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SHORT COMMUNICATION

INDIRECT SPECTROPHOTOMETRIC DETERMINATION OF PIROXICAM AND TENOXICAM THROUGH OXIDATION WITH POTASSIUM PERMANGANATE

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ABSTRACT. Three rapid, simple, accurate and selective validated spectrophtometric methods (A, B and C) for the determination of piroxicam (PX) and tenoxicam (TX) in bulk sample and in dosage forms are described. The methods are based on the oxidation of the studied drugs by a known excess of potassium permanganate in sulfuric acid medium and subsequent determination of unreacted oxidant by reacting it with Methylene Blue (Basic Blue 9) dye (method A), Acid Red 27 (Amaranth) dye (method B) and Acid Orange 7 (orange II) dye (method C), in the same medium at a suitable λ_{max} = 660, 520 and 485 nm, respectively. The reacted oxidant was found to be corresponding to the drug content. Regression analysis of Beer-Lambert plots showed good correlations in the concentration ranges 1.0-8.0, 1.0-9.0 and 1.0-7.2 μg mL^{-1} using methods A, B and C, respectively, for PX and 0.3-7.0, 0.3-1.6 and 0.3-2.5 μg mL^{-1} using methods A, B and C, respectively, for TX. The stoichiometric ratios for the cited drugs to oxidant were studied. The optimum reaction conditions and other analytical parameters were evaluated. The proposed methods were applied successfully to determine the examined drugs either in pure form or pharmaceutical formulations with good accuracy and precision. The relative standard deviations were ≤ 0.33 with recoveries 98.9-101.7% for PX and ≤ 0.49 with recoveries 99.4-102.0% for TX.

KEY WORDS: Spectrophotometry, Piroxicam assay, Tenoxicam assay, Redox reactions, Dosage forms

INTRODUCTION

Piroxicam, 4-hydroxy-2-methyl-N-(pyridine-2-yl)-2H-1,2-benzothiazine-3-carbox-amide-1,1-dioxide [36322-90-4], and tenoxicam, 4-hydroxy-2-methyl-N-(pyridin-2-yl)-2H-thieno[2,3e]1,2-thiazine-3-carboxamide-1,1-dioxide [59804-37-4], are non-steroidal anti-inflammatory drugs (NSAIDs) from oxicams family. The oxicam family of NSAIDs is derived from enolic acid, a carbon- and hydroxyl-containing molecule (C=C–OH) made from carboxylic acid. Piroxicam and tenoxicam are used as an effective analgesic and anti-inflammatory agent in rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and acute pain in musculoskeletal disorder and acute gout. It has been shown to be an effective analgesic in fracture, dental, postoperative and postpartum pain. The pharmacological actions of these oxicams are related to inhibition of cyclooxygenase (Cox), a key enzyme of prostaglandine biosynthesis at the site of inflammation [1]. Several methods have been reported for determination of piroxicam and tenoxicam including spectrophotometry for PX [2-10] and for TX [11-14], chromatography, electroanalytical and physicochemical methods for PX [15-20] and for TX [21-23]. Among the various methods available for the determination of drugs, spectrophotometry continues to be very popular, because of its simplicity, specificity and low cost. This study presents new spectrophotometric methods for the assay of PX and TX. The applicability of the developed methods was evaluated through the determination of these drugs in pure and in dosage forms. No attempts have been made to develop a spectrophotometric method for determination of piroxicam (PX) and tenoxicam (TX) by oxidation with potassium permanganate and using three different dyes (MB, AM, AO) which are preferable because they react with $KMnO_4$ simultaneously.

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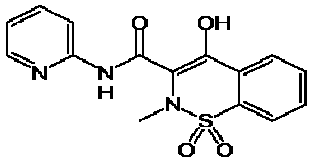
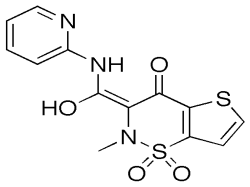


Piroxicam



Tenoxicam

EXPERIMENTAL

Apparatus. All the absorption spectral measurements were made using Jasco V-530 (UV-VIS) spectrophotometer (Japan) with scanning speed 400 nm min^{-1} and bandwidth 2.0 nm equipped with 10 mm matched quartz cells.

