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SPECTROFLUORIMETRIC METHOD FOR DETERMINATION OF SOME OXICAMS USING POTASSIUM BROMATE

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تم استحداث طريقة لصفية لتعيين ث مركبات صيدلية من مجموعة الأوكسيكام وهى تينوكسكام وبيروكسكام ولورنوكسكام. اعتمدت الطريقة المستخدمة على تحلل وأكسدة المركبات فى وسط حامضى باستخدام مركب برومات البوتاسيوم لكى تعطى امينوبيريدين الذى له وميض يمكن قياسه عند طول موجى قدرة نانوميتر بعد تعرضه ثارة بطول موجى قدره نانوميتر. تمت دراسة التغيرات فى ظروف التفاعل المستخدم منها تركيز العامل المؤكسد ونوع وتركيز الحامض ووقت التفاعل فى الحمام المائى و كذلك أنسب مذيب لتخفيف مخلوط التفاعل. تم أيضا دراسة عناصر صلاحية الطريقة وشملت مدى التركيزات التى تحقق علاقة خطية والحد الأدنى للكشف والحد الادنى للتقدير ومدى التكرارية لهذه الطريقة المقترحة واتضح أن الطريقة بسيطة وحساسة ودقيقة وتم تطبيقها فى تقدير المركبات المدروسة فى العديد من المستحضرات الصيدلية المتاحة فى السوق المحلى وثبت التوافق الكبير فى النتائج مع مثيلاتها من الطرق المنشورة.

A sensitive and selective spectrofluorimetric method has been developed for the determination of some non-steroidal anti-inflammatory oxicams derivatives namely: tenoxicam (Tx), piroxicam (Px) and lornoxicam (Lx) after their complete oxidative acidic hydrolysis to 2-aminopyridine. The hydrolytic product 2-aminopyridine exhibits fluorescence emission at 365 nm (excitation at 305 nm). The optimal conditions of the reaction were investigated. The method was found to be linear in the ranges of (0.015-0.500 µg/ml) for Tx (0.006-0.300 µg/ml) for Px and (0.060-0.200 µg/ml) for Lx. The suggested method was successively applied for the determination of the studied drugs in different dosage forms with a recovery percentages ranged 96.82-102.79 ± 0.614-2.578. The method was also applied for the determination of the drugs in spiked urine with a recovery percentages ranged 80.51-105.35 ± 1.067-5.338. The validity of the method was

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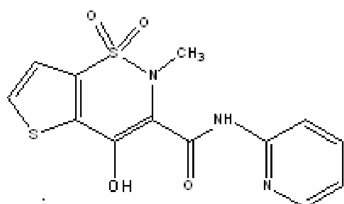
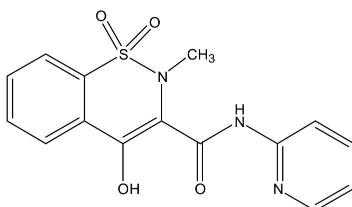
assessed according to USP guidelines. Statistical analysis of the results reveled high accuracy and good precision.

INTRODUCTION

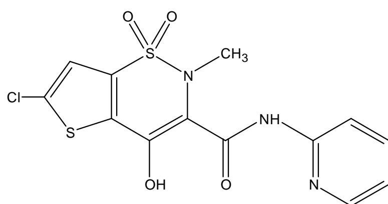
Tenoxicam, piroxicam and lornoxicam are potent nonsteroidal drugs with marked anti-inflammatory analgesic and antipyretic effects.



Tenoxicam



Piroxicam



Lornoxicam

Several analytical methods were developed for their assay in pharmaceutical formulations and biological fluids including: non aqueous titration[1] spectrophotometric[2-13], flow injection analysis[14-16], electrophoresis[17&18], electrochemical[6,19-25], GLC[26], HPTLC[27-30] and liquid chromatography[4,31-62]. On the other hand, spectrofluorimetric determination of Px and Tx in HNO_3 was studied[63-64] Also effect of β-cyclodextrin on the fluorescence of Px was investigated[65]. Tx and Lx were determined fluorimetrically after their reaction with 7-chloro-4-nitro-2-oxa-1,3-diazole (NBD-Cl)[2].

