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Development and Validation of a Stability Indicating RP-HPLC Method for the Determination of Piroxicam in Pharmaceutical Dosage Form

A. Chandra Prabha[1], M. Sankar[2] and A. Chenthilnathan[1]*

[1]*Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli–627012, Tamil Nadu, India.*
[2]*Caplin Point Laboratories Ltd., Gummidipoondi, Chennai–601201, Tamil Nadu, India*

A B S T R A C T

A stability-indicating RP-HPLC was developed and validated for the determination of Piroxicam in bulk and its injection dosage form. The separation was carried out on Waters Nova pack C18 coloum (150 × 3.9mm; 4µm) column at ambient temperature using buffer and methanol (50:50) as eluent. The flow rate was 1.0 ml/min and Piroxicam was quantified by absorbance at 240 nm. The retention time of Piroxicam was 4.50 min. The percentage recovery was within the range between 100.20 % and 100.70% for Piroxicam. The linear ranges were found in the range of 50µg/ml – 150µg/ml (r^2 = 0.999) of Piroxicam.. The percentage relative standard deviation for accuracy and precision was found to be less than 2%. Piroxicam was subjected to stress conditions including acidic, alkaline and oxidative degradation. It was found that, Piroxicam is more sensitive towards oxidative degradation. The proposed method was validated as per ICH guidelines.

Keywords: Reversed-phase HPLC, Validation, Stability-indicating

A R T I C L E I N F O

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***Corresponding Author**
A. Chenthilnathan
Assistant Professor
Department of Pharmaceutical Chemistry
Manonmaniam Sundaranar University,
Tirunelveli – 627 012, Tamil Nadu
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1. Introduction

Piroxicam (Fig.1), chemically (4-hydroxy-2-methyl-N-(pyridine-2-yl)- 2H-1,2-benzothiazine-3-carboxamide-1,2-dioxide), is a non-steroidal anti-inflammatory and analgesic drug of the oxicam class, often indicated in the treatment of rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, acute pain in musculoskeletal disorders and acute gout [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]. Piroxicam is official in Indian Pharmacopoeia [3], British Pharmacopoeia [4], European Pharmacopoeia [5] and United States Pharmacopeia [6].

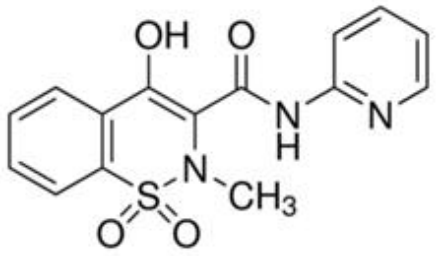

Figure 1: Structure of Piroxicam

A literature survey revealed that some methods had been developed for piroxicam alone or in combination with other drugs by using UV Spectrophotometry [7, 8], Spectrofluorimetry [9], TLC [10] and HPLC [11-13]. In this present work, an attempt was made to develop and validate HPLC method so as to obtain an economic, accurate, sensitive and precise for quantitative determination of piroxicam in injection dosage form. The proposed method was validated in accordance with International Conference on Harmonization (ICH) guidelines.

2. Experimental

Chemicals and reagents

Disodium hydrogen phosphate of AR grade and Citric acid (anhydrous) of AR grade were purchased from Qualigens Fine Chemicals Ltd., Mumbai. Methanol of HPLC grade was purchased from E. Merck (India) Ltd., Mumbai. Piroxicam was a gift sample by Caplin Point Laboratories Ltd., Chennai– 601201, Tamil Nadu, India. The commercially available injection containing Piroxicam was procured from the local market.

Instrumentation and chromatographic conditions

The chromatographic separation was carried out on Agilent 1260 Infinity HPLC system (Agilent Technologies, Santa Clara, CA) with UV- Visible dual absorbance detector (PDA), Waters Nova pack C18 coloum (150 × 3.9mm; 4µm). The mobile phase consisting of buffer (a mixture of 7.72 g anhydrous citric acid and 5.35 g of disodium hydrogen phosphate in 1000ml) and methanol water was filtered through 0.45µ membrane filter before use, degassed and was pumped from the solvent reservoir in the ratio of 50:50 v/v was pumped into the column at a flow rate of 1.0 ml/min. The detection was monitored at 240nm. The volume of injection loop was 20 µl prior to the injection of the drug solution; the column was equilibrated for at least 30 min. with the mobile phase following through the system.

Preparation of Standard solutions

50 mg of Piroxicam working standard was weighed accurately and transferred carefully in 100 ml volumetric flask. About 60 ml of mobile phase was added, sonicated to dissolve the drug completely and the volume was made up with mobile phase. 5ml of above solution was diluted to 25 ml with mobile phase. The resulting solution was mixed and filtered through 0.45 µm filter.

