Journal of Chromatography, 614 (1993) 103–108
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6719

Rapid and sensitive determination of piroxicam in rat plasma, muscle and skin by high-performance liquid chromatography

D. Cerretani*, L. Micheli, A. I. Fiaschi and G. Giorgi

Institute of Pharmacology, University of Siena, Via delle Scotte 6, 53100 Siena (Italy)

(First received July 28th, 1992; revised manuscript received December 21st, 1992)

ABSTRACT

A rapid and precise high-performance liquid chromatographic method for the determination of piroxicam in a variety of biological samples has been developed. A reversed-phase column, isocratic elution and ultraviolet detection were employed. Calibration curves were reproducible and highly linear, with correlation coefficients typically averaging over 0.992. The detection limit of the assay was 100 ng/ml for all biological samples examined (at a signal-to-noise ratio of 3:1). Validation of the method demonstrated a good sensitivity, accuracy and precision. The method has been adopted for a pharmacokinetic study in rats.

INTRODUCTION

Piroxicam [4-hydroxy-2-methyl-N-(2-pyridyl)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide] is a non-steroidal anti-inflammatory and analgesic agent. Its efficacy has been demonstrated in humans in the treatment of various inflammatory diseases and arthropathies, such as rheumatoid arthritis and osteoarthritis [1–5]. Piroxicam has been shown to be an inhibitor of prostaglandin synthesis *in vitro* and *in vivo* [2], in addition to inhibiting the secondary phase of platelet aggregation [3,4].

Several HPLC methods have been described [6–13] for piroxicam determination in plasma, urine and bile. The method we have developed is simple and precise, and allows the measurement of piroxicam also in tissues (skin and muscle). We have used it to study the kinetics after topical administration to rats.

* Corresponding author.

EXPERIMENTAL

Reagents and materials

HPLC-grade chemicals (mobile phase and extraction solvents) were purchased from Merck (Bracco, Milan, Italy). Piroxicam and isoxicam were purchased from MSD (Rome, Italy). Filters (FM 0.5 μm) were obtained from Millipore (Waters, Milford, MA, USA).

Standard solutions

A standard solution of 100 μg/ml piroxicam in methanol was prepared and stored in the dark at 4°C. All working solutions were made by diluting this stock solution with methanol. Piroxicam was stable in methanol for at least one week at 4°C. All stock standards were prepared weekly and all working standards were prepared daily from the appropriate stock standard. A solution of equivalent concentration (100 μg/ml) was prepared for isoxicam, to be used as the internal standard (I.S.).

0378-4347/93/$06.00

Sample preparation

Male albino Wistar rats (Morini, S. Polo d'Enza, Reggio Emilia, Italy) weighing 180–200 g, were epilated on the right hind leg by a blade razor, and 10 mg of piroxicam were topically administrated an this area. After 1, 2, 4, 8, 18, 24 and 48 h, the animals were sacrificed. The obtained plasma was frozen at −70°C until analysis. The skin and muscle of the leg were homogenized in a Polytron blades homogenizer (Kinematica, Littan, Lucerne, Switzerland) in 0.9% saline (1:4, w/v) and frozen at −70°C until analysis. A group of fifteen untreated animals were used as control and, after sacrifice, plasma, skin and muscle were used for the experiment. A 1-ml volume of homogenate was used for piroxicam determination, using the same analytical method as for plasma.

Calibration procedure

Blank plasma, muscle and skin plasma (1 ml) were spiked with piroxicam at concentrations within the studied range. I.S. solution (0.05 ml = 5 μg) was added to every calibration and unknown sample, and all tubes were vortex-mixed before extraction.

Extraction procedure

To 1 ml of plasma or tissue, 0.05 ml of I.S. solution, 700 mg of potassium carbonate, 1 ml of tetrahydrofuran (THF) and 0.5 ml of ethanol were added. The tubes were vortex-mixed for 30 s, then centrifuged for 5 min at 2000 *g*. The supernatant was placed in a second test-tube and evaporated to dryness at 70°C in a water-bath under a stream of nitrogen.

The residue was then reconstituted in 100 μl of THF, vortex-mixed for 5 s, and filtered with a 0.5-μm FH filter (Millipore) prior to injection (20 μl) onto the HPLC column.

Chromatography

The HPLC system consisted of a Model 302 solvent-delivery system with a Model 231 autoinjector (Gilson) and an Holochrome variable-wavelength UV detector (Gilson, Villiers le Bel, France). A Novapak C_{18} column (15 cm × 3.9 mm I.D., 3 μm particle size) (Waters) was used. The volume of the injection loop was 20 μl, and effluent was monitored at 313 nm. The mobile phase was THF–water (45:55, v/v) with 1% acetic acid and 0.005 *M* 1-heptansulphonic acid (PIC B-7, Waters) delivered at a flow-rate of 0.7 ml/min. All separations were achieved at room temperature.

