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Simultaneous determination of tenoxicam and 2-aminopyridine using derivative spectrophotometry and high-performance liquid chromatography

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Abstract

Derivative spectrophotometry and high-performance liquid chromatography (HPLC) were used to determine tenoxicam and one of its decomposition products (2-aminopyridine) simultaneously and in the presence of each other. The derivative procedure was based on the linear relationship between the tenoxicam concentration and the second derivative amplitudes at 390–348 nm (peak-to-trough) measurement. The 2-aminopyridine was determined through measuring the second derivative amplitude at 241 nm (zero-crossing for tenoxicam). For the HPLC procedure, a reversed-phase C_8 column with a mobile phase composed of 0.02 M sodium acetate–methanol–acetonitrile (11:8:1) with 0.005 M heptane sulfonic acid sodium salt, as an ion pair, was used to separate both compounds with 2,4-dinitrochlorobenzene, as an internal standard, in reasonable time. The flow rate was 1.5 ml min^{-1} with a programmable ultraviolet (UV) detection at 300 and 375 nm. Both UV derivative spectrophotometric and HPLC approaches were followed for confirming the purity of tenoxicam in bulk and tablets dosage form. © 1997 Elsevier Science B.V.

Keywords: 2-Aminopyridine; Bulk and tablets; Derivative spectrophotometry; Impurity in drug; High-performance liquid chromatography; Tenoxicam

1. Introduction

The oxicams, *N*-heterocyclic carboxamide derivatives of the benzothiazine-1,2-dioxide, a new chemical class of non-steroidal anti-inflammatory drugs, has recently been introduced to the market. Piroxicam and tenoxicam are the most famous members of this group. The high similarity of the structure of both drugs enabled the chemical synthesis of both drugs through almost the same route. The 2-aminopyridine (2AP) was one of the main impurities in the piroxicam bulk or pharmaceutical products [1,2]. It was considered as a synthesis precursor or a decomposition product through an acidic cleavage [3]. In the

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same manner, the 2AP can be considered as one of the impurities in tenoxicam (Tx) bulk and pharmaceutical tablets. Few methods have been reported for Tx determination in biological fluids, e.g., high-performance liquid chromatography (HPLC) [4–7] and voltametric techniques [8]. The chelating tendency of Tx was investigated using differential pulse polarographic and cyclic voltametric techniques [9]. Recently, the analytical profile of Tx, with a good number of references for its physical and chemical characteristics, has been reviewed [10]. The present paper describes derivative spectrophotometric and HPLC methods useful for the simultaneous determination of Tx and 2AP in bulk material and in pharmaceutical tablets.

2. Experimental

2.1. Materials

Methanol and acetonitrile were HPLC grade (Romile Chemicals, UK). Water was double glass distilled and filtered before use. The Tx and 2AP, utilized as standard, were kindly supplied by F. Hoffman-La Roche (Basel, Switzerland) accompanied by their certificates of analysis and used without further purification. The 2,4-dinitrochlorobenzene (internal standard) was purchased from BDH (Poole, UK). The commercial tablets was obtained from the local market.

2.2. Apparatus and conditions

Absorption and derivative spectra were recorded over the wavelength range 240–350 nm in 10 mm quartz cells using Perkin–Elmer Model 550S UV-VIS spectrophotometer connected to a Hitachi Model 561 recorder. The scan speed was 120 nm min^{-1}, chart speed 60 nm min^{-1}, and slit width 2 nm.

For HPLC, a Hewlett-Packard system consisting of a Model 1050 pump, with a HP 1050 programmable multiple wavelength detector and a Model HP 1050 autosampler were used. The chromatograms were recorded and the peaks were quantitated using an HP 3396A automatic integrator. The separation was carried out, at ambient temperature, on RP-C_8 Lichrosorb Column (Alletech Associate, USA) of 250 × 4.6 mm ID containing 10 μm modified silica. The mobile phase consisted of a ternary mixture of 0.02 M sodium acetate–methanol–acetonitrile (11:8:1) and the aqueous phase contains 0.005 M heptane sulfonic acid sodium salt, the pH being adjusted to 3.5. The flow rate was set at 1.5 ml min^{-1} with 10 μl as injection volume. The mixture was quantitated with programmable wavelength change. The wavelength of detection was 300 nm from the start of the run to 4 min, then changed to 375 nm till the end of the run. The sensitivity of the detection was set at 0.1 AUFS. The integrator parameters were set to be: attenuation 5; threshold 3; peak width 0.04; and chart speed 0.2 cm min^{-1}.

