ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
28 (2002) 1201–1204

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Short communication

LC determination of piroxicam in human plasma

S. Dadashzadeh *, A.M. Vali, N. Rezagholi

Department of Pharmaceutics, Faculty of Pharmacy, Shaheed Beheshti University of Medical Sciences, P.O. Box 14155-6153, Tehran, Iran

Received 11 October 2001; received in revised form 4 November 2001; accepted 1 December 2001

Keywords: Piroxicam; HPLC; Plasma

1. Introduction

Piroxicam [4-hydroxy-2-methyl-*n*-(2-pyridyl)-2H-1, 2-benzothiazine-3-carboxamide-1, 1-dioxide] is a non-steroidal anti-inflammatory drug with analgesic and anti-pyretic activity. It is widely used for the treatment of various inflammatory diseases and arthropathies, such as rheumatoid arthritis and osteoarthritis [1,2].

Early studies on the pharmacokinetics of piroxicam employed a degradative fluorometric 'wet' chemical analytical technique [3]. This technique lacked sensitivity and selectivity and was replaced by a number of HPLC methods with UV detection [4–14]. Most of these methods require liquid–liquid extraction with consecutive evaporation. The extraction procedure is prone to complications because it involves several separate steps, which not only make the method tedious and time consuming but also increase the potential of introducing a bias in the results.

Few HPLC methods using simple method of plasma protein precipitation for sample preparation have been reported [15,16]. These methods require a high temperature of column to be maintained during the run time. Moreover the method proposed by Macek and Vacha suffers from lack of sufficient sensitivity (limit of detection, LOD = 0.15 μg ml^{-1}) [15].

This paper describes a simple and reliable HPLC method for the determination of piroxicam in plasma. The sample preparation only involves protein precipitation; no evaporation and extraction steps are required. The simplicity along with higher sensitivity and avoiding the requirement for a high temperature of column are the main advantageous of this method over the previously reported ones.

2. Experimental

2.1. Materials

Piroxicam and naproxen (internal standard) were obtained from Sigma chemical co. (St. Louis, MO).

* Corresponding author. Tel.: +98-21-8773521; fax: +98-21-8795008.
E-mail address: dadashzadeh5@yahoo.com (S. Dadashzadeh).

PII: S0731-7085(02)00025-0

HPLC grade acetonitrile and methanol as well as analytical grade sodium acetate and glacial acetic acid were purchased from Merck (Darmstadt, Germany).

2.2. Instruments

The HPLC system consisted of a model 590 Waters solvent delivery pump, an auto injector system (Rheodyne) equipped with a 50 μl loop and a model 486 Waters variable wavelength UV detector. Chromatographic separation was achieved at ambient temperature using a Novapak C_{18} column (4 μm, 25 cm × 4.6 mm) and a C_{18} precolumn (10 μm, 20 × 3.9 mm^2) (all from Waters Assoc. MA).

2.3. Chromatographic conditions

The mobile phase consisted of 0.1 M sodium acetate–acetonitrile–triethylamine (61:39:0.05 V/V) adjusted to pH 4 with glacial acetic acid. All separations were carried out at room temperature and a flow-rate of 1.5 ml min^{-1}, detector sensitivity of 0.01 absorbance units and a chart speed of 0.25 cm min^{-1}. Eluted peaks were detected at 330 nm.

2.4. Sample preparation

To 250 μl of plasma sample were added, methanolic solution of naproxen (20 μl; 50 μg ml^{-1}) as I.S., 200 μl of acetonitrile and 50 μl of methanol in a micro tube. The mixture was vortex-mixed for 1 min. After centrifugation at 8000 rpm for 10 min the supernatant was separated and a sample of 50 μl was injected into the liquid chromatograph.

2.5. Standard preparation

Known amounts of piroxicam were added to pooled drug free plasma to achieve standard solutions containing 0.1, 0.25, 0.5, 1, 1.5, 2.5, 4 and 6 μg ml^{-1} that were used to constructing standard curves.

2.6. Validation procedure

A full validation of the assay consisting of linearity, lower limit of quantitation (LOQ), LOD, recovery of the analytes from plasma, intra-day and inter-day accuracy and precision of the method was performed.