Analysis of Sample Preparation

5 ml of sample containing Piroxicam in the concentration of 20mg/ml was transferred into 100 ml volumetric flask. About 60 ml of mobile phase was added and the volume was made up with mobile phase. 5ml of above solution was diluted to 50 ml with mobile phase. The resulting solution was mixed and filtered through 0.45 µm filter.

Procedure:

About 20 µl each of the test and the standard solutions were injected separately into the chromatograph and the chromatograms were recorded and the responses for the major peaks were then measured.

The quantity of Piroxicam in mg/ ml was calculated by using the formula:

$$\frac{A_T}{A_S} \times \frac{D_S}{D_T} \times \frac{P}{100}$$

The percentage labeled amount was calculated by using the formula:

$$\frac{\text{Quantity (mg/ml)}}{LC} \times 100$$

Where,

AT: Average area of Piroxicam peak obtained from the Sample Chromatogram

AS: Average area of Piroxicam peak obtained from the Standard Chromatogram

DS: Dilution factor for Standard Preparation

DT: Dilution factor for Sample Preparation

P: Percentage Purity of Piroxicam working Standard

LC: Label claim for Piroxicam in Piroxicam Injection (20 mg/ml)

3. Results and Discussion

All of the analytical validation parameters for the proposed method were determined according to International Conference on Harmonization (ICH) guidelines [14].

Specificity

The specificity of the HPLC method is illustrated in Fig. 2, where complete separation of Piroxicam was noticed in presence of other inactive excipients used in injection dosage form. In addition, there was no any interference at the retention time of in the chromatogram of placebo solution. In peak purity analysis with PDA, purity angle was always less than purity threshold for the analyte. This shows that the peaks of analyte were pure and excipients in the formulation does not interfere the analyte. The data were presented in the Table 1.

Table 1: Specificity for Piroxicam

S.No.	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	563842214
4.	Sample	1	552822898

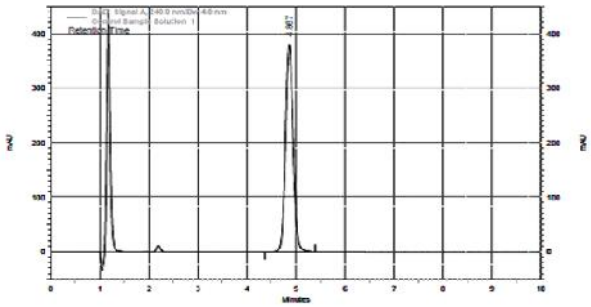

Figure 2: Typical HPLC Chromatogram of Piroxicam Injection

Linearity and Range

The Linearity of this method was determined at five levels from 50%– 150% of operating concentrations for Piroxicam and it was shown in Table 2. The plot of peak area of each sample against respective concentration of Piroxicam was found to be linear (Figure 3) in the range of 50%– 150% of operating concentrations. Beer's law was found to be obeyed over this concentration range. The linearity was evaluated by linear regression analysis using least square method. The regression equations were found to be Y= 68676x + 54678 for Piroxicam and correlation coefficient of the standard curve was found to be 0.999 for Piroxicam. It observed that correlation coefficient and regression analysis are within the limits.

Table 2: Linearity of response for Piroxicam

S.No	Target Level (%)	Conc. (µg/ml)	Area**
1.	50	50	33826327
2.	80	80	54553673
3.	90	90	61125847
4.	100	100	67913544
5.	110	110	74957178
6.	120	120	82063416
7.	150	150	102359424

***** Operating concentration

**Mean area of two replicate injections

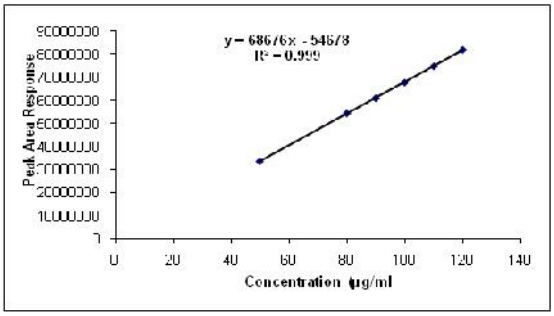

Figure 3: Linearity of response for Piroxicam

Accuracy: Accuracy of the method was found out by recovery study by standard addition method. The known amounts of standard, Piroxicam was added to pre-analyzed samples at a level from 80% up to 120% and then subjected to the proposed HPLC method individually. The results of recovery studies were shown in Table 3. It was observed that the mean percentage recoveries were found to be for Piroxicam which demonstrated that the method was highly accurate.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under the prescribed conditions.

Reproducibility

Examines the precision between laboratories and is often determined in collaborative studies. Reproducibility data for Piroxicam was shown in Table 4. This indicated that method was highly precise.

Repeatability

Repeatability is the precision of a method under the same operating conditions over a short period of time. One aspect of this is instrumental precision. A second aspect is sometimes termed intra-assay precision and involves multiple measurements of the same sample by the same analyst under the same conditions. Repeatability data for Piroxicam was shown in Table 5. This indicated that method was highly precise.

