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Original Article

A novel RP-UPLC method development and validation for quantification of piroxicam tablet dosage form

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ABSTRACT

Objective: A simple, novel, sensitive and rapid ultra-performance liquid chromatographic (RP-UPLC) method has been developed and validated for quantitative determination of piroxicam (PIROX) in bulk and tablet formulations.

Method: The chromatographic development was carried out on Water C18 (3.6mm X 50 mm; 3 microns) column, with mobile phase consisting of Buffer: methanol 400ml:600 ml v\v. The flow rate was 0.6 ml/min and the effluents were monitored at 254 nm.

Results: The retention time was found to be 2.876 min. The method was validated as per International Conference on Harmonization Guideline with respect to linearity, accuracy, precision, and robustness. The calibration curve was found to be linear over a range of 25–75 µg/mL with a regression coefficient of 0.9999. The method has proved to be of high sensitivity and specificity.

Conclusion: The results of the study showed that the proposed RP-UPLC method was simple, rapid, precise and accurate which is useful for the routine determination of piroxicam in bulk drug and in its pharmaceutical dosage form.

Introduction

The piroxicam (PIROX) is the non- steroidal anti-inflammatory drug (NSAIDS) approved by the United States FDA for symptomatic treatment of anti-inflammatory, analgesic, and antipyretic properties. Osteoarthritis, rheumatoid arthritis Chemically, it is [4-hydroxy-2-methyl-N-(2-pyridyl) H-1,2-benzothiazine-3-carboxamide-1,1-di-oxide] (Figure1) Its molecular formula is $C_{15}H_{13}N_3O_4S$ and its molecular weight is 331.35g/mol [1,2].

The literature survey shows that several analytical techniques such as high- performance liquid chromatographic (HPLC) LC-MS, HPTLC, chemiluminescence and UV have been reported for its determination in plasma and tablet dosage forms. The present work reports simple, rapid, sensitive and economical rapid UPLC (RP-UPLC) method with UV detection, useful for the routine analysis of PIROX in

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bulk and pharmaceutical formulations. The method parameters such as linearity, accuracy, precision, robustness, stability and system suitability were validated as per International Conference on Harmonization (ICH) guidelines.

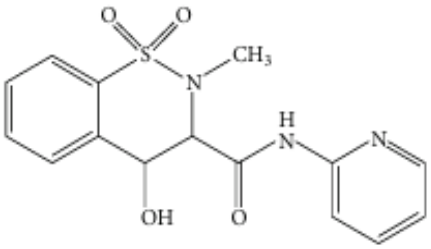

Figure 1: Chemical structure of piroxicam.

Materials and Methods

Instrumentation

UPLC analysis was carried out on water C18 (3.6 mm X 50 mm; 3 microns), reversed phase column. (Agilent Chemstation software), pump (1290 infinity II flexible pump), ultra sonicater (BVK enterprises), analytical balance (Metler) The mobile phase consisting of Buffer: methanol 400ml:600ml V/V. was used at a flow rate of a 0.6 mL/min. The detection was carried out by at 254 nm. All analysis was carried out at a temperature of 30°C under isocratic conditions.

Method development

The RP-UPLC method was developed by conducting number of trails in which the values of chromatographic parameters like wavelength, mobile phase composition and ratio, flow rate, stationary phase etc were altered to determine the effect of them on separation and identification of selected drug, finally the chromatographic parameters were optimized as follows.

Selection of wavelength

The wavelength was optimized by scanning the 50µg/ml concentrated solutions prepared by using dilutions of selected drugs through UV detector of UPLC system. The optimum identification was achieved at 254 nm, hence the wavelength 254 nm was used throughout the method development and validation.

Selection of chromatographic conditions

Preliminary trials were conducted by injecting the diluted standard solution of analyte to get the optimized chromatographic condition which favour the optimum ionization of selected drug in suitable mobile phase by changing the various solvent and solvent compositions. The effective separation and symmetrical peak shapes were achieved by changing the nature of column with different type, and manufacturers, the flow rate was adjusted to get the proper peak resolution and shape.

Optimized Chromatographic conditions

Chromatographic separations of piroxicam was performed on Waters C18 (3.6 x 50 mm, 3 microns) column with a mobile phase consisting methanol: buffer (3.86g of citric acid in 200 ml of water and 2.675g sodium phosphate in 50 ml of water and finally make up with 500 ml of water), having pH 3.0 in the ratio of 600:400 v/v, pump flow rate of 0.6 ml/min and detection wavelength of 254 nm was set at room temperature. The injection volume of 5µl and 5 min of runtime was used for effective separation of selected drug.

