Asian Journal of Pharmaceutical and Health Sciences
www.ajphs.com

Analytical method development and validation of piroxicam by high performance liquid chromatography and ultraviolet spectroscopy technique

ShashankSoni *[1,2], Veerma Ram [1], AnuragVerma[2]

1 School of Pharmaceutical Sciences,SardarBhagwan Singh PG Institute of Biomedical Sciences and Research, Balawala, Dehradun, India
2 School of Pharmaceutical Sciences, IFTM University, Moradabad, India.

ARTICLE HISTORY

Received: 11.10.2017

Accepted: 12.02.2018

Available online: 30.03.2018

Keywords:

PRX, HPLC, UV, International Conference on Harmonization, Quality control

***Corresponding author:**

Email : shashank_soni64@yahoo.com
Tel.: +91 - 9410572306

ABSTRACT

Purpose: A simple, precise, specific, and accurate High Performance Liquid Chromatographic (HPLC) and Ultraviolet (UV) spectrophotometer method was developed and validated for determination of Piroxicam (PRX) in pure and pharmaceutical dosage forms. **Method :** The different analytical performance parameters such as linearity, accuracy, specificity, precision, and sensitivity (limit of detection and limit of quantification) were determined according to International Conference on Harmonization ICH Q2 (R1) guidelines. HPLC was conducted on Water Spherisorb® analytical column used having the dimension of 5 µm, 4.6*250mm. The mobile phase was consisting of buffer(containing 0.1 M potassium dihydrogen phosphate solution having pH 3.0) and acetonitrile (ACN) in the ratio 1:3 v/v, and the flow rate was maintained at 1.0 mL/min. PRX was monitored using Water Breeze 2 system equipped with photo diode array detector (λ = 333 nm) and also byUV spectrophotometer (λ = 333 nm) by Shimadzu UV 1800. **Result :** Linearity was observed in concentration range of 1050*µg/mL*by HPLC and 0-10 *µg/mL* by UV spectroscopy method. Correlation coefficient was found to be 0.9967 and 0.9962 respectively by HPLC and UV method. Linearity, accuracy, specificity, precision, and sensitivity (limit of detection and limit of quantification) were determined and value find within the range as specified by ICH guidelines. **Conclusion :** All the system suitability parameters were found within the range. The performed method is rapid, cost-effective and can be used asa quality-control tool for routine quantitative analysis of PRX in pure and pharmaceutical dosage forms.

INTRODUCTION

Piroxicam (PRX) is chemically 4-Hydroxy-2-methyl-N-(2-pyridyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide having an average molecular weight of 331.346, monoisotopic of 331.062676609 and chemical formula $C_{15}H_{13}N_3O_4S$ [1]. PRX is a nonsteroidal anti-inflammatory drug (NSAIDs) belonging to oxicam group. They exhibit a potent analgesic and anti-inflammatory activity effective in the treatment of rheumatoid arthritis, and other joint diseases. They exhibit cyclooxygenase (COX) at the peripheral end, which is an important enzyme for the biosynthesis of prostaglandins (PG) at the site of inflammation. They are well absorbed from the oral route and from stomach mucosal cell. They are strongly bound in protein plasma (usually > 95 %) so their volume of distribution typically approximates to plasma volume (0.14 L/kg) [2]. They are a weak acid having a pKa value of 6.3. This shows the water solubility 23 mg/L at room temperature, permeability in human epithelial Colorectal adenocarcinoma cells (Caco2) -4.45 and according to the Biopharmaceutical Classification System (BCS) belonging to class II drug characterized by a low water solubility and dissolution rate. It has an intrinsic solubility (log So) expressed as average log molar concentration ± standard deviation is 4.03 ± 0.001. It is having cLogP 1.89 cm/s. Intrinsic dissolution rate at pH 1.2 was 0.088 ± 0.022 mg/min/cm^2 [3].

