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Determination of piroxicam and its major metabolite 5-hydroxypiroxicam in human plasma by zero-crossing first-derivative spectrophotometry

A. Klopas, I. Panderi, M. Parissi-Poulou *

Department of Pharmacy, Division of Pharmaceutical Chemistry, University of Athens, Panepistimiopolis, Zografou 157 71, Athens, Greece

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Abstract

A zero-crossing first-derivative spectrophotometric method for the determination of piroxicam and its major metabolite 5-hydroxypiroxicam (5-HP) in human plasma is described. This technique permits the quantification of compounds with closely overlapping spectral bands without any separation step. The method consists of direct extraction of the less-ionised forms of piroxicam and 5-hydroxypiroxiam with pure diethyl ether. First derivative values at 343.5 and 332.5 nm for piroxicam and 5-HP, respectively, were obtained. The absolute recovery of the method was found to be 89.4% for piroxicam and 90.3% for 5-HP. Calibration graphs are linear (r better than 0.9998), with zero-intercept, in the concentration range 0.5–12.0 μg ml^{-1} for both compounds. The limits of quantification attained according to the IUPAC definition were 0.29 and 0.27 μg ml^{-1} for piroxicam and 5-HP, respectively. The results obtained by the proposed method were in good agreement with those found by the high-performance liquid chromatographic method (HPLC).

Keywords: Zero-crossing derivative spectrophotometry; Human plasma; Piroxicam; 5-Hydroxypiroxicam

1. Introduction

Piroxicam, 4-hydroxy-2-methyl-2H-1,2-benzothiazine-1-(*N*-(2-pyridyl)carboxamide) 1,1-dioxide, belongs to the chemical group of oxicams which are *N*-heterocyclic benzothiazine carboxamides. It is used in the treatment of rheumatoid arthritis, osteoarthritis and other inflammatory disorders [1].

Piroxicam is readily absorbed after oral or rectal administration. It is usually given in doses of 20 mg daily since it shows long plasma half life of 35–60 h [2]. It is extensively metabolised by hepatic cytochrome P450 enzyme, principally to the hydroxyl metabolite. Hydroxylation occurs at the 5-position of the pyridyl ring and the hydroxylated metabolite undergoes subsequent glucuronidation. About 2–5% of an oral dose is excreted unchanged in urine, and, under steady state conditions, 75% of a dose is excreted as either 5-hydroxypiroxicam (5-HP) or 5-hydrox-

* Corresponding author.

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ypiroxicam glucuronide in urine and faeces [3]. 5-HP is at least 1000 times less active than piroxicam in inhibiting prostagladin synthetase [4]. After a single oral dose of 20 mg, peak plasma concentrations of piroxicam are of the order of 4.5 μg ml^{-1}. No conjugates of piroxicam have been detected in plasma [5].

Several reports have been developed for the determination of piroxicam in biological fluids, including a degradative fluorometric technique[6] and several reversed-phase high-performance liquid chromatographic method (HPLC) method with UV detection [7–11]. Chromatographic procedures allowing the simultaneous determination of piroxicam and 5-HP have also been reported [12–14]. Although these methods provide high sensitivity, most of them are complicated and time consuming. The interest for the determination of piroxicam and its major metabolite, 5-HP, has prompted us to develop a simple, rapid and reliable method for their determination in human plasma. For this purpose a zero-crossing [15] first-derivative UV spectrophotometric method was developed for the quantitation of both compounds.

Derivative spectrophotometry, is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands. It consists of calculating and plotting one of the mathematical derivatives of a spectral curve. Thus, the information content of a spectrum is presented in a potentially more useful form, offering a convenient solution to a number of analytical problems, such as resolution of multi-component systems, removal of sample turbidity, matrix background and enhancement of spectral details. Several papers on the theoretical aspects of derivative spectrophotometry have been reported [16–20]. Moreover, the recognised resolution enhancement potential of derivative UV spectrophotometry has been used advantageously in the determination of drugs in biological fluids [21–24], in the analysis of multicomponent mixtures in pharmaceutical preparations [25–30], and in stability studies of drugs [31].

The method yielded accurate, rapid and reproducible results for plasma samples spiked with these compounds. The results obtained by the proposed method were compared with those obtained by a HPLC method with spectrophotometric detection, similar to that proposed by other investigators [32].

