

Method Development and Validation of a Stability Indicating RP-UPLC Method For the Determination of Piroxicam in 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 Stability indicating nature of the method was analysed by stress degradation study by acid, alkaline, oxidative, thermal condition, photolytic condition and humidity. The degradation Product of Piroxicam in Thermal Condition is characterized in LC-MS, NMR, IR and UV. The LC-MS study helped to identify the degraded product. Toxicity prediction of the thermally degraded product of Piroxicam was carried out by using OSIRIS. The method has been proved to be of high sensitivity and specificity. **Conclusion:** The results of the study showed that the proposed RP-UPLC method is simple, rapid, precise and accurate which is useful for the routine determination of Piroxicam in bulk drug and in its pharmaceutical dosage form.

Keywords: Piroxicam, UPLC, Method Validation, Stability indicating, ICH guideline, Degradation Study.

INTRODUCTION

The Piroxicam (PIROX) is a non-steroidal anti-inflammatory drug (NSAIDS) approved by the United States FDA for symptomatic treatment of anti-inflammatory, analgesic, and antipyretic properties. Chemically, it is [4-hydroxy-2-methyl-N-(2-pyridyl) H-1,2-benzothiazine-3-carboxamide-1,1-di-oxide] (Figure 1). Its molecular formula is C₁₅H₁₃N₃O₄S 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) [3,4], LC-MS, HPTLC, chemiluminescence and UV have been reported for its

determination in plasma and tablet dosage forms. The present work reports a simple, rapid, sensitive and Stability indicating UPLC method with UV detection, useful for the routine analysis of PIROX in 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.

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

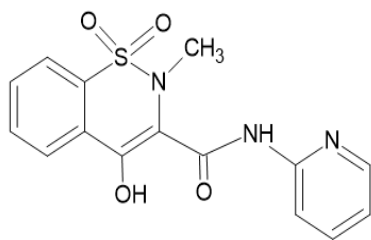


Figure 1: Chemical structure of Piroxicam

2. 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 sonicator (BVK enterprises), analytical balance (Mettler) 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 their effect of 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 drug by UV detector of UPLC system. The optimum identification was achieved at 254nm, hence the wavelength 254nm 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 x50mm, 3microns) 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 made 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 to the final volume with 500ml of milliQ water, and adjusted the pH to 3.0. 400ml of the above prepared buffer was mixed with HPLC grade methanol, degassed in the mixture by ultrasonication, and was filtered through 0.45µm membrane filter using vacuum filtration assembly.

Preparation of standard stock solution

20mg of piroxicam was accurately weighted, transferred to 100ml 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 µg.

Preparation of sample stock solution

330.25 mg of piroxicam was accurately weighted and transferred to a 100ml 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 µg.

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.

3. 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 was found to be 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.

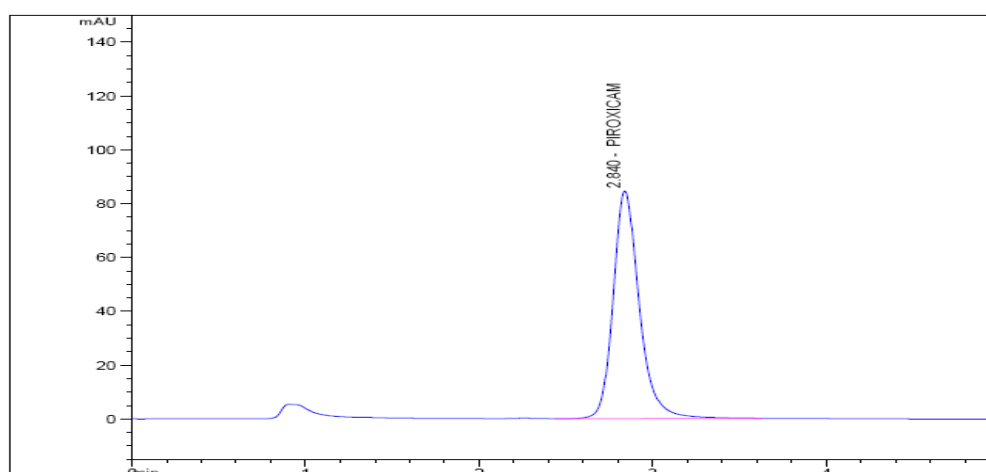


Figure 2: Chromatogram of Piroxicam

Table -1. System suitability parameters of piroxicam

S. No.	Parameter	Piroxicam	
		Mean \pm SD	%RSD
1	Rt (min)	2.87 \pm 0.014	0.50
2	Peak area	862.566 \pm 8.403	0.97
3	Plate count	2081 \pm 11.13	0.53
4	Tailing Factor	1.260 \pm 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. The linearity result is given in Table -2.



