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Simultaneous determination of piroxicam and its main metabolite in plasma and urine by high-performance liquid chromatography

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Summary

A rapid and sensitive high-performance liquid chromatographic procedure is described for the simultaneous determination of piroxicam and its main metabolite, such as 5′-hydroxypiroxicam, in plasma and urine. Acidified plasma (pH 3.0) was extracted with ethyl ether and indomethacin was used as an internal standard. The organic extract was reduced to dryness, the resultant residue redissolved in the mobile phase, and aliquots of this solution chromatographed on a Lichrosorb RP-18 (7 μm) column using a mobile phase consisting of an acetonitrile–water–acetic acid (58 : 38 : 4) mixture. The flow rate was 1.2 ml/min and the effluent was monitored at 365 nm with 0.02 aufs. The sensitivities of this method were 0.05 μg/ml levels of piroxicam and 5′-hydroxypiroxicam in the plasma and urine samples.

Introduction

Piroxicam is a non-steroid anti-inflammatory, antipyretic and analgesic agent used in the treatment of rheumatoid arthritis. Numerous approaches have been described for the analysis of this substance. Among these, spectrophotometric and fluorometric (Hobbs and Twomey, 1979; Milne and Twomey, 1980), high-performance thin-layer chromatography (Riedel and Laufen, 1983), high-performance liquid chromatograph (Schiantarelli et al., 1981) methods have been applied for the determination of piroxicam in biological fluids. However, no proprietary methods

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have been reported for simultaneous determination of piroxicam and its main metabolite by high-performance liquid chromatography (HPLC).

Although Twomey et al. (1980) described an efficient quantitative method for piroxicam by HPLC, in this method the peak of piroxicam and its metabolite were shown to significantly overlap and so the recovery of the metabolite was relatively poor (< 20%). For present pharmacokinetic work, a HPLC method of fast, good recovery and complete separation of the components for the simultaneous determination of piroxicam and 5′-hydroxypiroxicam from a single plasma and urine extract is described.

Materials and Methods

Materials

Indomethacin (Sumitomo Chemicals, Osaka, Japan), piroxicam (Pfizer, U.S.A) and 5′-hydroxypiroxicam (Pfizer, U.S.A.) were dried in a desiccator under vacuum for 24 h before use. Acetonitrile, acetic acid, ethyl ether, citric acid and sodium phosphate dibasic-2-hydrate were of guaranteed reagent grade.

Internal standard solution

About 100 mg of indomethacin was accurately weighed out, transferred to a 100-ml volumetric flask and diluted with methanol to make up to full volume.

Chromatographic conditions

A Water Associates (Milford, MA, USA) high-performance liquid chromatograph (HPLC) equipped with a Waters Model 440 UV detector and a 25 cm × 4.0 mm i.d. 7-μM Lichrosorb RP-18 column was used. The mobile phase consisted of an acetonitrile–water–acetic acid (58 : 38 : 4) mixture. The operating temperature was ambient, and the flow rate was 1.2 ml/min with an operating pressure of 1.5 psi. The column effluent was monitored continuously at 365 nm with a full-scale deflection of 0.02 aufs, and the chart speed of the recorder was maintained at 0.5 cm/min.

In vivo tests

Male rabbits were fasted overnight before dosing. A 0.2% solution of piroxicam in biocarbonate buffer pH 9 (5 ml/kg) or a 0.1% solution of 5′-hydroxypiroxicam in bicarbonate buffer pH 9 (2.5 ml/kg) was injected into the marginal vein of the ear. All rabbit blood specimens were collected from the carotid artery by puncture using disposable plastic syringes pre-rinsed with a 1% solution of heparin sodium in normal saline. Blood samples were withdrawn at 12 specific time (Fig. 5) intervals after drug administration. The blood samples were centrifuged at 3000 rpm for 5 min to obtain plasma. Rabbit urine specimens were collected at 7 h after oral dose of 10 mg/kg of piroxicam (suspended in 1% CMC solution).

Human plasma and urine samples were obtained from healthy male subjects who had received an oral dose of 20 mg piroxicam with water following an overnight fast.

Food was withheld for an additional 4 h. Plasma samples were obtained at 2, 4, 6, 8, 24, 48, 72 and 96 h after drug ingestion. Urine samples were obtained at 10 h after injection.

