Simultaneous determination of piroxicam and its major metabolite 5′-hydroxypiroxicam in human plasma by derivative spectrophotometry

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A first-derivative spectrophotometric method for the simultaneous determination of piroxicam (PX) and its major metabolite 5′-hydroxypiroxicam (OH PX) in human plasma is described. The method consists of direct extraction of the two drugs from the plasma samples with hydrochloric and trichloroacetic acid prior to their determination by measuring the first-derivative signals at 337.0 nm for PX and at 327.0 nm for OHPX (zero-crossing wavelength). The calibration graphs were linear up to 10.0 and 8.0 mg l^{-1} of PX and OH PX, respectively, and the limits of quantification were 0.27 and 0.56 mg l^{-1}. The possible interfering effects of other substances were studied.

Keywords: *Piroxicam; 5′-hydroxypiroxicam; human plasma; derivative spectrophotometry*

Piroxicam, 4-hydroxy-2-methyl-*N*-(2-pyridyl)-2-*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide (PX), is a non-steroidal anti-inflammatory drug (NSAID) belonging to a class of compounds called oxicams.[1] Its anti-inflammatory action is caused by its inhibition of prostaglandin synthetase, and it is the most widely used drug in the treatment of patients suffering from rheumatological disoders. It may be administered systematically or topically and its once-daily administration has made it widely appreciated when NSAID treatment is required for a chronic condition.

It has been suggested[2] that PX is completely absorbed after both oral and rectal administration, peak plasma concentration (C_{max}) being achieved 2–3 h after a single oral dose and 5–6 h after a single rectal dose. Mean peak plasma concentrations are roughly related to dosage, being 1.5–3 and 6–8 mg l^{-1} after a single 20 or 60 mg dose, respectively. Because of the long $t_{1/2}\beta$ (terminal elimination half-life), once-daily administration keeps the plasma PX concentration relatively stable. The main metabolic pathway of PX involves the hydroxylation of the 5-pyridine ring, resulting in 5′-hydroxypiroxicam (OHPX) as oxidation product. This metabolite is less active than the drug itself in inhibiting prostaglandin biosynthesis.

The pharmacology and pharmacokinetic and clinical efficacy of PX have recently been reviewed.[2] In the earliest studies of PX in humans, plasma levels were measured by spectrophotometric or fluorimetric techniques,[3–5] although such methods lacked selectivity and sensitivity. Since then, several chromatographic methods have been reported for the determination of PX in biological fluids.[6–14] However, most of these methods, while providing a high degree of sensitivity, are complicated and time consuming, which led us to search for a simple, rapid, reliable and specific method for the determination of PX and its major metabolite OHPX in human plasma. The classical UV/VIS spectrophotometric method presents the severe problem of overlapping spectral bands of PX, OHPX and the plasma background, which makes the simultaneous determination of these drugs impossible in this matrix.

This paper describes the application of derivative UV/VIS spectrophotometry to the simultaneous determination of PX and its metabolite OHPX in human plasma. This technique, which involves calculating and plotting one of the mathematical derivatives of a spectral curve, offers an alternative approach to drug analysis and shows greatest sensitivity and specificity. Although the derivative transformation does not increase the information content of a spectrum, it does permit discrimination against broad band interferences which arise from turbidity or non-specific matrix absorption. It also emphasizes subtle spectral features of the data by presenting them in a new and visually more accessible way. Several papers on the theoretical aspects of derivative spectrophotometry have been published.[15–19] The recognized resolution enhancement of derivative UV/VIS spectrophotometry has been used advantageously in the determination of drugs in biological fluids[20–25] and binary mixtures of drugs.[26–30]

Experimental

Apparatus

A Shimadzu (Kyoto, Japan) UV 240 double-beam spectrophotometer with an optional program unit (Model OPI-2) and 1 cm quartz cell was used. The most suitable settings were slit width 2 nm (the response time was automatically adjusted according to the slit width), scan speed 45 nm min^{-1} and derivative wavelength difference 4 nm. The recorder scale expansion was optimized to facilitate readings on the recorder trace.

Standard solutions and sample preparation

All chemicals and solvents were of analytical-reagent grade and solutions were prepared with doubly distilled water.

Piroxicam and 5′-hydroxypiroxicam stock standard solutions (400.0 mg l^{-1}) were prepared by dissolving 40.0 mg of PX (Sigma, St. Louis, MO, USA) or OHPX (kindly provided by Lab. Pfizer, Madrid, Spain) in 100 ml of methanol A series of working standard solutions containing equal amounts of PX and OHPX between 15.0 and 150.0 mg l^{-1} were prepared by appropriate dilution with saline solution of the stock standard solutions.