2. Experimental

2.1. Apparatus

A Perkin-Elmer, Model Lambda 7, double-beam UV-visible spectrophotometer, with the capability of applying the derivative mode, was used. The optimized operating conditions for recording the first-order derivative spectra were scan speed 30 nm min^{-1}, response 5 s, spectral slit width 2 nm, delta wavelength 8 nm and ordinate maximum–minimum ± 10. All measurements were carried out using quartz microcells (volume: 1 ml; path-length: 10.0 mm).

The HPLC system consisted of a Waters Model 501 pump and a Rheodyne Model 7125 injector with a 5 μl loop, which were coupled to a Waters Model 486 UV-Vis detector with an 8 μl flow cell operated at 330 nm. The chromatograms were obtained by using a Hewlett-Packard Model HP3394A integrator.

2.2. Materials

All experiments were performed with analytical-reagent grade chemicals and water purified using a Milli-Q system (Millipore). Piroxicam and 5-HP were kindly provided by Pfizer Hellas and were used without further purification.

2.3. Standard solutions and sample preparation

Stock standard solutions of piroxicam (1.0 mg ml^{-1}) and 5-HP (0.5 mg ml^{-1}) were prepared by dissolving the compounds in methanol. Working standard solutions of piroxicam (10.0 and 100.0 μg ml^{-1}) and 5-HP (10.0 and 100.0 μg ml^{-1}) were prepared by the appropriate dilutions of the above mentioned stock standard solutions with methanol. The stock and standard solutions were stored in the dark under refrigeration.

Standard aqueous solutions of piroxicam and 5-HP in the range of 0.5–12.0 μg ml^{-1} were prepared daily by the addition of the appropriate working standard solutions of the compounds in a mixture of 1.0 M HCl-acetonitrile (1:1, v/v). Mixed standard aqueous solutions containing 0.5–12.0 μg ml^{-1} of piroxicam and 0.5–12.0 μg ml^{-1} of 5-HP in the ratio 1:1, were also prepared in the same solvent.

Spiked plasma samples of piroxicam were prepared by the addition of the appropriate dilutions of the working standard solutions of the drug in 1 ml plasma. The final concentrations of piroxicam in the spiked plasma samples were: 0.5, 1.0, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 10.0 and 12.0 μg ml^{-1}. Spiked plasma samples containing 0.5–12.0 μg ml^{-1} of 5-HP were prepared in a similar way. Mixed spiked plasma samples containing 0.5–12.0 μg ml^{-1} of both compounds in a ratio 1:1, were also prepared.

3. Procedure

3.1. Plasma samples

Appropriate aliquots of piroxicam or/and 5-HP working standard solutions were placed in 10 ml centrifuge tube. The aliquots were evaporated to dryness under a gentle stream of air and 1 ml human plasma was added. The plasma samples were acidified with 0.3 ml of 1.0 M HCl and 5 ml of pure diethyl ether were added. The samples were then vortex-mixed for 1 min and the aqueous and organic phases were separated by centrifugation at 3500 rev min^{-1} ($2890 \times g$) for 10 min. After refrigeration of the aqueous phase at -17°C, the organic layers were placed in 10 ml glass vials and evaporated to dryness under a gentle stream of air. The residues were reconstituted in 1 ml of a mixture of 1.0 M HCl-acetonitrile (1:1, v/v). The first order derivative UV spectra of these solutions were recorded over the wavelength range 325–360 nm and the derivative values at the 343.5 and 332.5 nm were measured for the determination of piroxicam and 5-HP, respectively.

3.2. Calibration procedure

Four calibration curves were constructed by assaying unextracted samples of piroxicam, 5-HP and their mixture in 1.0 M of HCl-acetonitrile (1:1, v/v). The first-order derivative spectra of these samples were recorded over the wavelength range 325–360 nm against a blank of a mixture of 1.0 M HCl-acetonitrile (1:1, v/v). The derivative values at 343.5 nm, $D_{1(343.5)}$ and at 332.5 nm $D_{1(332.5)}$ were measured for the determination of piroxicam and 5-HP, respectively.

In order to determine piroxicam and 5-HP in plasma, four other calibration curves were also constructed by assaying plasma samples spiked with piroxicam, 5-HP and their mixture in a ratio 1:1. Piroxicam and 5-HP were determined by measuring the derivative values at 343.5 nm, $D_{1(343.5)}$ and at 332.5 nm $D_{1(332.5)}$, respectively.