Materials and reagents. Pure piroxicam (PX), was kindly supplied by Pfizer Pharmaceutical Company S.A.E., Cairo, Egypt, the purity of the sample was found to be 98.5-101.0% and tenoxicam (TX), was obtained from Egyptian International of Pharmaceutical Industries Company (EIPICO), 10^{th} of Ramadan City, Egypt, the purity of the sample was found to be 99.0-101.0%. Stock solutions (100 µg mL^{-1}) of pure PX and TX were prepared by dissolving appropriate weight of pure drugs in acetone and made up to the mark in a 100 mL calibrated flask with the solvent. The working standard solutions were obtained by further dilution of the stock solution with acetone. All chemicals were of analytical grade and double distilled water was used throughout. A stock aqueous solution of 1.0 x 10^{-3} M of Methylene Blue (Basic Blue 9) [$C_{16}H_{18}ClN_3S$, M.Wt. 319.86] was prepared by dissolving 0.032 g of the dye (E-Merck, Germany, C.I. 52015) in 40 mL of double-distilled water in 100 mL measuring flask, then diluted to the mark with water. A stock aqueous solution of 1.0 x 10^{-3} M of Acid Red 27 (Amaranth dye) [$C_{20}H_{11}N_2Na_3O_{10}S_3$, M.Wt. 604.48] was prepared by dissolving 0.0605 g of the dye (E-Merck, Germany, C.I. 16185) in 40 mL of warm double-distilled water in 100 mL measuring flask, then diluted to the mark with water, whereas stock aqueous solution of 1.0 x 10^{-3} M of Acid Orange 7 (Orange II) [$C_{16}H_{11}N_2NaO_4S$, M.Wt. 350.33] was prepared by dissolving 0.0350 g of the dye (E-Merck, Germany, C.I. 15510) in warm double-distilled water then after cooling it was transferred to a 100 mL measuring flask, then diluted to the mark with water.

A stock solution of 2.0 M of sulfuric acid was prepared by transferring drop-wise addition of 55.6 mL of concentrated acid (Riedel-de-Haen, Germany) 18 M to 100 mL double-distilled water in 500 mL measuring flask and diluted to the mark. The resulting solution was standardized with 1.0 M Na_2CO_3 as recommended previously [24].

A stock solution of 5.0 x 10^{-3} M of $KMnO_4$ was prepared by dissolving 0.079 g of $KMnO_4$ (Aldrich, USA) in 10 mL of warm double-distilled water then after cooling it was transferred to a 100 mL measuring flask and diluted to the mark with double-distilled water, then standardized using sodium oxalate as a primary standard, secondary standards include metallic iron and ethylene-diamine ferrous sulfate [25]. The 5.0 x 10^{-4} M solution of $KMnO_4$ was prepared by diluting an appropriate volume of the previously stock solution with double-distilled water.

General procedures. These methods depend on oxidation of PX and TX performed by adding (0.5-12, 0.5-15 and 0.5-10 µg mL^{-1}, of PX) and (0.1-10, 0.1-2 and 0.1-3.5 µg mL^{-1}, of TX) using methods A, B and C, respectively, to an excess volume (2.0 mL, and 1.0 mL, of 5.0 x 10^{-4} M $KMnO_4$) for PX and TX, respectively, and adding the optimum volume of H_2SO_4 (1.0 mL of 0.2 M for PX and 0.3 mL of 2.0 M for TX). The mixture was heated in a water bath (5.0 min at

50 ^{o}C), then cooling the mixture. After oxidation process had been completed which is known by observing the disappearance of the color of potassium permanganate, known volume of dyes were added (0.45, 1.5 and 1.2 mL, 1.0 x 10^{-3} M) using methods A, B and C, respectively, for PX and (0.25, 1.0 and 0.8 mL, of 1.0 x 10^{-3} M) using methods A, B and C, respectively, for TX). The volume was made up to 10 mL with acetone, the decrease in color intensities of MB, AM and AO dyes were measured spectrophotometrically at their corresponding maximum wavelengths 660, 520 and 485, for MB, AM and AO, respectively. The concentration ranges were determined in each case by plotting the concentration of PX and TX against absorbance at the corresponding λ_{max}.