From the literature survey, it was found that various methods were used for the estimation of PRX such as spectrophotometric

method, High-Performance Liquid Chromatographic (HPLC) method [4], and Gas Chromatographic-Mass Spectrometric (GC-MS) method [5] in a laboratory-prepared mixture, pharmaceutical preparation, and biological matrices such as human plasma. However, the aim of the present work is to develop a simple, precise, specific, accurate, cost-effective, and validated HPLC and Ultraviolet (UV) method according to USP and ICH guidelines for the estimation and routine evaluation of PRX in pure and pharmaceutical formulations [6, 7].

MATERIALS AND METHODS

Materials, Equipments and Instruments

Piroxicam (PRX) was received as a gift sample from Akum's Drug and Pharmaceutical Limited, India. Orthophosphoric acid, Sodium hydroxide, Potassium dihydrogen phosphate, Acetonitrile used was of AR grade. 0.45µm membrane filter procured from Rankem. Ultrapure water (Maxima ultrapure water, UK) with a resistivity more than 18 MΩ/cm was used during the experimental procedure. Micropipette procured from modern scientific industries, India and high accuracy weighing balance; Shimadzu ATX 224 used during the experimental work. All the other chemicals and reagents used were of analytical grade.

For method validation, HPLC used is of the Water Breeze 2 system, Water Spherisorb® analytical column used having the dimension of 5 µm, 4.6*250mm. Ultraviolet spectroscopy (Shimadzu UV-1800) also used for validation purpose. Both HPLC and UV spectroscopy used integrated with two different computers having the latest version of Windows 7 for computation of data.

Preparation of mobile phase

The mobile phase consisted of Acetonitrile and Potassium dihydrogen phosphate was mixed in a ratio of 3:1 maintaining the pH of 3.0. Both the composition is degassed in a sonicator for a time period of 25 minutes. Injection volume was 20.00 µL and UV detection was at 333 nm.

Standard solution preparation for HPLC technique

Accurately weigh and transfer 25mg of Piroxicam into a 50 mL of the calibrated volumetric flask, add about 50 mL of diluent, sonicate to dissolve for 25 minutes, makeup 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.

Retention time of PRX by HPLC technique

Retention time (RT) is a measure of the time taken for a solute to pass through a chromatography column. It is calculated as the time from injection to detection [4].

Linearity

This is the method's ability to obtain results which may directly or after mathematical changes proportional to the concentration of the analyte in a given range. Linearity can be determined by the calculation of the regression line using a mathematical treatment of the results (i.e least mean squares) versus analyte concentration [4].

Accuracy

Accuracy is a measure of the closeness of the test results that obtained by a method to the true value. Accuracy is the deviation between the found mean value and the true value. It is determined by applying the method to the samples in which known amounts of the analyte have been added to the sample. These values should be analyzed against the standard and the blank solutions to ensure that there is no interference exists. The accuracy can be determined by the test results as a percentage of the analyte recovered by the assay of the sample [4, 8].

Precision

The precision of an analytical method described as the degree of agreement within the individual test results obtained when the method is applied to multiple sampling of a homogenous sample. Precision is a measure of the reproducibility of the whole analytical method (which includes the process of sampling, sample preparation & analysis) under the normal operating circumstances. Precision can be determined by using the method of assay of a sample for a sufficient number of times to obtain statistically valid results. A precision of the method was determined by repeatability (intraday precision) and intermediate precision (interday precision) of both standard and sample solutions [4, 8].

Ruggedness

Ruggedness is the degree of reproducibility of results obtained by the analysis of the same sample under a variety of normal test conditions i.e. different analysts, labs, instruments, reagents used, temperatures for assay, small variations in mobile phase and different days and some others. (i.e. from a laboratory to laboratory, from analyst to analyst [4, 8].

RESULTS

(A) Validation method of Piroxicamby HPLC method

Retention time

Retention time (RT) was studied for 5 µg/mL solution (Figure 1 and Table 1). A total number of six injections were run in HPLC column having a flow rate of 1 mL/min having run time of 5 minutes. The average RT and % RSD was found to be 3.249 ± 0.23, 1.30 respectively.