Four series of plasma samples containing various concentration of 5-HP (0.0, 0.5, 3.0, 4.0, 8.0 and 12.0 μg ml^{-1}) and a constant concentration of piroxicam (0.5, 4.0, 8.0 and 12.0 μg ml^{-1}) were constructed in order to investigate the effect of 5-HP in the determination of piroxicam. Moreover, the specificity of the method in the determination of 5-HP was also investigated by constructing four series of plasma samples containing various concentration of piroxicam (0.0, 0.5, 3.0, 4.0, 8.0 and 12.0 μg ml^{-1}) and a constant concentration of 5-HP (0.5, 4.0, 8.0 and 12.0 μg ml^{-1}).

The over-all precision of the assay was evaluated by analysing plasma samples spiked with three different concentrations of piroxicam (0.5, 6.0 and 12.0 μg ml^{-1}), 5-HP (0.5, 6.0 and 12.0 μg ml^{-1}) and their mixture in ratio 1:1.

Furthermore, the effect of the 1.0 M HCl on the extraction efficiency was examined by assaying spiked plasma samples containing a mixture of piroxicam and 5-HP in the ratio 1:1, using various aliquots (0.1, 0.2, 0.3 and 0.4 ml) of 1.0 M HCl.

4. Results and discussion

4.1. Spectrophotometric measurements

A thorough investigation was conducted in order to choose the optimum solvent medium for the spectrophotometric determination of piroxicam and 5-HP.

The zero-order absorption spectra of solutions of piroxicam, 5-HP and their mixture in 1.0 M NaOH are shown in Fig. 1 over the wavelength range 210–400 nm. Acidic pH exerts a large effect on molar absorptivity of both compounds (hyperchromic effect), as can be seen in Fig. 2, which presents the zero-order spectra of the compounds in 1.0 M HCl over the wavelength range 200–450 nm. It was essential that an organic solvent such as acetonitrile could further purify the extracted plasma samples from any remaining proteins. However, the molar absorptivity of both compounds was decreased when acetonitrile alone was

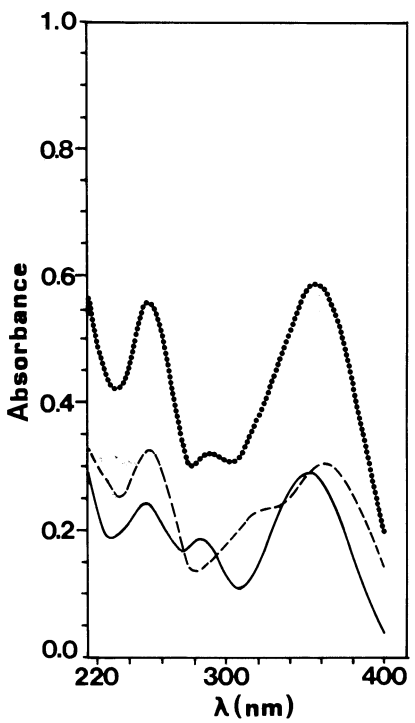

Fig. 1. Absorption (zero-order) UV spectra of 5.0 μg ml^{-1} piroxicam (——), 5.0 μg ml^{-1} 5-HP (- - -), and their mixture in the ratio 1:1 (···), in 1.0 M NaOH.

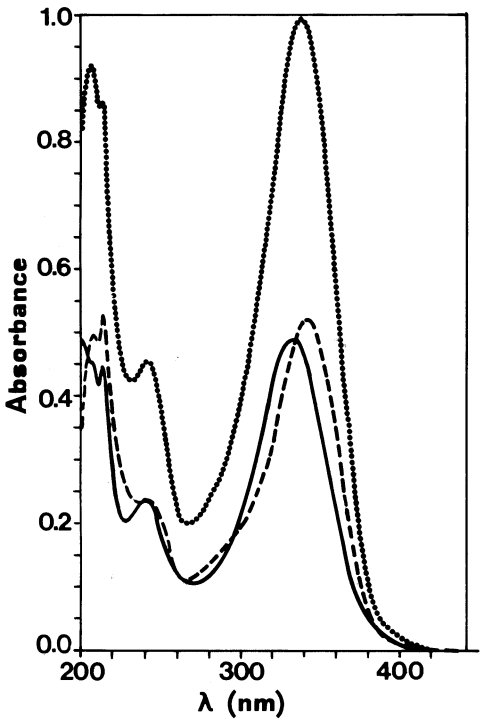

Fig. 2. Absorption (zero-order) UV spectra of 5.0 μg ml^{-1} piroxicam (——), 5.0 μg ml^{-1} 5-HP (- - -), and their mixture in the ratio 1:1 (···), in 1.0 M HCl.

used as solvent, Fig. 3. The addition of hydrochloric acid was essential in order to increase the molar absorptivity and the λ_{max} distance of both compounds. Therefore, the solvent chosen was a mixture of 1.0 M HCl-acetonitrile (1:1, v/v).