2.3. Standard solutions

Stock standard solutions were prepared in methanol with Tx and 2AP final concentrations of 0.2 and 0.25 mg ml^{-1}, respectively. For the derivative measurements, further dilution were made using 0.1 N methanolic HCl to give final concentrations in the ranges 2–10 and 0.3–2 μg ml^{-1} for Tx and 2AP, respectively. For the above prepared solutions, the ^2D amplitudes were measured at 390–348 nm (peak-to-trough) and 241 nm (zero-crossing) for the Tx and 2AP solutions, respectively. All the spectrophotometric measurements were performed against a similarly prepared blank.

For HPLC, synthetic mixtures were prepared containing Tx and 2AP in the ranges 2–10 and 0.3–2 μg ml^{-1} of Tx and 2AP, respectively, with a constant concentration of 2,4-dinitrochlorobenzene (5 μg ml^{-1}) as internal standard (IS). The samples were filtered through 0.45 μm membrane filter before injection into the chromatograph.

2.4. Samples solutions

Ten tablets were weighed and reduced to a fine powder. An amount corresponding to one tablet was accurately weighed, transferred to a 100 ml volumetric flask, stirred with methanol, made up

to volume with the same solvent and filtered. Further dilution was made using either 0.1 N HCl (for derivative measurements) or methanol (for HPLC procedure).

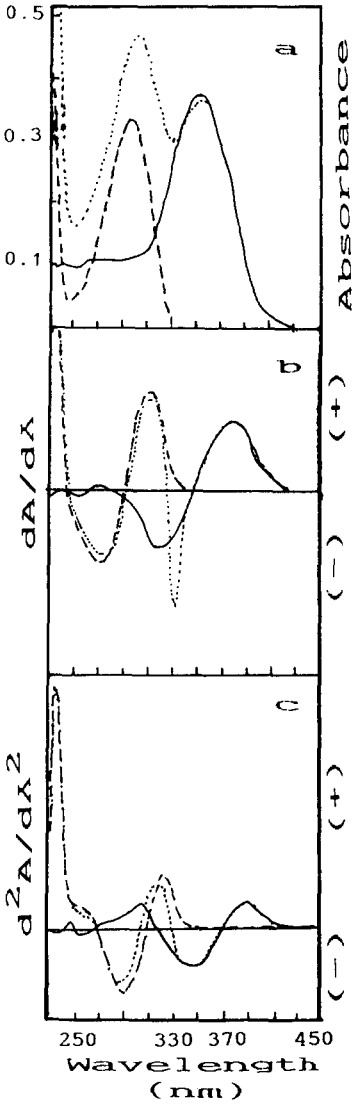

Fig. 1. (a) Zero-order, (b) first-order and (c) second-order derivative spectra of 5 μg ml^{-1} Tx (——), 5 μg ml^{-1} 2AP (– – –) and their synthetic mixture (· · ·) in 0.1 N methanolic HCl.

