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Short communication

Spectrofluorimetric determination of 2-aminopyridine as a potential impurity in piroxicam and tenoxicam within the pharmacopoeial limit

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Abstract

The *British Pharmacopoeia* defines 2-aminopyridine (2-AP) as a potential impurity in piroxicam (PX) and tenoxicam (TX). Selective spectrofluorimetric determination of 2-AP in PX and TX, within or near the pharmacopoeial level, 0.2%, was developed, based on the measurement of the native fluorescence either in aqueous 0.1N sulfuric acid or in dioxane. Accordingly, this approach was followed for confirming purity of PX and TX in bulk and pharmaceutical preparations. The study was also extended to include simultaneous determinations of PX/2-AP and TX/2-AP systems based on selective fluorescence measurements in the cited solvents.

Keywords: 2-Aminopyridine; Piroxicam; Tenoxicam

1. Introduction

Piroxicam and tenoxicam are non-steroidal anti-inflammatory drugs belonging to the chemical class of oxicams, *N*-heterocyclic carboxamide derivatives of benzothiazine-1,2-dioxide. They are used in musculoskeletal and joint disorders [1].

2-AP is one of the potential impurities in PX and TX bulk drugs and pharmaceutical preparations. It is considered as a synthesis precursor or a decomposition product through acid cleavage. The *British Pharmacopoeia 2001* [2] specifies the limit of 2-AP in PX and TX to be 0.2% in bulk drugs and 0.25% in pharmaceutical preparations.

Different analytical techniques have been described for PX assaying in pharmaceutical formulations, including UV-Vis spectrophotometry [3–7], spectrofluorimetry [8–10], flow injection spectrophotometry [11], voltammetry [12,13], capillary zone electrophoresis [14] and HPLC [15,16]. Stability indicating chromatographic methods [17–20] have been reported for PX determination with special interest to check for the potential impurities. Derivative spectrophotometric determination of 2-AP in PX bulk material and pharmaceutical preparations has been reported [21].

Of the analytical methods reported for TX determination in pharmaceutical preparations are spectrophotometry [4,5], spectrofluorimetry [22,23], HPLC [24],

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polarography [25], and flow injection spectrophotometry [26]. The stability indicating assays of interest include derivative spectrophotometric and HPLC methods [27], vis-spectrophotometric [28] and spectrofluorimetric [22] methods.

Based on the previous review, more attention in the area of fluorimetric analysis of PX and TX with special intent to evaluate the interference of 2-AP would be worthy. The native fluorescence characteristics of 2-AP in aqueous (0.1N sulfuric acid) and non-aqueous (dioxane) solutions were considered with the mutual interference from PX and TX. A sensitive and fast method to check for 2-AP, within the pharmacopoeial limit, in PX and TX samples was developed to be a favourite alternative to the *British Pharmacopoeia* chromatographic purity test. Simultaneous spectrofluorimetric analysis of PX/2-AP and TX/2-AP systems, based on selective measurements in the aforementioned solvents, was also described. The developed spectrofluorimetric methods were applied for determination of PX and TX in bulk drugs and pharmaceutical preparations.

2. Experimental

2.1. Apparatus

Fluorescence spectra and measurements were taken on a Perkin-Elmer 650-10S spectrofluorimeter, equipped with a 1 cm quartz cell and a 150 W Xenon arc lamp.

2.2. Standard/assay solutions

2.2.1. Preparation of standard solutions of piroxicam, tenoxicam and 2-aminopyridine

Stock standard solutions of PX, TX and 2-AP at a concentration level of 1 mg ml^{-1} were prepared in dimethylformamide. Two sets of working standard solutions were prepared by appropriate dilution steps with 0.1N sulfuric acid, to give a final concentration of 100 μg ml^{-1} for PX and 0.1 μg ml^{-1} for 2-AP, and with dioxane to give a final concentration of 10 μg ml^{-1} for PX or TX and 1 μg ml^{-1} for 2-AP.

2.2.2. Preparation of tablet assay solutions

Twenty tablets (Feldene tablets, labeled to contain 10 mg PX per tablet, and Epicotil tablets, labeled to contain 20 mg TX per tablet) were weighed and reduced to a fine powder. An amount equivalent to 25 mg of either PX or TX was accurately weighed, transferred to a 25 ml volumetric flask, stirred with dimethylformamide, made up to volume with the same solvent and filtered. Further dilution was made with dioxane to give a final concentration of 10 μg ml^{-1}.

2.2.3. Preparation of piroxicam ampoule assay solution

The contents of 10 ampoules (Feldene ampoules, labeled to contain 20 mg PX per ml) were mixed and a volume equivalent to 25 mg PX was diluted with 0.1N sulfuric acid to give a final concentration of 100 μg ml^{-1}.