Preparation of mobile phase

3.86 gm of citric acid was accurately weighted, transferred to 100ml volumetric flask, dissolved with milliQ water, and 2.675 gm of sodium phosphate dissolved with 50ml of milliQ water, made up the final volume with 500ml of milliQ water, and adjusted pH to 3.0. mix 400ml of above prepared buffer with HPLC grade methanol, degassed in the mixture by ultrasonication, and was filtered through the 0.45µm Membrane filter using vacuum filtration assembly.

Preparation of standard stock solution

20mg of piroxicam were accurately weighted, transferred to 100ml of clean and dry flask and made up to volume with 0.1M methanolic HCl, it was sonicated and filtered through the 0.45µm membrane filter using vacuum filtration assembly. It was further diluted to obtain a concentration of 50 mcg.

Preparation of sample stock solution

330.25 mg of piroxicam was accurately weighted and transferred to 100ml of clean and dry flask and made up to volume with 0.1M methanolic HCl, it was sonicated and filtered through 0.45µm membrane filter using vacuum filtration assembly. It was further diluted to obtain a concentration of 50 mcg.

Assay of formulation

5 ml of standard stock solution and sample stock solutions were transferred separately into 20 ml volumetric flasks, diluted and made up to the final volume with diluent, the resulting solutions were sonicated for about 15 min, and filtered through 0.45mm filter. Standard and sample solutions were injected separately into the chromatographic system in triplicate.

Method Validation

Validation of the optimized method parameters includes linearity, system suitability, accuracy, precision, ruggedness, robustness, limit of detection and limit of quantification according to ICH guidelines.

System suitability

This parameter was tested by giving five replicate injections of the standard solution to check the system suitability parameters like asymmetry, theoretical plates (NLT 2000), tailing factor (NMT 2.0) etc. These tests are carried out based on the concept that the equipment, analytical method and samples are integral part of the system that needs to be evaluated. The test results was not more than 2% RSD.

Linearity

The linearity of the developed method was determined by preparing the aliquots of standard drug solutions with concentrations of 25-75 µg/ml of piroxicam from the standard stock solution, the linearity parameters like regression value (r^2), slope, y-intercept, were evaluated by plotting the calibration curve taking concentration in µg/ml on x-axis, and peak area on y-axis for linearity concentrations. The correlation coefficient (r^2) should not be less than 0.9999, % RSD of peak areas for replicated individual linearity concentrations should not be more than 2.0% to accept the linearity of the method.

Precision

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision. Solutions of piroxicam were prepared as per test method and injected 6 times. The mean SD and RSD were checked for precision. Method precision is done by preparing six samples as per the method of a single batch representing 100% of test concentration. For intermediate precision six samples were prepared by different analysts by using different column and different systems on different days. The system suitability criteria were evaluated. % RSD of for above 6 preparations was calculated and the overall % RSD for above experiment results was recorded.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is also termed as trueness. It was done by recovery study.

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Sample solutions were prepared with 100% in triplicate. To the formulation, the working standards of the drug were added at the level of 80%, 100%, 120%. each level was Prepared in triplicate injection and the average was taken to calculate the percentage relative standard deviation.

Robustness

The robustness of the proposed method was determined by triplicate injection of working stock solution from homogenous lots by differing physical parameter like flow rate and mobile phase composition, temperature variation which may differ, but the response was still within the specified limits of the assay. Acceptance criteria: % Relative standard deviation of peak areas and Rt should not be more than 2.0%.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection and limit of quantification was calculated by the average value of standard deviation and slope. The LOD and LOQ were determined by the linearity studies.

LOD = 3.3 x (SD / SLOPE)

LOQ = 10 x (SD / SLOPE)

Where,

SD = standard deviation of y- intercept

S = slope

Results and Discussion

Method development and optimization

The current research work enumerates a validated RP-UPLC method development for piroxicam in tablet formulation. The effective separation and good peak symmetry was achieved by the mobile phase Methanol: Buffer having pH3.0 [600:400, v/v] as mobile phase, on water C18 (3.6 x 50mm, 3 microns), analytical column under isocratic conditions.