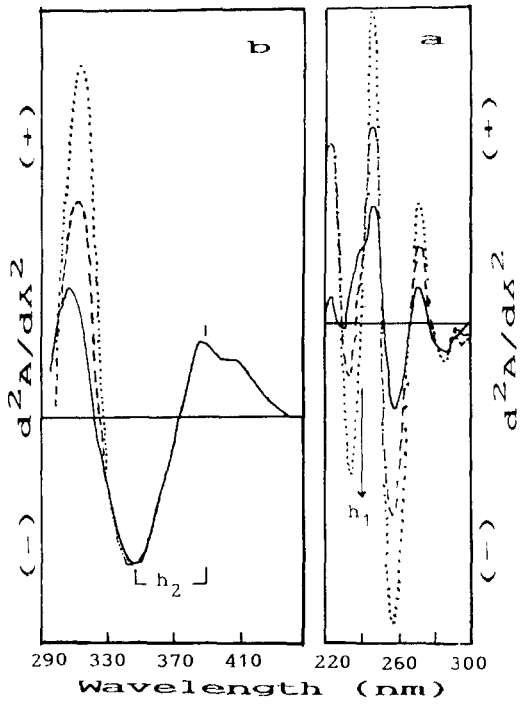

Fig. 2. Second derivative spectra, in 0.1 N methanolic HCl, of (a) 10 μg ml^{-1} Tx (——) in the presence of 1.0 μg ml^{-1} (– – –) and 10 μg ml^{-1} (· · ·) 2AP; (b) 1 μg ml^{-1} 2AP (——) in the presence of 25 μg ml^{-1} (– – –) and 125 μg ml^{-1} (· · ·) Tx.

3. Results and discussion

3.1. Spectrophotometric procedure

Fig. 1(a) shows the absorption spectra of Tx and 2AP at similar concentration in methanolic 0.1 N HCl. The spectra clearly display considerable overlap. The 2AP exhibits nil absorbance over the wavelength range 340–420 nm, while the Tx shows a maximum at 368 nm. Despite this feature, the absorption spectrum of the mixture shows a negative sum of the absorbance which made the absorption spectrum useless. The first-derivative (^{1}D) spectra (Fig. 1(b)) showed a little improvement where Tx can be quantitated at 378 nm (nil contribution of 2AP). On the other hand, the second-derivative spectra (^{2}D) (Fig. 1(c)) offered an extremely valuable mean for the simultaneous determination of both compounds. The

Table 1
Beer's law data and statistical analysis for the calibration graphs of tenoxicam (Tx) and 2-aminopyridine (2AP) using the derivative procedure

Compound	Coexisting compound (μg ml^{-1})	Regression equation			
		a (S.E.)	*b* (S.E.)	*r*	R.S.D.
Tx[a]: ^{2}D measured at 390–348 nm	—, —	0.11 (2.24×10^{-3})	6.795 (1.15×10^{-3})	0.9999	0.62
	2AP, 1.0	0.19 (8.29×10^{-3})	6.784 (1.34×10^{-3})	0.9998	1.46
	2AP, 10.0	0.20 (2.88×10^{-2})	6.800 (4.65×10^{-3})	0.9991	0.16
2AP[b]: ^{2}D measured at 241 nm	—, —	-0.35 (1.5×10^{-1})	20.225 (1.4×10^{-1})	0.9999	0.75
	Tx, 25.0	-0.12 (6.30×10^{-3})	20.275 (5.84×10^{-3})	0.9999	1.46
	Tx, 125.0	-0.29 (9.05×10^{-2})	20.217 (8.39×10^{-2})	0.9992	0.72

a, Intercept; *b*, slope; *r*, correlation coefficient; S.E., standard error; R.S.D., relative standard deviation.
[a] Concentration range 2–10 μg ml^{-1} ($n=5$).
[b] Concentration range 0.3–2 μg ml^{-1} ($n=5$).

wavelength at 390 nm shows a maximum and an inflexed maximum at 348 nm (peak-to-trough) where the 2AP shows no interference. At the same time, Tx exhibits a zero crossing at 241 nm where 2AP can be determined.

In order to test the mutual independence of the derivative signals of Tx and 2AP, i.e., to show that h_1 and h_2 (Fig. 2(a) and (b)) are independent of the Tx and 2AP concentrations, respectively, the following experiments were performed. Three calibration graphs were constructed from ^{2}D signals for standards containing between 2 and 10 μg ml^{-1} Tx, in the absence of 2AP and in the presence of 1 and 10 μg ml^{-1} 2AP. Similarly, three calibration graphs were prepared for standards containing between 0.1 and 2 μg ml^{-1} 2AP in the absence of Tx and in the presence of 25 and 125 μg ml^{-1} Tx.

A critical evaluation of the proposed method was performed by the statistical analysis of the experimental data. The obtained slopes, intercepts and correlation coefficients obtained are summarized in Table 1. The linearity of the calibration graphs and the adherence of the systems to Beer's law are validated by the high value of the correlation coefficient of the regression equations and by the value of the intercepts on the ordinate, which is close to zero.