Analytical procedures

A 0.5-ml aliquot of plasma or 2-ml urine sample (spiked or from dosed rabbits and human) was pipetted into a 15-ml glass-stoppered centrifuge tube, along with 1 ml of Sørensens citrate buffer (pH 3). The mixture was shaken for 10 s and extracted with 5 ml of ethyl ether by mechanical shaking for 20 min. After centrifugation for 3 min at 3000 rpm, 4 ml of the ether phase was transferred to another tube and evaporated to dryness on a water bath at 50°C. The residue was redissolved in 1 ml of mobile phase and 10 ml of internal standard at a concentration 1 mg/ml added and mixed for 15 s by a vortex mixer; then 50 μl of this solution was injected into the column for HPLC through a stop-flow injection port.

A calibration curve was prepared, using known concentrations of piroxicam and 5′-hydroxypiroxicam with plasma, by plotting the concentration of piroxicam and 5′-hydroxypiroxicam (μg/ml of plasma) against the respective peak ratios.

Results and Discussion

Fig. 1 gives typical chromatograms for 5′-hydroxypiroxicam and piroxicam extracted from rabbit-spiked plasma (Fig. 1X), dosed plasma samples (Fig. 1Y) and dosed urine samples (Fig. 1Z). Fig. 2 gives the typical chromatograms for piroxicam and 5′-hydroxypiroxicam extracted from dosed human plasma (Fig. 2A) and urine (Fig. 2B) samples. Under the chromatographic conditions described above, the retention times of these compounds were 3.8, 4.8 and 6.1 min, respectively. As indicated in Fig. 1D, no interfering peaks were found in the control plasma specimen. Fig. 1Z showed an unknown peak prior to the peak 'a', but it did not interfere with the peak of piroxicam and 5′-hydroxypiroxicam. The complete separation of components in the chromatograms permitted accurate measurements of small quantities of 5′-hydroxypiroxicam in the presence of a large quantity of piroxicam.

Fig. 3 illustrates the linearity of the calibration curves for piroxicam and 5′-hydroxypiroxicam in rabbit plasma at concentrations ranging from 0.5 to 25 μg/ml. The assay precision and reproducibility are summarized in Table 1. The coefficient of variation (CV) ranged from 1.2 to 5.26%.

The present method employed a solvent of ether for the extraction of piroxicam and its metabolite from acidified plasma samples. This solvent gave a good recovery of these compounds while experiencing essentially no interference from the plasma. The acidification of the plasma samples enhanced the recovery by solvent extraction due to the acidic nature of these compounds (Helleberg, 1976). The extraction of piroxicam and 5′-hydroxypiroxicam with ether were pH-dependent. As shown in Fig. 4, it was found that the recovery of piroxicam and 5′-hydroxypiroxicam can be improved when the plasma was adjusted to pH 2–3 with Na_2HPO_4–citric acid

