Pergamon

0731-7085(95)01289-3

Journal of Pharmaceutical & Biomedical Analysis, Vol. 13, No. 6, pp. 785–789, 1995
Copyright © 1995 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0731–7085/95 $9.50 + 0.00

Short Communication

A reproducible and rapid HPLC assay for quantitation of piroxicam in plasma

L. EDNO,† F. BRESSOLLE,*†‡ B. COMBE§ and M. GALTIER†

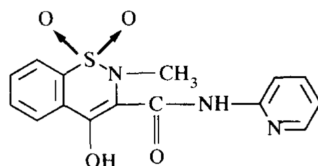
† *Laboratoire de Pharmacocinétique, Hôpital Carémeau, 30900 Nîmes Cedex, France*
‡ *Laboratoire de Pharmacocinétique, Faculté de Pharmacie, 34060 Montpellier Cedex 01, France*
§ *Service de Rhumatologie, Hôpital Carémeau, 30900 Nîmes Cedex, France*

Keywords: *Piroxicam; HPLC method; plasma.*

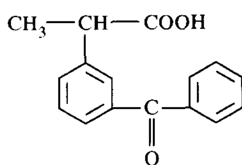
Introduction

Piroxicam (4-hydroxy-2-methyl-N-(2-pyridyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide) (Fig. 1) is a non-steroidal anti-inflammatory and analgesic agent. Its efficacy has been demonstrated in humans for the treatment of various inflammatory diseases and arthropathies, such as rheumatoid arthritis and osteoarthritis. Several methods for the detection and quantitation of piroxicam in biological fluid have been developed. These methods involve reversed-phase HPLC with UV detection [1–9]. Most of these methods required liquid–liquid extraction with evaporation of the extract, an on-line solid-phase extraction procedure has also been described [2]. Furthermore, rapid HPLC procedures have been developed to quantify piroxicam in human plasma [9] and in plasma and blood of small laboratory animals [1].

This report describes a rapid and reproducible method for the determination of piroxicam in plasma. The sample preparation only involves protein precipitation, lipid extraction and centrifugation, no evaporation step is required. This method was validated according to GLP guidelines [10–13].



Piroxicam



Ketoprofen

Figure 1
Chemical structures of prioxicam and ketoprofen.

Experimental

Materials

Piroxicam was obtained from Pfizer laboratories (Paris, France). The internal standard (ketoprofen) was obtained from the Sigma Chemical Company (St Quentin Fallavier, France) (Fig. 1). Stock solutions of piroxicam and ketoprofen (0.1 and 4 mg ml^{-1}, respectively) were prepared in methanol. Acetonitrile, dichloromethane and methanol were of HPLC grade (SDS, France). Disodium hydrogenphosphate, orthophosphoric acid were all analytical grade and were purchased from Merck (Darmstadt, Germany). The buffer (pH 8) consisted of 0.04 M disodium hydrogen-

* Author to whom correspondence should be addressed.

phosphate in aqueous solution, adjusted with orthophosphoric acid to pH 8.

Pooled plasma samples from healthy volunteers were used for the validation of the method.

Instrumentation

Analysis by HPLC was performed using a Gilson instrument with a model 805 manometric module, a model 305 pump, an automatic sample injection system (Gilson 232) equipped with a 50-μl loop, a stainless-steel column (250 × 4.6 mm i.d.; SFCC, Neuilly Plaisance, France) packed with Spherisorb ODS (5 μm), and a guard column (20 × 4.6 mm i.d.; SFCC, Neuilly Plaisance, France) packed with Spherisorb C8 (10 μm) placed just before the inlet of the analytical column. All the chromatographic conditions were controlled using the GME 712 Gilson software. The eluent was monitored with a variable wavelength UV detector (model SPD-6AV; Shimadzu Instruments, Touzart Matignon, France) operated at 360 nm.

Chromatographic conditions

The mobile phase, containing 60 parts buffer solution and 40 parts methanol, was degassed ultrasonically before use. Buffer solution and methanol were filtered through a membrane filter (0.45 μm; Millipore, Molsheim, France). The oven temperature was 50°C, and the flow rate was 1 ml min^{-1}, which corresponds to a pressure of about 175 bars.