The working wavelength region was chosen to be between 280 and 380 nm, because in lower wavelengths there was considerable interference from the plasma proteins, which make the spectra very complicated. The zero-order UV spectra of solutions of 5.0 μg ml^{-1} piroxicam, 5.0 μg ml^{-1} 5-HP and their mixture (1:1) in a mixture of 1.0 M HCl-acetonitrile (1:1, v/v) over the wavelength range 280–380 nm are shown in Fig. 4. Due to extensive overlap of the spectral bands, conventional UV spectrophotometry cannot be used for the quantification of both substances in the presence of each other. However, zero-crossing first-order derivative spectrophotometry permits a more selective identification and determination of the two compounds in a mixture. The zero-cross-

ing method, involves measurements of the absolute value of the total derivative spectrum at an abscissa value corresponding to the zero-crossing wavelengths of the derivative spectra of the individual compound.

Fig. 5(a) shows the first-order derivative spectra of piroxicam, 5-HP and their mixture (the zero-crossing wavelengths are indicated). The selection of the optimum wavelength is based on the fact that the absolute derivative value of the total derivative spectrum at the selected wavelength exhibited the best linear response to the analyte concentration, gives a zero or near to zero intercept on the derivatives axis of the calibration curve and it is the least affected by the concentration of any other component.

Preliminary experiments showed that the signals at 332.5 nm, $D_{1(332.5)}$, (zero-crossing wavelength point of piroxicam) are proportional to the concentration of 5-HP and the signals at 343.5 nm, $D_{1(343.5)}$, (zero-crossing wavelength point of 5-HP) are proportional to the concentration of piroxicam.

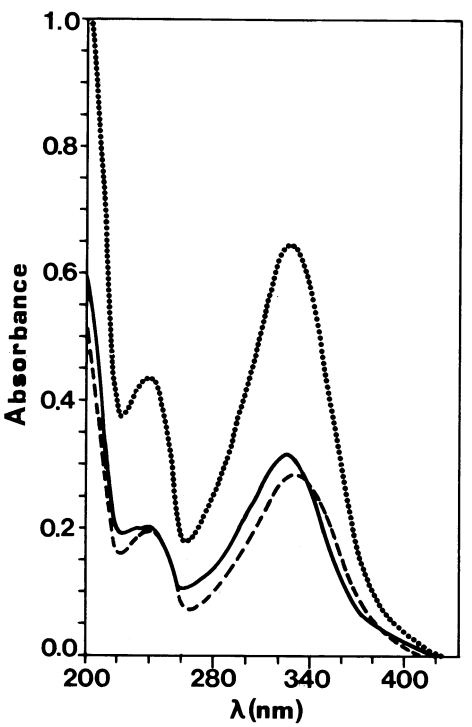

Fig. 3. Absorption (zero-order) UV spectra of 5.0 μg ml^{-1} piroxicam (——), 5.0 μg ml^{-1} 5-HP (- - -), and their mixture in the ratio 1:1 (···), in acetonitrile.

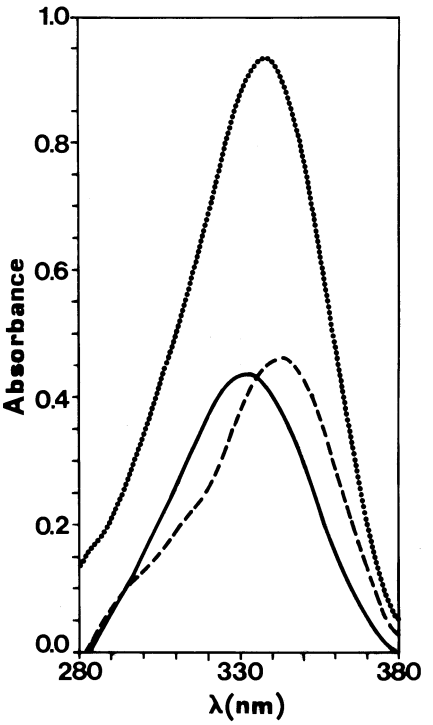

Fig. 4. Absorption (zero-order) UV spectra of 5.0 μg ml^{-1} piroxicam (——), 5.0 μg ml^{-1} 5-HP (- - -), and their mixture in the ratio 1:1 (···), in a mixture of 1.0 M HCl-acetonitrile (1:1, v/v).