Plasma samples simultaneously spiked with PX and OHPX were prepared by diluting 0.2 ml aliquots of working standard solutions in a 1.8 ml serum pool. The final concentration of both PX and OHPX in the plasma standard were 1–10 mg l^{-1}.

Triglycerides were supplemented with Intralipid (Pharmacia and Upjohn, Madrid, Spain). Hemoglobin and bilirubin were purchased from Sigma.

Calibration procedure

Aliquots of 0.2 ml of the working standard solution were placed in 10 ml centrifuge tubes and 1.8 ml of human plasma was added. The spiked plasma samples were acidified with 1.0 ml of

1.0 M HCl. After 15 min, 1.0 ml of 20% trichloroacetic acid was added. The mixture was shaken for 1 min and centrifuged at 3500 rev min^{-1} (2270*g*) for 10 min. The aqueous layer was separated and the first-order derivative spectrum of this solution was recorded over the wavelength range of 310–400 nm. The amplitudes (mm) were measured at 337.0 nm for PX and 327.0 nm for OHPX.

Results and discussion

Spectrophotometric measurements

Fig. 1 shows the zero-order absorption spectra of blank plasma taken from one individual (A), blank plasma spiked with 2.5 mg l^{-1} 5′-hydroxypiroxicam (B) or piroxicam (C), or both mixed in a 1 : 1 ratio (D). PX exhibits an absorbance maximum at 333 nm and OHPX at 340 nm; the blank plasma has a very low absorbance at these wavelengths. Similar spectra were obtained when the same procedure was repeated with individual plasma samples taken from 10 volunteers. There is a clear overlapping of the spectra, which prevents the simultaneous determination of the compounds by direct UV/VIS absorbance measurements in plasma.

Derivative spectrophotometry is a suitable technique for overcoming this problem, with the zero-crossing method being the most common procedure for the preparation of calibration graphs. In practice, the measurements selected are those which exhibit the best linear response, give a zero or near zero intercept on the ordinate of the calibration graphs and are least affected by the concentration of any other component.

Fig. 2 shows the first-derivative UV/VIS spectra of the same solutions as in Fig. 1. Derivatization of the zero-order spectra leads to an improvement of the spectral details and the resulting first-derivative spectra present spectral features which can be used for the simultaneous determination of PX and OHPX. Owing to the overlapping spectra, the zero-crossing method is clearly the most appropriate approach for resolving mixtures of these compounds and it was used in this work with satisfactory results. Preliminary experiments with 10 different blank plasma samples and one taken from a serum pool showed that the first-derivative signals at 337.0 nm (working blank plasma zero-crossing wavelength of OHPX) are proportional to the PX concentration and the first-derivative signals at 327.0 nm (working blank plasma zero-crossing wavelength of PX) are proportional to the OHPX concentration.

Selection of optimum instrumental conditions

The main instrumental parameters that affect the shape of the derivative spectra are the wavelength scanning speed, the

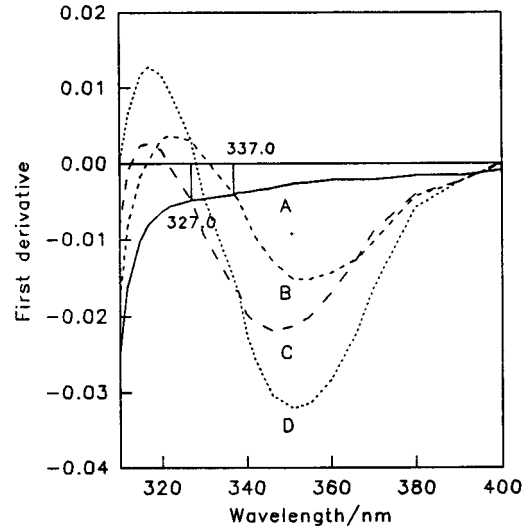

Fig. 2 First-derivative spectra ($\Delta\lambda$ = 4 nm) of (A) blank plasma, (B) blank plasma spiked with 2.5 mg l^{-1} of OHPX, (C) blank plasma spiked with 2.5 mg l^{-1} of PX and (D) blank plasma spiked with 2.5 mg l^{-1} of PX and 2.5 mg l^{-1} of OHPX.