Table 1
Experimental and analytical features for the determination of piroxicam, tenoxicam and 2-aminopyridine

Analyte	Measurement $\lambda_{ex}/\lambda_{em}$ (nm) sensitivity settings	Linearity range (μg ml^{-1})	Regression data			$S_{y/x}$	S_a	S_b	LOD (μg ml^{-1})	LOQ (μg ml^{-1})
			a	*b*	*r*					
Measurements in 0.1N sulfuric acid										
Piroxicam	345/470 10	0.2–2.0	−0.622	30.88	0.9999	0.271	0.232	0.189	0.034	0.113
2-Aminopyridine	306/366 1	0.001–0.02	0.100	4384.0	0.9999	0.118	0.145	10.583	0.00016	0.00053
Measurements in dioxane										
Piroxicam	340/470 3	0.2–1.2	1.80	33.86	0.9993	0.303	0.322	0.477	0.045	0.150
Tenoxicam	360/480 10	0.3–2.4	−2.22	38.45	0.9999	0.412	0.434	0.342	0.041	0.137
2-Aminopyridine	300/345 1	0.01–0.1	−0.08	777.40	0.9996	0.416	0.324	5.338	0.0023	0.0078

$S_{y/x}$: standard error of estimates; S_a: standard deviation of intercept; S_b: standard deviation of slope; LOD: limit of detection; LOQ: limit of quantification.

2.2.4. Preparation of tenoxicam vial assay solution

The content of five vials (Epicotil vials, labeled to contain 20 mg TX per vial) were transferred to a 50 ml volumetric flask, dissolved in dimethylformamide and made up to volume with the same solvent. Further dilution was made with dioxane to give a final concentration of 10 $\mu g\,ml^{-1}$.

2.3. Calibration graphs

Further dilutions of the working standard/assay solutions, previously prepared in 0.1N sulfuric acid or in dioxane, were made using the respective solvent to give final concentrations in the ranges listed in Table 1. The fluorescence intensities were measured at the specified wavelengths (Table 1) against solvent blank.

3. Results and discussion

3.1. Fluorescence spectral characteristics

The fluorescence excitation and emission spectra of PX, TX and 2-AP in 0.1N sulfuric acid are shown in Figs. 1 and 2. On the basis of the fluorescence spectral characteristics of the analytes, two aspects should be considered. First, the emission wavelength of 2-AP is quite apart from that of PX (~100 nm separation), while the separation between 2-AP and TX emission maxima is only 45 nm. Second, the intrinsic fluorescence intensity of 2-AP in 0.1N sulfuric acid is about one thousand times that of either PX or TX.

These previous facts allowed selective measurement of 2-AP in PX and TX at a level down to 0.05%. Also, with careful instrumental adjustment of sensitivity settings (Table 1), it was possible to selectively determine PX in the presence of 2-AP. However, spectral interference of 2-AP (excited at 315 nm, λ_{ex} of TX) and the relatively poor fluorescence intensity of TX (Fig. 2) made the selective determination of the latter in 0.1N sulfuric acid unworthy.

In dioxane, the fluorescence excitation and emission spectra of PX, TX and 2-AP are shown in Fig. 3. Studying the fluorescence spectral features of the analytes, the previous concepts could be considered with the only exception that 2-AP and TX emission maxima are quite separated by 135 nm. Accordingly,

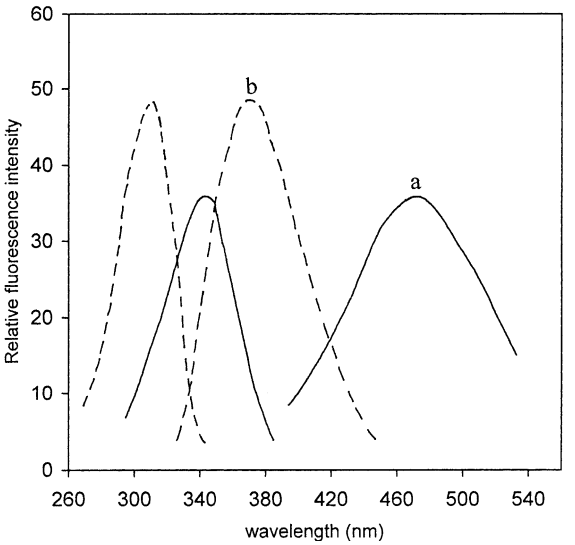

Fig. 1. Excitation and emission spectra of (a) 1 $\mu g\,ml^{-1}$ PX and (b) 0.01 $\mu g\,ml^{-1}$ 2-AP measured in 0.1N sulfuric acid solution (uncorrected spectra for blank). The sensitivity range was 10 and 1, respectively.