Fig. 5(b) shows a typical set of the first-order derivative spectra of spiked plasma extracts in a mixture of 1.0 M HCl-acetonitrile (1:1, v/v), containing mixture of both compounds at concentrations 0.5, 1.0, 3.0, 6.0 and 10.0 μg ml^{-1}.

4.2. Selection of optimum instrumental conditions

The zero-crossing technique, chosen for the binary mixture assay is more sensitive to small drifts of the band of one of the components measured, compared with the graphical measurements. A low scan speed of 30 nm min^{-1} was chosen in order to achieve better reproducibility of the zero-crossing wavelengths, while at such a low scan speed noise was more pronounced [33]. Therefore, several other parameters need to be optimized in order to achieve an adequate signal to noise ratio.

The main instrumental parameter that affects the shape of the derivative spectra and the signal to noise ratio is the wavelength increment over which derivatives are obtained, $\Delta\lambda$. Increasing $\Delta\lambda$ improves the signal to noise ratio, thus decreasing the fluctuation in a derivative spectrum. Several $\Delta\lambda$ values were tested and $\Delta\lambda = 8$ nm was chosen as the optimum in order to give an adequate signal to noise ratio. A satisfactory reduction of noise was also obtained by selecting a slow response time of 5 s.

4.3. Statistical analysis of data

Under the experimental conditions described above, linear relationships between the selected derivative values and the corresponding concentrations of the compounds tested were observed, as shown by the equations presented in Table 1. The slopes and intercepts of the regression equations obtained from the analysis of binary mixtures do not differ significantly from those obtained from the analysis of each of the compounds. Therefore, it can be deduced that the value of the derivative signal of the mixture, measured at the zero-crossing point of the first-order derivative spectrum of one of the two components, is a function only of the concentration of the other component.

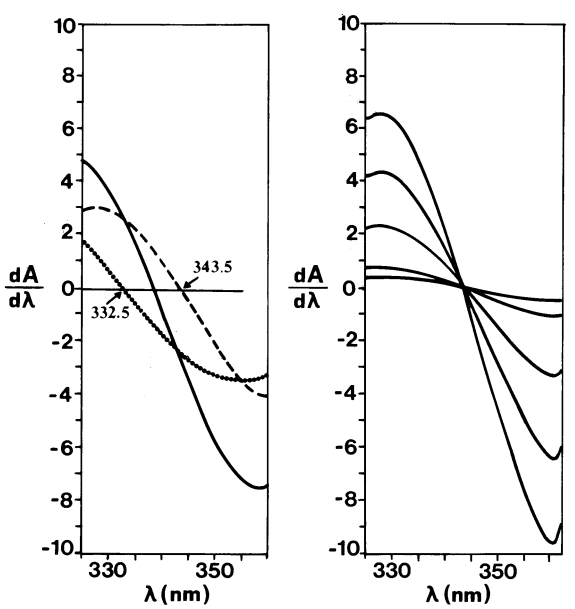

Fig. 5. (a) First-order derivative spectra of 5.0 μg ml^{-1} piroxicam (···), 5.0 μg ml^{-1} 5-HP (- - -), and their mixture in ratio 1:1 (——), in a mixture of 1.0 M HCl-acetonitrile (1:1, v/v); (b) First-order derivative spectra of spiked plasma extracts containing mixture of piroxicam and 5-HP in ratio 1:1 at concentrations 0.5, 1.0, 3.0, 6.0 and 10.0 μg ml^{-1}.

A Student's t-test was performed to determine whether the experimental intercepts (a) of the above mentioned regression equations were significantly different from the theoretical zero value. The test is based on the calculation of the quantities $t = a/S_a$, where a is the intercept of the regression equations and S_a is the standard deviation of a, and their comparison with tabulated data of the t-distribution. The calculated t-values are presented in Table 1, these values do not exceed the 95% criterion of $t_p = 2.31$ for $f = 8$ df, which denotes that the intercepts of all regression lines are not significantly different from zero.

A further interaction study was performed by keeping the concentration of each one of the compounds constant at 0.5, 4.0 and 12.0 μg ml^{-1} while the concentration of the other one was varied from 0.0 to 12.0 μg ml^{-1}. The results in Fig. 6a and b indicate that the derivative values at $D_{1(332.5)}$ and $D_{1(332.5)}$ are not affected by the presence of up to 12.0 μg ml^{-1} of piroxicam and 5-HP, respectively. Thus, the proposed assay can be used for their simultaneous determination in human plasma.

