Pergamon

0731-7085(95)01282-6

Journal of Pharmaceutical & Biomedical Analysis, Vol. 13, No. 3, pp. 319–322, 1995

0731-7085/95 $9.50 + 0.00

Short Communication

A rapid method for the determination of piroxicam in plasma by high-performance liquid chromatography

MANUELA T. MAYA,*† JOÃO P. PAIS‡ and JOSÉ A. MORAIS‡

†*Centro de Metabolismos e Genética, Faculdade de Farmácia da Universidade de Lisboa, Av. Forças Armadas, 1600 Lisboa, Portugal*
‡*Faculdade de Farmácia de Universidade de Lisboa, Av. Forças Armadas, 1600 Lisboa, Portugal*

Keywords: *High-performance liquid chromatography; piroxicam; plasma.*

Introduction

Piroxicam is a non-steroidal anti-inflammatory drug (NSAID) used in the treatment of rheumatoid arthritis, osteoarthritis and other inflammatory disorders [1, 2]. Several sensitive methods by high-performance liquid chromatographic (HPLC) techniques have been developed for piroxicam determination in plasma for pharmacokinetic studies, as very low plasma levels of piroxicam are usually obtained [3–9]. Nevertheless, there is a need to improve these methods in terms of ease of sample handling and/or analysis time. With that purpose, buffer solutions were avoided as a component of the mobile phase.

Since droxicam is converted into piroxicam in the gastrointestinal tract and plasma levels of unchanged droxicam are usually not found [10] only plasma prioxicam concentrations were determined. Nevertheless, a method which ensures no interference from droxicam is needed.

The aim of the present study was to develop a rapid, simple but sensitive HPLC isocratic method for the quantitative determination of piroxicam in plasma for use in a comparative bioavailability study after a single oral dose of droxicam and droxicam concurrently administered with an antacid.

Materials and methods

Apparatus

A Merck–Hitachi liquid chromatograph equipped with a reversed-phase column, LiChrospher 60 RP-Select B (250 × 4 mm i.d. particle size 5 μm) (Merck, Darmstadt, Germany) was used.

The solvent was delivered by means of a Merck–Hitachi pump, Model L-6000, which was coupled to a Merck–Hitachi automatic injector, Model AS-2000 A (Merck, Darmstadt, Germany).

A variable wavelength UV–VIS detector, Merck–Hitachi, Model L-4200, operated at 340 nm and at a sensitivity of 0.005 a.u.f.s., was used.

Peak heights were measured by a Hewlett–Packard integrator, Model 3390 A (Avondale, PA, USA) with a chart speed at 0.1 cm min^{-1}.

Reagents and standards

Purified water (Barnstead E-pure purification system, Barnstead Thermolyne, IA, USA) and HPLC-grade methanol (Merck, Darmstadt, Germany) were used throughout. Dichloromethane, 100% acetic acid, hydrochloric acid, sodium sulphate anhydrous and sodium hydroxide were of analytical grade (Merck, Darmstadt, Germany).

Piroxicam and droxicam (supplied by Lab. OM) were used as reference substances at 1 mg ml^{-1} in methanol (stock solution). Isoxicam (the Sigma Chemical Company, St Louis, MO, USA) was used as internal standard at 1 mg ml^{-1} in methanol (stock solution).

The standard solutions were stored protected from light at 4°C. Control plasmas were

* Author to whom correspondence should be addressed.

prepared from working solutions diluted with blank plasma at the concentrations of 0.400 and 0.800 μg ml^{-1}. Blank plasma was obtained from healthy subjects undergoing no drug therapy.

Mobile phase

The mobile phase was a mixture of methanol–water–acetic acid (48:45:7, v/v/v) (pH 2.47) delivered at a flow rate of 1.1 ml min^{-1}. The mobile phase was prepared and degassed daily by passing through a 0.45-μm membrane filter (S-Pak filter, Millipore).

Sample preparation

To plasma (1 ml) in a 16 × 125 mm screw-cap tube, with teflon lining, methanol (100 μl) containing 2.0 μg of internal standard (Isoxicam), and 200 μl of hydrochloric acid 1 N were added.

The tubes were shaken for 30 s at low speed (vortex), and 10 ml of dichloromethane were added. The tubes were capped and vigorously shaken (30 s) and then centrifuged at 2500 r.p.m. for 5 min at room temperature.

The aqueous layer was removed by aspiration and sodium sulphate anhydrous (20 mg) was added to the organic phase.

After filtration (Whatman no. 4) the organic phase was completely evaporated to dryness under nitrogen (35°C). The residue was reconstituted with 200 μl of methanol and fully dissolved by a 30 s vortexing.