Table 1: Standard andRetention time table for standard PRX

Injection No.	Peak Name	RT (min)	Area	Mean	Standard Deviation	% RSD
1	Piroxicam	3.253	2166162	2170011.5	1.32	1.30
2		3.253	2115183			
3		3.249	2190818			
4		3.248	2176060			
5		3.249	2190399			
6		3.244	2181447			

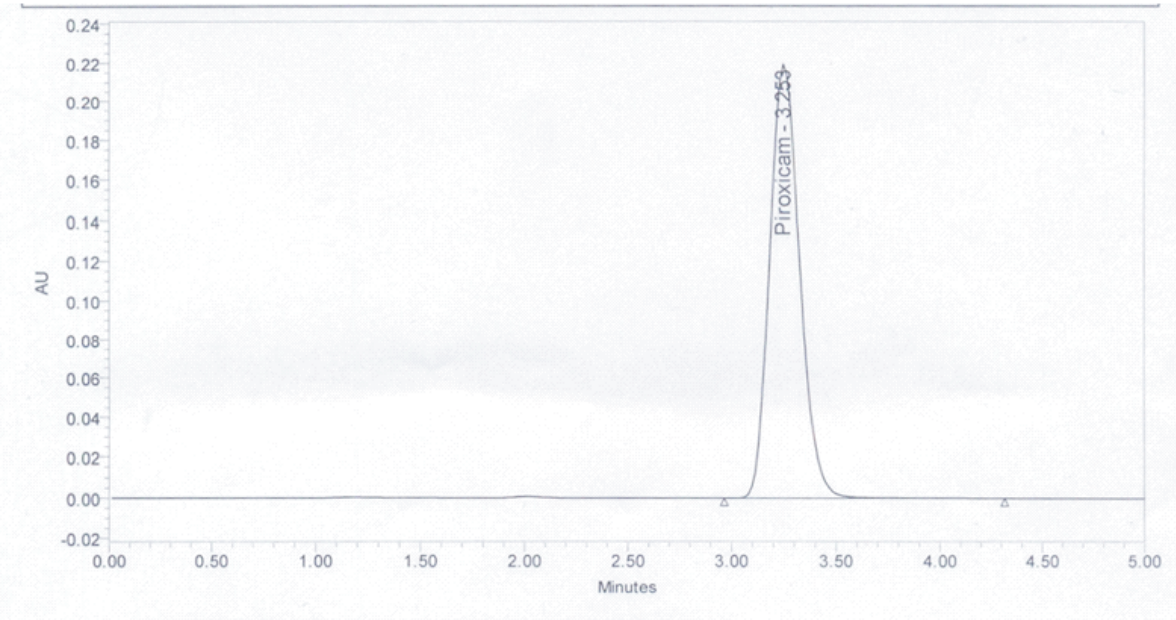

Fig. 1 : Chromatogram for Standard of PRX

Table 1(a) : Optimized Condition for HPLC method

S.NO	Parameter	Optimized Condition
1	Chromatograph	Water Breeze 2 HPLC system
2	Column	Water Spherisorb® 5 µm, 4.6*250mm Analytical column
3	Mobile Phase	ACN :Buffer
4	Flow rate	1 mL/min
5	Injection Volume	20.00 µl
6	Run time	5 minutes
7	Detection	UV at 333 nm
8	Column Temperature	Ambient

Table 2 : Linearity

Linearity No.	Conc. (µg/mL)	Injection No.	Peak Name	RT (min)	Area	Mean Area
1	10	1	Piroxicam	3.243	810162	810162
		2		3.241	810161	
		3		3.242	810161	
2	15	1		3.241	1101150	1101151
		2		3.244	1101151	
		3		3.244	1101151	
3	20	1		3.243	1401590	1401590
		2		3.244	1401590	
		3		3.241	1401589	
4	30	1		3.241	2021733	2021733
		2		3.241	2021734	
		3		3.240	2021733	
5	40	1		3.240	2609405	2609405
		2		3.243	2609404	
		3		3.243	2609404	
6	50	1		3.245	3205965	3205965
		2		3.244	3205964	
		3		3.232	3205965	

