ASSAY METHOD OF ACTIVE PHARMACEUTICAL INGREDIENT PIROXICAM BY HIGH PERFORMANCE LIQUID CHROMATOGRAFIC TECHNIQUE

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ABSTRACT:
A simple, linear and reproducible assay method was developed for the quantification of piroxicam. The method was developed by using Phenomenox Kinetex C18, (100 x 4.6) mm, 2.7 micron column. Mobile phase consisted of Water:Methanol:Orthrophosphoric acid in ratio 50:50:0.1 with flow rate of 0.7 ml/min at 240 nm and column oven temperature at 35°C. RSD for standard preparation under system precision was observed 0.10%. RSD for retention time was observed 0.48% which shows reproducibility during replicate injections. The linearity range was achieved from 50 to 150 ppm level for piroxicam. Comparative data of method and intermediate precision shows average assay value 99.81% on. The method was applied for quantification of assay of piroxicam drug substance. The proposed method is very easy to apply having low-cost, non polluting reagents and requires relatively inexpensive equipments.

KEY WORDS: HPLC, Assay, Piroxicam API, Methanol.

INTRODUCTION:

Validation of method was performed to demonstrate system suitability, precision, linearity, range, accuracy study and stability of analytical solution and robustness of chromatographic method by using high performance liquid chromatography instrument. The validation procedures followed were as per ICH (International Conference on Harmonization) guidelines evaluating the parameters like linearity, precision, accuracy, detection limit and quantitation limit[1]. Piroxicam is nonsteroidal anti inflammatory drug (NSAID) and it is official in Indian Pharmacopoeia[2], British Pharmacopoeia[3], European Pharmacopoeia[4] and United States Pharmacopeia[5]. Piroxicam helps to reduce pain in human body, inflammation and stiffness caused by rheumatoid arthritis and osteoarthritis. The anti inflammatory effect of this drug may result from the reversible inhibition of cyclooxygenase which causes the peripheral inhibition of prostaglandin synthesis. The prostaglandins are produced by an enzyme called as Cox-1. Piroxicam blocks the Cox-1 enzyme which results in to the growth and production of prostaglandins. It also inhibits the migration of leucocytes in to sites of inflammation and prevents the formation of thromboxane A2. Piroxicam is chemically: (3E)-3-[hydroxy-(pyridine-2-yl amino) methylidene]-2-methyl-1,1dioxobenzo [e]thiazin-4-one (fig 1). It is analgesic and anti-inflammatory agent[6-10].

HPLC is a versatile, reproducible chromatographic technique for the estimation of drug products, drug substance and raw materials. It has wide applications in different fields in term of quantitative and qualitative estimation of active molecules[11]. Madhukar A. et al[12] also developed a method for piroxicam but had Retention time of around 5.183 minutes. This method is time consuming hence we developed a new method having less retention time and having good peak shape.

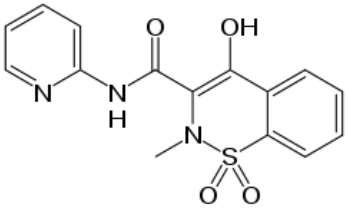

Fig.1: Chemical structure of drug Piroxicam

MATERIALS AND METHODS

Chemical and reagents:
Piroxicam test sample was received from Ramdev Chemical as a gift sample. HPLC grade methanol as a solvent (Spectrochem), orthrophosphoric acid AR grade (Rankem) and HPLC grade water (Merck) were used.

Instrument:
HPLC instrument of make Shimadzu LC 2010C HT with a quaternary gradient pump system and a fixed dual wavelength UV detector having LC-solution software with auto sampler tray having cooling facility and column oven temperature compartment available was used for measurements.

Preparation of stock solution:
25 mg of piroxicam was weighted accurately and transferred to 25 ml volumetric flask. Sample was dissolved in methanol, sonicated it for 5 min and diluted it with diluent up to the mark (stock solution). 5 ml of stock solution were further diluted to 25 ml with diluent making solution of concentration 200 ppm.