A 150-μl volume was transferred to an Eppendorf tube (1.5 ml), centrifuged (5 min, 15 000*g*) and the supernatant was injected onto the HPLC System (20 μl).

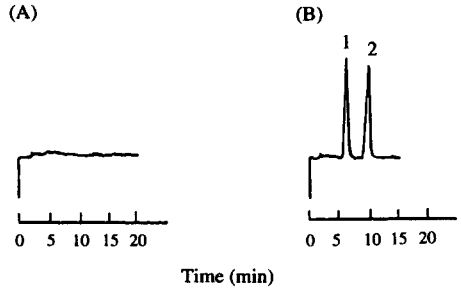

Figure 1
HPLC of piroxicam: (1) piroxicam; (2) isoxicam (internal standard). UV detection: 340 nm. Column: LiChrospher 60 RP-Select B (5 μm). Mobile phase: methanol–water–acetic acid (48:45:7, v/v/v). (A) blank extracted plasma; (B) extracted spiked plasma with 0.800 μg ml^{-1} of piroxicam and 2.00 μg ml^{-1} of internal standard.

Results

Chromatography

Figure 1 shows the chromatograms obtained for blank plasma (A) together with blank plasma spiked with piroxicam (0.800 μg ml^{-1}) and the internal standard (2.00 μg of isoxicam) (B) obtained by means of the described methodology.

Quantitation

Concentrations of piroxicam in unknown samples were determined from the slope of calibration plots of the peak height ratio of piroxicam/internal standard vs the calibration standard piroxicam concentrations.

Linearity

The lineratiry of the method was checked for piroxicam in plasma (0.300–2.00 μg ml^{-1}). Peak height ratios (reference to internal standard) and analyte concentrations were found to be linearly related over this range (Table 1).

Between-day precision

Analysis of pooled plasma spiked with piroxicam (0.400 and 0.800 μg ml^{-1}) gave relative standard deviations (RSD) of 5.82 and 3.91, respectively (Table 2).

Recovery

The mean relative recovery was calculated by dividing the concentrations obtained for the drug-supplemented plasma by the same nominal concentrations utilized for calibration curve. The mean relative recovery for piroxicam in plasma was 100% and ranged from 94.0 to 108% (Table 3) with a RSD of 3.67%.

Extraction reproducibility

Plasma samples (n = 6), prepared by spiking blank plasma with 0.400 and 0.800 μg ml^{-1} of piroxicam, were extracted and injected once on the same day. Mean values of 0.400 and

Table 1
Results of linear regression analysis of calibration data

	Plasma Piroxicam
Slope (b)	0.001272
Intercept (a)	0.0005471
Standard error of slope (S_b)	0.00001670
Standard error of intercept (S_a)	0.03691
Range (μg ml^{-1})	0.300–2.00
Correlation coefficient (r)	0.9988

Table 2
Between-day precision study of Piroxicam in plasma

	Nominal concentration ($\mu g\ ml^{-1}$)	Mean* ($\mu g\ ml^{-1}$)	Measured value RSD %	n†
Piroxicam	0.400	0.400	5.82	9
	0.800	0.804	3.91	18

*Mean values of different spiked plasmas analysed on different days.
†Number of plasma samples analysed for each value. Experimental conditions as described in the text.

Table 3
Percentage of nominal piroxicam concentrations from calibration data

	Spiked value ($\mu g\ ml^{-1}$)	Inversely estimated concentration (I.E.C.) ($\mu g\ ml^{-1}$)	Mean relative recovery* (%)
Piroxicam	0.300	0.302	101
	0.400	0.413	103
	0.500	0.541	108
	0.600	0.593	98.8
	0.800	0.752	94.0
	1.00	0.988	98.8
	1.20	1.205	100
	1.50	1.485	99.0
	2.00	2.018	101
Mean			100
RSD (%)			3.67

*[(IEC)/(Spiked value)] × 100. Two replicate determinations for each concentration. Experimental conditions as described in the text.

Table 4
Mean values of extraction reproducibility

	Spiked value ($\mu g\ ml^{-1}$)	Mean recovered ($\mu g\ mlm^{-1}$)	RSD (%)	n*
Piroxicam	0.400	0.400	3.61	6
	0.800	0.804	2.15	6

*Number of plasma samples analysed for each value. Experimental conditions as described in the text.

0.804 $\mu g\ ml^{-1}$ were obtained with a RSD of 3.61 and 2.15%, respectively (Table 4).

