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Short communication

Extractionless high-performance liquid chromatographic method for the simultaneous determination of piroxicam and 5′-hydroxypiroxicam in human plasma and urine

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Abstract

A simple and rapid (extractionless) high-performance liquid chromatographic method with UV detection, at 330 nm, was developed for the simultaneous determination of piroxicam and its major metabolite, 5′-hydroxypiroxicam, in human plasma and urine. Acidified plasma and alkali-treated urine samples are used and naproxen is added as internal standard. The separation is performed at 40°C on a C_{18} Spherisorb column with acetonitrile–0.1 *M* sodium acetate (33:67, v/v, pH 3.3) as mobile phase. The retention time is 2.2 min for 5′-hydroxypiroxicam, 2.6 min for piroxicam and 3.2 min for naproxen. The detection limit is 0.05 μg/ml using a 100-μl loop.

1. Introduction

The non-steroidal anti-inflammatory drug (NSAID) piroxicam is widely used in the treatment of rheumatoid arthritis and other inflammatory disorders. It is given in usual doses of 20 mg daily since it shows long plasma half-life (50 h). Piroxicam (Fig. 1) is extensively metabolized to 5′-hydroxypiroxicam and is mainly excreted in urine either as 5′-hydroxypiroxicam or 5′-hydroxypiroxicam glucuronide and less than 5% as parent drug [1]. Consequently, several high-performance liquid chromatographic (HPLC) methods have been developed for the determination of piroxicam alone or together with its metabolites in body fluids and tissues (serum/plasma, urine, bile, muscle and skin) [2–14]. All these methods require liquid extraction and are time-consuming, except of two concerning piroxicam alone [8,12], in which the serum proteins are precipitated prior to centrifugation and injection. Three HPLC methods, which pay attention both to piroxicam and its major metabolite (5′-hy-

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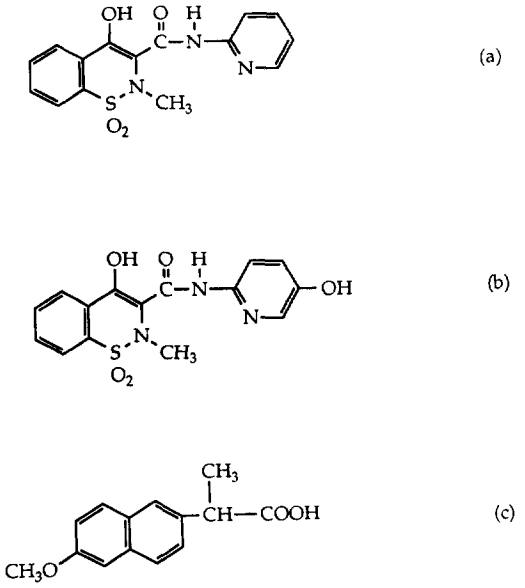

Fig. 1. Chemical structures of (a) piroxicam, (b) 5′-hydroxypiroxicam and (c) naproxen.

droxypiroxicam), involve liquid extraction with diethyl ether [6,7,13]. In this report a simple and rapid, extractionless method with minimum sample preparation is proposed for the determination of piroxicam together with its major metabolite, in both human plasma and urine.

2. Experimental

2.1. Apparatus and chromatographic conditions

The HPLC system consisted of a Gilson 802G high-performance liquid chromatograph (Gilson, Villiers-le-Bel, France) equipped with a variable-wavelength detector (set at 330 nm) and a Gilson NI chart recorder.

The separation was performed at 40°C on a stainless-steel column (25 cm × 4.5 mm I.D.) packed with Spherisorb 5 μm (C_{18} reversed-phase, Perkin-Elmer, Norwalk, CT, USA). A stainless-steel precolumn (100 mm × 2.0 mm I.D.) packed with pellicular reversed-phase material (Spherisorb) was used as a guard column. Analytical samples were introduced onto the column using a 100-μl loop valve (Rheodyne, Cotari, CA, USA).

The mobile phase was acetonitrile–0.1 *M* sodium acetate (33:67, v/v) with a final pH of 3.3 which was adjusted by the dropwise addition of glacial acetic acid. The flow-rate was 2.5 ml/min and the inlet pressure ca. 18.00 MPa (2600 p.s.i.). Absorbance of the effluent from the column, at 330 nm, was monitored at a sensitivity of 0.02 a.u.f.s. by using a 10-mV strip recorder (5 mm/min.)