Extraction procedure

After thawing, plasma samples (0.5 ml) were spiked with internal standard solution (20 μl) and homogenized. Acetonitrile (0.5 ml) was added to all samples and the mixture was mixed by vortex for 10 s, then all vials were centrifuged at 1500 *g* for 10 min. An aliquot (0.8 ml) of the supernatant was pipetted into a 10 ml glass tube then 5 ml dichloromethane was added. The extracts were vortex mixed for 30 s then centrifuged for 10 min. The aqueous solution was separated and 50 μl of this solution were injected into the chromatograph.

Instrument calibration

Calibration standards for control plasma were prepared using concentrations of 0.3, 0.4, 1, 2, 3, 4 and 8 μg ml^{-1} in human plasma. The standard samples were prepared by adding appropriate volumes of piroxicam solution. The volume added was always smaller than or equal to 2% of total volume of the sample, so that the integrity of the sample was maintained.

These standards were treated concurrently and in the same manner as the unknown samples to be analysed.

Data analysis

For plasma, the ratio of the peak height of piroxicam to that of internal standard was used as the assay parameter. Peak height ratios were plotted against theoretical concentrations. Standard calibration lines were obtained from unweighted least-squares linear regression analysis of the data.

The linearity of the method was statistically tested.

Precision and accuracy

Between- and within-run accuracy and precision in human plasma were assessed by performing replicate analysis of spiked samples (0.6, 3, 6 μg ml^{-1}) against calibration standards. The precision and accuracy of the method were calculated as percentage deviation of observed concentration from theoretical concentration and absolute error, respectively.

Recovery

The extraction efficiency (recovery) was determined by computing the ratio of the amount of the extracted compound from drug-free plasma spiked with known amounts of piroxicam (0.3, 2, 8 μg ml^{-1}) to the amount of the compound added at the same concentrations to water just before HPLC injection.

Determination of the limit of quantitation (LOQ)

The LOQ was determined from the peak and the standard deviation of the noise level. The LOQ was defined as the sample concentration of piroxicam resulting in a peak height of 10-times the noise level. The estimate of noise level was determined by extrapolation to zero. To determine the analytical error on the LOQ, spiked plasma was used.

Stability study

The stability of piroxicam was assessed during all the storage steps and during all steps of the analytical method.

During the first days of the study, quality control samples (in plasma) were spiked with standard solutions of piroxicam (0.6, 3 and 6 μg ml^{-1}). Then the aliquoted quality control samples were placed in freezer storage at −20°C and randomly removed at various times in each analytical sequence during a 1 month period. The ambient stability in the auto-sampler was assessed for all concentrations of the calibration curve after 12 and 24 h.

Results

Retention times

Observed retention time were 4.4 and 8.6 min for piroxicam and ketoprofen, respectively. The capacity factors were 2.10 for piroxicam and 5.06 for internal standard. There were no interfering peaks in control plasma at the retention time of the respective analytes (Fig. 2a). An HPLC chromatogram of plasma sample is shown in Fig. 2(b).

Linearity

The peak height ratio of piroxicam to internal standard varied linearly with the concentration over the range used (0.3–8 μg ml^{-1}) (Table 1). The linearity of this method was statistically confirmed. For each calibration curve, the intercept was not statistically different from zero. The correlation coefficients (r) for calibration curves were equal to or better than 0.997. The relative standard deviation (RSD) values of the slope were equal to or better than 6%. For each point of calibration standards, the concentrations were recalculated from the equation of the linear regression curves (experimental concentrations). Inter- and intraday variabilities in plasma containing piroxicam at concentrations of calibration