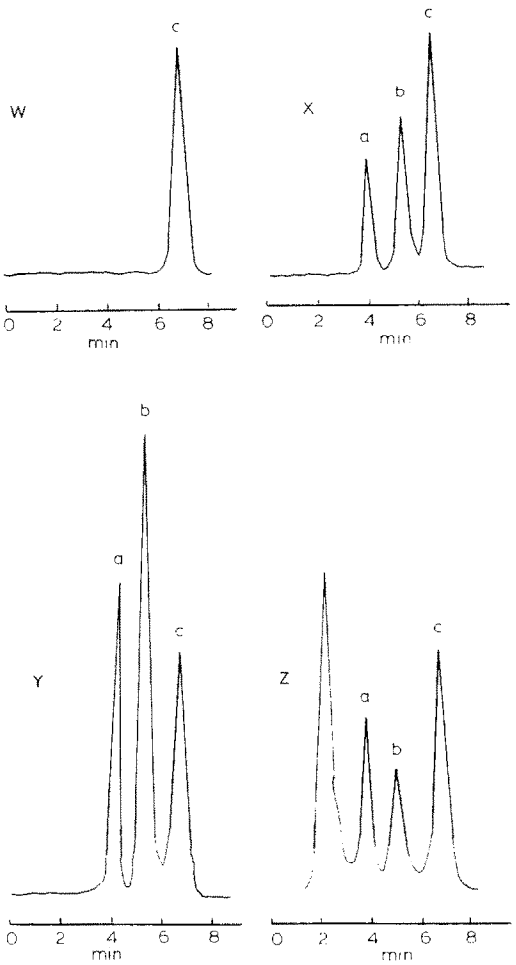

Fig. 1. High-pressure liquid chromatograms of rabbit plasma and urine extracts. Key: W = control plasma containing internal standard (c); X = plasma containing internal standard (c), piroxicam (b), 5′-hydroxy-piroxicam (a); Y = plasma sample at 4 h after i.v. administration of 10 mg/kg of piroxicam; Z = urine sample at 7 h after p.o. administration of 10 mg/kg of piroxicam.

TABLE 1

ASSAY PRECISION AND REPRODUCIBILITY

Compound	Concentration in rabbit plasma	Compound/internal standard		
		Mean height ratio (n = 4)	S.D.	CV (%)
5′-Hydroxy-piroxicam	0.5	0.060	0.0025	4.17
	5.0	0.469	0.0176	3.76
	15.0	1.442	0.0173	1.20
	25.0	2.442	0.0980	4.02
Piroxicam	0.5	0.092	0.0047	5.00
	5.0	0.545	0.0260	4.90
	15.0	1.594	0.0840	5.26
	25.0	2.662	0.1320	4.97

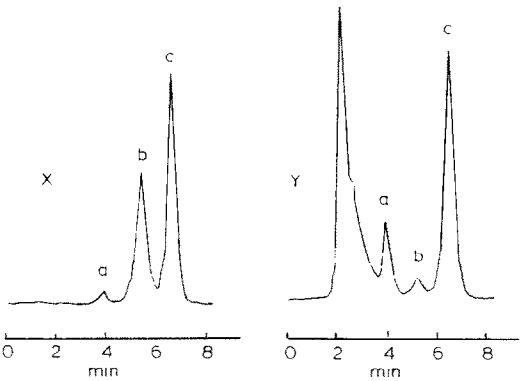

Fig. 2. High-pressure liquid chromatograms of human dosed plasma and urine extracts. Key: plasma samples at 6 h after oral administration of 20 mg piroxicam; Y = urine samples at 10 h after oral administration of 20 mg piroxicam.

buffer solution. Identical curves were found for the extraction of piroxicam and 5′-hydroxypiroxicam from plasma and an equal portion of deproteinized plasma which was previously added 5 N HCl, incubated at 37°C for 2 h and adjusted to pH 7.0 with 5 N NaOH. This result suggested that the determination of plasma levels of piroxicam and 5′-hydroxypiroxicam were its total concentration including both free and protein-bound forms. Assay recovery was determined by comparing the response from known amounts of drug and the metabolite with processed, fortified plasma samples. Recoveries for piroxicam and 5′-hydroxypiroxicam were 70.16% and 64.9%, respectively.

Time courses for the concentration of piroxicam and 5′-hydroxypiroxicam in the

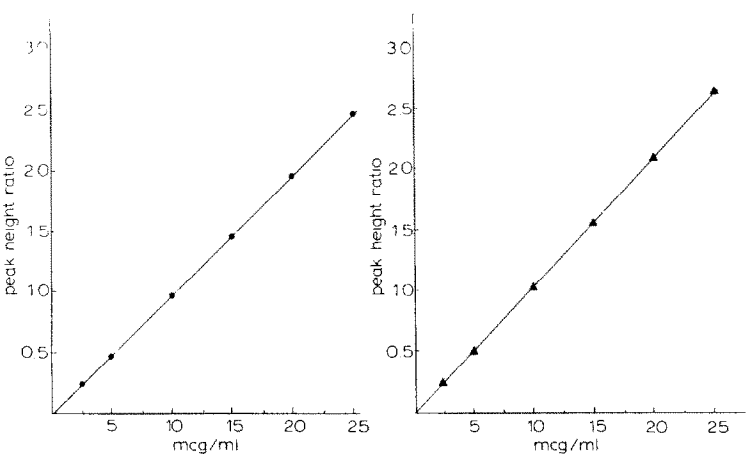

Fig. 3. Calibration curves for 5′-hydroxypiroxicam (●, 0.5–25 μg/ml) and piroxicam (▲, 0.5–25 μg/ml) extracted from rabbit plasma. Each point represents the mean of 4 determinations. Linear regression lines: ●, $y = -0.0015 + 0.097x$; $r = 0.99985$. ▲, $y = 0.0357 + 0.1035x$, $r = 0.9992$.