System suitability

The retention time (Rt) for piroxicam were found as 2.8 min respectively with consistent reproducibility represented by % RSD as shown in figure-2. The chromatographic parameters like USP plate count, tailing factor, resolution were found to be within the limits as given in table-1 which indicates the system suitability of the developed method

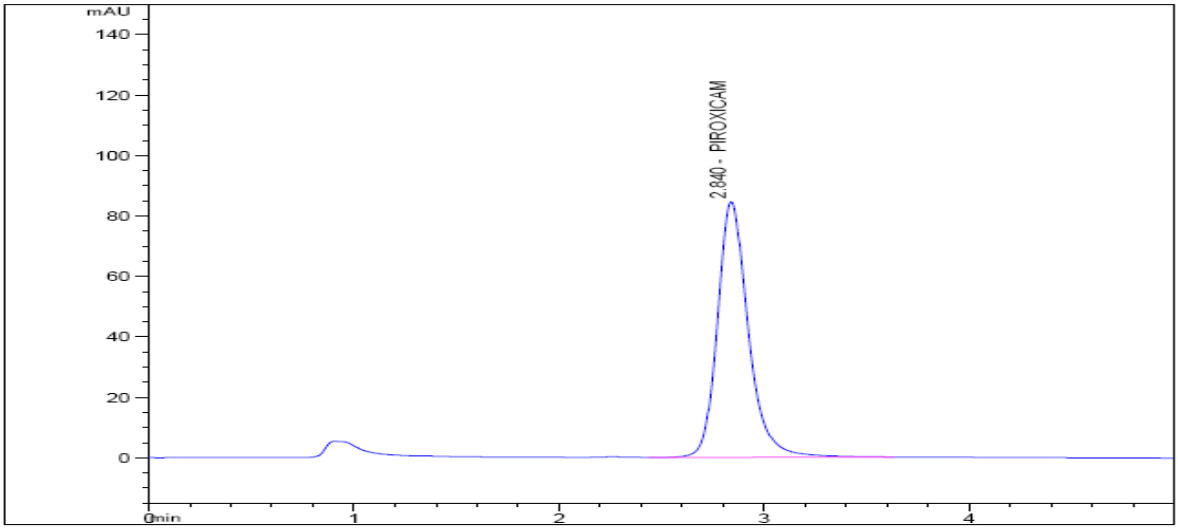

Figure 2: Chromatogram of piroxicam

Table 1: System suitability parameters of piroxicam.

S. No.	Parameter	Piroxicam	
		Mean ± SD	%RSD
1	Rt (min)	2.87 ± 0,014	0.50
2	Peak area	862.566 ± 8.403	0.97
3	Plate count	2081 ± 11.13	0.53
4	Tailing Factor	1.260 ± 0.011	0.88

Linearity

The developed method showed the proportional relationship between peak area and concentration at different level of standard drug in the range 25-75µg/ml with regression coefficients (r^2) 0.9999 for piroxicam. Respectively the linearity result is given in the Table-2. the linearity plots were shown in Figure-3.

Table 2. Calibration data of piroxicam.

Linearity level (%)	Piroxicam	
	Conc.(µg/ml)	Peak area
50	25.06	422.57
70	35.09	598.568
100	50.13	845.332
125	62.66	1051.925
150	75.19	1266.066
Correlation coefficient		0.9999