The linear regression equations calculated for spiked plasma samples, containing mixture of piroxicam and 5-HP, are presented in Table 2. On increasing the amount of 1.0 M HCl used for the acidification, the slopes of the equations increase up to 0.3 ml of 1.0 M HCl. Therefore, an aliquot of 0.3 ml of 1.0 M HCl proved to be adequate for the acidification of plasma samples.

The efficiency of the extraction procedure was determined by calculating the ratio of the slopes of the regression equations obtained from spiked plasma extracts to those for unextracted standard solutions, containing binary mixture of piroxicam and 5-HP. The absolute recovery of the method was found to be 89.4% for piroxicam and 90.3% for 5-HP.

Table 1
Calibration equations for the determination of piroxicam and 5-HP by zero-crossing first-derivative spectrophotometry

Fluid	Concentration range of piroxicam (μg ml^{-1})	Concentration range of 5-HP (μg ml^{-1})	Regression equations[a]	r[b]	SE[c]	a/S_a[d]
Plasma	0.5–12.0		$D_{1(343.5)} = 0.348\ (\pm 1.9\times10^{-2})C_p - 0.014\ (\pm 0.012)$	0.9998	0.23	1.17
Plasma	0.5–12.0	0.5–12.0	$D_{1(343.5)} = 0.346\ (\pm 2.3\times10^{-2})C_p - 0.011\ (\pm 0.014)$	0.9998	0.27	0.79
Plasma		0.5–12.0	$D_{1(332.5)} = 0.345\ (\pm 2.1\times10^{-2})C_{5HP} - 0.011\ (\pm 0.013)$	0.9998	0.25	0.85
Plasma	0.5–12.0	0.5–12.0	$D_{1(332.5)} = 0.342\ (\pm 2.8\times10^{-2})C_{5HP} - 0.014\ (\pm 0.017)$	0.9997	0.32	0.82
Aqueous solution[e]	0.5–12.0		$D_{1(343.5)} = 0.387\ (\pm 2.6\times10^{-2})C_p - 0.015\ (\pm 0.016)$	0.99990	0.30	0.94
Aqueous solution[e]	0.5–12.0	0.5–12.0	$D_{1(343.5)} = 0.387\ (\pm 2.4\times10^{-2})C_p - 0.011\ (\pm 0.015)$	0.99991	0.29	0.69
Aqueous solution[e]		0.5–12.0	$D_{1(332.5)} = 0.376\ (\pm 2.6\times10^{-2})C_{5HP} + 0.005\ (\pm 0.016)$	0.9998	0.30	0.31
Aqueous solution[e]	0.5–12.0	0.5–12.0	$D_{1(332.5)} = 0.379\ (\pm 2.3\times10^{-2})C_{5HP} + 0.009\ (\pm 0.014)$	0.9998	0.28	0.64

[a] Derivative value at the zero-crossing wavelength versus amount of the concentration of the compound measured in μg ml^{-1}; ten standards.
[b] Correlation coefficient.
[c] Standard error of the estimate.
[d] Theoretical value of t at $P = 0.025$ level of significance, for $f = n-2 = 8$ df, 2.31.
[e] Mixture of 1.0 mol l^{-1} HCl and acetonitrile (1:1, v/v).

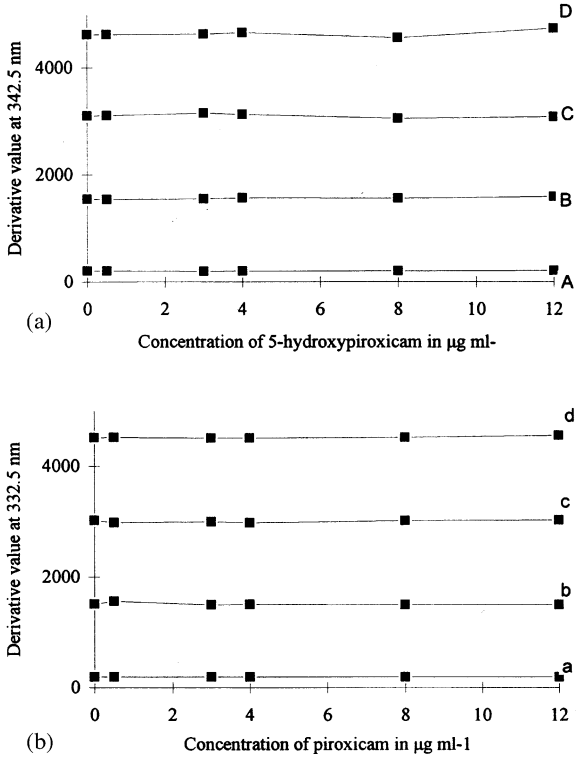

Fig. 6. (a) Interaction graphs of 0.5, 4.0, 8.0 and 12.0 μg ml^{-1} piroxicam (A, B,C and D, respectively); (b) Interaction graphs of 0.5, 4.0, 8.0 and 12.0 μg ml^{-1} 5-HP (a, b, c and d, respectively).