3. Results and discussion

3.1. Sample preparation and chromatography

Several HPLC methods have been reported for quantitation of piroxicam in plasma [4–16]. Most of these methods require liquid–liquid extraction with consecutive evaporation, which is time-consuming and tedious. Therefore, we evaluated various protein precipitation methods for sample preparation to make easier and speed up the analysis. Although these techniques are simple compared to extraction procedures but most of them yielded low recovery of piroxicam that may be due to its strong and high plasma protein binding. Employing a combination of acetonitrile and methanol for protein precipitation resulted in a high recovery of analyte without increase of endogenous peaks. The composition and pH of the mobile phase were varied to achieve the optimum chromatographic conditions.

The chromatograms of a blank plasma, a plasma spiked with piroxicam to a concentration of 1 μg ml^{-1} and a plasma sample taken from a volunteer 24 h after oral administration of 20 mg piroxicam are illustrated in Fig. 1. The chromatographic method yields sharp, well-resolved and symmetrical peaks without interferences from endogenous plasma components.

The average retention times of piroxicam and I.S. were 6.6 and 11.3 min, respectively.

3.2. Linearity

The calibration curve for the detection of piroxicam was linear over the concentration range of 0.1–6 μg ml^{-1} and the corresponding regression equation was $y = 0.972x - 0.011$ ($r^2 = 0.9996$), where y is the peak height ratio of piroxicam to

I.S. and x is the concentration of piroxicam ($\mu g\,ml^{-1}$) in plasma. The relative standard deviation (RSD) values of the slope and intercept were less than 5.8 and 6.4%, respectively.

3.3. Assay recovery

Analytical recovery from plasma for four different concentration of piroxicam was determined. A mean recovery of $100.09 \pm 6.52\%$ was observed in the concentration range of 0.1–3 $\mu g\,ml^{-1}$.

3.4. Limit of detection and limit of quantitation

The LOD with considering signal to noise ratio of 3 was 0.02 $\mu g\,ml^{-1}$ and the LOQ was 0.06 $\mu g\,ml^{-1}$. At this level the RSD was less than 10%.

3.5. Assay precision and accuracy

The intra-day and inter-day precision and the accuracy of the method were determined by analyzing multiple sets of spiked plasma samples. For inter-day evaluation spiked plasma samples at four concentrations (0.1, 0.5, 1 and 2.5 $\mu g\,ml^{-1}$) were prepared and analyzed every other day for 11 days. For intra-day assessment replicate measurements were made on the same samples on the same day. The results presented in Table 1 indicate that overall C.V. of the method ranges between 3.17 and 5.37% and corresponding R.E. is in the range of 3.48–6.11%.

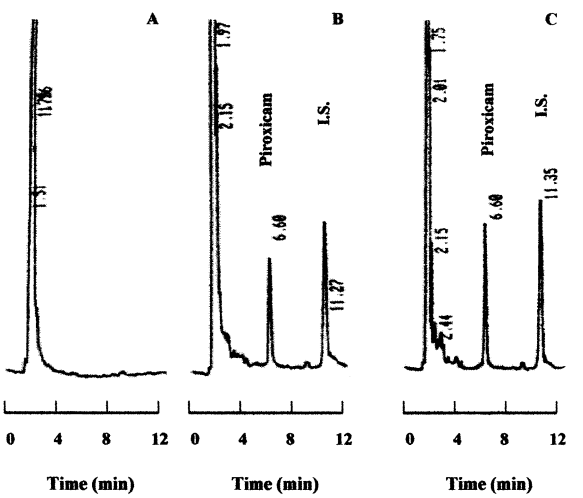

Fig. 1. Chromatograms obtained from blank plasma (A), a plasma spiked with 1 $\mu g\,ml^{-1}$ of piroxicam and 4 $\mu g\,ml^{-1}$ naproxen as I.S. (B) and a plasma sample taken 24 h after administration of a single oral dose of 20 mg piroxicam (C).

