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Original Article

Development and Validation of UV/visible Spectrophotometric Method for Estimation of Piroxicam from Bulk and Formulation

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ABSTRACT

Background: Piroxicam (PRX) is a potent anti-inflammatory medicine used both orally and topically to treat arthritis, gout, and other inflammatory disorders. Developing and testing an effective analytical technique is critical for quantitative estimations. **Materials and Methods:** This study provides a straightforward, exact, repeatable, accurate, and cost-effective UV-visible spectrophotometric method for quantifying PRX in bulk and formulation using Phosphate Buffer Saline (PBS) pH 7.4. In compliance with the International Conference on Harmonization (ICH) guidelines, the developed method was validated for several aspects, including robustness, specificity studies, accuracy, linearity, precision, and range. **Results:** PRX showed absorbance Maximum (λ_{max}) at 354 nm in PBS pH 7.4. The value of the correlation coefficient (R^2) was found to be 0.995 in the range of 2-10 µg/mL. The percent recovery of PRX was found in the range of 98.86-101.44% with less than 2 percentage Relative Standard Deviation (% RSD). The percent recovery from nanosuspension and PRX solution was found to be 99.83% and 100.6% respectively. This indicates that there was no excipient interference. **Conclusion:** The % RSD for every parameter was less than 2%, which indicated that the developed method was accurate, precise, specific, and suitable for the analysis of commercial samples.

Keywords: PRX, UV/visible Spectrophotometry, Nanosuspension.

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INTRODUCTION

The first step in the systematic development of dosage forms for pharmacological substances is preformulation testing. A preformulation scientist examines the mechanical, chemical, and physical characteristics of a new pharmaceutical ingredient during the stage of preformulation in the procedures of research and development in order to create a stable, secure, and efficient dosage form.[1,2] Pharmacologically, PRX is an oxicam derivative used to relieve aches that are not associated with the system of muscles or bones and include moderate-to-severe inflammatory disorders such as osteoarthritis, rheumatoid arthritis, bursitis, tendinitis, and ankylosing spondylitis, also known as Bechterew's disease, as well as major postoperative or dysmenorrhea pains.[3,4] It is commonly used topically and orally to treat other inflammatory diseases and gout. It is classified as a Biopharmaceutical Classification System (BCS) Class II medicine, having 99% protein binding, poor solubility, and high permeability.[4-6] PRX is odourless, off-white to light brown powder. PRX has a melting point between 198°C-200°C. The distribution volume of PRX is 0.141/kg. PRX is taken by mouth in doses of 20 mg and topically at 0.5% weight. PRX has a log p=1.8 and is lipophilic in nature. PRX is chemically 4-hydroxy-2-methyl-2H-1,2-benzothiazine-3-carboxylic acid 1,1-dioxide as shown in Figure 1.[7,8]

A review of the literature found publications on analytical techniques for determining PRX from bulk and formulation, including UV-visible, HPTLC, LC-MS, and HPLC. There were not many published analytical techniques for PRX.[4,9] Furthermore, in terms of solvent usage, the disclosed methods were not very cost-effective. The accurate and precise method developed in PBS pH 7.4 would have wider applications in determining the entrapment efficiency of the drug-containing nanoformulations and in estimating drug content. Also, the development of the analytical method in simulated body fluids

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such as PBS 7.4 would be applicable to determine the amount of drug penetrated in topical drug delivery systems. Therefore, current study aimed to build a quick, fast, economical, and precise UV-visible spectrophotometric method for measuring PRX from bulk and nanosuspension formulations utilizing PBS pH 7.4 as a medium. The results were analyzed and validated statistically. This could further demonstrate its suitability for determining the application of PRX in skin permeation experiments, considering that PRX has been used more often in topical formulations as an anti-inflammatory gel in recent years.[4,9]

MATERIALS AND METHODS

Instrument

UV/vis spectrophotometer (Shimadzu UV-1800) equipped with two identical 1 cm quartz cells was used to measure the UV spectra and absorbance of the PRX.

