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Technical note

Rapid method for the determination of piroxicam in rat plasma using high-performance liquid chromatography

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Abstract

A previously published method was used for the determination of piroxicam in plasma samples obtained from rat. The sample preparation involved liquid extraction, centrifugation and evaporation. Separation of piroxicam from internal standard occurred on a reversed-phase C_{18} column with a mobile phase consisting of methanol–phosphate buffer pH 2 (45:55). The detection limit of the assay was 0.02–20 μg/ml. The assay linearity was good (typically $r=0.9992$). The method was applied for determination of piroxicam in rats after administration of an oral dose of 2 mg/kg piroxicam. © 1997 Elsevier Science B.V.

Keywords: Piroxicam

1. Introduction

Piroxicam, a non-steroidal anti-inflammatory (NSAID) drug is used in the treatment of rheumatoid arthritis and osteoarthritis [1]. Several high-performance liquid chromatographic (HPLC) methods have been developed for measurement of piroxicam in human plasma [2–5]. However, few methods are presented for detection of piroxicam in rat [6–8]. We encountered some practical difficulties when using the above mentioned methods to detect piroxicam in rat plasma.

The present paper reports a sensitive, rapid and accurate high-performance liquid chromatographic (HPLC) method for quantitation of piroxicam in plasma samples obtained from rat.

2. Experimental

2.1. Materials and equipment

Piroxicam USP reference standard, tenoxicam, methanol (HPLC grade), anhydrous citric acid, phosphoric acid, sodium dihydrogen phosphate and ethyl ether were of analytical reagent grade and were obtained commercially. Deionized water was used for preparation of all aqueous standard and buffer solutions. The high-performance liquid chromato-

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graph (HPLC) system consisted of a Waters (Milford, MA, USA) analytical liquid chromatograph equipped with a 300 mm×3.9 mm I.D., 10 μm, μBondapak ODS (C_{18}) column, Waters 510 HPLC pump, Waters 490E multi-wavelength programmable detector and Waters 746 data module.

2.2. Chromatographic separation

A mobile phase of methanol–10 m*M* phosphate buffer pH 2 (45:55, v/v) was filtered, degassed and used at a flow-rate of 1.5 ml/min. The elutes were monitored at 361 nm with detector range setting fixed at 0.01 AUFS. Under these conditions, the retention times were 5.81 min for tenoxicam and 9.85 min for piroxicam (Fig. 1).

2.3. Standard solution

Stock solutions of 1 mg/ml piroxicam and internal standard (tenoxicam) were prepared in methanol. Appropriate dilution of these solutions were made with water to produce working solutions containing 200, 10, 2, 0.2 μg/ml for piroxicam and 40 μg/ml for internal standard. Plasma calibration standards containing 0.02–20 μg/ml piroxicam were prepared by diluting the working solution with blank plasma; the final volume of all plasma calibration standards was 1.0 ml.

2.4. Sample preparation

Determination of piroxicam was performed by an adaptation and modification of the method described