Table 2: Calibration data of piroxicam

Linearity level (%)	Piroxicam	
	Con.(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

Precision

found was calculated. The % RSD was found to be 0.48.

Repeatability

Six working sample solution of 50ppm were injected and the percentage 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 %

Accuracy

Three injection 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 ($\pm 2\text{nm}$) and flow rate ($\pm 0.025\text{ml}$) which

shall not much affect 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 (-) _253	0.53%
4	Wavelength (+) _255	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. The result are tabulated in (Table5).

Table 5: Assay of Formulation

Standard	Sample	%Assay
903.350	896.590	99.46%

STABILITY INDICATING UPLC METHOD FOR PIROXICAM

Piroxicam was subjected to acid and alkali hydrolysis, oxidation, photo degradation and thermal stress condition. Stress testing of Piroxicam was done under different conditions using the mobile phase that consists of a mixture of the Buffer: Methanol in the ratio (40:60).

Forced Degradation Study**Acidic degradation**

5mL of sample stock solution was taken into a 20mL standard measuring flask, to this solution 2mL of 0.1 M hydrochloride acid was added at a temperature condition of 35°C and then injected in UPLC for peak estimation.

Alkali degradation

5mL of sample stock solution was taken into a 20mL standard measuring flask, to this solution 2mL of 0.1 M sodium hydroxide was added at a temperature condition of 35°C and then injected in UPLC for peak estimation.

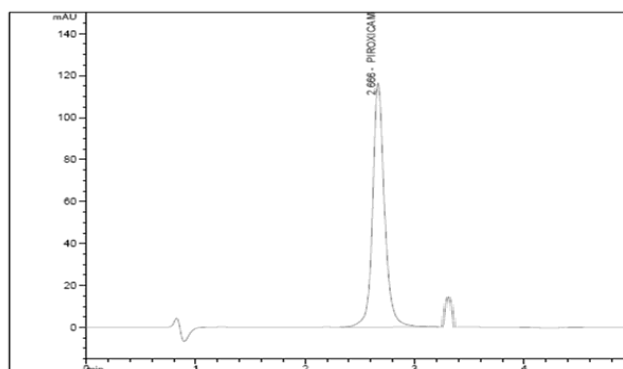
Oxidative degradation

The forced degradation of Piroxicam was studied under the influenced of 3% hydrogen peroxide (H₂O₂). 5mL of sample stock solution was taken into a 20mL standard measuring flask and then 2mL of 3% hydrogen peroxide (H₂O₂) was added and then the diluents was added and it was made up the volume.

The sample was subjected to UPLC run for estimation.

Thermal degradation

A volume of 5mL of Piroxicam was pipette out in a 20mL volumetric flask and then it was subjected to heat at 105°C for 2hr. then this solution is made-up using diluents. This solution was subject to UPLC run for estimation. The chromatogram obtain from the study is shown in (Figure 3)

**Figure 3: Thermal degradation of piroxicam at 105°C at hot air oven****Photo stability study**

A volume of 5 ml of stock solution of Piroxicam was pipetted out to a 20 ml volumetric flask. Then this solution was subjected to UV light in the range of 254nm and 365nm for 7 days, then made-up using diluents 20ml of the final sample solution was subjected to UPLC run for estimation.

Humidity Stress

Analytical solution was prepared as per method. and then placed in open container at room temperature $25 \pm 5^\circ\text{C}$ for 48 Hours. The result of degradation study are shown in (Table 6).

Table 6: Forced degradation study of Piroxicam

Sr.no	Condition	Experiment	Total degradation
1	Acid Hydrolysis	Simulate degradation in acidic environment using 0.1N Hydrochloric acid	89.53%
2	Basic Hydrolysis	Simulate degradation in alkaline environment using 0.1M Sodium hydroxide	91.54%
3	Oxidative Degradation	Check degradation under oxidizing conditions using 3% Hydrogen peroxide	87.23%
4	Photolytic Degradation	Simulate exposure to light at 254nm for 7days	86.06%
		Simulate exposure to light at 365nm for 7days	100.12%
5	Thermal Degradation	Simulate heat stress in hot air oven at 105°C for 2hours	82.68%
6	Humidity Stress	Assess stability under humidity stress at $25 \pm 5^\circ\text{C}$ and 60–75% RH for 48 hours	100.20%

Characterization of degradation product

UV spectrum of Thermal Degradation of Piroxicam is shown in (Figure 4)