Chemical and reagents

A free sample of PRX (USP) was given out by Zydus Cadila Healthcare Ltd., located in Goa, India. The deionized water was supplied by Millipore's New Milli-Q filtering technology (Bedford, Massachusetts, and the United States of America). All other chemicals were used of an analytical grade.

Preparation of Standard stock solution

20 mg of the PRX was carefully measured and transferred into a 100 mL volumetric flask. The drug was then dissolved in 15 mL of methanol. To achieve a 200 µg/mL concentration, the volume was increased to 100 mL by using PBS pH 7.4. 10 mL of the generated stock solution were extracted using PBS pH 7.4 and added to a second 100 mL volumetric flask, which had a volume of 100 mL and a concentration of 20 µg/mL.[10] The resulting solution was used for absorbance and results were recorded.

Selection of wavelength

The UV spectrum of pure drug PRX was measured between 200-400 nm using a UV-visible spectrophotometer (Shimadzu UV-1800).

Method Development

The maximum absorbance wavelength was recorded by scanning a 20 µg/mL solution in the range of 200-400 nm. From the stock solution (20 µg/mL), aliquots of 1, 2, 3, 4, and 5 mL were taken and poured into 10 mL volumetric flasks separately. Subsequently, the volume was adjusted using PBS (pH 7.4) to obtain concentrations of 2, 4, 6, 8, and 10 µg/mL. The prepared samples were examined at a wavelength of 354 nm (λ_{max}). Every study was carried out five times, and the average data was noted.[11,12]

Method validation

To ascertain the analyte's ruggedness, robustness, linearity, and accuracy, according to the ICH guidelines for the validation of analytical techniques, the technique was confirmed in compliance with the analytical method guidelines for the validation of analytical techniques. The technique was verified in compliance with the analytical procedure.[13]

Linearity and range linearity

Solutions of 2, 4, 6, 8, and 10 µg/mL, respectively, were made five times to access it using a calibration curve. To calculate linearity, the regression approach was applied. The difference between the solution's upper and lower PRX concentrations determined in the analytical procedure's range.[14,15]

Accuracy

The usual addition method was used to assess the accuracy. The pre-analysed samples were treated with a known concentration of an analyte. Recovery experiments were conducted at 3 different levels such as 50, 100 and 150% using standard concentrations of 3 µg/mL, 6 µg/mL, and 9 µg/mL respectively considering 6 µg/mL as 100%.[16-19] Absorbance was recorded and % recovery was estimated by using calculated amount of drug in the following formula: % Recovery=A-B/C×100, where, A represents total amount of drug estimated, B represents amount of drug found on pre-analysed basis, C represents amount of pure drug added.

Precision

Six distinct solutions containing 6 µg/mL PRX were analyzed as part of a repeatability measurement, and the % RSD was calculated. Analysing several identical PRX samples allowed for the determination of the method's reproducibility. Precision was achieved by the use of intraday and interday variations. Three days in a row were used to analyse the interday variance sample. Six times a day, the intraday fluctuation in absorbance was observed. 6 µg/mL concentration was used to calculate interday and intraday precision.[12,20]

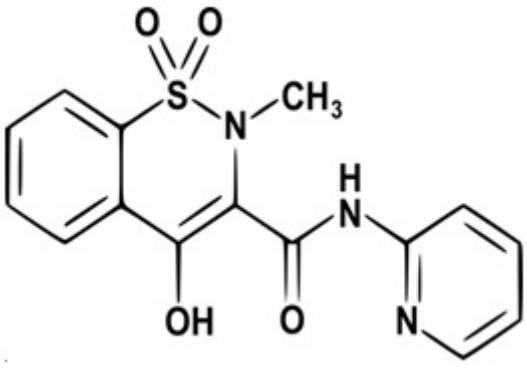

Figure 1: Structure of PRX.

Intraday precision

Six analyses were conducted over the course of six consecutive days to identify the solution (6 µg/mL) for the intraday fluctuation research (at morning, afternoon, and evening). The % RSD and mean standard deviation were calculated.[21]

Interday precision

The inter-day precision of the solution (6 µg/mL) was identified after three separate analyses over several days. The % RSD was calculated.