RESULT AND DISCUSSION:

Selection of detection wavelength:

Analytical wavelength was selected by taking absorption spectra of piroxicam which gave max of piroxicam. It was investigated by using stock solution of piroxicam which was scanned in range 200-400nm. UV spectrum shows maximum absorption at 240nm. Hence wavelength 240nm was selected

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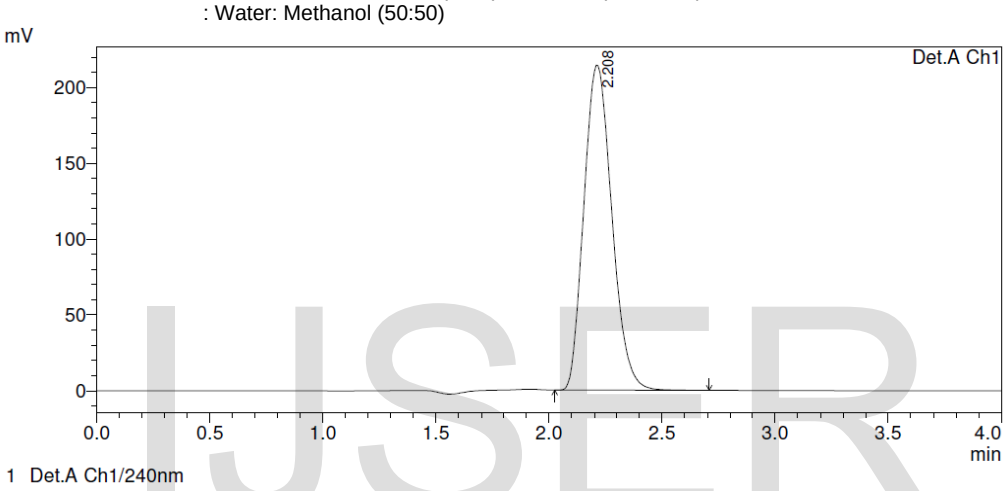
Optimization of chromatographic conditions:

The HPLC method was optimized with a view to develop an assay method for piroxicam. Initially different combination of mobile phases such as methanol, water and ortho-phosphoric acid in different proportions were tried at different flow rates. Finally 0.1% OPA in water: methanol (50:50), flow rate 0.7 ml/min was found suitable. The analysis was performed at 35°C column oven temperature with UV detection at 240 nm.

Chromatographic conditions:

Column name	: Phenomenox Kinetex C18, (100 x 4.6) mm, 2.6 micron
Flow rate	: 0.7 ml/min
Wavelength	: 240 nm
Column Oven temperature	: 35°C
Run time	: 7 minutes.
Injection volume	: 5 µl
Mobile phase	: Water: Methanol: Orthrophosphoric acid (50:50:0.1)
Diluent	: Water: Methanol (50:50)



PeakTable

Detector A Ch1 240nm

Peak#	Ret. Time	Area	Tailing Factor	Name
1	2.208	1846014	1.230	Piroxicam
Total		1846014		