Discussion and Conclusions

Different mobile phase compositions were evaluated in the present study for their ability to separate Px from the internal standard (Ix). One of the principal aims on its selection was to avoid the use of salt buffer solutions, reported by some authors [5, 6, 9]. They are more time consuming on its preparation and can easily cause disturbances in the analytical instrumentation and/or in the life time of the column. Methanol, water and acetic acid were used as components of the mobile phase. No systematic method of optimization was followed except for a trial and error procedure, following the general rules of solvent dependent order of elution.

The HPLC method described is selective and no endogenous interfering peaks were visible in blank plasma (Fig. 1A). The two peaks of piroxicam and isoxicam (internal standard) are well separated with an isocratic mobile phase with retention times of 6.10 and 9.75 min, respectively (Fig. 1B). Moreover, no interference from droxicam was observed by direct injection of a mixture of droxicam and piroxicam which exhibited a resolution factor of 1.82. To achieve clean samples an extraction procedure was used with dichloromethane. Recently, Saeed [8] proposed an on-line solid-phase extraction where the plasma is directly injected onto the HPLC system. Nevertheless, a special column-switching system is necessary.

To prolong the lifetime of the column by protecting it from some interfering substances a guard-column was used. The calibration plot of peak height ratio (piroxicam/internal standard) is linear over the range of 0.300–2.00 $\mu g\ ml^{-1}$ and the precision of the assay is acceptable as shown in Table 2. The precision of the method, calculated from the calibration curve, shows a relative standard deviation (RSD) of 3.67%.

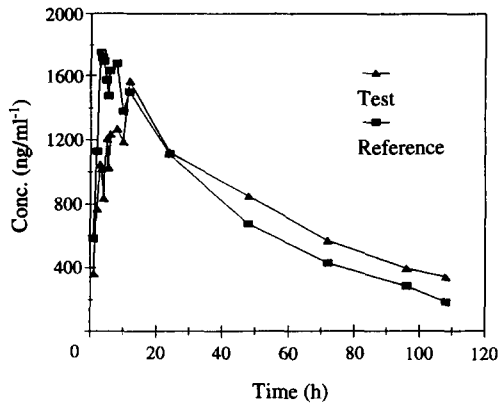

Figure 2
Concentration vs time curve of piroxicam in plasma of a healthy volunteer who was given a single oral dose of 20 mg of droxicam (test and reference preparations), in a bioavailability study.

The limit of quantification of the assay is 2.0 ng when a signal-to-noise ratio of 5 was used as criterion for a significant response. This corresponds to a concentration of 20 ng ml^{-1} in plasma under the assay conditions which is substantially lower than the one reported by Milligan [9] but higher than Boudinot's [6].

The simplicity of the method with the simultaneous utilization of an automatic injector allows the processing of over 100 samples in 1 day, which is an advantage in a bioavailability study as a large number of samples have to be analysed.

The method reported here shows good characteristics (linearity, recovery, sensitivity and precision) and is adequate for the evaluation of piroxicam in plasma, in pharmacokinetic studies over the collection period, in order to clearly define the absorption and elimination phase of piroxicam (Fig. 2).

Acknowledgement — This work was supported by OM — Portuguesa, Laboratórios de Especialidades Farmacêuticas S.A.

References

[1] N.E. Pitts and R.R. Proctor, in *Piroxicam* (W.M. O'Brien and E.H. Wiseman, Eds), p. 97. Academic Press, London (1978).
[2] R.N. Brogden, R.C. Heel, T.M. Speight and G.S. Avery, *Drugs* **28**, 292–296 (1984).
[3] J.S. Dixon, J.R. Lowe and D.B. Galloway, *J. Chromatogr.* **310**, 455–459 (1984).
[4] C.J. Richardson, S.G. Ross, K.L. Blocka and R.K. Verbeeck, *J. Chromatogr.* **382**, 382–386 (1986).
[5] J. Macek and J. Vacha, *J. Chromatogr.* **420**, 445–449 (1987).
[6] F.D. Boudinot and S.S. Ibrahim, *J. Chromatogr.* **430**, 424–428 (1988).
[7] R.B. Gillilan, W.D. Mason and C.H. Fu, *J. Chromatogr.* **487**, 232–235 (1989).
[8] K. Saeed and M. Becher, *J. Chromatogr.* **567**, 185–195 (1991).
[9] P.A. Milligan, *J. Chromatogr.* **576**, 121–128 (1992).
[10] L. Martinez, J. Sanchez, R. Roser *et al.*, *Meth. Find. Exptl. Clin. Pharmacol.* **10**, 729–737 (1988).

[Received for review 6 July 1994; revised manuscript received 24 October 1994]