Table 1
Intra-day and inter-day precision and accuracy of HPLC method ($n = 6$)

Concentration ($ng\,ml^{-1}$)	C.V. (%)		Error (%)	
	a	b	a	b
100	4.19	5.37	6.11	6.63
500	3.17	3.23	3.78	5.50
1000	5.21	4.32	6.42	6.13
2500	4.78	4.87	3.78	3.89

a, intra-day; b, inter-day.

3.6. Sample stability

The stability of piroxicam was assessed during all the storage steps. Samples containing known concentrations of piroxicam maintained at 4 and -20 °C for a 1-month period.

These samples were randomly removed at various times and analyzed by the assay method. No significant trend in sample concentration change was seen following storage at the 4 and -20 °C over 1-month period.

3.7. Application of the assay to a pharmacokinetic study

The method was applied to a pharmacokinetic study of piroxicam in healthy volunteers. Mean plasma concentration–time profile of piroxicam following administration of a 20 mg oral dose to 12 subjects is shown in Fig. 2. The maximum plasma concentration of piroxicam was on average 20 times higher than the LOQ, which indicates the suitability of the analytical method for single dose pharmacokinetic study of piroxicam.

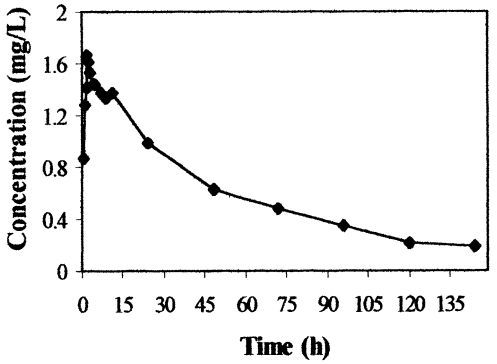

Fig. 2. Mean plasma concentration–time profile of piroxicam following administration of a 20 mg oral dose to 12 healthy subjects.

4. Conclusions

A reliable HPLC method is described for quantitation of piroxicam in plasma, which utilizes a short and simple extraction less sample preparation technique with adequate sensitivity required for pharmacokinetic studies. The validation data demonstrates good precision and accuracy with lower LOD value than most of the previously published methods. 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 analyzed.

References

[1] R.K. Verbeeck, C.J. Richardson, L.N. Blocka, J. Rheumatol. 13 (1986) 789–796.
[2] R.N. Brogden, R.C. Heel, T.M. Speight, G.S. Avery, Drugs 28 (1984) 292–296.
[3] C.H. Donald, T.M. Twomey, J. Clin. Pharm. 19 (1979) 270–276.
[4] J.S. Dixon, J.R. Lowe, D.B. Galloway, J. Chromatogr. 310 (1984) 455–459.
[5] C.J. Richarson, S.G. Ross, J. Chromatogr. 382 (1986) 382–388.
[6] F.D. Boudinot, S.S. Ibrahim, J. Chromatogr. 430 (1988) 424–428.
[7] R.B. Gillilan, W.D. Mason, C.H.J. Fu, J. Chromatogr. 487 (1989) 232–235.
[8] K. Saeed, M. Becher, J. Chromatogr. 567 (1991) 185–195.
[9] S. Wanwimorluk, S.Z. Wanwimorluk, A.R. Zoest, J. Liq. Chromatogr. 14 (1991) 2373–2381.
[10] P.A. Milligan, J. Chromatogr. 576 (1992) 121–128.
[11] J.I.F. Troconiz, L. Bustamante, D. Fos, Arzneim-Forsch. Drug Res. 43 (1) (1993) 679–681.
[12] D. Cerretani, L. Micheli, A.I. Fiaschi, G. Giorgi, J. Chromatogr. 614 (1993) 103–108.
[13] T. Maya, J.P. Pais, J.A. Morais, J. Pharm. Biomed. Anal. 13 (1995) 319–322.
[14] L. Endo, F. Bressolle, B. Combe, M. Galtier, J. Pharm. Biomed. Anal. 13 (1995) 785–789.
[15] J. Macek, J. Vacha, J. Chromatogr. 420 (1987) 445–449.
[16] A. Avgerinos, S. Axarlis, J. Dragatsis, T. Karidas, S. Malamataris, J. Chromatogr. B 673 (1995) 142–146.