Figure: - 1 Chromatogram of Standard preparation

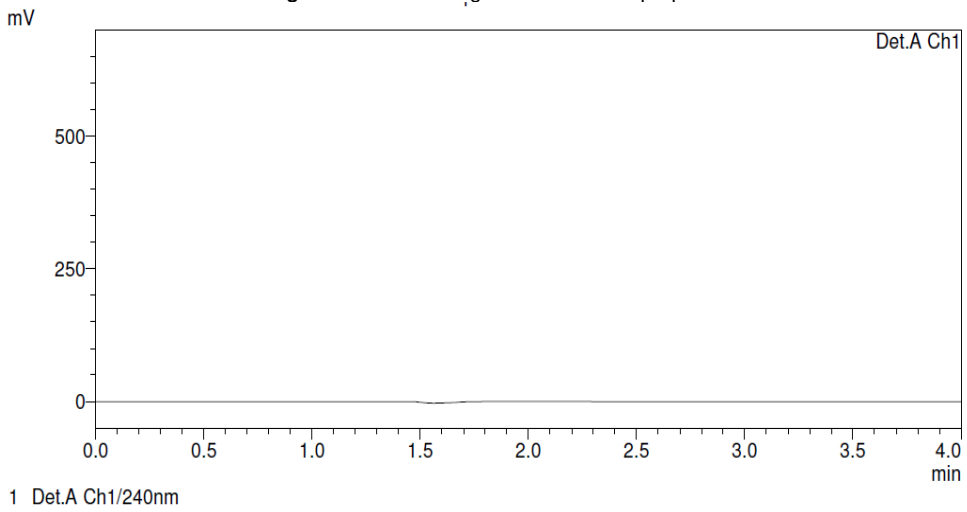


Figure: - 2 Chromatogram of Blank

From the chromatogram (Fig. 1) it was concluded that the Retention time for Piroxicam was 2.2 ± 0.05 minutes and tailing factor was 1.20 which as per USP should not be more than 1.5.

Table- 1: System precision

Sr.No	RT (min)	Area
1	2.208	1846014
2	2.208	1845638
3	2.209	1848423
4	2.209	1852423
5	2.211	1855522
Average	2.209	1849604
% RSD	0.06	0.23

Linearity is an important parameter in validation which needs to be performed to obtain test results which are directly proportional to the concentration of analyte in test sample. Linearity was performed from 50% to 150% level with respect to test concentration level in which linearity range was covered from 50 ppm to 150 ppm level. It was concluded that linearity graph of test concentrations are linear and observed value for correlation coefficient is 0.9995. The result shows that an excellent correlation existed between the peak area and concentration of the analyte (Fig 3)

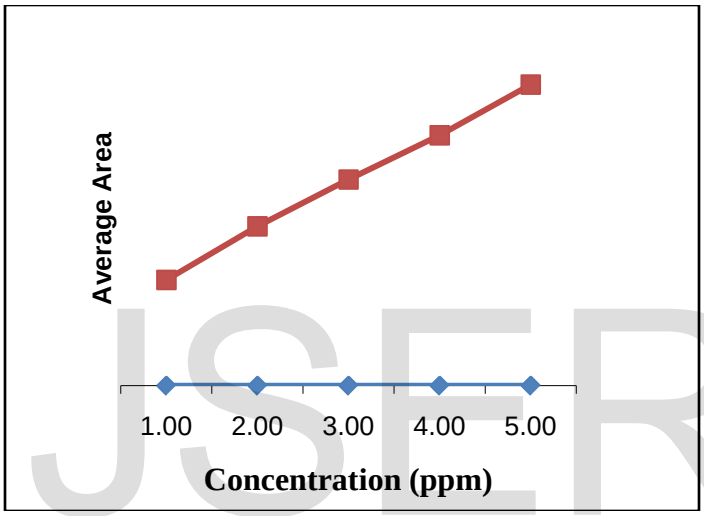


Figure: 3 Linearity graph

Reproducibility of method was checked by performing method precision in which same test preparation was performed six times and each of the preparations was injected in duplicate. Results were calculated against average area of five replicate injections of standard preparation. Reproducibility of method was checked by performing intermediates precision, in which same experiment was performed by different worker, different days etc. From the data obtained it is concluded that our method is reproducible. This is depicted in table 2 for comparative results.

Table: 2 Comparison between method precision and intermediate precision.

Sr.No	Method Precision	Intermediate Precision
	99.7	99.9
	100.9	99.8
	100.2	100.0
	99.5	100.4
	100.6	99.5
	100.1	100.8
Average	100.1	
% RSD	0.48	

Robustness[13] of method was checked by altering chromatographic conditions that is column oven temperature, mobile phase composition and flow rate. Result found that the method is robust in all condition and there is no merging of any peaks and peak shape is also found good. The evaluation of robustness should be considered during the developmental phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. Stability of analytical solution was checked and found that it is stable up to 14 days at room temperature. For that freshly prepared solution was injected and same solution was kept for 14 days at room temperature and injected. Area difference found is within acceptance criteria.

CONCLUSION:

The results obtained from the validation parameter inferred that the method was found to be simple, specific, precise and linear 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 arecision. The method can be used successfully for identification and quantification of the active pharmaceutical ingredient Piroxicam from other pharmaceutical ingredient. Hence this method can be used for the routine analysis of Piroxicam .

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CONFLICT OF INTEREST:

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