2.2. Chemicals

Piroxicam and naproxen were obtained from Esteve Quimica (Barcelona, Spain) and 5′-hydroxypiroxicam was a gift from Pfizer (Groton, USA). Solvents for HPLC were purchased from Carlo Erba (Milan, Italy).

Internal standard stock solution was prepared by dissolving 100 mg naproxen in 100 ml of methanol and further dilutions were made with methanol to obtain a 15 μg/ml standard solution.

2.3. Sample preparation

Plasma samples were prepared by the addition of 200 μl of trichloroacetic acid (10% w/v), 100 μl of diluted internal standard solution (15 μg/ml) and 0.7 ml of methanol to 0.5 ml of heparinized plasma. The samples were vortex-mixed for 30 s, centrifuged at 2105 *g* for 10 min and a 100-μl aliquot of the supernatant was injected onto the HPLC column.

For the determination of piroxicam and its major metabolite in urine, samples of 1.0 ml were analysed after the hydrolysis of glucuronides by the addition of 1 *M* sodium hydroxide (400 μl). After vortex-mixing, the alkali-treated samples were left to stand for 2 h at room temperature and 300 μl of methanol as well as 100 μl of diluted internal standard solution (15 μg/ml)

were added. No precipitate was formed on the addition of sodium hydroxide and methanolic internal standard solution. After mixing, 100 μl were injected onto the column.

3. Results and discussion

The retention times of 5′-hydroxypiroxicam, piroxicam and naproxen were 2.2, 2.6 and 3.2 min, respectively. Investigation of the influence of oven temperature on the retention behaviour showed that an oven temperature of 40°C was the best compromise between a low enough viscosity of the solvent mixture, minimized degassing problems, less organic solvent employed and optimum separation power achieved by the system.

An acid buffer system was used to suppress ionization and to take advantage of the properties of reversed-phase systems. The composition and pH of the mobile phase were varied to achieve the optimum chromatographic conditions. A mobile phase consisting of acetonitrile–0.1 *M* sodium acetate (33:67, v/v) adjusted to pH 3.3 with glacial acetic acid gave optimum resolution of piroxicam, 5′-hydroxypiroxicam and naproxen (I.S.) and no interference was observed due to endogenous constituents in plasma and urine chromatograms. Particularly in urine, no interfering peaks appear either with or without the alkali treatment applied in order to hydrolyse the glucuronide conjugates.

The peak-height ratio method was used to calculate the concentration of piroxicam and its metabolite, by reference to internal standard, from a series of previously prepared calibration graphs. The calibration graphs were constructed by treating drug-free plasma and urine spiked with piroxicam and 5′-hydroxypiroxicam over the ranges of 0.05–20.0 and 0.05–10.0 μg/ml, respectively.

All the calibration graphs displayed good linearity over the range examined and almost passed through the origin. The linear regression equations, in plasma and urine, respectively, were: $x = 2.86y + 0.04$ and $x = 3.64y - 0.01$ for piroxicam, and $x = 1.04y + 0.16$ and $x = 1.89y - 0.02$ for 5′-hydroxypiroxicam, where x is the concentration (μg/ml) and y is the peak-height ratio. The correlation coefficients were 0.999 or better for at least eight points.

The reproducibility of the method was examined for both plasma and urine by the repeated analysis of samples spiked with piroxicam and 5′-hydroxypiroxicam at certain concentration. The results for inter-day assay variation are presented in Table 1. Using the criterion of minimum detectability as three times the system

Table 1
Reproducibility for inter-day determinations of piroxicam and 5′-hydroxypiroxicam in plasma and urine samples ($n = 5$)

Sample	Spiked concentration (μg/ml)	Determined concentration (μg/ml)			
		Piroxicam		5′-Hydroxypiroxicam	
		Mean	R.S.D. (%)	Mean	R.S.D. (%)
Plasma	0.20	0.21	4.5	0.19	2.9
	2.00	2.01	2.4	1.99	3.4
	4.00	4.01	2.7	4.00	4.2
Urine	0.20	0.20	1.5	0.22	3.5
	2.00	2.00	2.8	1.99	2.5
	4.00	3.99	1.9	4.01	4.3

noise, the detection limit was 0.05 μg/ml by using a 100-μl loop.

