IJPSR (2023), Volume 14, Issue 3 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 21 April 2022; received in revised form, 02 February 2023; accepted 14 February 2023; published 01 March 2023

DUAL WAVELENGTH SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF PARACETAMOL AND PIROXICAM IN THEIR COMBINED TABLET DOSAGE FORM

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Keywords:

Piroxicam, Paracetamol, Dual wavelength method, UV spectrophotometric method, Accuracy, Precision

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ABSTRACT: A simple, accurate, and precise dual-wavelength spectrophotometric method was developed to determine Paracetamol and Piroxicam in combined pharmaceutical dosage form simultaneously. The principle for the dual wavelength method is "the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest". The pre-requisite for the dual wavelength method is the selection of two such wavelengths where the interfering component shows the same absorbance. In contrast, the component of interest shows a significant difference in absorbance with concentration. The wavelengths selected for paracetamol were 257nm, whereas the wavelengths selected for the determination of Piroxicam were 353nm. 0.5M NaOH was taken as a solvent. The regression analysis of Beer's plots showed good correlation in a concentration range of 2-10 µg/ml for paracetamol and 2-10 µg/ml for Piroxicam. The accuracy method was found to be between 97.33-101.5%. The method's precision (intra-day, inter-day, and repeatability) was found within limits. The proposed method was successfully applied to determine these drugs in commercial tablets.

INTRODUCTION: Chemically, Paracetamol (PCM) is N-(4-hydroxyphenyl) acetamide. It is a benzylisoquinoline derivative [1]. It is analgesics and antipyretics, a medication used to treat fever and mild to moderate pain [2]. Paracetamol may relieve pain in acute mild migraine. Paracetamol inhibits prostaglandin synthesis by reducing the active form of COX-1 and COX-2 enzymes [3]. Piroxicam (PIX) is 4-hydroxy – 2 – methyl -N-(pyridin-2yl)-2H-1, 2-benzothiazine- 3-carboxamide-1,1-dioxide [3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(3).1339-43

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.14(3).1339-43

It is a non-steroidal anti-inflammatory drug (NSAID) taken to reduce inflammation and as an analgesic reducing pain in conditions such as arthritis or acute injury. It is a non-selective COX inhibitor possessing both analgesic and antipyretic properties [4]. Literature survey reveals that PCM and PIX in bulk and tablet dosage form is official in Indian Pharmacopoeia 2018 and British Pharmacopoeia 2019.

Several analytical methods have been reported for estimation of PCM which include spectro-photometry [5-7], HPLC [8], thin layer chromatography [9, 10] and voltammetry [11]. The analytical methods reported for estimation of PIX are spectro-photometry [12-14], HPLC [15-17], LC-MS [18] and fluorimetry [19].

In the present work, a successful attempt has been made to estimate both these drugs simultaneously using the dual wavelength UV spectrophotometric method. This study attempts to develop a simple, accurate, and precise analytical spectrophotometric method, which can quantify these drugs simultaneously from a combined tablet dosage form. The developed method was validated as per ICH Q2 r1 guidelines and found to comply with the acceptance criteria [20]. Structures of both the drugs (PCM and PIX) are shown in **Fig. 1.**

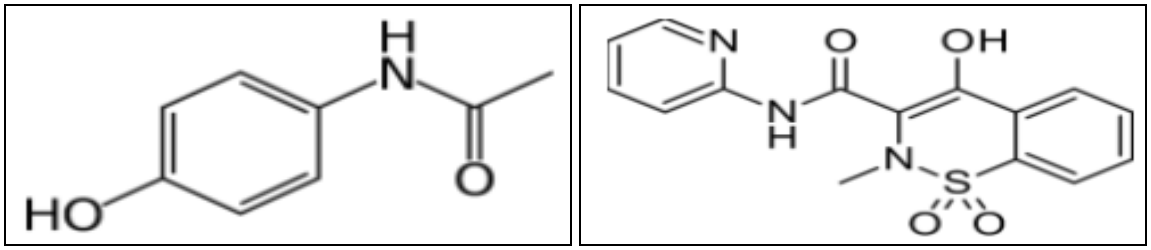

FIG. 1: CHEMICAL STRUCTURE OF THE ANALYTES

MATERIALS AND METHOD: The apparatus Instrument used was a UV-Visible double beam spectrophotometer, make: SHIMADZU (model UV-1800), with a pair of 1 cm matched quartz cells. All weighing was done on Shimadzu analytical balance (Model AU-220). Calibrated glassware were used throughout the work.

Reagents and Chemicals: Pure drug samples of PCM and PIX were obtained from medicinal chemistry Labs, Vadodara. 0.5 M NaOH was used as a solvent. The marketed formulation studied was Mixi Plus tablet manufactured by MEDOZ Pharmaceuticals. Each tablet contains 325 mg Paracetamol and 20 mg Piroxicam.