The present work aims to develop a simple, sensitive, precise and inexpensive fluorimetric procedure for assay of Px, Tx and Lx based on their hydrolysis with $KBrO_3$ in acidic medium to give 2-aminopyridine[66].

EXPERIMENTAL

Apparatus

(a) Fluorescence measurements were performed using a Shimadzu (Kyoto, Japan) RF-3501 PC Spectrofluorimeter equipped with a 150 w xenon lamp, using 1.00 cm quartz cells, slit width of 3 nm, excitation at 305 nm and fluorescence emission at 365 nm.

(b) Thermostatically controlled water bath (Memment GmbH, Schwabach, Germany).

Materials

Drugs and chemicals

All chemicals were used without further purification.

(a) Drugs: Tenoxicam was obtained from Hoffmann LaRoche (Basel, Swizerland). Piroxicam was obtained from Pfizer (Sandwich, Kent, UK). Lornoxicam was obtained from Nycomed (Linz, Austria) and meloxicam were obtained from Boehringer Ingelheim (Rrims, France).
(b) Chemicals: Potassium bromate (Aldrich, U.S.A.). 2-Amino-pyridine was obtained from F. Hoffmann LaRoche (Basel, Swizerland). Analytical grade chemicals and solvents were used in all experiments.
(c) Water: Double-distilled, used throughout.

Dosage forms

Commercial dosage forms of tenoxicam; Epicotil® tablet 20 mg, Epicotil® suppository 20 mg and Epicotil lyophilized vial 20 mg (E.I.P.I.Co., Cairo, Egypt). The pharmaceutical formulations of piroxicam; piroxidin® capsule 20 mg (El-Nasr Pharmaceutical Chemical Co, ADWEC, Abu Zaabal, Egypt), dispercam tablet 20 mg and dispercam ampoules IM 20 mg (Abu-Soltan, Ismailia, Egypt). Commercial dosage forms of lornoxicam; Xefo® film coated tablet 8 mg and Xefo® lyophilized vial 8 mg (October Pharmo S.A.E., 6 October city, Egypt). All these dosage forms were purchased from local market.

Reagent Solutions

(a) Potassium bromate: An accurately weighed amount of 10 mg of potassium bromate was transferred into a 100-ml standard flask containing about 50 ml water. The contents were shaked well and completed to the mark with water. Several dilutions were made to obtain concentration ranges (0.005-0.500 mg/ml).
(b) Acid solutions: Aqueous solutions 1.5% v/v of each of HCl, H_2SO_4, HNO_3, CH_3COOH, $HClO_4$ and H_3PO_4 were prepared.

Preparation of standard solutions

About 10 mg of accurately weighed amount of each of the studied drugs (Tx, Px and Lx) was transferred into a 100-ml standard flask containing 5 ml methanol. The contents were shaked well till dissolution completed to the mark with water to provide a standard solution of 100 µg/ml. Since the drugs are sensitive to light, the flask was protected from light by aluminum foil and stored at 4°C. More diluted solutions were prepared daily by accurate dilution with water just before use.

Preparation of samples

Tablet

Twenty tablets were accurately weighed and finely powdered. An amount of powdered tablet equivalent to 10 mg of drug was transferred to a 100-ml standard flask and dissolved in 5 ml methanol. The mixture was sonicated for five minutes and completed to the mark with water. The solution was filtered and the first portion of the filtrate was discarded. One milliliter portion of the filtrate was transferred to a 100-ml standard

flask and diluted with water to obtain the required concentration for determination.

Capsules

The drug content of ten Px capsules was weighed, finely powdered and mixed. A quantity of the powder equivalent to 10 mg Px was transferred accurately into a 100-ml standard flask containing 5 ml methanol. The content of the flask was sonicated for five minutes and then completed to the volume with water. The procedure was completed as mentioned under tablets.