Procedure for dosage forms. An accurately weighed amount of ten tablets, capsules and equivalent milliliters of six ampoules, equivalent to 100 mg of the cited drugs, dissolved in 10 mL acetone, and then they were transferred into 100 mL volumetric flask, and diluted to the mark with the solvent, shaken well for 15 min, and then filtered through a sintered glass crucible G4. A 1.0 mL aliquot of the test solution was diluted to 100 mL in a measuring flask with the solvent. Aliquot of the diluted drug solution was then treated as described above in general procedure. The above stated procedure described was applied to determine the drug concentration.

RESULTS AND DISCUSSION

This study was used to establish simple, rapid, accurate and sensitive spectrophotometric methods for the determination of PX and TX in pure forms and pharmaceutical preparations. The methods involve two stages, oxidation of drug by $KMnO_4$ then estimation of unconsumed $KMnO_4$ with three different dyes (MB, AM and AO) which are preferable, because they react with $KMnO_4$ simultaneously. However they are used as indicators to estimate PX and TX. $KMnO_4$ reacts with PX and TX resulting in oxidation depending upon the functional group (-OH) present in PX and TX. The absorption spectra of the reaction products in methods A, B and C, show characteristic λ_{max} value as shown in Figure 1.

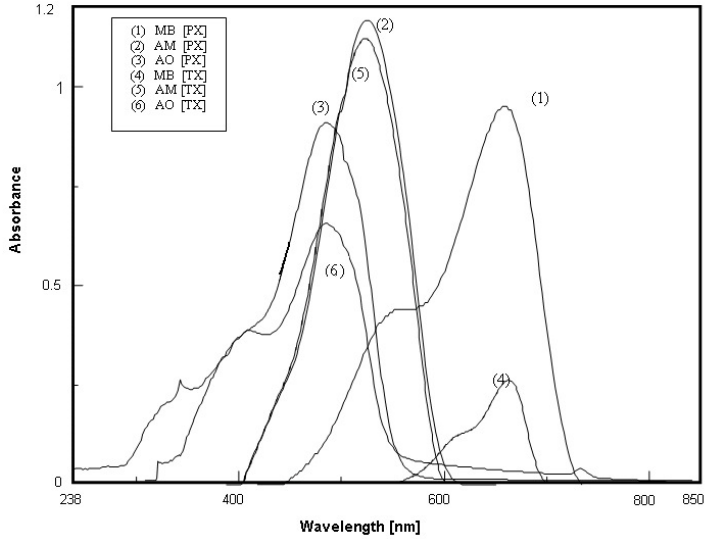


Figure 1. Absorption spectra for the reaction product of 5.0 µg mL^{-1} of PX and 1.6 µg mL^{-1} of TX with $KMnO_4$ (5.0 x 10^{-4} M) and MB, AM and AO dyes (1.0 x 10^{-3} M).

Effect of potassium permanganate concentration. The influence of the concentration of $KMnO_4$ on the color development was studied using different concentrations ranging from 5.0 x 10^{-3} to 5.0 x 10^{-5} M. The optimum concentration was obtained with 5.0 x 10^{-4} M; higher concentrations of $KMnO_4$ caused the color disturbed. The optimum concentration was found to be 2.0 mL and 1.0 mL, of 5.0 x 10^{-4} M $KMnO_4$, for PX and TX, respectively.

Effect of sequence of additions. Oxidant-acid-drug-dye is the optimum sequence of addition; other sequences gave lower absorbance values under the same experimental conditions.

Effect of acid concentration. The optimum type of the acid examined (H_2SO_4, H_3PO_4 and CH_3COOH) was sulfuric acid. Moreover, various volumes of sulfuric acid were tested, the optimum volume was found to be (1.0 mL of 0.2 M for PX and 0.3 mL of 2.0 M for TX).