Preparation of Standard Stock Solution: Accurately weighed quantity of PCM (100 mg) and PIX (100 mg) was transferred to two separate 100 ml volumetric flasks, dissolved in little amount of 0.5 M NaOH and diluted to the mark with NaOH (stock solutions: 1000 µg/ml of PCM and PIX). 10 ml is pipetted out and transferred into two separate 100 ml volumetric flasks 0.5 M NaOH diluted up to 100ml (stock solution 100 µg/ml of PCM and PIX). Sonicate for 10 min.

Preparation of Working Standard Solution: 100µg/ml of PCM solution was prepared by diluting 10 ml of stock solution to 100 ml with 0.5 M NaOH. 100 µg/ml of PIX solution was prepared by diluting 10 ml of stock solution to 100 ml using 0.5 M NaOH.

Dual Wavelength Method: The utility of dual-wavelength data processing program is to calculate the unknown concentration of a component of interest present in a mixture containing both the components of interest and an unwanted interfering component by the mechanism of the absorbance difference between two points on the mixture spectra. This is directly proportional to the concentration of the interest component, independent of the interfering components.

The pre-requisite for the dual-wavelength method is the selection of two such wavelengths where the interfering component shows the same absorbance. In contrast, the interest component shows a significant difference in absorbance with concentration.

Study of Overlain Spectra and Selection of Wavelength: By appropriate dilutions from the standard working solutions of 100µg/ml of PCM and PIX, the solutions of PCM (10 µg/ml) and PIX (10 µg/ml) were prepared respectively and scanned over the range of 200-400 nm. The overlain spectra were observed for the development of a suitable analysis method.

The overlain spectra of PCM and PIX are shown in **Fig. 2**. From the overlay spectra, two wavelengths 257.0 nm and 353.0 nm were selected as λ1 and λ 2 for the estimation of DV. AF shows the same absorbance at these wavelengths.

Similarly, wavelengths 301.5 nm and 311.0 nm were selected as □1 and □2 for the estimation of AF. Mixed standards were prepared in the ratio of 9:1, as the formulation contains PCM and PIX 325 mg and 20 mg, respectively.

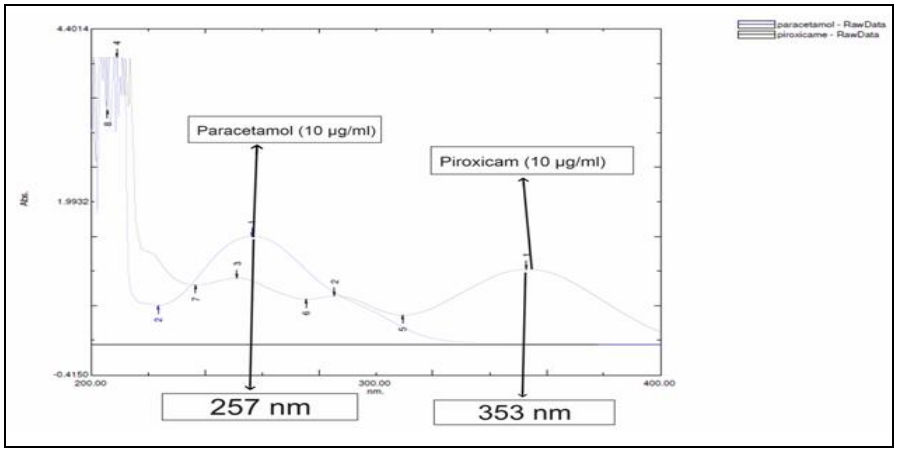

FIG. 2: UV-VIS SPECTRUM OF PARACETAMOL, PIROXICAM

Assay of Tablet Formulation by Dual Wavelength: Spectrophotometry Ten tablets were weighed and crushed to obtain a fine powder. An accurately weighed tablet powder equivalent to about 235 mg of PCM and 20 mg of PIX was transferred to 100 ml volumetric flask and dissolved in 50 ml of 0.5 M NaOH. The volume was made up to the mark using NaOH as solvent. The resulting solution was filtered through Whatman filter paper no. 42 and 10 ml of this filtrate was appropriately diluted to get the concentration of 235 µg/ml of PIX and 20 µg/ml of PCM. From the above-prepared solution, further dilutions were prepared to get the concentration of PCM and PIX. The absorbance was measured at the selected wavelengths, and concentrations were determined. The analysis was done in triplicate.

Method Validation:

Linearity and Range: Aliquots of standard stock solutions of PCM and PIX were diluted with 0.5 M NaOH to get final concentrations in range of 2-10 µg/ml for PCM and 2-10 µg/ml for PIX. This calibration range was prepared five times, and absorbance was measured at respective wavelengths for each drug separately.

Precision: The precision of the methods was determined by performing interday variation, intraday variation, and method repeatability studies. In an interday variation, the absorbance of standard solutions of PCM (2-10 µg/ml) and PIX (2-10 µg/ml) were measured on three consecutive days. In an intraday variation, the absorbances were measured three times in a day. In a repeatability study, three concentrations of both drugs were analyzed in triplicate.

Recovery Studies: To study the accuracy of the proposed method, recovery studies were carried out by standard addition method at three different levels. A known amount of the two drugs was added to pre-analyzed tablet powder, and percentage recoveries were calculated.