Ruggedness

Different UV spectrophotometers (Shimadzu-1800, and Systronics-119) in different laboratories were used to conduct analysis with several analysts and the degree of reproducibility assessed by measuring its absorbance (6 µg/mL).[15,22] The percentage RSD was calculated.

Robustness

A robustness test was used to assess how a small but intentional change in the spectrometric condition affected the PRX result. Table 6 displays the robustness data for the PRX absorbance, analytical performance parameters, and wavelength of detection fluctuation (354±5 nm).[15,23] The robustness was expressed as amount recovered in %RSD.

Specificity Studies

A nanosuspension equivalent to 10 mg of PRX USP was dissolved in 15 mL of methanol, and diluted to 100 mL using PBS pH 7.4. Then sonicated the above solution for 15 min, centrifuged for 15 min at 5000 rpm, and took 1 mL from the supernatant. Using PBS at pH 7.4, the volume was made up to 10 mL. In a similar manner, pure PRX alone was used to make solutions of standard PRX, and the amount recovered was determined. The % recovery was

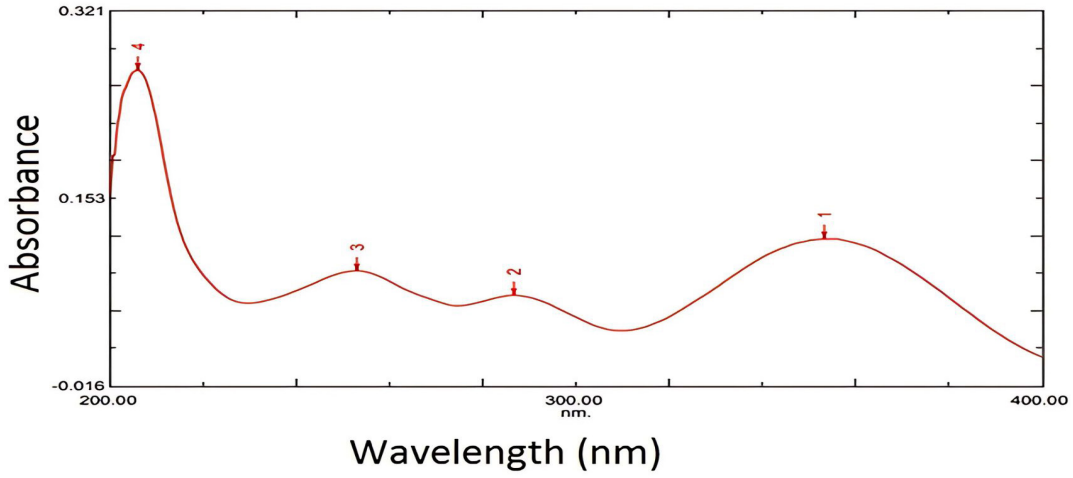

Figure 2: λ_{max} for the drug PRX in PBS (pH 7.4).

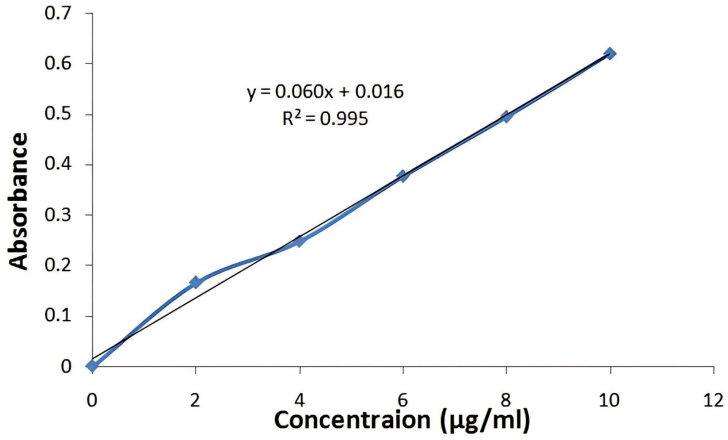

Figure 3: Calibration curve of PRX in PBS pH 7.4 at 354 nm.

calculated using a formula after the solution mentioned above was tested for absorbance at 354 nm.[10,24]

RESULTS AND DISCUSSION

Various conditions were tested to improve the UV parameters and obtain a suitable absorption and peak shape for PRX. The current work offers an easy and precise method for analysing PRX in bulk drugs $y = mx + c$.....(1)using a UV/visible spectrophotometer. $x = \frac{y - c}{m}$

Table 1: Linearity Study for PRX.