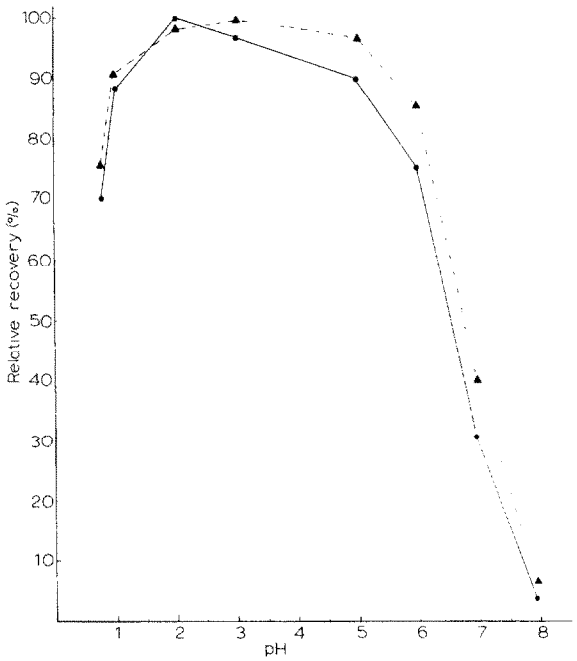

Fig. 4. Effect of pH on the extraction of piroxicam and 5′-hydroxypiroxicam from rabbit plasma. The pH of the plasma was adjusted by the addition of different Na_2HPO_4–citric acid buffers. Key: ●, 5′-hydroxypiroxicam; ▲, piroxicam.

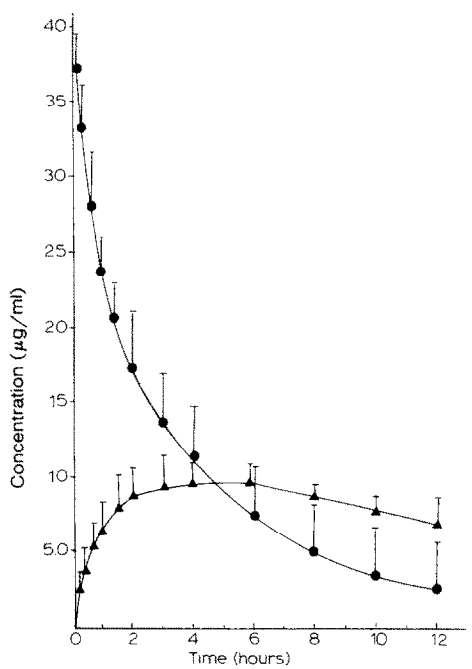

Fig. 5. Plasma concentration–time curve for piroxicam (●) and 5′-hydroxypiroxicam (▲) in rabbit plasma after i.v. administration of 10 mg/kg of piroxicam. Each point represents the mean of 4 determinations. ———, curve for piroxicam calculated from equation $C = 26.03 \cdot e^{-0.555t} + 14.39 \cdot e^{-0.0876t}$, where C is piroxicam concentration in plasma.

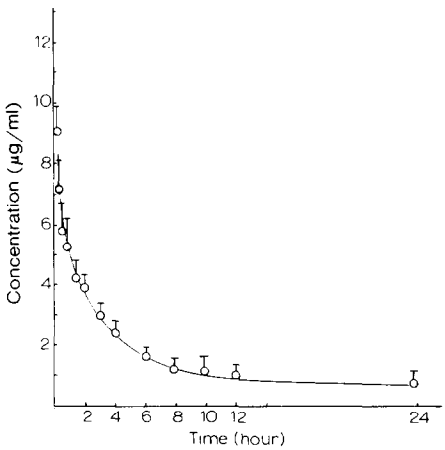

Fig. 6. Plasma concentration–time curve for 5′-hydroxypiroxicam (○) in rabbit plasma after i.v. administration of 2.5 mg/kg of 5′-hydroxypiroxicam. Bars are standard deviations (n = 4). Equation curve: $C = 7.36 \cdot e^{-0.559t} + 1.76 \cdot e^{-0.032t}$.