3.2. HPLC procedure

The reversed-phase HPLC method was developed to provide a specific procedure suitable for the rapid quality control analysis of Tx, and as referee method for the developed derivative assay. The mobile phase was chosen after several trials with acetonitrile–water and methanol–water in various proportions and at different pH values with different internal standards. The chromatographic system described above allows complete base line separation for every two adjacent peaks (Fig. 3). The presence of heptane sulfonic acid sodium salt was essential to increase the retention time of the 2AP and to reduce tailing of the peaks. The partial replacement of methanol with acetonitrile improves the peaks symmetry. Due to the low concentration of the 2AP relative to Tx and the difficulty in changing the attenuation of the integrator during the run, the option of changing the wavelength of detection was chosen to improve the sensitivity towards 2AP. At a flow of 1.5 ml min^{-1}, the retention times for 2AP, Tx and (IS) were 2.90, 5.88 and 10.45 min, respectively. For quantitative analysis, linear calibration graphs ($Y=-1.9\times10^{-4}+2.90C$, $\text{S.E.}_a=2.17\times10^{-2}$, $\text{S.E.}_b=3.28\times10^{-3}$, $r=0.9997$ for Tx; and $Y=-2.2\times10^{-3}+2.88C$, $\text{S.E.}_a=$

Table 2
Assay results of tenoxicam (Tx) and 2-aminopyridine (2AP) in laboratory-made mixtures and commercial tablets using second derivative and HPLC methods

Preparation	Recovery (mean ± S.D.)[a] (%)			
	Tenoxicam		2-Aminopyridine	
	$^2D_{390-348}$	HPLC	$^2D_{241}$	HPLC
Laboratory-made mixture	100.04 ± 0.87 $t = 0.90$ $F = 1.02$	100.57 ± 0.88	100.35 ± 1.03 $t = 0.20$ (2.31)* $F = 0.13$ (6.39)*	100.49 ± 1.18
Commercial[b] tablets	99.73 ± 0.45 $t = 0.76$ $F = 4.18$	99.56 ± 0.22	—	

[a] Mean of five determinations ± S.D.
[b] Tilcotil tablets were labeled to contain 20 mg per tablet, made in Switzerland by F. Hoffman-La Roche, Basel, Switzerland.
* Theoretical values for t and F at P = 0.05 level.

3.57×10^{-2}, S.E.$_b = 2.89 \times 10^{-3}$, $r = 0.9998$ for 2AP, where Y, C, S.E.$_a$, S.E.$_b$ and r are the detector response, concentration in μg ml^{-1}, standard error of the intercept, standard error of the slope, and the correlation efficient, respectively) were obtained over the working concentration.

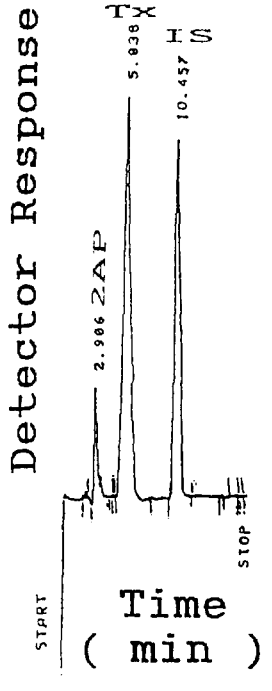

Fig. 3. Typical chromatogram for Tx and 2AP separation using 2,4-dinitrochlorobenzene as internal standard (IS).

In order to demonstrate the validity and applicability of the proposed methods, recovery studies were performed by analyzing synthetic mixtures of Tx and 2AP which reproduced different composition ratios. The percentage recoveries of Tx and 2AP from spiked excipient solutions were summarized as follows: 99.6 and 100.7% (derivative method), 99.5 and 100.2% (HPLC method), respectively. Such results encourage the use of the methods described for the assay of tenoxicam commercial tablets, the results of which (Table 2) show no sign for the existence of any 2AP in the tablets.

In conclusion, the proposed methods could be applied with great success to the Tx assay in tablets without the interference of 2AP, the degradation product. The HPLC method may be considered more specific than the derivative procedure, but also more expensive. Accordingly, the methods are considered as indicating stability.

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