Sl. No.	Conc. (µg/mL)	Absorbance
1	2	0.166
2	4	0.248
3	6	0.377
4	8	0.495
5	10	0.620
Mean		0.3812
SD		0.1637
RSD		0.429
% RSD		42.95
Correlation coefficient (R^2)		0.995
Slope		0.060

Calibration curve and λ_{max} of PRX in PBS pH 7.4

As depicted in Figure 2, the PRX was examined between 200 and 400 nm wavelength using PBS pH 7.4, and its maximum absorption was detected at 354 nm. This aligns with the documented findings from previous studies.[1]

Linearity and range Linearity

A spectrophotometric technique for PRX detection was developed and verified with a regression coefficient (R^2) close to 1, or 0.999 depicted in Figure 3. The Beer-Lambert rule was applied in the concentration range of 2-10 µg/mL. The reliability and validity of this approach were confirmed by the low RSD values for each parameter. Similar result has been reported in earlier research as per ICH guideline.[1,14,15] It illustrates the linear absorbance range that was shown to exist between 0.166 and 0.620, as indicated in Table 1.

Accuracy

To ensure the accuracy of the proposed method, the usual addition method was employed. The recovery test was performed at three different concentrations (50, 100, and 150%). The recovery for the PRX was found between the ranges of 98.86-101.44% with less than 2% RSD, as depicted in Table 2. The results were found to be well within the acceptable limits of 98-102%, indicating the accuracy of the method being used. Similar results were reported by Singh *et al.*[11]

Table 2: Accuracy studies of PRX with statistical validation (*n*=6).

Sl. No.	Standard Conc. (µg/mL)	Added Conc. (µg/mL)	Percent of spiked sample	Amount recovered (µg/mL)	Percent recovery	% RSD
1	6	3	50%	2.966	98.86	1.157
2	6	6	100%	6.030	100.50	1.332
3	6	9	150%	9.130	101.44	0.798

Table 3: PRX intraday precision (*n*=6).

Sl. No.	Conc. (µg/mL)	Abs 1 (Morning)	Abs 2 (Evening)	Abs 3 (Afternoon)
1	6	0.322	0.324	0.328
2	6	0.320	0.319	0.327
3	6	0.322	0.322	0.325
4	6	0.316	0.316	0.325
5	6	0.312	0.320	0.328
6	6	0.310	0.322	0.322
Average		0.317	0.3205	0.3258
SD		0.0051	0.0028	0.0023
RSD		0.0163	0.0087	0.0071
% RSD		1.633	0.8769	0.71
Average % RSD		1.07		

Table 4: PRX interday precision (*n*=6).

Sl. No.	Conc. (µg/mL)	Day 1	Day 2	Day 3
1	6	0.322	0.300	0.301
2	6	0.320	0.303	0.302
3	6	0.322	0.306	0.307
4	6	0.316	0.302	0.306
5	6	0.312	0.307	0.310
6	6	0.310	0.306	0.306
Average		0.317	0.304	0.305
SD		0.0051	0.0027	0.0033
RSD		0.0163	0.0090	0.0108
% RSD		1.633	0.9068	1.0895
Average % RSD		1.20		

Table 5: Statistical validation for ruggedness studies of PRX.

Sl. No.	Parameter	Set 1	Set 2
1	System	Shimadzu-1800	Systronics-119
2	Sample	Batch no A	Batch no B
3	Day	Monday	Tuesday
4	Date	23/05/2022	24/05/2022
5	Time	10:20 AM	11:45 AM
6	Lab	Analysis	Chemistry
7	Analyst	Kunal Yadav	Ravindra Dudhal
8	Sample	6 µg/mL	6 µg/mL
9	Mean Absorbance	0.317	0.320
% RSD		1.63	0.87

Table 6: Statistical validation for PRX robustness studies.