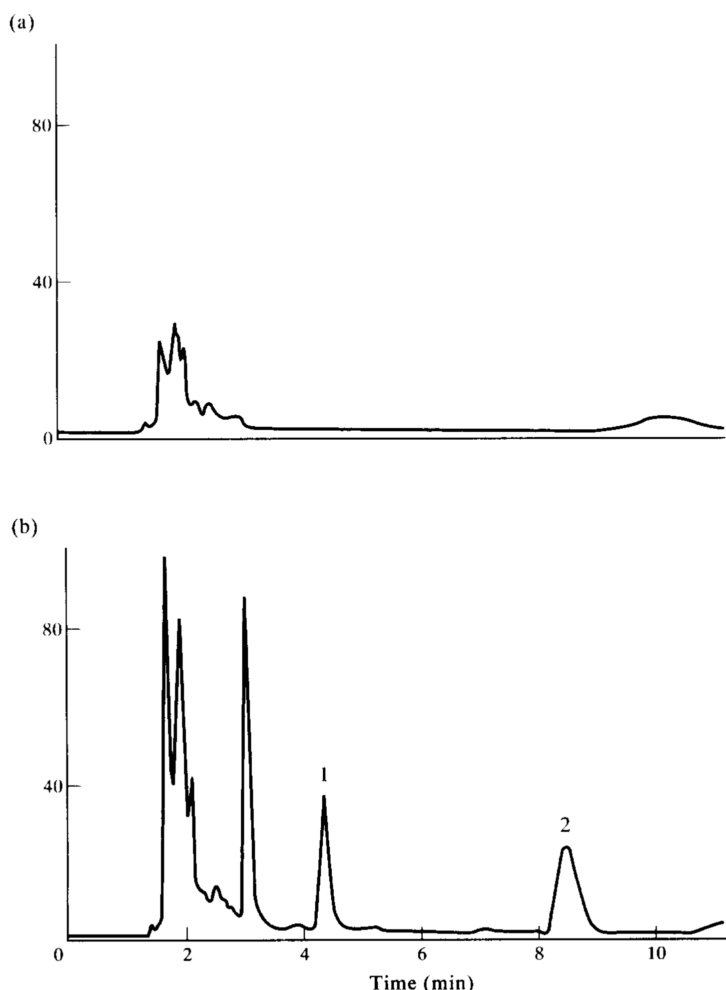


Figure 2
Representative chromatograms of blank plasma (a) and of plasma sample from an arthritis patient containing 4.39 μg ml^{-1} of piroxicam. Peaks: (1) piroxicam; (2) ketoprofen. For chromatographic conditions, see text. Analysis: 20 mV FS.

Table 1
Assay linearity

	Coefficient of the linear regression analysis ($r \pm$ SD)	Slope ± SD	Intercept ± SD
Intra-assay $n = 6$	$0.9991 \pm 5.79 \times 10^{-4}$ RSD = 0.0579%	0.255 ± 0.0145 RSD = 5.69%	$0.0129 \pm 7.031 \times 10^{-3}$
Inter-assay $n = 15$	$0.999 \pm 9.585 \times 10^{-4}$ RSD = 0.0959%	0.242 ± 0.0146 RSD = 6.03%	0.0176 ± 0.0120

Table 2
Inter-assay reproducibility of the HPLC analysis ($n = 15$)

Theoretical concentration μg ml^{-1}	Experimental concentration (mean ± SD), μg ml^{-1}	RSD %
0.3	0.332 ± 0.020	6.0
0.4	0.456 ± 0.054	11.7
1	1.06 ± 0.067	6.33
2	2.02 ± 0.018	8.79
3	2.83 ± 0.20	6.94
4	4.14 ± 0.037	0.88
8	8.01 ± 0.074	0.92

Table 3
Intra-assay reproducibility of the HPLC analysis ($n = 6$)

Theoretical concentration μg ml^{-1}	Experimental concentration (mean ± SD), μg ml^{-1}	RSD %
0.3	0.356 ± 0.036	10.0
0.4	0.443 ± 0.060	13.5
1	0.978 ± 0.042	4.31
2	1.87 ± 0.097	5.21
3	3.09 ± 0.076	2.47
4	4.04 ± 0.154	3.81
8	7.97 ± 0.097	1.21

Table 4
Accuracy and precision of HPLC method

Theoretical concentration μg ml^{-1}	n	Experimental concentration (mean ± SD), μg ml^{-1}	RSD %	Deviation from theoretical value %
Within-day				
0.6	4	0.595 ± 0.027	4.54	0.8
3	5	3.11 ± 0.153	4.92	3.7
6	4	6.32 ± 0.482	7.63	5.3
Between-day				
0.6	6	0.601 ± 0.063	10.45	0.2
3	6	3.19 ± 0.209	6.56	6.3
6	6	5.78 ± 0.709	12.26	3.7

standards are given in Tables 2 and 3, respectively. The small percentage differences between nominal and found concentrations of the standards in the standard curves for both intra and interday data confirmed that the assay was linear over the concentration range investigated.