Vials

An accurately weighed quantity of the powder from the vial, equivalent to about 10 mg drug was transferred into a 100-ml standard flask. The procedure was completed as mentioned under tablets.

Suppositories

An accurately weighed quantity of suppository equivalent to 10 mg of drug was placed in a 50-ml beaker, melted in a water bath at 50-60°C. Five milliliters of methanol was added and placed again in the water bath for few minutes with gentle shaking. The solution was quantitatively transferred into a 100-ml standard flask with the aid of water and completed to the volume with the same solvent. The solution was centrifuged and filtered. The procedure was completed as under tablets.

General procedures

One milliliter of the working standard or sample solution was transferred into a 10-ml test tube. One milliliter of potassium bromate (50 µg/ml for Tx or 10 µg/ml for Px or 5 µg/ml for Lx) and 1.5 ml of acid 1.5% v/v (HCl in case of Tx and Px or H_2SO_4 in case of Lx) were added to the test tube. The contents of the test tube were put in a boiling water bath for 40 min. The test tube was cooled and quantitatively transferred into a 10-ml standard flask and completed to the mark with distilled water. The emission fluorescence was measured at 365 nm (excitation at 305 nm) against reagent blank treated similarly.

Determination of drugs in urine

Urine sample was collected from healthy male volunteer and stored in a deep freezer at -20°C. Before use the urine sample was kept at room temperature to be liquefied. One ml of standard drug solution (500-5000 ng/ml in case of Tx, 500-3000 ng/ml in case of Px and 600-3000 ng/ml in case of Lx) in methanol was transferred into a test tube and evaporated to dryness. One ml urine was added and shaken well on a shaker for 2 min. Dichloromethane (4 ml) was added and transferred to a 50-ml separator funnel, shaken well and extracted with further portion of dichloromethane (4 ml). The organic layer was collected into a 10- ml standard flask, evaporated on a water bath till dryness and the analysis was completed as under General procedure.

RESULTS AND DISCUSSION

Choice of the oxidant

Oxicam compounds, which contain 2-aminopyridine side chain bonded to the carbonyl group have no native fluorescence, when treated with oxidizing agent in acid medium, upon heating in boiling water bath gave intensely fluorescent product. Different oxidizing agents were tested KIO_3, KIO_4 and $KBrO_3$. The latter was chosen because it gave the most intense fluorescent product.

Excitation and emission spectra

Upon addition of aqueous solution of $KBrO_3$ to the drug solution in acid medium and after heating in boiling water bath, the drugs undergo hydrolysis to give 2-aminopyridine compound which have a native fluorescence at $_{emission}$ 365 nm ($_{excitation}$ 305 nm) (Fig. 1). British pharmacopoeia defines 2-aminopyridine (2-AP) as a potential impurity in Tx and Px[1].

Optimization of the reaction conditions

1- Effect of $KBrO_3$ concentration

Different concentrations of $KBrO_3$ were prepared (0.005-0.500 mg/ml) and tested for their effect on the relative fluorescence intensity (RFI). Concentrations of: 0.05, 0.01 and 0.005 mg/ml were selected for determination of Tx, Px and Lx respectively.

2- Effect of type and volume of acid

Different acids were tested for their effect on the RFI include HCl, H_2SO_4, $HClO_4$, H_3PO_4, HNO_3 and CH_3COOH. It was found that HCl gave the highest intensities in case of Tx and Px, while H_2SO_4 gave the highest intensity in case of Lx (Table 1).

Table 1: Effect of type of acid on the relative fluorescence intensity of the studied drugs (300 ng/ ml).

Acid	Relative fluorescence intensity		
	Tx	Px	Lx
HCl	660	576	389
H_2SO_4	420	488	504
$HClO_4$	55	105	336
H_3PO_4	72	27	96
HNO_3	556	140	141
CH_3COOH	249	261	150

Different volumes (0.2-2.0 ml) of the selected acids were tested for their effect on the relative fluorescence intensities. It was found that 1.5 ml of either of HCl or H_2SO_4 gave high and precise results (Fig. 2).