Set No.	Wavelength	Conc. (µg/mL)	Absorbance	Average Abs	SD	RSD	% RSD
I	359	6	0.337	0.3393	0.0020	0.00621	0.613
	359	6	0.341				
	359	6	0.340				
II	354	6	0.345	0.3473	0.0020	0.0059	0.599
	354	6	0.349				
	354	6	0.348				
III	349	6	0.340	0.342	0.0020	0.0058	0.584
	349	6	0.344				
	349	6	0.342				

Precision

Replication injections were used to analyse the drug formulation in order to evaluate the method's precision, and mixed standard solutions were used to determine the system's precision. The proposed technique was determined to have a high degree of accuracy since the analyte. It was determined that the percent RSD was within the 2% limit, as shown in Tables 3 and 4. The suggested method's repeatability is indicated by the low percent RSD results.[11]

Ruggedness and Robustness

The test for ruggedness was carried out under the same conditions on several days by various analysts, using different instruments, and at different times, as indicated in Table 5. The ruggedness of

Table 7: Specificity Studies (% Recovery of formulation).

Parameters	Results
Absorbance of PRX nanosuspension 10 µg/mL.	0.615
Absorbance of PRX drug at 10 µg/mL.	0.620
Concentration of PRX nanosuspension at 10 µg/mL.	9.98
Concentration of PRX at 10 µg/mL.	10.06
% Recovery of PRX nanosuspension at 10 µg/mL.	99.83
% Recovery of PRX at 10 µg/mL.	100.6

the method for the standard solution of the above drugs shows that the RSD for both the analysts falls within the limits i.e. within 2%.

The robustness of the method was evaluated following a wavelength change. Table 6 displays the percentage RSD was found to be within the range i.e. within 2%. Similar results were noted in previous research.[15]

Specificity Studies (% Recovery study)

Table 7 summarizes the results of the specificity studies. There was not a significant variance in the percent mean recovery of the PRX solution and nanosuspension when tested using the UV/vis-Spectrophotometric method. This indicates that there was no excipient interference. This aligns with the documented findings from previous studies.[10]

CONCLUSION

The results suggest that the established method was statistically validated for linearity, accuracy, precision recovery, ruggedness, and robustness. This indicates that the UV/visible spectrophotometric method is simple, precise, accurate, and economical. PBS pH 7.4 was chosen as a cheap and readily accessible solvent and is typically used to replicate a biological system fluid. The results of the validation showed that it was effective in estimating the content of PRX in bulk and their formulations without interference from commonly used excipients and related substances. However, the UV method has some limitations, including its comparatively low selectivity and sensitivity. As a result, it could be challenging to identify an analyte at extremely low concentrations or to separate it from other materials that absorb light at the same wavelength. The proposed method utilizes inexpensive solvents. Thus, the proposed method will be suitable for determining the entrapment efficiency of PRX-containing nanoformulations and estimating drug content. Also, developing the analytical process in simulated body fluids such as PBS 7.4 would apply to determining the amount of drug penetrated in topical drug delivery systems.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

SD: Standard deviation; **RSD:** Relative standard deviation; **UV:** Ultraviolet; **PRX:** Piroxicam; **HPLC:** High performance liquid chromatography; **ICH:** International Council on Harmonization.

SUMMARY

This study provides a straightforward, exact, repeatable, accurate, and cost-effective UV-visible spectrophotometric method for quantifying PRX in bulk and formulation using Phosphate Buffer Saline (PBS) pH 7.4. PRX showed absorbance maximum (λ_{max}) at 354 nm in PBS pH 7.4. The value of the correlation coefficient (R^2) was found to be 0.995 in the range of 2-10 µg/mL. The percent recovery of PRX was found in the range of 98.86-101.44%. The % RSD for every parameter was less than 2%, which indicated that the developed method was accurate, precise, specific, and suitable for the analysis of commercial samples.

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