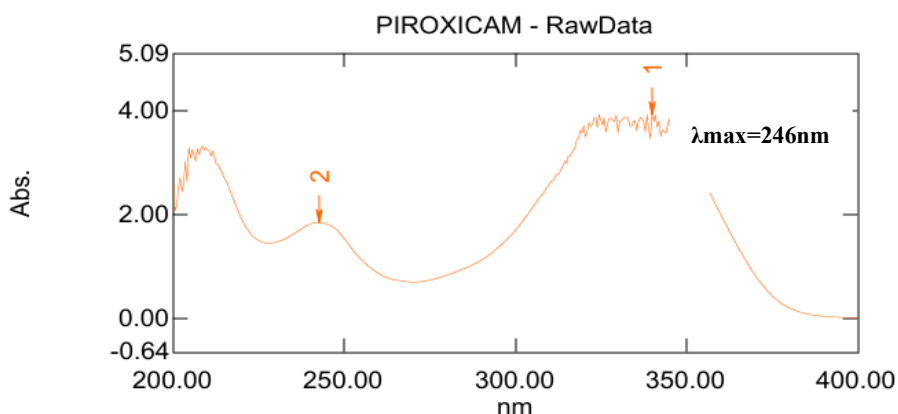


Figure 4: λ_{max} of Thermal Degradation product of PIROX

IR spectrum of Thermal Degradation of Piroxicam is shown in (Figure 5) and interpretation shown in (Table 7)

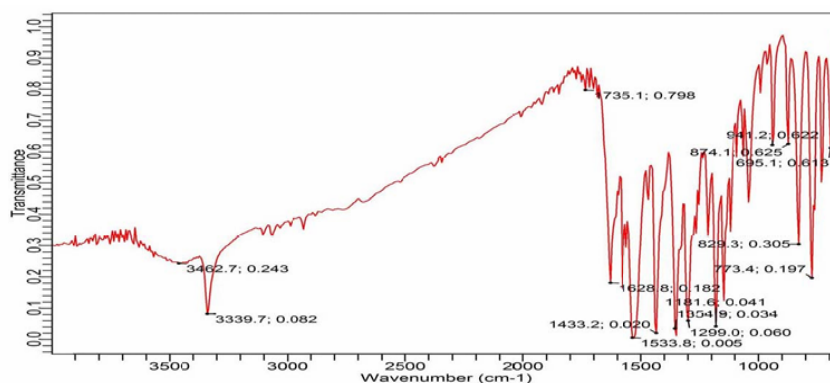


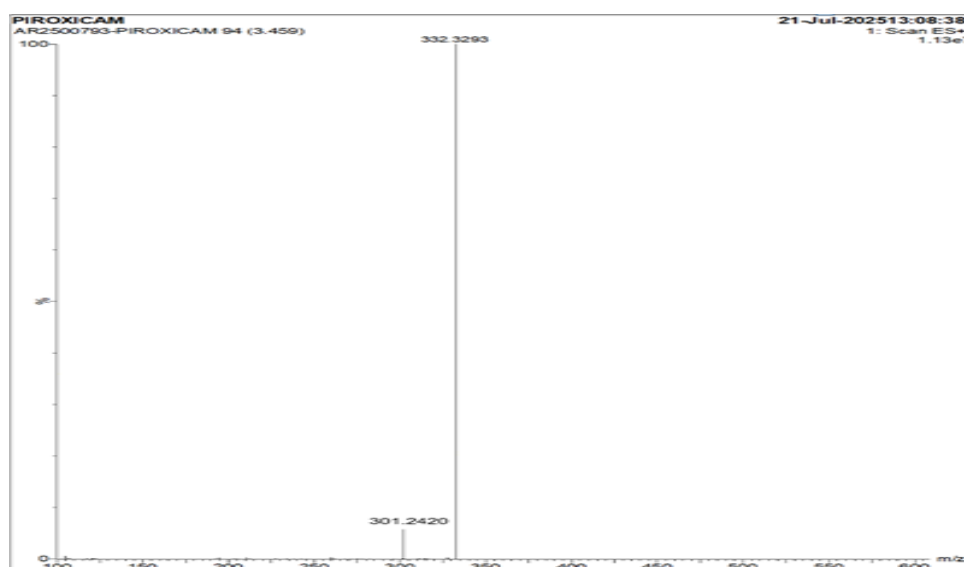
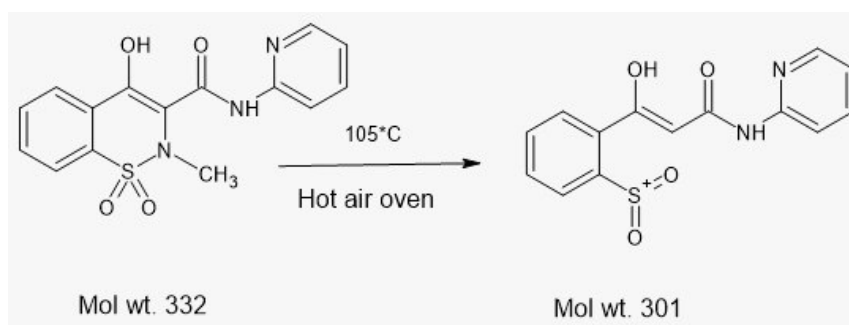
Figure 5: IR degradation spectrum of Piroxica