The limits of quantification attained, as defined by IUPAC [34], $C_{L(k=10)} = k\ S_a/b$ (where b is the slope of the calibration graph and S_a is the standard deviation of the blank signal) were found to be 0.29 and 0.27 μg ml^{-1} for piroxicam and 5-HP, respectively.

Data for the variation of precision and accuracy given in Table 3, indicate for piroxicam RSD% = 0.13–2.00 and $E_r\% = -0.1$–2.0 and for 5-HP RSD% = 0.17–2.04 and $E_r\% = -2.00$–0.25.

A number of drugs were tested for possible interference in the assay of piroxicam and 5-HP. The results presented in Table 4 shows that the selected drugs cause no serious interference.

The first-order zero-crossing derivative spectrophotometric method was evaluated by comparison with an HPLC method, proposed by other investigators [32] and performed in our laboratory. The separation was performed on a reversed phase Hypersil BDS C-18 column (250 × 4.6 mm i.d., 5 μm particle size), Shandon HPLC, UK and a BDS C-18 precolumn placed just before the inlet of the analytical column. A variable wavelength UV detector was used set at 330 nm. The mobile phase, methanol-acetonitrile-sodium acetate 0.1 M adjusted to pH 3.3 with glacial acetic acid (25:18:57 v/v/v), was filtered through a 0.45 μm Millipore filter and degassed under vacuum prior to use. A flow rate of 1.4 ml min^{-1} with a column inlet pressure at 2100 psi was used in order to separate piroxicam and 5-HP.

Nine samples containing a mixture of both compounds, covering the concentration range of interest (0.5–12.0 μg ml^{-1}) were analysed in duplicate by HPLC and the proposed method. Data pairs were plotted on a scatter diagram, with the abscissa for the HPLC procedure (assumed to be more precise) and the ordinate for the first-order zero-crossing derivative spectrophotometric procedure. Linear regression analysis of data gave the following regression equations:

$$^{p}C_D = 0.99(\pm 0.01) \times {}^{p}C_{HPLC} + 0.01(\pm 0.07)$$

$$r = 0.9997;\ SE = 0.15$$

$$^{5-HP}C_D = 0.997(\pm 7.3 \times 10^{-3}) \times {}^{5-HP}C_{HPLC} - 3.2 \times 10^{-3}(\pm 0.04)$$

$$r = 0.99991;\ SE = 0.25$$

where $^{p}C_D$ and $^{5\text{-HP}}C_D$, are the concentrations of piroxicam and 5-HP, respectively, determined by derivative spectrophotometry, while $^{p}C_{HPLC}$ and $^{5\text{-HP}}C_{HPLC}$ are the concentrations of piroxicam and 5-HP, respectively, determined by HPLC. A Student's t-test was performed to determine whether the experimental intercepts (a) of the above mentioned regression equations were different from the theoretical zero value. The absolute values calculated for t are 0.19 for piroxicam and 0.09 for 5-HP (these values do not exceed the 95% criterion of $t_p = 2.36$ for n = nine samples), so the intercepts are not sig-

Table 2
Calibration equations for the determination of binary mixture of piroxicam and 5-HP in human plasma using different aliquots of 1.0 M HCl

HCl added/ml	Compound	Regression equations[a]	r[b]	SE[c]
0.1	Piroxicam	$D_{1(343.5)} = 0.306\ (\pm 8.1\times10^{-2})C_p - 0.052\ (\pm 0.049)$	0.98	0.75
0.2	Piroxicam	$D_{1(343.5)} = 0.315\ (\pm 9.2\times10^{-2})C_p - 0.052\ (\pm 0.055)$	0.992	0.55
0.3	Piroxicam	$D_{1(343.5)} = 0.346\ (\pm 2.3\times10^{-2})C_P - 0.011\ (\pm 0.014)$	0.9998	0.27
0.4	Piroxicam	$D_{1(343.5)} = 0.246\ (\pm 11.3\times10^{-2})C_P - 0.095\ (\pm 0.068)$	0.97	0.65
0.1	5-HP	$D_{1(332.5)} = 0.284\ (\pm 11.5\times10^{-2})C_{5HP} - 0.061\ (\pm 0.070)$	0.97	0.98
0.2	5-HP	$D_{1(332.5)} = 0.320\ (\pm 5.3\times10^{-2})C_{5HP} + 0.025\ (\pm 0.032)$	0.991	0.73
0.3	5-HP	$D_{1(332.5)} = 0.342\ (\pm 2.8\times10^{-2})C_{5HP} + 0.014\ (\pm 0.017)$	0.9997	0.32
0.4	5-HP	$D_{1(332.5)} = 0.181\ (\pm 2.5\times10^{-2})C_{5HP} - 0.019\ (\pm 0.014)$	0.91	0.64