Robustness

The Measure of a method capacity remains unaffected by small, but deliberate variation in method Robustness of the above method was carried out by purposefully varying some chromatographic method parameters. The sample preparations were analyzed as per methodology by changing the ratio of solvents in the mobile phase by means of +10% or -10%. The robustness data of Piroxicam by changing the ratio of solvents present in the mobile phase medium, column temperature and flow rate. It was shown Table 6, and it was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was robust.

Ruggedness: Five duplicates sample preparations were analyzed as per the methodology by a different analyst on a different instrument on a different day. The Ruggedness data for Piroxicam was shown in Table 7. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was ruggedness.

Forced Degradation: The study was intended to ensure the effective separation of Piroxicam and its degradation peaks of formulation ingredients at the retention time of Piroxicam. Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method. In this method, the samples were subjected to various forced degradation conditions and percentage of degradation under various treatment conditions were shown in Table 8. Piroxicam is more sensitive towards oxidative degradation. The evaluation of chromatographic peak response of the analyte from every degradation method showed homogeneous and free of co-eluting peaks.

Table 3: Accuracy for Piroxicam

S.No.	Spike Level (%)	Amount added (mg)	Amount recovered (mg)	Drug recovery (%)
1.	80	80	80.84	101.3
2.	80	80	80.82	101.2
3.	80	80	80.74	101.3
4.	100	100	101.27	101.5
5.	100	100	101.25	101.7
6.	100	100	101.22	101.6
7.	120	120	120.88	101.2
8.	120	120	120.94	101.2
9.	120	120	120.92	101.2
			Mean	**101.35**
			Standard deviation	**0.1943**
			RSD in %	**0.19**

Table 4: Precision - Reproducibility for Piroxicam

S.No.	Name	Amount (µg/ml)	Area
1.	Standard -1	100	68269255
2.	Standard -2	100	68278843
3.	Standard -3	100	68322881
4.	Standard -4	100	68319473
5.	Standard -5	100	68331075
6.	Standard -6	100	68338018
		Mean	**68309924**
		Standard deviation	**28690.4**
		RSD in %	**0.04**

Table 5: Precision - Repeatability for Piroxicam

S.No	Sample Name	Area	Average area	Amount of drug present (mg)	Drug Recovered (%)
1.	Sample -1	65912481	65953950	19.5893	97.9
2.	Sample -2	65995419			
3.	Sample -3	66060916	65996435	19.6020	98.0
4.	Sample -4	65931954			
5.	Sample -5	66066162	66082216	19.6274	98.1
6.	Sample -6	66098270			
7.	Sample -7	65939924	65953147	19.5891	97.9
8.	Sample-8	65966369			
9.	Sample-9	65898717	65937669	19.5980	97.9
10.	Sample-10	65976621			
				Mean	**97.96**
				Standard deviation	**0.089**
				RSD in %	**0.09**

Table 6: Robustness data for Piroxicam

S.No	Parameter Name	Results obtained	
		Amount of Drug obtained (mg)	Amount of drug obtained (%)
1.	Change in Flow rate (-)	19.7213	98.6
2.	Change in Flow rate (+)	19.8611	99.3
3.	Change in Column Temperature (-)	19.8030	99.0
4.	Change in Column Temperature (+)	19.7498	98.7
5.	Change in Organic (-)	19.5617	97.8
6.	Change in Organic (+)	19.6564	98.3

Table 7: Ruggedness data for Piroxicam -Change of analyst

S.No.	Sample Name	Area	Average area	Amount of drug present (mg)	Drug Recovered (%)
1.	Sample -1	546203594	546887427	19.5359	97.7
2.	Sample -2	547571259			
3.	Sample -3	548175443	548269450	19.5852	97.9
4.	Sample -4	548363457			
5.	Sample -5	551011409	551659303	19.7063	98.5
6.	Sample -6	552307196			
7.	Sample -7	551291811	550741286	19.6735	98.4
8.	Sample-8	550190760			
9.	Sample-9	540074927	543359099	19.4098	97.0
10.	Sample-10	546643271			
				Mean	**97.9**
				Standard deviation	**0.6041**
				RSD in %	**0.62**

Table 8: Forced degradation data for Piroxicam

S.No	Sample treatment conditions	Area	Amount of drug present (mg/unit)	Amount of Drug (%)	Degradation (%)
1	Acid treated sample	501690943	19.4225	97.1	1.2
2	Base treated sample	503853136	19.5062	97.5	0.8
3	H_2O_2 treated sample	118355011	4.5820	22.9	76.7

4. Conclusion

The proposed study describes a new, economical and simple reversed phase - HPLC method for the analysis of Piroxicam in pharmaceutical dosage form. The method was validated as per ICH guidelines and found to be linear, sensitive, accurate and precise. Therefore the proposed method can be successfully employed for the routine analysis of Piroxicam in pharmaceutical dosage form without interference.

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