Linearity

The linearity was analyzed through the standard curves ranging from 10 - 50 µg/mL by diluting appropriate amounts of PRX stock solution with acetonitrile and buffer which is prepared in triplicate. Three calibration plots were prepared on the same day with the following concentrations (10, 15, 20, 30, 40 and 50 µg/mL). The linearity was evaluated by linear regression analysis, which was calculated by the least-square regression analysis. The regression equation was found to be (y=62370x + 114200) and r^2 value 0.9967 (table 2 and 3) was highly significant. It obeys the Beer - Lambert's law in a concentration range of 10-50 *µg/mL*. The validity of the assay was verified by means of the ANOVA (Graph-pad prism ®). According to it, there is linear regression and there is no deviation from linearity ($P < 0.05$).

Accuracy by recovery method

The accuracy of the method was estimated by addition recovery method. In this, known amount of standard PRX was added to the pre-analyzed sample. This was done for 20, 30, 40 µg/mL and reading was performed in triplicate mean area ± S.D was found to be 3417880 ± 2.84 with low % RSD value (Table 5).

Ruggedness analysis

The ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective RT with peak area was noted. The result was indicated (Table 7 and 8).

Method Precision

The precision of the assay was determined by repeatability (intraday) and intermediate precision (interday) and reported as

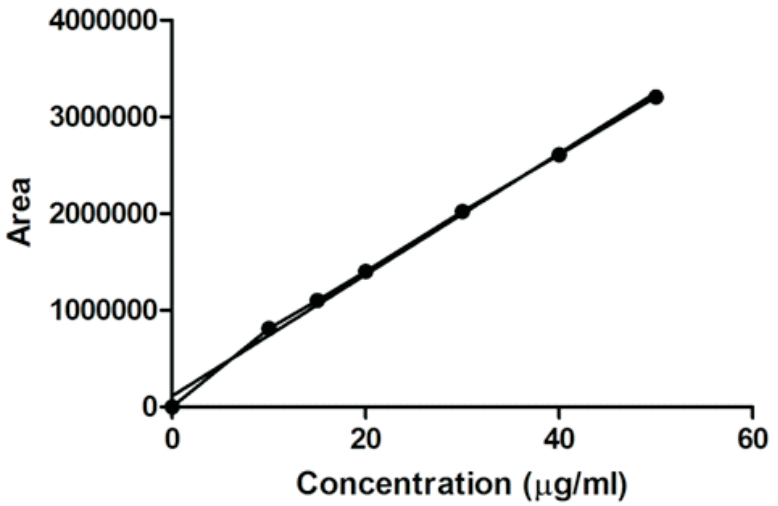

Fig. 2 : Linearity of PRX

Table 3 : Statistical Parameter Related to Calibration Plot

Best-fit values	Results
Equation	Y=62370x+114200
Slope	62730 ± 1610
Y-intercept when X=0.0	114200 ± 46050
X-intercept when Y=0.0	-1.821
1/slope	0.00001594
95% Confidence Intervals	
Slope	58590 to 66870
Y-intercept when X=0.0	-4194 to 232600
X-intercept when Y=0.0	-3.927 to 0.06341
Goodness of Fit	
r^2	0.9967
Sy.x	68990
Is slope significantly non-zero?	
F	1518
DFn, DFd	1.000, 5.000
P value	< 0.0001
Deviation from zero?	Significant

Table 4 : Bracketing standard

Injection No.	Peak Name	RT (min)	Area	Mean	Standard Deviation	% RSD
1	Piroxicam	3.228	4883231	4233197	0.225	1.999
2		3.228	3583163			
3		3.228	3593163			

Table 5 : Accuracy by recovery method

Recovery %	Injection No.	Peak Name	RT (min)	Area	Mean	Standard Deviation	% RSD
50	1	Piroxicam	3.225	3424416	3417880	2.84	0.83
	2		3.225	3420827			
	3		3.228	3408398			
100	1		3.224	4437259			
	2		3.225	4489532			
	3		3.223	4516858			
150	1		3.229	5666209			
	2		3.229	5754629			
	3		3.229	5639390			