RESULTS AND DISCUSSION

Validation

Excellent linearity was observed between the peak-height ratios (piroxicam/I.S.) in plasma, muscle and skin concentrations over the exam-

TABLE I

INTRA- AND INTER-ASSAY COEFFICIENTS OF VARIATION OF PIROXICAM IN RAT PLASMA SAMPLES

Piroxicam added (μg/ml)	Intra-assay (n = 6)		Inter-assay (n = 6)					
	Mean found (μg/ml)	C.V. (%)	Day 1		Day 2		Day 3	
			Mean found (μg/ml)	C.V. (%)	Mean found (μg/ml)	C.V. (%)	Mean found (μg/ml)	C.V. (%)
0.1	0.105	8.97	0.098	8.16	0.096	7.60	0.11	7.73
1	1.030	6.70	0.99	7.27	1.03	6.52	1.06	5.75
4	4.030	1.64	4.02	2.26	3.98	2.12	4.10	2.20
10	10.100	1.78	9.98	1.72	10.30	1.63	10.00	1.70
60	59.980	1.59	60.25	1.38	60.70	1.81	59.90	2.10

TABLE II

INTRA- AND INTER-ASSAY COEFFICIENTS OF VARIATION OF PIROXICAM IN RAT MUSCLE SAMPLES

Piroxicam added (μg/ml)	Intra-assay (n = 6)		Inter-assay (n = 6)					
	Mean found (μg/ml)	C.V. (%)	Day 1		Day 2		Day 3	
			Mean found (μg/ml)	C.V. (%)	Mean found (μg/ml)	C.V. (%)	Mean found (μg/ml)	C.V. (%)
0.1	0.097	8.2	0.103	8.7	0.100	8.0	0.104	6.7
4	3.990	2.3	4.020	2.2	4.030	2.2	4.000	2.5
10	10.130	1.9	9.990	2.6	10.000	2.1	9.980	2.0
20	20.300	1.8	20.910	2.3	20.680	1.9	19.950	2.1
40	40.050	2.8	39.900	2.5	40.100	2.6	39.970	2.8

ined concentration range (0.5–60 μg/ml for plasma; 4–80 μg/ml for muscle; 4–250 μg/ml for skin). The standard curve concentrations were chosen to encompass the full range of piroxicam concentrations that are expected in the various biological matrices examined.

Standard curves for plasma, muscle and skin can be described by $y = -0.0554 + 0.365x$ ($r = 0.9919$), $y = 0.0412 + 0.136x$ ($r = 0.9993$) and $y = 0.465 + 0.125x$ ($r = 0.9991$), respectively.

Inter- and intra-assay variability

The intra-assay variability was determined by HPLC analysis of six samples spiked with a known amount of piroxicam during a single day. The inter-assay variability was determined by HPLC analysis of six samples spiked with a known amount of piroxicam on different days. Data on the analytical method variability are presented in Tables I–III.

Sensitivity

The limit of detection, at signal-to-noise ratio of 3:1, of the proposed method was 100 ng/ml for all biological samples examined.

Recovery

Blank samples of plasma, muscle and skin homogenate were spiked with a known amount of piroxicam and extracted as previously described.

TABLE III

INTRA- AND INTER-ASSAY COEFFICIENTS OF VARIATION OF PIROXICAM IN SKIN PLASMA SAMPLES

Piroxicam added (μg/ml)	Intra-assay (n = 6)		Inter-assay (n = 6)					
	Mean found (μg/ml)	C.V. (%)	Day 1		Day 2		Day 3	
			Mean found (μg/ml)	C.V. (%)	Mean found (μg/ml)	C.V. (%)	Mean found (μg/ml)	C.V. (%)
0.1	0.102	6.7	0.103	7.8	0.099	7.1	0.102	6.9
8	8.030	2.7	7.990	2.4	8.010	2.5	7.980	2.8
40	40.000	2.6	40.200	2.7	40.000	2.4	39.970	2.5
100	99.870	2.3	100.300	2.1	100.200	2.0	100.000	2.0
250	250.200	2.6	249.600	1.8	250.910	1.6	250.700	1.9