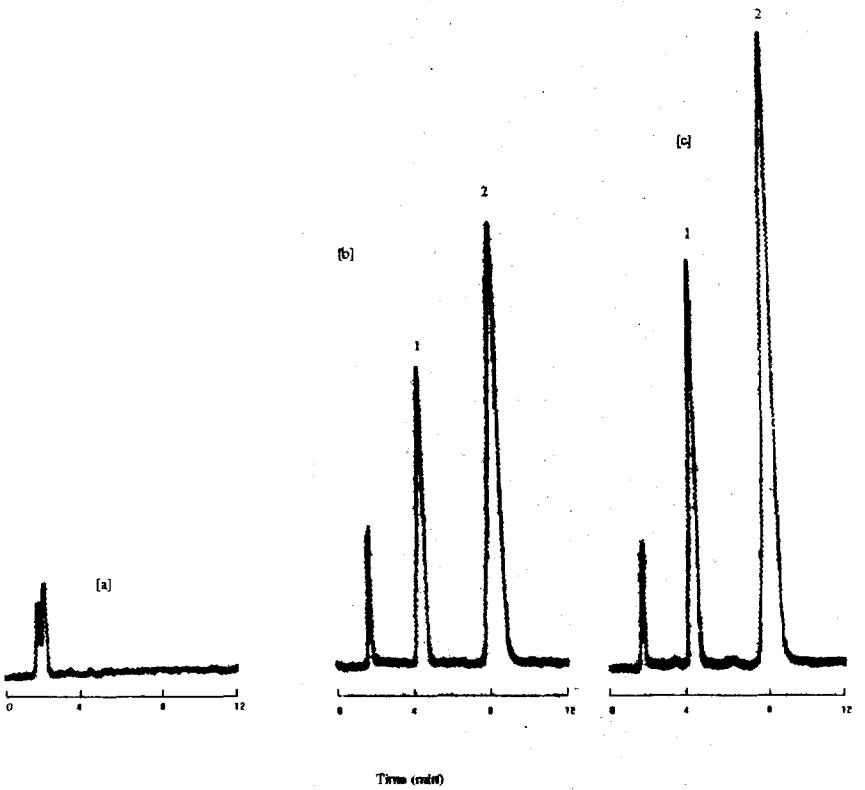

Fig. 1. Chromatograms of blank plasma (a), spiked plasma containing 1 μg/ml piroxicam (b), rat plasma 4 h after receiving single 2 mg/kg oral dose of piroxicam (c). Peaks: 1-tenoxicam (I.S.), 2=piroxicam.

by Heizmann et al. [4] and the method reported by Dixon et al. [3]. To 1 ml of plasma in 15 ml conical glass tubes with screw caps (PTFE-lined) were added 50 μl of 40 μg/ml tenoxicam solution (as internal standard), 1 ml phosphate buffer pH 2 and 10 ml diethyl ether. The tubes were capped, vortex-mixed for 1 min and centrifuged (5 min, 1300 *g*). The solvent layer was transferred to another tube and evaporated to dryness under a stream of nitrogen. The residue was reconstituted in 100 μl hydrochloric acid (0.01 *M*) in methanol and 40 μl of this solution was injected onto the HPLC system.

3. Results and discussion

Chromatograms of extracts of blank plasma, blank plasma spiked with piroxicam and internal standard and a plasma sample taken 4 h after a single oral dose of 2 mg/kg piroxicam in rat are shown in Fig. 1. No endogenous interfering peaks were visible in blank plasma. The two peaks are well separated with retention times of 5.18 and 9.85 for internal standard and piroxicam, respectively. Analysis of a series of five known piroxicam concentrations in plasma, ranging from 0.2–20 μg/ml, yielded a straight calibration curve by a weighted ($1/y$) linear regression line (r=0.9992) when peak area ratio (piroxicam/internal standard) was plotted against the concentrations of piroxicam. The mean extraction recovery for piroxicam was found to be 82±6% (S.D.). Replicate determinations of 1.0 and 10 μg/ml samples of piroxicam gave within-day coefficients of variation (C.V.) of 2.3 and 2.2% and day-to-day C.V. of 7.9 and 4.6%, respectively (n=5 in each case), which indicates good precision for the assay. The detection limit for piroxicam at a signal-to-noise ratio of 3:1 was found to be 0.02 μg/ml which is consistent with the findings of Twomey et al. [2].

The present HPLC technique provides a simple, sensitive and rapid procedure to measure plasma concentration of piroxicam. Therefore, it is a suitable method for determination of piroxicam level in rat plasma after single oral dose.

References

[1] P.A. Insel, in J.G. Hardman, L.E. Limbird, P.B. Molinof and R.W. Ruddon (Editors), The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, 1996, ch. 21, p. 617.
[2] T.M. Twomey, S.R. Bartolucci, D.C. Hobbs, J. Chromatogr. 183 (1980) 104.
[3] J.S. Dixon, J.R. Lowe, D.B. Galloway, J. Chromatogr. 310 (1984) 455.
[4] P. Heizmann, J. Korner, K. Zinapold, J. Chromatogr. 374 (1996) 95.
[5] K.D. Riedel, H. Laufen, J. Chromatogr. 276 (1983) 243.
[6] Y.H. Tsai, L.R. Hsu, S.I. Maito, Int. J. Pharm. 24 (1985) 101.
[7] R.B. Gillilan, W.D. Mason, C.H.J. Fu, J. Chromatogr. 487 (1989) 232.
[8] D. Cerretani, L. Micheli, A.I. Fiaschi, G. Giorgi, J. Chromatogr. 614 (1993) 103.