Table 6 : Bracketing Standard

Injection No.	Peak Name	RT (min)	Area	Mean	Standard Deviation	% RSD
1	Piroxicam	3.235	5408949	5397360	7.12	1.32
2		3.228	5385772			
3		3.227	5375774			

Table 7 : Ruggedness analysis by analyst I

Standard no.	Injection No.	Peak Name	RT (min)	Area	Mean	Standard deviation	% RSD
1	1	Piroxicam	3.387	3044619	2902307	1.56	0.539
	2		3.389	2687345			
	3		3.391	2960876			
	4		3.391	3086675			
	5		3.391	3057798			
	6		3.390	3074193			
1	1		3.390	3139365			
	2		3.391	3139367			
2	1		3.400	2827468			
	2		3.397	2827469			
3	1		3.402	2895853			
	2		3.392	2895855			
4	1		3.405	2724598			
	2		3.402	2724596			
5	1		3.405	2772989			
	2		3.403	2772990			
6	1		3.403	2755043			
	2		3.402	2755041			

Table 8 : Ruggedness analysis by analyst II

Standard no.	Injection No.	Peak Name	RT (min)	Area	Mean	Standard deviation	% RSD
1	1	Piroxicam	3.386	3044620	2902301	1.51	0.539
	2		3.388	2687343			
	3		3.392	2960878			
	4		3.390	3086671			
	5		3.390	3057793			
	6		3.390	3074191			
1	1		3.390	3139366			
	2		3.392	3139361			
2	1		3.401	2827468			
	2		3.398	2827463			
3	1		3.402	2895854			
	2		3.392	2895856			
4	1		3.405	2724596			
	2		3.402	2724592			
5	1		3.404	2772990			
	2		3.403	2772991			
6	1		3.401	2755044			
	2		3.402	2755042			

% relative standard deviation (RSD). For this, 20, 30, 40 µg/mL concentration solutions were measured three times in a day and the same was measured in the next 3 days. The %RSD was calculated (Table 9 and 10).

(B) Validation method of Piroxicam by UV spectroscopy method

Standard stock solution of PRX in 0.1 M HCl solution

Standard drug solution of PRX was prepared by dissolving 10 mg of PRX in 5 mL 0.1 M HCl in a 10 mL volumetric flask, shaken well, followed by vortexing for 5 minutes and finally the volume was adjusted to get a solution of a concentration of 1 mg/mL. This 1 mg/mL solution was used as a stock solution.

Calibration plot of PRX in 0.1 M HCl solution

Five milliliters of 1 mg/mL aliquot solution was further diluted up to 50 mL by 0.1 N HCl in a 100 mL volumetric flask and the final volume was adjusted up to 100 mL. This was scanned spectrophotometrically in the wavelength region 200-400 nm to determine the wavelength of absorption maximum (λ_{max}). The

Table 9 : Method Precision by intraday precision

Standard no.	Injection No.	Peak Name	RT (min)	Area	Mean	Standard Deviation	% RSD
1	1	Piroxicam	3.414	2938515	2521888	1.49	0.593
	2		3.408	2929085			
	3		3.402	2868270			
	4		3.404	2952992			
	5		3.401	2912076			
	6		3.399	2926546			
2	1		3.398	2961523			
	2		3.399	2961522			
3	1		3.395	2821356			
	2		3.397	2821347			
4	1		3.391	2891910			
	2		3.392	2891918			
5	1		3.389	3073795			
	2		3.387	3073797			
6	1		3.387	3090622			
	2		3.386	3090624			

Table 10 : Method Precision by intraday precision

Standard no.	Injection No.	Peak Name	RT (min)	Area	Mean	Standard Deviation	% RSD
1	1	Piroxicam	3.414	2938516	2521876	1.12	0.781
	2		3.408	2929084			
	3		3.402	2868271			
	4		3.404	2952993			
	5		3.401	2912076			
	6		3.399	2926545			
2	1		3.398	2961521			
	2		3.399	2961520			
3	1		3.395	2821356			
	2		3.397	2821346			
4	1		3.391	2891911			
	2		3.392	2891917			
5	1		3.389	3073791			
	2		3.387	3073798			
6	1		3.387	3090621			
	2		3.386	3090623			

$\lambda_{max}$was found to be 333 nm against blank [Figure 3]. From 1 mg/mL stock solution, the serial dilution pattern was followed to obtain aliquots of 0-10 *µg/mL*concentration. The calibration plot was plotted between concentration and absorbance. The optical characteristics of different aliquots are depicted in Table 11.