Table 1 Calibration data for the simultaneous determination of PX and OHPX in plasma

Compound	Equation	*r*
PX	$^1D_{337}$ = 1.50 × 10^{-3} (± 2.19 × 10^{-4})* + 6.04 × 10^{-3} (± 3.9 × 10^{-5}) [PX]†	0.9998
OHPX	$^1D_{327}$ = −3.14 × 10^{-3} (± 1.60 × 10^{-4})* + 3.31 × 10^{-3} (± 3.9 × 10^{-5}) [OHPX]†	0.9994

* ± *s*; *n* = 12. † Concentration in mg l^{-1}.

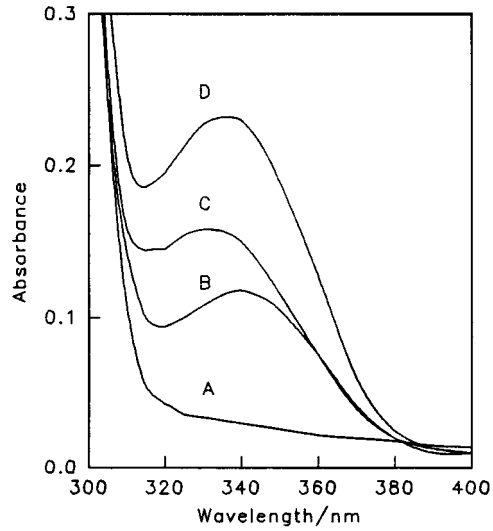

Fig 1 Absorption (zero-order) spectra of (A) blank plasma, (B) blank plasma spiked with 2.5 mg l^{-1} of OHPX, (C) blank plasma spiked with 2.5 mg l^{-1} of PX and (D) blank plasma spiked with 2.5 mg l^{-1} of PX and 2.5 mg l^{-1} of OHPX.

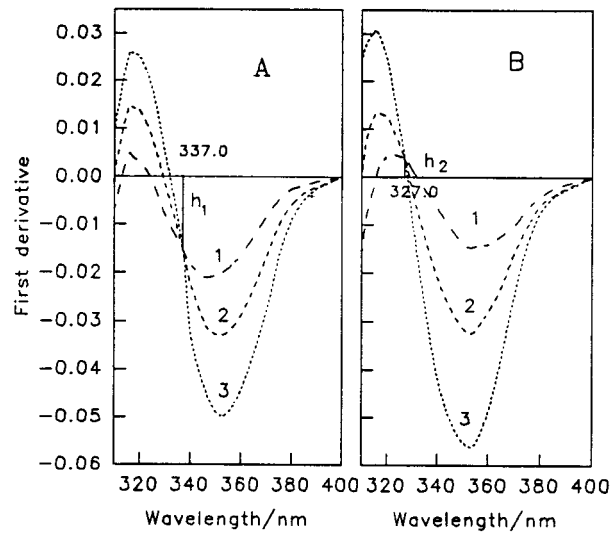

Fig 3 First-derivative spectra ($\Delta\lambda$ = 4 nm) of plasma samples containing (A) different concentrations of OHPX [(1) 0.0; (2) 2.5; (3) 5.0 mg l^{-1}] and 2.5 mg l^{-1} of PX and (B) different concentrations of PX [(1) 0.0; (2) 2.5; (3) 5.0 mg l^{-1}] and 2.5 mg l^{-1} of OHPX.

Table 2 Simultaneous determination of PX and OHPX in human plasma

	PX			OHPX		
Plasma Sample No.	Added/ mg l^{-1}	Found*/ mg l^{-1}	Recovery* (%)	Added/ mg l^{-1}	Found*/ mg l^{-1}	Recovery* (%)
1	2.50	2.39 ± 0.09	95.6 ± 3.6	2.50	2.49 ± 0.10	99.6 ± 4.0
2	5.00	5.21 ± 0.16	104.2 ± 3.2	5.00	5.16 ± 0.15	103.2 ± 3.0
3	2.50	2.31 ± 0.09	92.4 ± 3.6	5.00	5.24 ± 0.10	104.8 ± 2.0
4	7.50	7.65 ± 0.17	102.0 ± 2.3	2.50	2.69 ± 0.13	107.6 ± 5.2
5	5.00	5.30 ± 0.13	106.0 ± 2.6	5.00	5.10 ± 0.18	102.0 ± 3.6
6	7.50	7.40 ± 0.19	98.7 ± 2.5	2.50	2.61 ± 0.12	104.4 ± 4.8
7	8.00	7.68 ± 0.25	96.0 ± 3.1	2.00	1.90 ± 0.08	95.0 ± 4.0
8	7.50	7.56 ± 0.20	100.8 ± 2.7	1.50	1.45 ± 0.08	96.7 ± 5.3