Effect of time and temperature. The reaction takes place completely after 5 min and at 50 ^{o}C in a water bath. Raising the temperature more than 50 ^{0}C did not accelerate the oxidation process. The effect of time after addition of the dye indicated that shaken for 1-3 min is sufficient to give reliable results.

Effect of dye concentration. To establish the optimum concentration of the reagent, different volumes (0.1-2.0 mL) were added and tested. The optimum volumes used for production of maximum color intensity are (0.45, 1.5 and 1.2 mL, of 1.0 x 10^{-3} M, using MB, AM and AO, respectively, with PX) and (0.25, 1.0 and 0.8 mL, of 1.0 x 10^{-3} M, using MB, AM and AO, respectively with TX), were used as optimum dye volume.

Molar ratio. The molar ratio between oxidant and dye [Dye]/[O] at the selected conditions was carried out, by keeping the concentration of the oxidant constant (2.0 mL, of 5 x 10^{-4} M), and the drug PX (5.0 µg mL^{-1}) and (1.0 mL, of 5 x 10^{-4} M), and the drug TX (1.6 µg mL^{-1}) and variable concentrations of dye (1.0 x 10^{-3} M, for MB, AM and AO) were added. The absorbance values were then plotted against the molar ratio [Dye]/[O]. Experimental results showed that the inflection of the lines at (0.4, 1.2 and 1.5 in case of MB, AM and AO, respectively) for PX, whereas for TX, the inflection of the lines at 0.5, 2.0 and 1.6 in case of MB, AM and AO, respectively. Thus the stoichiometric ratios of the dye to the oxidant were calculated and recorded in Table 1.

In order to investigate the molar ratio between PX or TX and oxidant at the selected conditions, the molar ratio method described by Yoe and Jones was carried out [26]. In this method 2.0 mL of 5.0 x10^{-4} M $KMnO_4$ is kept constant and different volumes (0.1-0.9 mL, of 5.0 x 10^{-4} M) for PX and 1.0 mL of 5.0 x 10^{-4} M $KMnO_4$ is kept constant and different volumes (0.1-1.0 mL) of TX 5.0 x 10^{-4} M were added. The absorbance was measured at λ_{max} (660, 520 and 485 nm using MB, AM and AO, respectively) against blank solution prepared by the same manner. The absorbance values were then plotted against the molar ratio [D]/[O]. Experimental results showed that the inflection of the three straight lines at (0.6, 0.5 and 0.7 in case of MB, AM and AO, respectively) for PX, whereas for TX the inflection of the three straight lines at 0.5 for MB and 0.6 for AM and AO. Thus the molar ratios of PX to oxidant are 1.0:1.66, 1.0: 2.0 and 1.0:1.43 for methods A, B and C, respectively, whereas for TX, the molar ratios of TX to oxidant are 1.0: 2.0, 1.0: 1.66 and 1.0:1.66 for methods A, B and C, respectively (Table 1).

Quantification. Beer-Lambert law limits, molar absorptivities, Sandell sensitivities, regression equations and correlation coefficients obtained by linear squares treatment of the results are given in Table 1 for the PX and TX. The detection and quantitation limits were calculated from the standard deviation (SD) of absorbance measurements obtained from a series of 13 blank solutions for each procedure. The limits of detection (K = 3) and of quantitation (K = 10) were established according to IUPAC definitions [27]. In order to determine the accuracy and

precision of the proposed methods, solutions containing three different concentrations of PX and TX were prepared and analyzed in six replicates. The percentage standard deviations and the percentage range of error at 95% confidence level were calculated. The results can be considered to be very satisfactory, at least for the level of concentrations examined [28].

Table 1. Optical and regression characteristics.