Linearity

The linearity of the drug was obtained for 0-10 µg/mL concentration range of PRX. The calibration plot was obtained by plotting absorbance versus concentration and linear regression analysis was performed to get a linear equation. The linear equation found was y = 0.0422x + 0.012 and r^2 was 0.9962. The calibration curve was found to be linear in stated concentration.

Table 11 : Calibration Data for calibration plot in 0.1 M HCl solution in pH 1.2 at 333 nm

Concentration (µg/mL)	Mean Absorbance
0	0
2	0.101
4	0.186
6	0.277
8	0.351
10	0.422

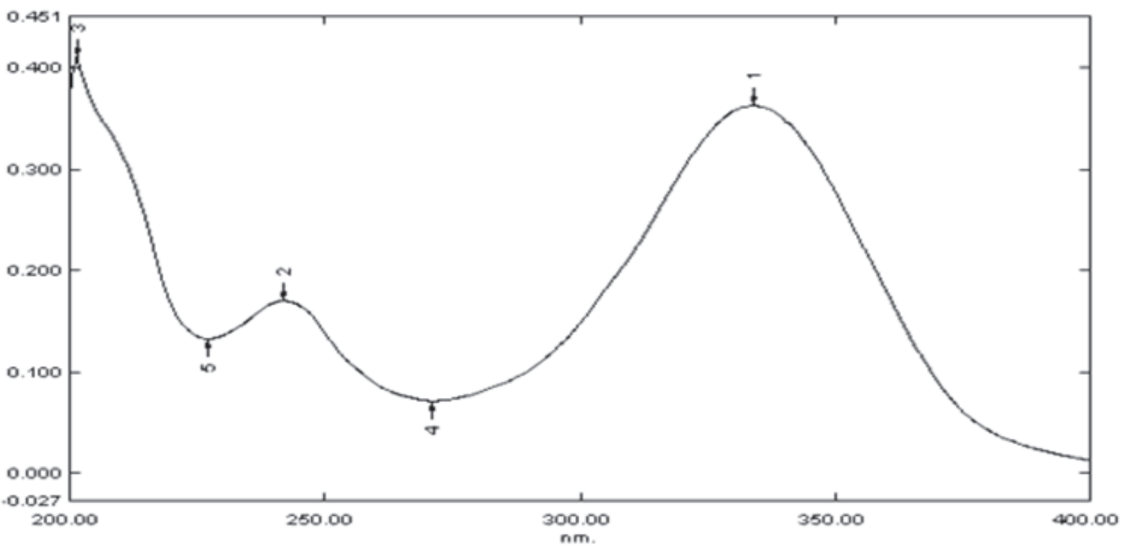

Fig. 3 : Estimation of $_{max}$of PRX in 0.1 M HCl

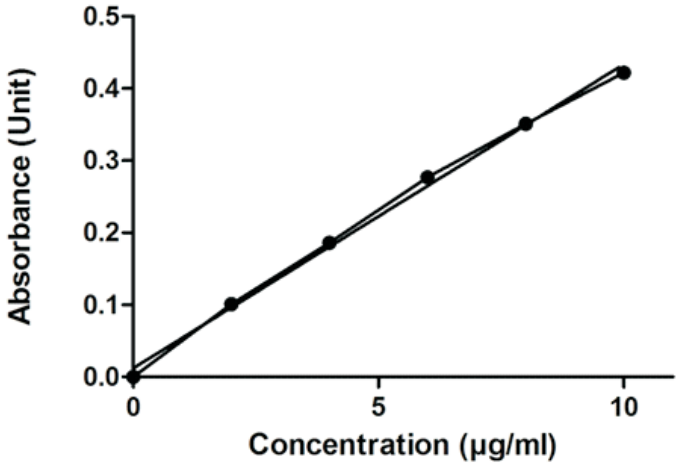

Fig. 4 : Calibration plot of PRX in 0.1 M HCl at 333nm

Table 11(a) : Statistical parameters related to calibration plot of PRX

Best-fit values	Results
Slope	0.04216 ± 0.001305
Y-intercept when X=0.0	0.01205 ± 0.007904
X-intercept when Y=0.0	-0.2858
1/slope	23.72
95% Confidence Intervals	
Slope	0.03853 to 0.04578
Y-intercept when X=0.0	-0.009895 to 0.03399
X-intercept when Y=0.0	-0.8695 to 0.2193
Goodness of Fit	
r^2	0.9962
Sy.x	0.01092
Is slope significantly non-zero?	
F	1043
DFn, DFd	1.000, 4.000
P value	< 0.0001
Deviation from zero?	Significant
Data	
Number of X values	6
Maximum number of Y replicates	1
Total number of values	6
Number of missing values	0

Repeatability analysis

Repeatability analysis was performed with concentration range 6 µg/mL. Mean concentration was found to be 6.28 ± 0.0335 with % RSD 0.0053. The reading was performed in triplicate (Table 13).

Method precision by interday and intraday analysis