[a] Derivative value at the zero-crossing wavelength versus the concentration of the compound measured in μg ml^{-1}.
[b] Correlation coefficient.
[c] Standard error of the estimate.

Table 3
Accuracy and precision for the determination of piroxicam, 5-HP by zero-crossing first derivative spectrophotometry

Nominal concentration (μg ml^{-1})		Assayed concentration (μg ml^{-1})					
Piroxicam	5-HP	Piroxicam			5-HP		
		Mean ± SD ($n = 3$)	RSD%[a]	E_r%[b]	Mean ± SD ($n = 3$)	RSD%[a]	E_r%[b]
0.5	—	0.51 ± 0.01	2.00	2.0	—		
—	0.5	—			0.50 ± 0.01	2.00	0.2
0.5	0.5	0.51 ± 0.01	1.28	2.0	0.51 ± 0.01	2.00	0.2
6.0	—	6.06 ± 0.04	0.12	1.0	—		
—	6.0	—			5.98 ± 0.02	0.25	−0.33
6.0	6.0	6.03 ± 0.05	0.82	0.5	5.94 ± 0.01	0.22	−1.00
12.0	—	11.99 ± 0.02	0.13	−0.1	—		
—	12.0	—			11.97 ± 0.02	0.17	−0.25
12.0	12.0	11.90 ± 0.03	0.37	0.25	12.03 ± 0.04	0.37	0.25

[a] Percentage relative standard deviation.
[b] Relative percentage error.

nificantly different from zero. Another t-test was carried out in order to determine whether the slopes differ significantly from unity. The test is based on the measurement of the quantities $t = 1.0 - b/s_b$, where b is the slope of the regression line and s_b is the standard deviation of b. The absolute values calculated for t are 0.30 for piroxicam and 0.36 for 5-HP (these values do not exceed the 95% criterion of $t_p = 2.36$ for $n =$ nine samples), so the slopes are not significantly different from unity.

The proposed procedure was successfully applied to the determination of the studied compounds in human plasma. It is a simple and accurate procedure requiring inexpensive reagents that could be used for rapid and reliable clinical and pharmacokinetic studies of piroxicam and its major metabolite 5-HP.

Table 4
Possible interference to the assay of piroxicam and 5-HP

Drug added (μg ml^{-1})[a]		Assayed concentration of piroxicam (μg ml^{-1})[b]			Assayed concentration of 5-HP (μg ml^{-1})[c]		
		Mean ± SD ($n = 3$)	S_r%[d]	E_r%[e]	Mean ± SD ($n = 3$)	S_r%[d]	E_r%[e]
Bromazepam	0.15	4.02 ± 0.02	0.52	0.50	4.02 ± 0.02	0.50	0.50
Caffeine	4.0	3.98 ± 0.01	0.25	−0.50	3.99 ± 0.01	0.25	−0.25
Chlorpheniramine maleate	0.04	4.02 ± 0.02	0.49	0.50	3.87 ± 0.01	0.25	−3.25
Lorazepam	0.2	3.97 ± 0.02	0.50	−0.75	4.02 ± 0.01	0.25	0.50
Paracetamol	15.0	4.03 ± 0.01	0.24	0.75	3.97 ± 0.02	0.52	−0.75
Pindolol	0.5	3.86 ± 0.02	0.52	−3.50	3.96 ± 0.02	0.50	−1.00
Pseudoephedrine HCl	0.8	3.94 ± 0.01	0.25	−1.50	3.91 ± 0.01	0.26	−0.22
Prazepam	0.30	4.03 ± 0.01	0.14	0.74	3.98 ± 0.01	0.25	−0.50

[a] Drug concentrations are mean plasma concentrations.
[b] Piroxicam concentration added was 4.0 μg ml^{-1}.
[c] 5-HP concentration added was 4.0 μg ml^{-1}.
[d] Relative standard deviation.
[e] Relative standard error.

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