Precision and accuracy

Between-day and within-day precision in human plasma were assessed by performing replicate analyses of spiked samples against calibration standards. The procedure was repeated the same day and for different days on the same spiked standards at concentrations

in range of the standard series (0.6, 3, 6 mg l^{-1}). The precision and accuracy of the method are presented in Table 4.

Recovery

In the range of calibration standards, the mean recovery of piroxicam was 95 ± 3.8% (n = 9).

Limit of quantitation

The limit of quantitation was 0.3 μg ml^{-1} for piroxicam. At this level, the relative standard deviation (RSD) was lower than 15%.

Limit of detection

The limit of detection which represents a signal noise ratio of 3:1 was 0.1 μg ml^{-1}.

Stability

The stability of piroxicam in the autosampler was checked after 12 and 24 h at room temperature, for each point of calibration standards in plasma. For all concentrations, no significant difference appeared between $t = 0$, $t = 12$ h and $t = 24$ h.

The stability of piroxicam in plasma was also assessed after 30 days of cold storage (frozen at −25°C). No significant difference appeared.

Discussion and Conclusion

The present HPLC method involves a rapid assay for the determination of piroxicam in plasma. Its limit of quantitation is similar to that reported by Troconiz *et al.* [1] in rat plasma but better than the one reported by Michotte *et al.* [9] (0.3 μg ml^{-1} instead of 0.5 μg ml^{-1}). This technique is extremely simple and has good reproducibility, recovery and accuracy. It has been validated according to the report of the conference on analytical method validation [10].

This method is sufficiently sensitive to monitor plasma levels of piroxicam following administration of a 20-mg dose. It has been successfully applied to check the compliance to treatment for patient with rheumatological disorders.

Acknowledgement — The authors gratefully acknowledge support of this work by Pfizer Laboratories, Paris, France.

References

[1] J.I.F. Troconiz, L.G. Lopez-Bustamante and D. Fos, *Arzneim-Forsch./Drug Res.* **43**, 679–681 (1993).
[2] K. Saeed and M. Becher, *J. Chromatogr.* **567**, 185–193 (1991).
[3] P.A. Milligan, *J. Chromatogr.* **576**, 121–128 (1992).
[4] F. Douglas Boudinot and S.S. Ibrahim, *J. Chromatogr.* **430**, 424–428 (1988).
[5] R.B. Gillilan and W.D. Mason, *J. Chromatogr.* **487**, 232–235 (1989).
[6] D. Cerretani, L. Micheli, A.I. Fiaschi and G. Giorgi, *J. Chromatogr.* **614**, 103–108 (1993).
[7] F. Lapicque, P. Netter, B. Bannwarth, P. Trechot, P. Gillet, H. Lambert and R.J. Royer, *J. Chromatogr.* **496**, 301–320 (1989).
[8] C.J. Richardson and S.G. Ross, *J. Chromatogr.* **382**, 382–388 (1986).
[9] Y. Michotte, H.P. Van Klaveren, M.R. Detaevernier, Ch.F. Gusdorf and L. Vanhaelst, *Arzneim-Forsch./Drug Res.* **41**, 244–246 (1991).
[10] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Yacohi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pittman and S. Spector, *J. Pharm. Sci.* **81**, 309–312 (1992).
[11] F. Bressolle, J. Brès and A. Moulin, *J. Pharm. Sci.* **79**, 534–539 (1990).
[12] J.M. Jacquet, M. Galtier, F. Bressolle and J. Jourdan, *J. Pharm. Biomed. Anal.* **10**, 343–348 (1992).
[13] J.M. Kinowski, F. Bressolle, D. Fabre, F. Goncalves, R. Panis and M. Galtier, *J. Pharm. Sci.* **83**, 738–741 (1994).

[*Received for review 7 July 1994*]