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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 (Water 2695 with PDA detector) model and a column, 150mm × 4.6 mm, 5µ (Inertsil, ODS- 3V, 150mm × 4.6mm, 5µ). The mobile phases were comprised of A, B of Methanol and Buffer pH 3.0 (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 5-150 µg/ML^{-1}. The method showed good recoveries (98.0 – 99.8%).

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

INTRODUCTION

Piroxicam (PRX), (3E)-3-[hydroxy-(pyridine-2-yl amino) methylidene]-2-methyl-1, 1-dioxobenzo[e]thiazin-4-one ($C_{15}H_{13}N_3O_4S$). Piroxicam is a class of drug called nonsteroidal anti-inflammatory drugs (NSAIDs). Piroxicam works by reducing hormones that cause inflammation and pain in the body. Piroxicam is used to reduce the pain, inflammation, and stiffness caused by rheumatoid arthris and osteoarthritis. The anti-inflammatory effect of Piroxicam 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. Piroxicam blocks the Cox-1 enzyme, resulting in to the disruption of production of prostaglandins. 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. Piroxicam, 4-Hydroxy-2-methyl-N-(pyridin-2yl)-2H-1, 2-benzothiazine-3-carboxamide 1, 1-dioxides is analgesic and anti-inflammatory agent [1-5]. Piroxicam is official in Indian Pharmacopoeia [6], British Pharmacopoeia [7], European Pharmacopoeia [8] and United States Pharmacopeia [9].

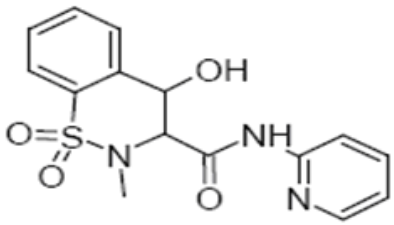


Fig. 1: Piroxicam

Piroxicam is Oxicam derivative having analgesic and anti-inflammatory action [10-11]. Various analytical methods had reported in literature for estimation of Piroxicam individually and in combination with other drugs [12-17]. Pharmaceutical validations among these methods undergo the world 'Validation' means 'Assessment' of validity or action of providing effectiveness [18, 19], and validation as per ICH guidelines [20].

MATERIALS AND METHODS

Apparatus

The analysis was performed by using the analytical balance G285 (Mettler Toledo), pH meter 744 (metrohm), the HPLC used is of Water 2695 with PDA detector. Column used in HPLC is of 150mm × 4.6mm 5µ(Inertsil, ODS-3V, 150mm ×4.6mm, 5µ is suitable) with a flow rate of 0.8 ml/min (Isocratic). The mobile phase consists of A & B with mixture of Methanol and the Buffer pH (3.0) at different proportions A & B which are degassed in a sonicator for about 10 minutes the injection volume is 10µL and the ultra violet detection was at 240 nm.

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 Ortho Phasphoric acid, Sodium hydroxide, Potassium Di-hydrogen phosphate used were of AR grade. Optimized chromatographic conditions are listed in table no.1.

Standard preparation

Accurately weigh and transfer 25mg of Piroxicam into a 50 mL of volumetric flask, add about 50 mL of diluent, sonicate to dissolve, make up to volume with diluent. Transfer 5.0 mL of the above solution into 50 mL volumetric flask, dilute to the volume with mobile phase and mix well. Filter the solution through the 0.45 µm filter.

Accuracy

Accuracy for the assay of Piroxicam tablets is determined by applying the method in triplicate samples of mixture of placebo to which known amount of Piroxicam standard is added at different levels (80%, 100% and 120%). The sample were filtered through 0.45µm 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 0.60% in the results of precision.

Linearity and Range

The Linearity of detector response is established by plotting a graph to concentration versus area of Piroxicam standard and determining the correlation coefficient. A series of solution of Piroxicam standard solution in the concentration ranging from about 5ppm to 150ppm levels of the target concentration (50µg/ml of Piroxicam) were prepared and injected into the HPLC system.

RESULTS AND DISCUSSION

Piroxicam standard having concentration 50µg/ml was scanned in UV- region between 200-400 nm. λ max of Piroxicam was found to be at 240 nm. The Piroxicam peak in the sample was identified by comparing with the Piroxicam standard and the Retention time was found to be around 7.0 minutes. The estimation Piroxicam was carried out by RP-HPLC using Mobile phase having a composition volumes of phosphate buffer, 55 volumes of Methanol and 45 volumes of buffer (55 : 45 v/v). The ratio pH was found to be 3.0. Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10 minutes. The column used was C18 Inertsil ODS 3V (150 mm x 4.6 mm x 5 µ particle size). Flow rate of Mobile phase was 0.8 ml/min, System suitability parameters such as RSD for six replicate injections were found to be 0.4%, theoretical plates – 8881.6, and tailing factor – 1.20.

The acceptance criteria of System Suitability is RSD should be not more than 2.0% and the method show System Suitability 0.4% 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 1.91% which shows that the method is precise. 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 5 to 150 µg/ml of target concentration for Piroxicam standard with a correlation coefficient value is greater than 0.999. The correlation coefficient of (r^2) = 0.9991, which shows that the method is capable of producing good response in PDA-detector.

The Accuracy limit is the % recovery should be in the range of 98.0% to 99.8%. 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.