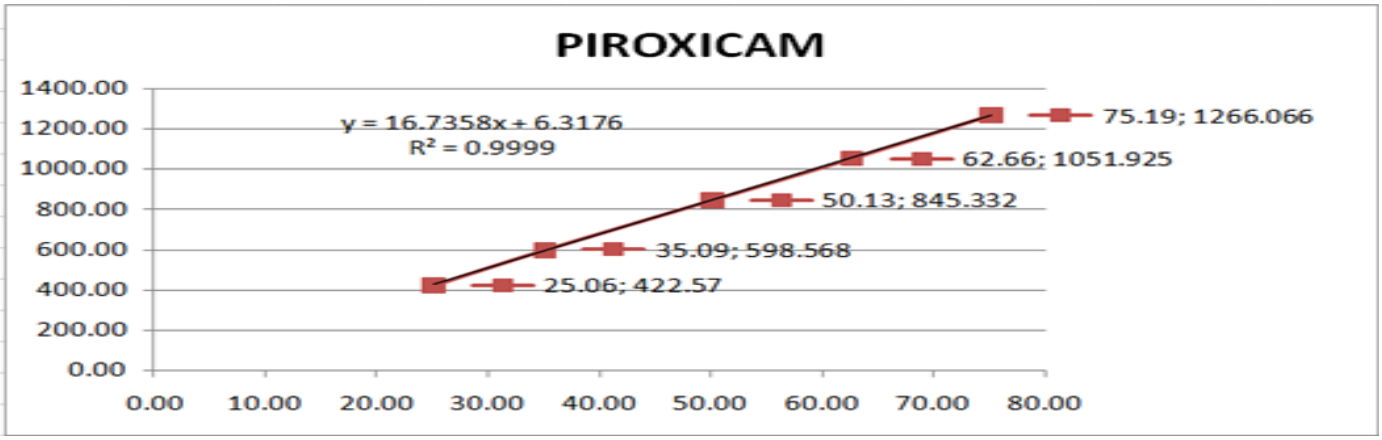

Figure 3: Calibration curve of piroxicam.

Precision

Repeatability

Six working sample solution of 50ppm were injected and the percentage amount was calculated. The %RSD was found to be 0.77.

Six working sample solution of 50ppm are injected on the next day of the preparation of sample and the % amount found was calculated. the % RSD was found to be 0.77.

Accuracy

Three injections of 80%, 100%, and 120% concentration were made in triplicate, and the recovery percentage was calculated. The accuracy result of piroxicam is shown in table-3.

Table 3: Accuracy of piroxicam.

% Level	Sample wt.(mg)	Area	Content (mg)	Content (%)
80%	269.02	695.351	19.775	98.99
	267.14	698.935	20.017	100.09
	268.52	698.548	19.903	99.52
100%	330.24	860.360	19.932	99.66
	331.58	859.309	19.827	99.14
	330.88	863.968	19.977	99.89
120%	396.25	1030.213	19.891	99.46
	396.83	1036.031	19.947	99.87
	395.24	1028.398	19.906	99.53

Robustness

Robustness of the method was checked by small deliberate changes in the method parameters such as wavelength (±2nm) and flow rate (±0.025ml) which shall not much affect in theoretical plates and peak asymmetry.

The robustness was tested by changing the wavelength and flow rate in the chromatographic system. The result are tabulated in table-4.

Table 4: Result of Robustness study.

S. No	Condition	% RSD
1	Flow rate (-) _0.35	0.43%
2	Flow rate (+) _0.55	0.43%
3	Wavelength (-) _228	0.53%
4	Wavelength (+) _232	0.50%

Limit of Detection (LOD)

Detection limit of the piroxicam in this method was found to be 0.65 µg/ml.

Limit of quantification (LOQ)

Quantification limit of the piroxicam in this method was found to be 1.97 µg/ml.

Assay of marketed formulation

Standard solution and sample solution were injected separately into the system and chromatograms were recorded and drug present in sample was calculated using before mentioned formula. The result are tabulated in table-5.

Table 5: Assay of Formulation.

Standard	Sample	%Assay
903.350	896.590	99.46%

Discussion

The aim in developing the UPLC method was to achieve separation and estimation of drug in tablet dosage under common conditions that are capable for routine quality control, research and development of the drug in ordinary laboratories. Recently the RP-UPLC method development for the determination of drug has received more attention because of it speed, sensitivity, resolution, less solvent consumption, cost effectiveness and more productivity which is important in the quality control of drug product. This work was intended to develop a precise, less time consuming and a rapid RP-UPLC method for estimation of piroxicam. as The LOD and LOQ values indicated that the method was more sensitive, %RSD values indicated that the method was precise, accurate and robust in nature.

Conclusion

Till date most RP-UPLC reported methods were estimated as the piroxicam with high retention time. Hence attempts which should separates the development of a method which should separate the compounds with good resolution and less retention times. different logical modification was tried to get good separated symmetrical peaks with less retention time this is achieved by changing the mobile phase composition. The result of developed method it is rapid, simple, accurate, precise, and robust in nature. This method can be utilized for routine estimation of piroxicam drug in bulk and pharmaceutical dosage form.

Author Contributions

All authors contributed equally to this research. All authors read and approved the final manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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Ethical Approvals

This study does not involve experiments on animals or human subjects.

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