The applicability of the proposed method was tested out by analysing blood and urine samples from a volunteer after the administration of 20 mg of piroxicam daily (one Feldene dispersible tablet) for three days. On the third day blood and urine samples were collected at scheduled intervals. Blood samples were collected from a forearm vein and the plasma was separated by centrifugation and then was frozen. Urine samples (5 ml) were frozen immediately after their collection. A typical chromatogram of plasma, 5 h after the third oral ingestion of 20 mg of piroxicam is shown in Fig. 2A. The chromatogram from the analysis of corresponding urine samples is shown in Fig. 2B.

No measurable concentrations of piroxicam were found in urine due to the extensive metabolism and the very small (less than 5%) excretion as parent drug in urine [1]. Also, unconjugated 5′-hydroxypiroxicam was not measurable in urine, since the majority of it is glucuronated [13]. The plasma concentration–time profiles of piroxicam and 5′-hydroxypiroxicam are illustrated in Fig. 3 together with the urinary excretion rate–time profile of 5′-hydroxypiroxicam.

The results in Fig. 3 together with the detection limits of 0.05 μg/ml, the relative standard deviations in Table 1 and the correlation coefficients for the regression equations ($r > 0.999$) indicate that: (a) this simple and rapid (extractionless) method is sufficiently sensitive to follow accurately blood levels of piroxicam after a single oral dose for at least four half-lives after peak time (t_{max}), as it is required in determinations of pharmacokinetic parameters for drug formulation studies; (b) the method is sufficient for the detection of 5′-hydroxypiroxicam in both human plasma and urine after three consecutive doses of 20 mg piroxicam daily.

The method utilizes standard HPLC equipment and is compatible with automation. About fifty samples (and with the help of an autosampler up to 100 samples) can be examined within a working day, since the typical assay time was 4 min. For the determination, as little as 0.1 ml of plasma can be used, with a proportional reduction in the volume of the internal standard and the methanol added to the samples. The precolumn could be used for at least 200 samples and the analytical column for 400 samples.

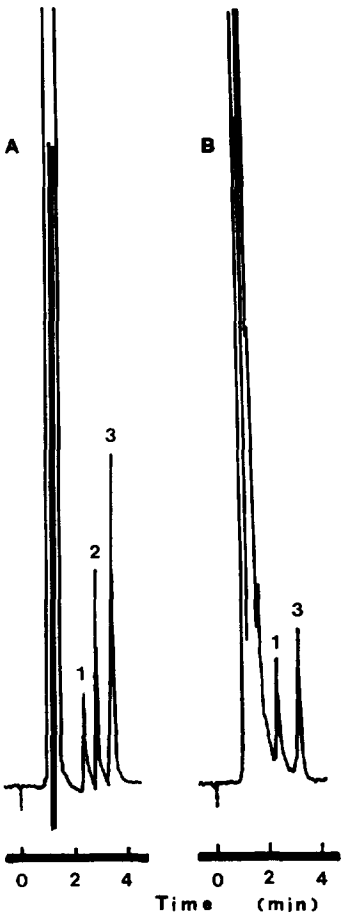

Fig. 2. Chromatograms of (A) plasma and (B) urine samples from a volunteer, 5 h after the third daily oral administration of 20 mg piroxicam. Peaks: 1 = 5′-hydroxypiroxicam (0.44 μg/ml in plasma and 1.5 μg/ml in urine); 2 = piroxicam (2.0 μg/ml in plasma); and 3 = internal standard (naproxen).