plasma of rabbit treated with 10 mg/kg of piroxicam i.v. are given in Fig. 5. Time courses for the concentration of 5′-hydroxypiroxicam in the plasma of rabbit treated with 2.5 mg/kg of 5′-hydroxypiroxicam i.v. are given in Fig. 6. The plasma levels of piroxicam and 5′-hydroxypiroxicam appear to be consistent with a two-compartment model, respectively. Table 2 summarizes the pharmacokinetic parameters generated from analysis of the data. The elimination half-life (7.9 h) of piroxicam differs from that reported (5 h) by Wiseman et al. (1976) and the $V_c = 247.4$ ml · kg^{-1} and

TABLE 2

VALUES FOR THE PARAMETERS OF A PHARMACOKINETIC MODEL DESCRIBING THE METABOLISM AND EXCRETION OF PIROXICAM AND 5′-HYDROXYPIROXICAM IN RABBIT FOLLOWING i.v. ADMINISTRATION OF 10 mg/kg AND 2.5 mg/kg, RESPECTIVELY (n = 4)

Parameter	Value	
	Piroxicam	5′-Hydroxypiroxicam
α (h^{-1})	0.555	0.559
β (h^{-1})	0.0876	0.032
$t_{1/2\beta}$ (h)	7.911	21.66
K_{el} (h^{-1})	0.191	0.134
K_{12} (h^{-1})	0.198	0.324
K_{21} (h^{-1})	0.254	0.133
V_c (ml·kg^{-1})	247.4	274.12
V_T (ml·kg^{-1})	192.8	667.78

α and β are hybrid first-order rate constant and $t_{1/2\beta}$ is the half-life associated with the terminal exponential process; K_{el} = elimination rate constant from the central compartment; K_{12} = rate constant from the central to tissue compartment; K_{21} = rate constant from the tissue to central compartment; V_c = distribution volume of the central compartment; and V_T = distribution volume of the tissue compartment.

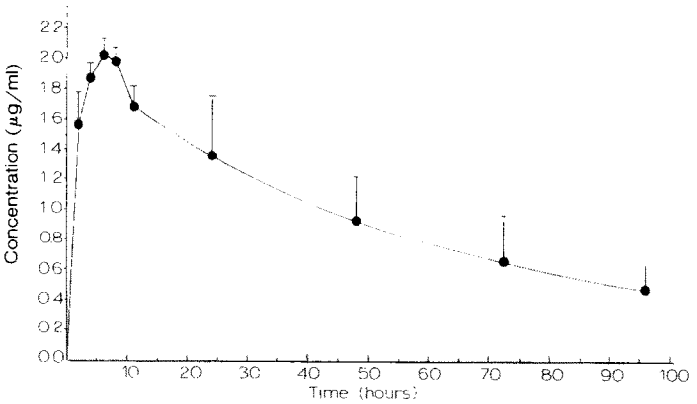

Fig. 7. Plasma concentration in human subjects after oral administration of 20 mg of piroxicam. Each point represents the mean of 4 determinations. Bars are standard deviations.

$V_T = 192.8$ ml · kg^{-1} are similar to that reported ($V_c = 235.1$ ml · kg^{-1} and $V_T = 160.2$ ml · kg^{-1}) by Schiantarelli et al. (1981). The 5′-hydroxypiroxicam level is relatively higher than that found by Wiseman et al. (1976); the time needed to reach maximum concentration is 6 h.

Fig. 7 gives the plasma piroxicam concentration in human subjects after oral administration of 20 mg of piroxicam. The elimination half-life was 45 h.

In conclusion, it can be said the HPLC procedure described here provides a rapid, sensitive and precise method for the simultaneous determination of plasma and urine levels of piroxicam and its main metabolite. Further applications of the procedure to pharmacokinetic studies of piroxicam and 5′-hydroxypiroxicam will be described at a later date.

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