TABLE IV

EXTRACTION RECOVERIES OF PIROXICAM FOR PLASMA, MUSCLE AND SKIN SAMPLES

Concentration (μg/ml)	Recovery (mean ± S.D., n = 3) (%)		
	Plasma	Muscle	Skin
0.1	84.9 ± 1.7	85.2 ± 1.6	85.9 ± 1.2
0.5	85.6 ± 1.9	–	–
1.0	87.3 ± 1.6		–
2.0	87.9 ± 2.0	89.9 ± 1.8	
4.0	–	89.2 ± 1.3	89.7 ± 1.4
5.0	89.1 ± 1.8	–	–
8.0	–	90.6 ± 1.5	91.1 ± 1.9
10.0	90.2 ± 1.4	91.3 ± 1.2	91.9 ± 1.8
20.0	–	91.9 ± 1.3	92.6 ± 2.0
40.0	–	92.4 ± 1.4	
60.0	93.0 ± 1.6	–	
80.0	–	–	94.2 ± 1.9
150.0	–	–	94.8 ± 1.7
250.0	–	–	100.8 ± 2.1

Comparing the spiked plasma, muscle and skin samples with the non-extracted standards gave the mean percentage recoveries and standard deviations shown in Table IV.

Applications

The procedure described above has been employed in a pharmacokinetic study of rats after topical administration of piroxicam (10 mg). The pharmacokinetic parameters calculated in this study, elimination half-life (t_1), area under the curve ($AUC_{0-\infty}$), peak-time (t^2_{max}), maximum observed concentration (C_{max}), are given in Table V. Fig. 1 shows a typical plasma, muscle and skin

TABLE V

PHARMACOKINETIC PARAMETERS OF PIROXICAM FOLLOWING A 10-mg TOPICAL DOSE

Sample	C_{max}	t_{max} (h)	$t_{1/2\alpha}$[a] (h)	$t_{1/2\beta}$[a] (h)	$AUC_{0-\infty}$ (μg/ml/h)
Plasma	58.3 μg/ml	4	11.6	10.9	950
Muscle	23.2 μg/g	1	0.4	185.0	912
Skin	257.4 μg/g	2	1.2	29.5	2710

[a] α and β are rate constants indicating the distribution and elimination phase, respectively.

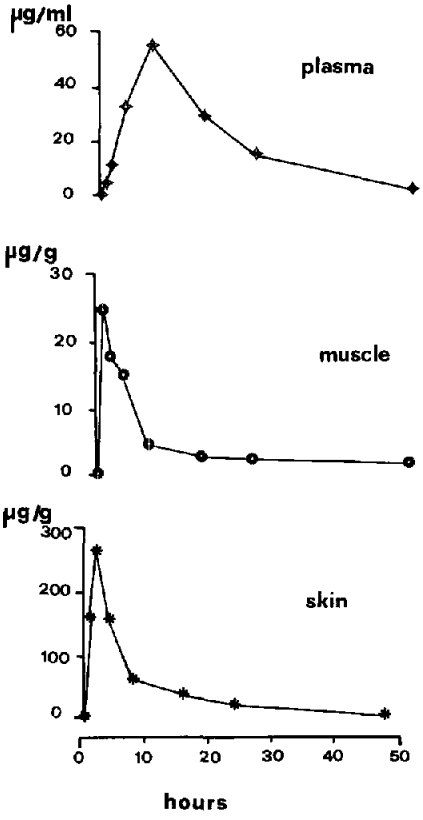

Fig. 1. Plasma, muscle and skin concentration–time curves after topical administration of a 10-mg dose of piroxicam. Each point is expressed as the mean of five animals.

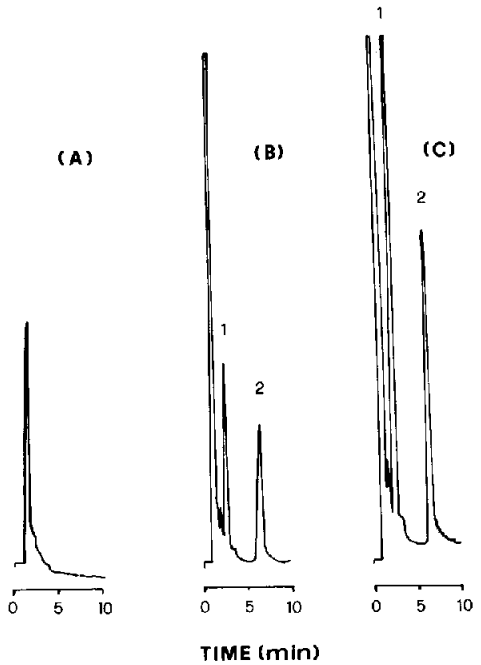

Fig. 2. Representative chromatograms of rat plasma extracts. (A) Blank control plasma; (B) plasma spiked with 2 μg/ml piroxicam; (C) plasma after topical administration of 10 mg of piroxicam. Peaks: 1 = piroxicam; 2 = internal standard.