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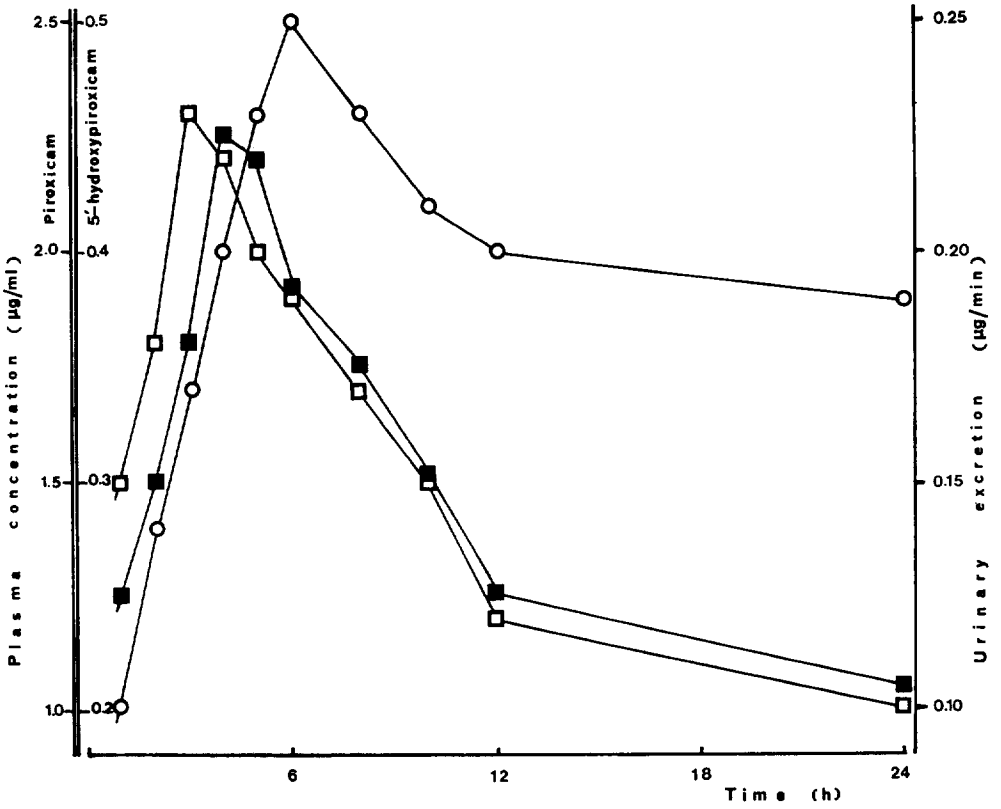

Fig. 3. Plasma concentration of piroxicam (□) and 5'-hydroxypiroxicam (■) and urinary excretion rate of 5'-hydroxypiroxicam (○) vs time in a volunteer after the third daily oral administration of a 20-mg dispersible piroxicam tablet.

References

[1] P.A. Insel, in A.G. Gilman, J.W. Rall, A.S. Nies and P. Taylor (Editors), The Pharmacological Basis of Therapeutics, Pergamon Press, New York, NY, 8th ed., 1990, p. 668.
[2] T.M. Twomey, S.R. Bartolucci and D.C. Hobbs, J. Chromatogr., 183 (1980) 104.
[3] K.D. Riedel and H. Laufen, J. Chromatogr., 276 (1983) 243.
[4] A.D. Fraser and J.F.L. Woodbury, Ther. Drug Monit., 5 (1983) 239.
[5] J.S. Dixon, J.R. Lowe and D.B. Galloway, J. Chromatogr., 310 (1984) 455.
[6] Y.H. Tsai, L.R. Hsu and S.I. Naito, Int. J. Pharm., 24 (1985) 101.
[7] C.J. Richardson, S.G. Ross, K.L. Blocka and R.K. Verbeeck, J. Chromatogr., 382 (1986) 382.
[8] J. Macek and J. Vacha, J. Chromatogr., 420 (1987) 445.
[9] F.D. Boudinot and S.A. Ibrahim, J. Chromatogr., 430 (1988) 424.
[10] R.B. Gillilan, W.D. Mason and C.H.J. Fu, J. Chromatogr., 487 (1989) 232.
[11] K. Saeed and M. Becher, J. Chromatogr., 567 (1991) 185.
[12] S. Wanwimolruk, S.Z. Wanwimolruk and A.R. Zoest, J. Liq. Chromatogr., 14 (1991) 2373.
[13] P.A. Milligan, J. Chromatogr., 576 (1992) 121.
[14] D. Cerretani, L. Micheli, A.I. Fiaschi and G. Giorgi, J. Chromatogr., 614 (1993) 103.