Parameters	Piroxicam			Tenoxicam		
	Methods			Methods		
λ_{max} (nm)	A	B	C	A	B	C
Beer's law limits (µg mL^{-1})	660	520	485	660	520	485
Ringbom limits (µg mL^{-1})	1.0-8.0	1.0-9.0	1.0-7.2	0.3-7	0.3-1.6	0.3-2.5
Molar absorptivity (L mol^{-1} cm^{-1})	1.2-7.7	1.3-8.9	1.1-7.05	0.35-6.8	0.4-1.55	0.45-2.3
Sandell sensitivity (ng cm^{-2})	5.04×10^4	5.59×10^4	4.46×10^4	4.28×10^4	2.35×10^5	1.39×10^5
Detection limits (µg mL^{-1})	6.57	5.92	7.42	7.87	1.43	2.42
Quantitation limits (µg mL^{-1})	0.0185	0.024	0.019	0.025	0.027	0.036
Regression equation*: Slope (b)	0.062	0.08	0.065	0.084	0.09	0.12
Intercept (a)	0.1521	0.1689	0.1347	0.127	0.697	0.413
Correlation coefficient (r)	0.01	0.019	0.007	0.02	0.015	0.018
RSD** %	0.9998	0.9999	0.9999	0.9999	0.9996	0.9998
Stoichiometric ratio: [Dye]/[O]	0.25	0.33	0.27	0.34	0.37	0.49
Stoichiometric ratio: [D]/[O]	1.0:2.2	1.0:0.83	1.0:0.66	1.0:2.0	1.0:0.5	1.0:0.625

*With respect to A = a + b C where C is concentration of drug in µg mL^{-1} and A is absorbance. **Relative standard deviation for six determinations.

Interferences. A systematic quantitative study was performed by measuring the absorbance of solutions containing 5.0 µg mL^{-1} of PX and 1.6 µg mL^{-1} of TX with varying concentrations of the additives and excipients such as lactose, magnesium stearate, propylene glycol and starch. The additives and excipients commonly used and presents in all dosage forms (tablets, capsules and ampoules) do not interfere in the examined methods A, B and C.

Table 2. Determination of PX and TX in pharmaceutical formulations using the proposed and official methods.

Preparation	Supplier	Nominal value	Recovery ± SD(%) [a]			Official method
			A	B	C	
Feldene capsules (PX)	Pfizer[1]	20 (mg/capsule)	100.1±1.0	99.9±0.9	100.7±1.1	100.2±1.3
Dispercam tablets (PX)	MUP[2]	10 (mg/tablet)	100.2±0.9	100.1±0.8	99.7±1.0	99.9±1.2
Feldoral ampoules (PX)	SEDICO[3]	20 (mg/ampoule)	99.7±0.9	100.4±1.0	99.9±0.7	99.8±1.3
Epicotil tablets (TX)	EIPICO[4]	20 (mg/tablet)	99.7±0.7	100.6±1.1	100.3±0.9	100.4±1.2
Soral capsules (TX)	GNP[5]	20 (mg/capsule)	99.6±0.9	99.8±1.0	100.2±0.8	99.8±1.0

[a]The average of six determination ± SD. [1]Pfizer Pharmaceutical Company S.A.E, Cairo, Egypt. [2]Medical Union Pharmaceuticals Company, (MUP) Abu Sultan, Ismalia, Egypt. [3]South Egypt Drug Industries Company (SEDICO), 6th October City, Egypt. [4]Egyptian International of Pharmaceutical Industries Company (EIPICO), 10th of Ramadan City, Egypt. [5] Global Napi Pharmaceuticals Company (GNP) under license from Merck & Co. Inc. USA, Egypt.

Analytical applications. The proposed methods were successfully applied to determine PX and TX in its dosage forms. The results obtained were compared statistically by student's *t*-test (for accuracy) and variance ratio *F*-test (for precision) with the official methods of PX and TX (non-aqueous titration with perchloric acid determining the end point potentiometrically) [29] at 95% confidence level with five degrees of freedom (Table 2). The results showed that there was no

significance difference between the proposed and official methods. So the proposed methods can be recommended for routine analysis in the majority of drug quality control laboratories.

CONCLUSIONS

The proposed spectrophotometric methods provided sensitive, specific, inexpensive and stability indicating analytical procedure for the determination of PX and TX. The proposed methods have higher sensitivity with apparent high molar absorptivity, simplicity, reproducibility, precision, accuracy and higher stability of color species. The proposed methods can be applied for routine analysis and in quality control laboratories for the quantitative determination of the studied drug in raw materials and pharmaceutical formulations.

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