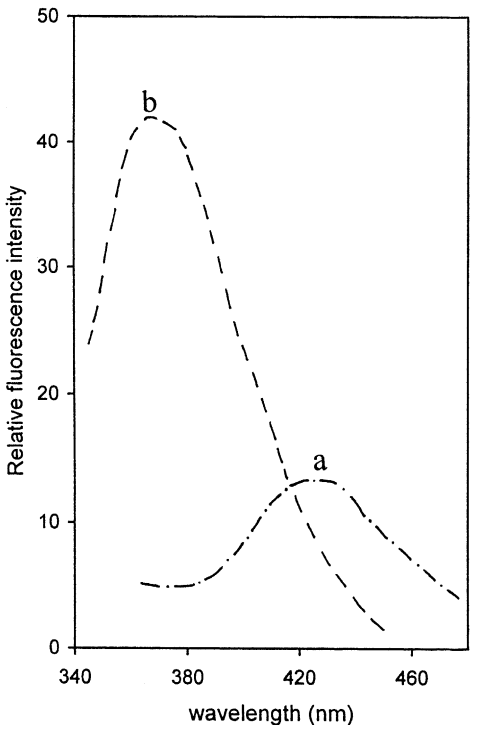

Fig. 2. Emission spectra of (a) 10 $\mu g\,ml^{-1}$ TX and (b) 0.01 $\mu g\,ml^{-1}$ 2-AP measured in 0.1N sulfuric acid solutions (uncorrected spectra for blank). The sensitivity range was 1.

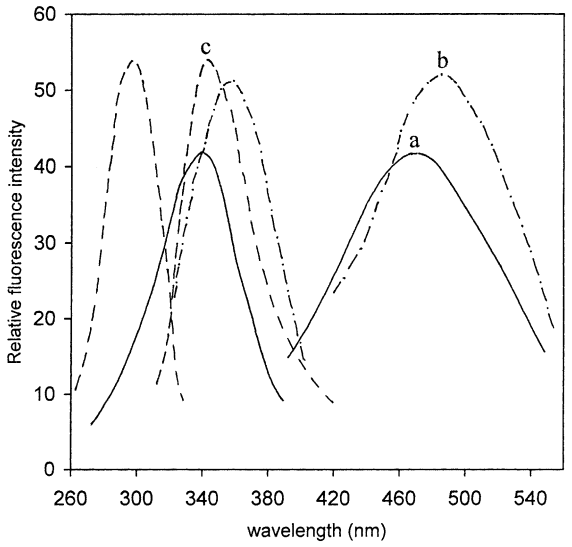

Fig. 3. Excitation and emission spectra of (a) 1.1 μg ml^{-1} PX, (b) 1 μg ml^{-1} TX and (c) 0.05 μg ml^{-1} 2-AP measured in dioxane solutions (uncorrected spectra for blank). The sensitivity range, in the same sequence, was 10, 3 and 1.

fluorimetric determination of 2-AP in PX and TX at a level down to 1% with nil interference from PX or TX and also selective fluorimetric determinations of PX and TX (in presence of 2-AP as a decomposition product) were possible.

3.2. Quantitative aspects

The selective fluorimetric determination of 2-AP, PX and TX was based on the measurement of fluorescence intensity of standard solutions, prepared in 0.1N sulfuric acid and in dioxane (not for TX), at the specified wavelengths (Table 1). Instrument sensitivity settings used in this work are given in Table 1.

Recovery experiments were carried out to check for the selective fluorescence measurements of 2-AP, PX and TX in PX/2-AP and TX/2-AP synthetic mixtures. The precision and accuracy of the methods were assessed through the statistical analysis of the experimental data (Table 2). From the previous work, it could be concluded that simultaneous determination of the fluorescence of PX/2-AP or TX/2-AP systems was possible, with instrument adjustment at the specified wavelengths and sensitivity settings of the respective analyte, taking into consideration that 2-AP could be determined in PX and TX bulk drugs at a concentration down to 0.05% and PX and TX could be determined in presence of 2-AP at a concentration level up to 50 and 20%, respectively.

3.3. Concentration ranges and calibration graphs

Using the optimized instrumental sensitivity settings, the relative fluorescence intensities measured at the specified working wavelengths were found to be linearly correlated to the PX, TX and 2-AP concentrations. Data recorded in Table 1 summarizes the characteristics of the calibration plots. These include linear regression equations, concentration ranges, correlation coefficients (r), and standard deviations of the intercept (S_a) and slope (S_b).

3.4. Detection and quantification limits

The limit of detection, LOD ($3sb^{-1}$, where s is the standard deviation of replicate blank readings, under the same conditions as for sample analysis), and the limit of quantification, LOQ ($10sb^{-1}$), [29] are given in Table 1.