* Mean of five determinations ± *s*.

wavelength increment over which the derivative is obtained ($\Delta\lambda$) and the smoothing, all of which need to be optimized to give a well resolved, large peak. Generally, the noise level decreases with increase in $\Delta\lambda$, which leads to less pronounced fluctuations in the derivative spectrum. Since the spectral resolution is very poor at excessively high $\Delta\lambda$ values, the optimum value of $\Delta\lambda$ should be determined by taking into account the noise level, the resolution of the spectrum and the sample concentration. Several values of $\Delta\lambda$ were tested and 4.0 nm was selected as the optimum for a satisfactory signal-to-noise ratio.

After establishing the optimum experimental conditions, the first-derivative spectra of two series of plasma samples containing various concentrations of OHPX (0.0, 2.5 and 5.0 mg l^{-1}) and a constant concentration of PX (2.5 mg l^{-1}) or various concentrations of PX (0.0, 2.5 and 5.0 mg l^{-1}) and a constant concentration of OHPX (2.5 mg l^{-1}) were constructed in order to investigate the effect of PX and OHPX on the simultaneous determination of the two compounds in plasma samples. Fig. 3 shows that the heights (h_1 and h_2) were not affected by the presence of OHPX or PX over the range of concentration studied in the first-derivative mode.

Determination of PX and OHPX in human plasma

Some binary mixtures of PX and OHPX in the plasma samples were prepared from the working standard solution in the proportions 1 + 1, using the recommended procedure. The first-order derivative spectra of these sample were recorded over the wavelength range 310–400 nm against a blank consisting of saline solution. The calibration graphs were constructed by plotting the analytical signals $^1D_{PX}$ at 337.0 nm and $^1D_{OHPX}$ at 327.0 nm *versus* the PX or OHPX concentration (mg l^{-1}). The ordinate values ^1D of the equations were calculated from the amplitude measurements (mm) and standardized as follows: ^1D = recorder divisions (*h* mm) × scale expansion /150 mm full-scale.[31] Table 1 summarizes the statistical data obtained from two calibration graphs. Linear relationships were obtained for concentrations up to 10.0 and 8.0 mg l^{-1} of PX and OHPX, respectively.

The reproducibility of the endogenous background signal was evaluated from 10 different individual blank plasma samples. The values obtained were $1.38 \times 10^{-3} \pm 1.67 \times 10^{-4}$ and $1.90 \times 10^{-3} \pm 1.85 \times 10^{-4}$ (mean of analytical signal ± standard deviation) at 337.0 and 327.0 nm, respectively.

The detection and quantification limits achieved, as defined by IUPAC,[32] were 0.083 and 0.27 mg l^{-1} for PX and 0.16 and 0.56 mg l^{-1} for OHPX.

We demonstrated that the method permits the simultaneous determination of 0.40 mg l^{-1} of PX in the presence of up to 8.0 mg l^{-1} of OHPX and 0.66 mg l^{-1} of OHPX in the presence of up to 10.0 mg l^{-1} of PX.

The precision of the method was evaluated by analysing 10 replicate plasma samples obtained from different individuals spiked with 5.0 mg l^{-1} of each compound. The RSDs obtained were 2.01 and 2.18% for PX and OHPX, respectively.

Bilirubin, triglycerides and hemoglobin were tested for possible interference effects in the simultaneous determination of 5.0 mg l^{-1} of PX and OHPX. No interference was observed even when bilirubin was present at a concentration of 100 mg l^{-1}, triglycerides at 10 g l^{-1} and hemoglobin at 2.5 g l^{-1}.

In addition to the anti-inflammatory drug PX, some patients may be given other drugs to alleviate pain and other symptoms of arthritis. In the physiological concentration range obtained after the usual therapeutic doses of the drugs had been administered, no interference was observed from penicillamine, prednisolone, ranitidine, misoprostol and sodium aurothiomalate, but chloroquine and azathioprine did interfere.

The validity of the proposed method was confirmed by applying the standard additions technique to different blank plasma samples. The results are given in Table 2. Quantitative recoveries of 95.6–106 and 95.0–107.6% were obtained for PX and OHPX, respectively.

Conclusions

The proposed first-derivative UV/VIS spectrophotometric method is suitable for the simultaneous determination of piroxicam and its major metabolite 5′-hydroxypiroxicam in human plasma. It is a simple and precise procedure which requires inexpensive reagents and which can be used for rapid and reliable clinical and pharmacokinetic studies of PX and OHPX simultaneously.

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