The precision of the assay was determined by repeatability (intraday) and intermediate precision (interday) and reported as % relative standard deviation (RSD). For this, 2µg/mL, 3µg/mL, and 4µg/mL concentration solution were measured three times in a day and the same was measured in the next 3 days. The %RSD was calculated and %RSD was found to be least (Table 14 and 15).

Recovery Studies

Recovery study (spike method) was performed at 80%, 100%,

Table 12 : Statistical parameters related to calibration plot of PRX continuation to table 11

Parameters	Results
Absorption maxima (λ max)	333 nm
Linear equation	y = 0.0422x + 0.012
Correlation coefficient (r^2)	0.9962
Linearity	0-10 µg/mL
Limit of Detection (LOD) µg/mL	0.031
Limit of Quantification (LOQ) µg/mL	0.096

Table 13 : Data for Repeatability analysis

Concentration (µg/mL)	Absorbance (unit)	Concentration found (µg/mL)	Mean Conc. (µg/mL)	S.D	%RSD
6	0.277	6.27	6.28	0.0335	0.0053
	0.278	6.30			
	0.277	6.27			

Table 14 : Data for Intraday analysis

Concentration (µg/mL)	Absorbance (unit)			Concentration found (µg/mL)			Mean Conc.	S.D	%RSD
	1	2	3	1	2	3	(µg/mL)		
2	0.101	0.103	0.101	2.10	2.15	2.10	2.11	0.0113	0.0053
3	0.139	0.139	0.138	3.00	3.00	2.98	2.99	0.0112	0.0037
4	0.185	0.186	0.186	4.09	4.12	4.12	4.11	0.0115	0.0027

Table 15 : Data for Recovery studies

Concentration (µg/mL)	Absorbance (unit)			Concentration found (µg/mL)			Mean Conc.	S.D	%RSD
	1	2	3	1	2	3	(µg/mL)		
2	0.102	0.101	0.102	2.13	2.10	2.13	2.12	0.0114	0.0049
3	0.138	0.139	0.139	2.98	3.00	3.00	2.99	0.0113	0.0039
4	0.186	0.184	0.186	4.12	4.07	4.12	4.10	0.0113	0.0025

Table 15 : Data for Interday analysis

Concentration (µg/mL)	Absorbance (unit)	Concentration found (µg/mL)	% Recovery	Mean recovery	S.D	% RSD
5 (80 %)	0.233	5.23	99.66	99.54	0.43	1.23
5 (100 %)	0.238	5.35	99.10			
5 (120%)	0.240	5.40	99.90			

120%, and the mean recovery was found to be 99.54 ± 0.43 and % RSD were found to be 1.23 in limits as mentioned by ICH guidelines (Table 16).