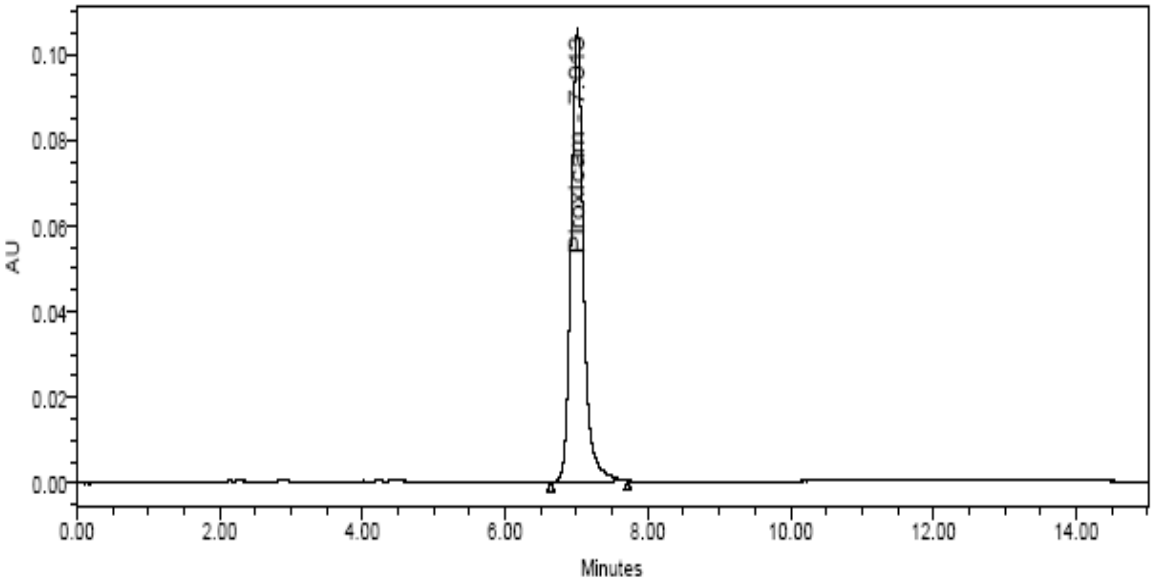
Table – 1: Optimized chromatographic conditions

Parameter	Optimized condition
Chromatograph	HPLC (Water 2695 with PDA detector)
Column	Inertsil ODS-3V, 150mm×4.6mm, 5µ is suitable
Mobile Phase*	Methanol and Buffer (55 : 45 v/v)
Flow rate	0.8 ml/min
Detection	PDA at 240 nm
Injection volume	10µl
Temperature column	25°C

Table – 2: System suitability parameters

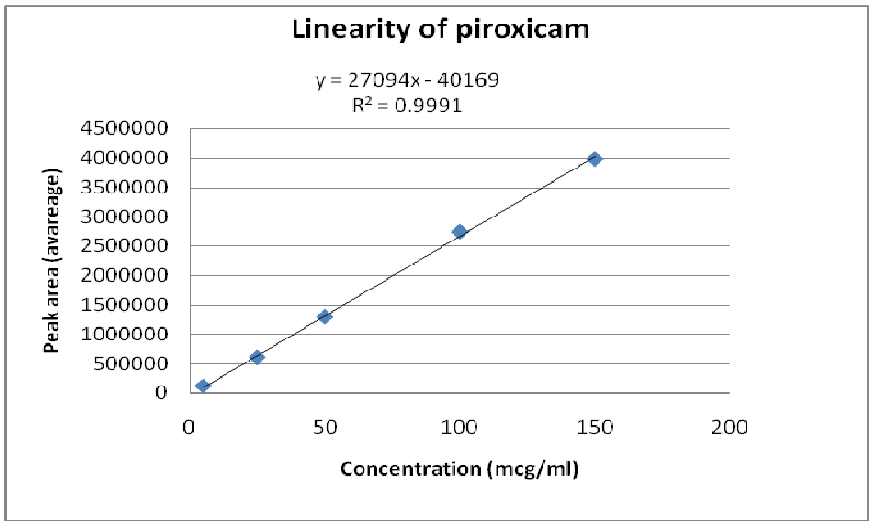
Parameter	Piroxicam
Calibration range (µg/ml)	5-150
Theoretical plates	8881.6
Tailing factor	1.20
Correlation Coefficient(r^2)	0.9991
% Recovery	98.0% - 99.8%
System Suitability %RSD	0.4%
Method Repeatability %RSD	1.9%

Chromatogram of standard for Piroxicam



	Peak Name	RT	Area	% Area	Height
1	Piroxicam	7.013	1288187	100.00	105602

Figure – 2: linearity of Piroxicam



Regression analysis of the calibration curve for Piroxicam 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 Piroxicam is done by RP-HPLC. The mobile phase consists of buffer (volumes of phosphate buffer, 55 volumes of Methanol and 45 volumes of buffer. The ratio pH was found to be 3.0. Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10 minutes). The detection is carried out using PDA detector set at 240nm. The solutions are chromatographer at the constant flow rate of 0.8 ml/min. The Retention time for Piroxicam was around 7.0 minutes. Linearity range for Piroxicam is 50 to 150µg/ml.

The quantitative estimation was carried out on the tablet by RP-HPLC taking a concentration of 50µg/ml. the quantitative results obtained is subjected to the statistical validation. The values of RSD are less than 2.0% indicating the accuracy and precision of the method. The % recovery 98.0% to 99.80% for Piroxicam.

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.

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