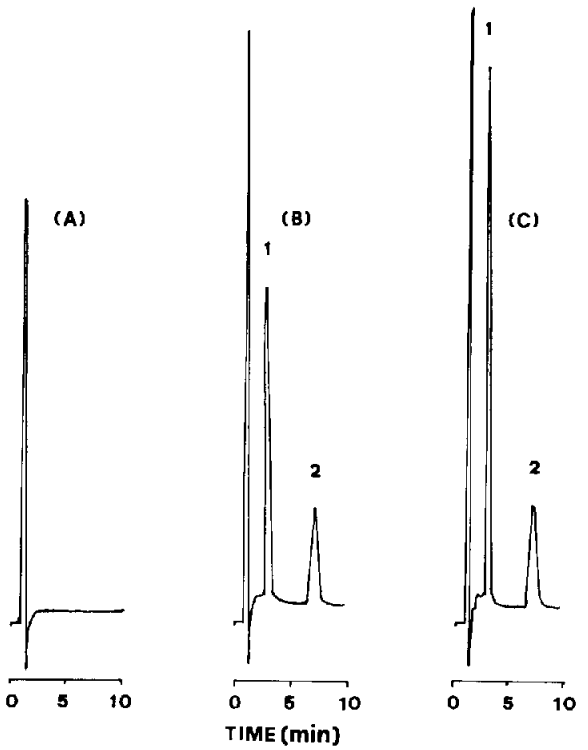

Fig. 3. Representative chromatograms of rat muscle extracts. (A) Blank control muscle; (B) muscle spiked with 2 μg/ml piroxicam; (C) muscle after topical administration of 10 mg of piroxicam. Peaks: 1 = piroxicam; 2 = internal standard.

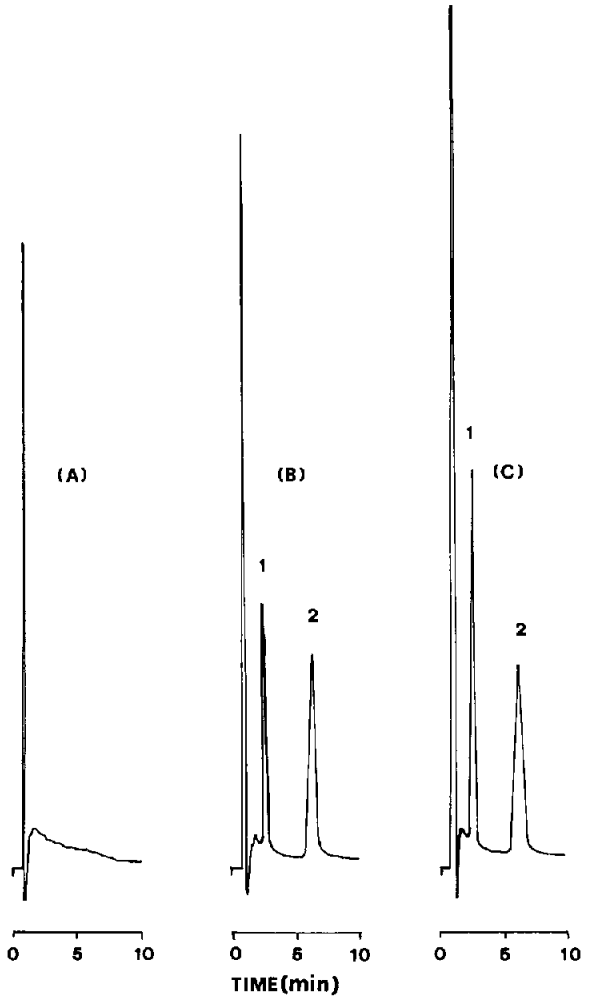

Fig. 4. Representative chromatograms of rat skin extracts. (A) Blank control skin; (B) skin spiked with 2 μg/ml piroxicam; (C) skin after topical administration of 10 mg of piroxicam. Peaks: 1 = piroxicam; 2 = internal standard.

profile obtained following topical administration of piroxicam. The chromatograms of blank control rat plasma, muscle and skin (Figs. 2A, 3A and 4A) show that no peaks corresponding to piroxicam or the I.S. were present in all chromatograms. Figs. 2B, 3B and 4B show chromatograms from rat plasma, muscle and skin samples spiked with 2 μg/ml piroxicam, which is well separated from the front peak; the retention times were 4.2 min for piroxicam and 8.0 min for the I.S. Finally, representative chromatograms of rat plasma, muscle and skin extracts following topical administration of 10 mg of piroxicam are shown in Figs. 2C, 3C and 4C.

CONCLUSION

An HPLC assay for the determination of piroxicam in rat plasma, muscle and skin has been validated. Sample preparation is rapid and provides clean chromatograms. The time required for the elution of piroxicam and the I.S. is less than 9 min. The method is fast and relatively inexpensive. The extraction procedure is simple and results in a good level of precision and extraction efficiency. In addition, the results described in this paper show that this assay is suitable for the study of piroxicam pharmacokinetics.

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