Table 7: IR SPECTRA INTERPRETATION

Functional group	Stretching frequency cm^{-1}
(Aromatic N-H)	3462
(O-H)	3339
(Aromatic C-H)	3062
(Ester)	1735
(C=C)	1433
(Amide)	1628
(SO ₂)	1354

The degraded product obtained in the thermal stress condition was further subjected to LC-MS study for characterization of the degraded Product. LC-MS spectrum obtained is shown in (Figure 6). The Possible degradation Pathway is shown in (Figure 7).

**Figure 6: LC-MS Spectrum of degradation Product of Piroxicam****Figure 7: Possible degradation pathway of Piroxicam**

NMR spectrum of Thermal Degradation of Piroxicam is shown in (Figure 8)

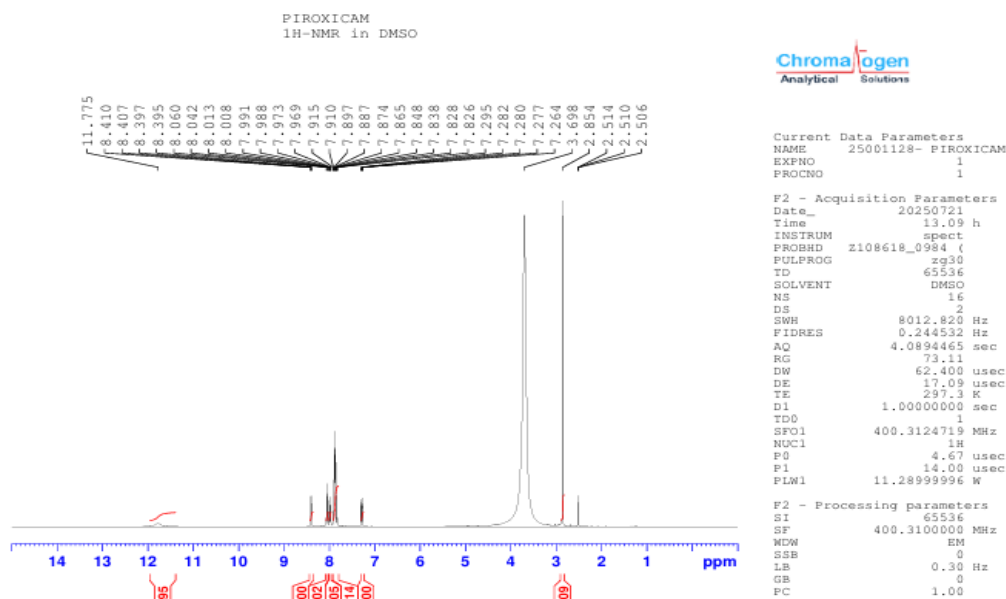


Figure 8: NMR degradation spectrum of Piroxicam

The toxicity of the thermally degraded product was predicted by OSIRIS software. It was found that the degraded product is non- mutagenic, non-tumorigenic and has no irritability and reproductive effects.

DISCUSSION

The aim of developing the UPLC method was to achieve separation and estimation of drug in tablet dosage under common conditions and Stress studies are carried out 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. 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 HPLC methods reported high retention time for Piroxicam. Hence by using UPLC which should separates the compounds with good resolution and less retention times, different logical modification were tried to get good separated symmetrical peaks with less retention time. This is achieved by changing

the mobile phase composition. The result is that the developed method is rapid, simple, accurate, precise, and robust in nature. Stress degradation studies were carried out to prove the Stability indicating nature of the method. 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 author read and approved the final manuscript.

ACKNOWLEDGEMENTS

The authors are thankful to the Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai, Tamil Nadu for helping in carrying out this work.

FUNDING

Non-funding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVALS

This study does not involves experiments on animals or human subjects



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HOW TO CITE: R. Hemanath*, P. G. Sunitha, N. Deattu, T. Hariharan, A. Mageshwari, R. Jawahar Samuvel, Method Development and Validation of a Stability Indicating RP-UPLC Method For the Determination of Piroxicam in Tablet Dosage Form, J. Pharm. Sci., 2025, 1 (10), 524-533. <https://doi.org/10.5281/zenodo.17390788>