3- Effect of reaction time and temperature

The fluorescence intensities of the studied drugs increased by increasing the heating time in boiling water bath. At about 40 min. the, drugs were completely hydrolysed to 2-amino-pyridine. So, 40 min. were selected as the suitable heating time (Fig. 3).

4- Effect of diluting solvent

Water, ethanol, methanol, dimethylformamide, acetonitrile and acetone were tested as diluting solvent for the reaction. It was found

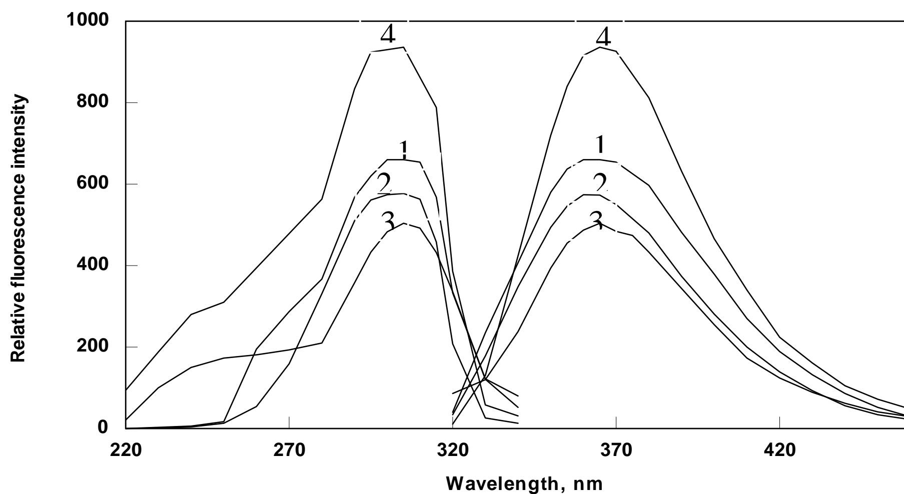


Fig. 1: Excitation and emission spectra of hydrolytic products of each of (1) Tx, (2) Px (3) Lx and (4) 2-aminopyridine.
Concentration of each drug and 2-aminopyridine was 300 ng/ml.

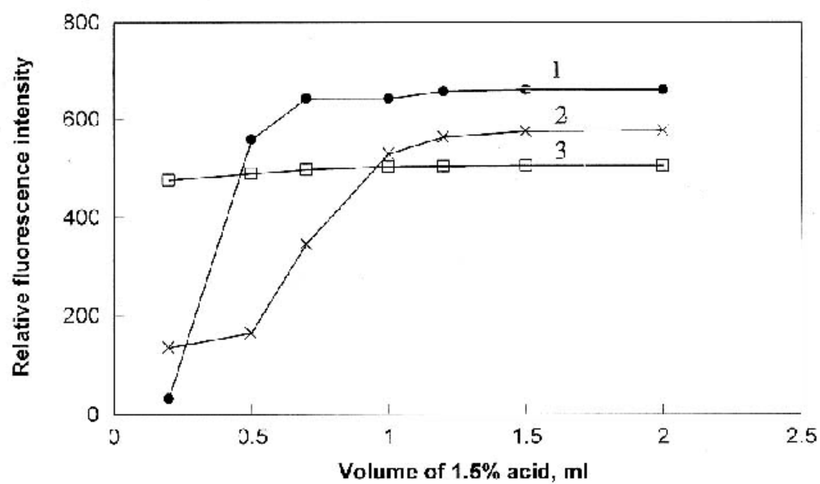


Fig. 2: Effect of volume of 1.5% acid on the relative fluorescence intensities of the hydrolytic products of (1) Tx, (2) Px and (3) Lx.
Concentration of each drug was 300 ng/ml.
The acid in cace of Tx and Px was HCl, while in case of Lx was H_2SO_4.