Ruggedness: The data for ruggedness were obtained from two different analysts.

RESULTS AND DISCUSSION:

Method Development and Validation: The overlain spectra of the drugs suggested that a dual-wavelength spectrophotometric method was suitable for the simultaneous determination of Paracetamol and Piroxicam. 0.5 M NaOH was taken as a solvent system, as both the drugs were soluble in this solvent. In the dual wavelength method, wavelengths 257nm were selected for the determination of paracetamol, whereas 353 nm were selected for the determination of Piroxicam. Optimized 0.5 M NaOH parameters for dual-wavelength spectrophotometry are shown in **Table 1.**

TABLE 1: OPTIMIZED METHOD PARAMETERS FOR DUAL-WAVELENGTH SPECTROPHOTOMETRY

Method parameter	Optimized parameter
Solvent	0.5 M NaOH
Scanning range	200 nm to 400 nm
Scan speed	Medium
Analytical wavelengths for determination of PCM	257 nm
Analytical wavelengths for determination of PIX	353 nm

Linearity: Paracetamol and Piroxicam calibration curves were linear in the range of 2-10 µg/ml and 2-10 µg/ml, respectively. The regression equations of calibration curves were

YPCM = 0.0641X - 0.0685, R^2 =0.9995 for Paracetamol and YPIX= 0.0197X + 0.0047, R^2 = 0.9984 for Piroxicam.

Precision: Relative standard deviations (% R.S.D.) for repeatability were found to be 0.10-0.16% and 0.06-0.18% for Paracetamol and Piroxicam, respectively. The intraday precision showed a % R.S.D. of 0.1-0.3% for paracetamol and 0.10-0.16% for Piroxicam. The inter-day precision showed % R.S.D. 0.10-0.16% and 0.06-0.18% for Paracetamol and Piroxicam, respectively. The results of repeatability and intra and inter-day precision of the method are illustrated in **Table 2.**

Accuracy: The percentage recoveries of drugs from marketed formulations were determined by the standard addition of pure drugs at three known concentrations, and excellent recoveries were obtained at each level. The percent recoveries for paracetamol at three levels were 97.22 ± 0.11, 96.25 ± 0.11, and 101.4 ± 0.11. The percent recoveries for Piroxicam at three levels were found to be 101.33 ± 0.11, 99.2 ± 0.11, and 98.36 ± 0.11. The results of the accuracy studies are shown in **Table 3.**

TABLE 2: VALIDATION PARAMETERS FOR THE DUAL-WAVELENGTH METHOD

Parameters	PCM	PIX
Linearity range	2 – 10 µg/ml	2 – 10 µg/ml
Correlation Coefficient (r^2)	0.9995	0.9984
Precision	%RSD	
Intraday	0.1263	0.1661
Interday	0.1324	0.1873
% Recovery	97.22 – 101.4	98.36 – 101.33
LOD	0.01287	0.04221
LOQ	0.03900	0.1279

TABLE 3: RECOVERY STUDIES

Name of Drug	Amount of Drug Added (µg/ml)	Dual Wavelength Method	
		%Recovery	SD
PCM	39	97.22 ± 0.11	0.010644404
	59	96.25 ± 0.11	0.002119748
	79	101.4 ± 0.11	0.047903479
PIX	39	101.33 ± 0.11	0.003350124
	59	99.2 ± 0.11	0.005940539
	79	98.36 ± 0.11	0.00569766

Application of the Method in Assay of Tablets: The proposed UV method was applied to determine Paracetamol and Piroxicam in their combined pharmaceutical formulation; the results are shown in **Table 4**. The high percentage recovery (98.33-101.5%) values confirm the suitability of the proposed method for the routine determination of these components in the combined formulation.

TABLE 4: RESULTS OF SIMULTANEOUS ESTIMATION OF DV AND AF IN MARKETED FORMULATION BY DUAL-WAVELENGTH SPECTROPHOTOMETRY METHOD

Method	Mg/tablet % of label claim* (± S.D.)			
	PCM	PIX	PCM	PIX
Dual Wavelength	328	22.1	100.92 ± 0.01	110.5 ± 0.03

CONCLUSION: The proposed dual wavelength method gives accurate and precise results for determining Paracetamol and Piroxicam in marketed formulation (tablet) without prior separation and is easily applied for routine analysis. The most striking feature of the dual-wavelength method is its simplicity and rapidity. Various tests have demonstrated method validation for linearity, accuracy, and precision. The proposed method was successfully applied to determine these drugs in commercial tablets.

ACKNOWLEDGEMENTS: The authors are thankful to Astran Lab, Ahmedabad, for providing pure gift samples of Paracetamol and Piroxicam. The authors also thank the Principal Parul Institute of Pharmacy and Research for providing the necessary facilities.

CONFLICTS OF INTEREST: Nil

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How to cite this article:
Parmar K and Patel S: Dual wavelength spectrophotometric method for simultaneous estimation of paracetamol and piroxicam in their combined tablet dosage form. Int J Pharm Sci & Res 2023; 14(3): 1339-43. doi: 10.13040/IJPSR.0975-8232.14(3).1339-43.