Ruggedness

The ruggedness of the method was determined by carrying out the analysis by different analysts and the respective absorbance of 2µg/mL was noted. The result was indicated as %RSD (Table 17).

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) for PRX were determined by using the standard deviation of response and slope [9]. The LOD and LOQ values are depicted in Table 12.

Table 17 : Data for Ruggedness

Concentration (µg/mL)	Absorbance by analyst I	Absorbance by analyst II	% RSD for Absorbance by analyst I	% RSD for Absorbance by analyst I
2	0.102	0.101	0.871	0.783
2	0.102	0.101		
2	0.101	0.102		
2	0.102	0.102		
2	0.101	0.102		

CONCLUSION

The developed HPLC and UV method for the determination of PRX is simple, precise, accurate, reproducible, and highly sensitive. The developed method was strictly based on USP and ICH guidelines [10, 11 and 12]. Hence, this method can be used for the routine determination of PRX in pure and pharmaceutical formulations.

CONFLICT OF INTEREST

None

ACKNOWLEDGEMENT

This research work supported by SardarBhagwan Singh PG Institute of Biomedical Sciences and Research, Dehradun, India. Authors are thankful to Dr. DivyaVerma for time to time suggestions to carry out the analytical research work.

REFERENCES

1. https://www.drugbank.ca/drugs/DB00554 [accessed on 19/02/2018]
2. Guttadauria M. The clinical pharmacology of piroxicam. *ActaObstetriciaetGynecologicaScandinavica*.1986: 65 (sup138):11-13.
3. Saharan VA, Choudhury PK. Dissolution rate enhancement of piroxicam by ordered mixing. *Pakistan journal of pharmaceutical sciences*. 2012: 25(3): 521-33
4. Bhadra S, Das SC, Roy S, Arefeen S, Rouf AS. Development and validation of RP-HPLC method for quantitative estimation of vinpocetine in pure and pharmaceutical dosage forms. *Chromatography Research International*. 2011: 1-7 doi:10.4061/2011/801656
5. El-Gindy A, Emara S, Mesbah MK, Hadad GM. Spectrophotometric and liquid chromatographic determination of fenofibrate and vinpocetine and their hydrolysis products. *Farmaco*. 2005: 60(5):425-38.
6. Vatsova M, Tzvetanov S, Drenska A, Goranscheva J, Tyutyulkova N. Improved gas chromatographicmass spectrometric method for the quantitative determination of vinpocetine in human plasma. *Journal of Chromatography B: Biomedical Sciences and Applications*. 1997: 702(1): 221-26.
7. Sahoo M, Syal P, Ingale S, Ingale K, Sindhe S, Sali M, Choudhari VP, Kuchekar BS. Development and Validation of a RP-HPLC-PDA method for Simultaneous Determination of Lornoxicam and Thiocolchicoside in Pharmaceutical dosage form and it's Application for Dissolution study. *Int J Res Pharm Sci*. 2011: 2(1):1-7.
8. Reviewer Guidance, Validation of Chromatographic technique. *Centre for Drug Evaluation and Research*. 1994: 1-33.
9. Argekar AP, Sawant JG. Determination of cisapride in pharmaceutical dosage forms by reversed-phase liquid chromatography. *Journal of pharmaceutical and biomedical analysis*. 1999: 21(1): 221-26.
10. The United States Pharmacopeia, *Validation of Compendial Methods, USP*, Rockville, Md, USA, 32nd edition, 2009.
11. International Federation of Pharmaceutical Manufactures & Associations (IFPMA), "Validation of analytical procedures: text andmethodology," in *Proceedings of the International Conference on Harmonization (ICH'96)*, Methodology Q2(R1), Geneva, Switzerland, 1996.
12. ICH. Q2A validation of analytical procedure-Guidelines, Methodology.*International Conference on Harmonization*.Steering Committee, Geneva: 1994.
13. Singh S, Mishra A, Verma A, Ghosh AK, Mishra AK. A simple Ultraviolet spectrophotometric method for the determination of etoricoxib in dosage formulations. *Journal of advanced pharmaceutical technology & research.* 2012: 3(4): 237-40.