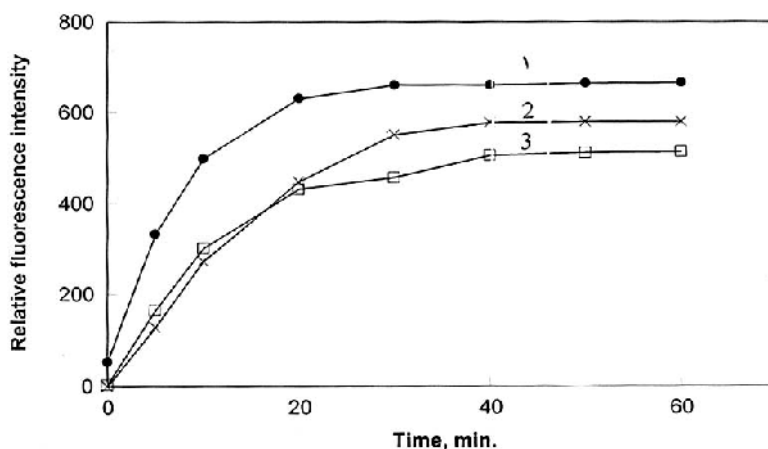


Fig. 3: Effect of heating time in boiling water bath on the relative fluorescence intensities of the hydrolytic products of each of (1) Tx, (2) Px and (3) Lx. Concentration of each drug was 300 ng/ml.

that water gave the highest fluorescence intensities with all studied drugs (Table 2).

Table 2: Effect of type of diluting solvent on the relative fluorescence intensity of the studied drugs (300 ng /ml).

Solvent	Relative fluorescence intensity		
	Tx	Px	Lx
H_2O	660	576	504
C_2H_5OH	493	485	488
CH_3OH	541	344	458
$(CH_3)_2NCOH$	300	242	400
CH_3CN	330	347	437
CH_3COCH_3	0	3	2

Validation of the method[67]

1- Linearity, quantitative and detection limits

By application of the suggested method, the relationship between the concentration and the relative fluorescence intensities was found to be linear over the concentration range 15:500, 6:300 and 60:400 ng/ml for Tx, Px and Lx respectively. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to USP 2002[67] as follows:

LOD or LOQ = K. σ a/b

Where, K = 3 for LOD and K= 10 for LOQ; σ a: is the standard deviation of the intercept (a) and b: is the slope.

The slope, intercept, correlation coefficient (r), determination coefficient (r^2), LOQ and LOD of the three studied drugs were listed in Table 3. The LOQ ranged from 5.46 to 57.21 ng/ml while LOD ranged from 1.63 to 17.16 ng/ml.

2- Selectivity

The suggested method was selective for the compounds which by

Table 3: Analytical data for the dtermination of tenoxicam, piroxicam and lornoxicam.

Analyte	Tx	Px	Lx
Linearity range (ng/ ml)	15-500	6-300	60-400
r	0.9999	0.9999	0.9987
r^2	0.9998	0.9999	0.9974
Slope ± SD	2.2004± 0.0013	1.9181± 0.0067	0.3562 ± 0.0090
Intercept ± SD	-0.0999 ±3.1845	1.3258 ± 1.0475	1.6672 ± 2.0381
LOD, ng/ml	4.342	1.638	17.165
LOQ, ng/ml	14.473	5.460	57.217

LOD: Limit of detection, LOQ: Limit of quantification.

oxidative hydrolysis gave 2-amino-pyridine. This is proved by application of the method for other oxicam compounds, doesn't posses the side chain 2-aminopyridine (e.g. meloxicam), the compound gave no fluorescence when exposed to oxidative hydrolysis. So the proposed method was considered to be selective.

3- Robustness

The effect of small variation in the reaction conditions e.g. acid volume, $KBrO_3$ concentration and reaction time was studied. From the optimization study, it was found that their was no significant change in the results by small change in the reaction conditions, so the method can be considered robust

4- Applications

(a) Determination of drugs in pharmaceutical dosage forms

The proposed procedures were applied for the determination of the studied drugs in different pharmaceutical formulations. The recovery percentages ranged from 96.82 to 102.79 ± SD 0.377: 2.494 (Table 4).

This indicated the suitability of the suggested procedures for the analysis in different dosage forms without significant interference from the excipients and additives as proved by F and t-testes.