3.5. Analysis of pharmaceutical formulations

The proposed spectrofluorimetric methods were applied to the determination of PX in tablets and ampoules and TX in tablets and vials. The results are shown in Table 3. The assay results show satisfactory recovery data and good precision of the proposed methods. For comparison, PX and TX preparations were analysed using the reported spectrophotometric A_{max} [3] and derivative [27] methods, respectively. The results of the proposed and reference methods were compared in accordance with Student's t-test and variance ratio F-test. There were no significant differences between the calculated and theoretical values at $P = 0.05$, demonstrating that the proposed methods are as accurate and precise as the respective reference method.

The fluorimetric methods described herein to quantify 2-AP were used to confirm the purity of PX and TX in dosage forms. The results show no sign for the existence of any 2-AP in the samples checked.

Table 2
Precision and accuracy for the determination of piroxicam, tenoxicam and 2-aminopyridine in synthetic mixtures

Analyte	Nominal value ($\mu g\,ml^{-1}$)	Found ± S.D.[a] ($\mu g\,ml^{-1}$)	R.S.D. (%)[b]	E_r (%)[c]
Measurements in 0.1N sulfuric acid				
Piroxicam				
4% 2-aminopyridine	0.50	0.507 ± 0.0061	1.20	1.40
1% 2-aminopyridine	2.00	1.982 ± 0.0222	1.12	−0.90
2-Aminopyridine				
0.05% in piroxicam	0.001	$0.00099 \pm 1.1 \times 10^{-5}$	1.08	−1.10
1% in piroxicam	0.02	$0.0197 \pm 1.8 \times 10^{-4}$	0.92	−1.50
0.05% in tenoxicam	0.001	$0.00101 \pm 9.8 \times 10^{-6}$	0.98	1.10
1% in tenoxicam	0.02	$0.0204 \pm 2.1 \times 10^{-4}$	1.02	1.75
Measurements in dioxane				
Piroxicam				
50% 2-aminopyridine	0.2	0.198 ± 0.0021	1.06	−1.00
10% 2-aminopyridine	1.0	0.995 ± 0.0090	0.91	−0.5
Tenoxicam				
20% 2-aminopyridine	0.50	0.499 ± 0.0044	0.88	−0.2
5% 2-aminopyridine	2.00	2.021 ± 0.0135	0.67	1.05
2-Aminopyridine				
2% in piroxicam	0.02	0.0198 ± 0.00026	0.33	−1.00
10% in piroxicam	0.10	0.0992 ± 0.0011	1.13	−0.80
1% in tenoxicam	0.02	0.0198 ± 0.00031	1.57	−1.00
5% in tenoxicam	0.10	0.101 ± 0.0010	1.01	1.00

[a] Mean ± standard deviation of five determinations.
[b] Percentage relative standard deviation.
[c] Percentage relative error.

Table 3
Assay results for the determination of piroxicam and tenoxicam in pharmaceutical preparations

Preparation	Recovery ± S.D.[a]	
	Fluorimetric method	Reference method
Feldene tablets[b]	100.47 ± 0.91 $t = 1.23$, $F = 1.40$[c]	99.81 ± 0.78[d]
Feldene ampoules[b]	100.33 ± 1.21 $t = 0.55$, $F = 1.33$[c]	99.93 ± 1.05[d]
Epicotil tablets[e]	100.37 ± 0.98 $t = 0.70$, $F = 1.12$[c]	99.92 ± 1.04[f]
Epicotil vials[e]	100.45 ± 1.16 $t = 0.07$, $F = 3.02$[c]	100.50 ± 0.67[f]

[a] Mean ± standard deviation of five determinations.
[b] Labeled to contain 10 and 20 mg piroxicam per tablet and per millilitre ampoule, respectively. It is manufactured by Pfizer-Egypt, Cairo, Egypt, under authority of Pfizer Inc., USA.
[c] Tabulated t-value for $P = 0.05$ and eight degrees of freedom is 2.306, tabulated F-value for $P = 0.05$ and $f_1 = f_2 = 4$ is 6.38.
[d] Ref. [3].
[e] Labeled to contain 20 mg tenoxicam per tablet or vial. It is manufactured by Egyptian Int. Pharm. Ind. Co (IPICO), Egypt.
[f] Ref. [27].

4. Conclusion

The article introduced a simple, sensitive and cheap spectrofluorimetric method valuable for selective quantitation of 2-AP, to be applied as a limit test to check the purity of PX and TX. It is a favourite alternative to chromatographic purity test specified by *British Pharmacopoeia 2001*.

Briefly, the analytical merit of this work is that one can simply and simultaneously carry out the purity

limit test and selective analyte assay on the same solution; only the instrumental settings have to be adjusted.

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