.

The results obtained on the validation parameter met the requirements. It inferred that the method was found to be Simple, Specific, Precision, and Linearity, Proportional i.e. it follows Lambert-Beer’s law. The method was found to have a suitable application in routine laboratory analysis with a high degree of Accuracy and Precision.

**REFERENCES**