(b) Determination of drugs in urine

By applying the proposed procedures for the determination of the studied drugs in spiked urine, it was found that, the method is satisfied with recovery percentage ranged from 85.01 to 105.35% ± SD ranged from 1.067 to 5.338, This indicates the suitability of the extraction procedure for the elimination of the interference of the substances already present in the urine (Table 5).

Table 4: Assay results for the determination of studied drugs in pharmaceutical preparations.

Preparation	Content mg	Recovery % ± SD[a]	
		Fluorimetric method	Reported method
Epicotil vials	20/vial Tx	99.27 ± 2.275 F= 1.537 t= 0.407	98.71 ± 2.111[64]
Epicotil suppositories	20/supp. Tx	102.79 ± 0.844 F= 1.081 t= 0.960	102.45 ± 1.658[64]
Epicotil tablets	20/tablet Tx	100.11 ± 1.269 F= 1.188 t= 0.526	100.51 ± 1.383[64]
Dispercam ampoules	20/ampoul Px	100.37 ± 1.031 F= 1.347 t= 0.524	100.08 ± 0.888[63]
Dispercam tablets	20/tablet Px	97.46 ± 0.995 F= 4.799 t= 0.112	97.49 ± 0.452[63]
Piroxidin® capsules	20/capsule Px	100.32 ± 2.494 F= 1.279 t= 0.318	99.83 ± 2.821[63]
Xefo® lyophilized vials	8/vial Lx	96.82 ± 0.787 F= 2.044 t= 2.137	96.99 ± 0.550[10]
Xefo® film coated tablets	8/tablet Lx	99.76 ± 0.377 F= 0.605 t= 1.095	99.51 ± 0.414[10]

[a] Mean ± standard deviation for sex determinations.

Tabulated t and F values are 2.228 and 5.050, respectively.

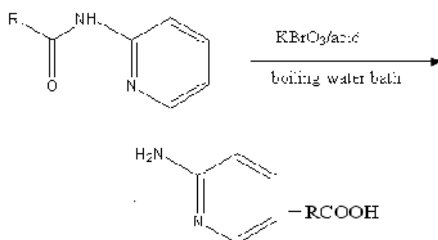
Table 5: Assay results for the determination of studied drugs in spiked urine.

Drug	Recovery % ± SD (concentration ng/ml)		
	a	b	c
Tenoxicam	105.35 ± 5.338	102.23 ± 2.775	97.00 ± 3.455
Piroxicam	104.18 ± 4.221	99.12 ± 3.019	99.16 ± 1.067
Lornoxicam	90.20 ± 5.123	85.01 ± 1.564	80.51 ± 1.355

For tenoxicam a, b and c were 50, 100 and 500 ng/ ml respectively.
For piroxicam a, b and c were 50, 100 and 300 ng/ ml respectively.
For lornoxicam, a, b and c were 60, 200 and 300 ng/ ml respectively.

Mechanism of the reaction

The studied drugs have no native fluorescence as it in the intact form. As previously mentioned[68], the oxicam compounds undergo hydrolysis to 2-amino pyridine under drastic conditions (heat with 6 M H_2SO_4 at 105°C for 20 hours). So addition of $KBrO_3$ was for acceleration of the reaction, as was mentioned for Hofmann preparation of the primary amine from the amide[69]. Under the optimal reaction conditions, the oxicam compounds which have a 2-aminopyridine side chain undergo oxidative hydrolysis to give 2-aminopyridine which have a native fluorescence can be measured at 365 nm (excitation at 305 nm) according to the following scheme.



Conclusion

A validated spectrofluorimetric procedures were described for quantification of bulk oxicam compounds. The suggested procedures were successfully applied for quantification of the three studied oxicam compounds in different pharmaceutical formulations and human urine. The procedures showed clear advantages such as simplicity cheapness where the used reagents are simple and available. Also the procedures could be used as a forced stability indicating assay as the procedures depended on the degradation of the intact drugs. The procedures could be recommended for analysis of Tx, Px and Lx in quality control and clinical laboratories.

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