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Note

Rapid method for the determination of either piroxicam or tenoxicam in plasma using high-performance liquid chromatography

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At least two methods for the determination of piroxicam [4-hydroxy-2-methyl-N-(2-pyridyl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide] concentrations in plasma using a high-performance liquid chromatographic (HPLC) technique have already been described [1, 2]. Piroxicam is an established non-steroidal anti-inflammatory drug (NSAID), and a new NSAID of similar chemical structure, tenoxicam {4-hydroxy-2-methyl-N-(2-pyridyl)-2H-thieno-[2,3-*e*]-1,2-thiazine-3-carboxamide 1,1-dioxide}, is currently undergoing clinical trials. We have previously reported a method for the determination of plasma concentrations of tenoxicam (Ro 12-0068) [3] which we have now modified so that the method can be used to measure either piroxicam or tenoxicam in plasma using the other as internal marker.

EXPERIMENTAL

Reagents and solvents

Acetonitrile and methanol (both HPLC grade) were obtained from Rathburn (Peebleshire, U.K.); dichloromethane (AnalaR) and monosodium phosphate from BDH (Poole, U.K.) and disodium phosphate (AnalaR) from Fisons

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(Loughborough, U.K.). For the preparation of aqueous solutions, single-distilled water was used.

Standard solutions

A stock solution of 1 mg ml^{-1} piroxicam (supplied by Pfizer) was prepared in methanol and stored in the dark at 4°C. This solution was prepared freshly each week. An aqueous dilution of 1:100 (10 $\mu g\ ml^{-1}$) was prepared daily and used for calibration purposes. Solutions of equivalent concentrations were prepared for tenoxicam (supplied by Roche) for use as internal marker.

Calibration procedure

Blank plasma samples (1 ml) were spiked with aqueous piroxicam in the concentration range 0.2—2.5 $\mu g\ ml^{-1}$ for single-dose studies or 1—20 $\mu g\ ml^{-1}$ for multiple-dose studies. Appropriate volumes of water were also added to ensure an equivalent total volume (e.g. 0.5 ml) was added in each case. Aqueous internal marker solution (100 μl) was added to every calibration and unknown sample, and all tubes were whirlymixed before extraction.

Extraction procedure

Water (0.5 ml) was added to 1 ml plasma in addition to 100 μl of internal marker for all unknown samples. All samples were acidified with 1 ml of 1 *M* hydrochloric acid, the mixture was whirlymixed and 8 ml dichloromethane were added. The stoppered tube was shaken for 3 min and centrifuged at 1800 *g* for 10 min at 10°C. The upper aqueous layer was then discarded and the organic layer decanted into a clean tube, blown dry with nitrogen at a temperature $\leqslant$ 35°C and the residue taken up into 0.5 ml of mobile phase [acetonitrile—water—0.1 *M* phosphate buffer pH 5.0 (30:30:40)].

Chromatography

The chromatographic system consisted of a Constametric III pump (Laboratory Data Control), Rheodyne injection system (20-μl loop) and LiChrosorb RP-18 column (150 × 3.2 mm I.D., steel), 5-μm particle size (Magnus Scientific). Detection was by means of a Spectromonitor III variable-wavelength dual-cell ultraviolet detector operated at 361 nm, coupled to a 308 computing integrator (both from Laboratory Data Control). A guard column (5 cm × 4.6 mm) of Whatman Co:Pell ODS was used between injector and analytical column. Samples could be automatically injected at 8-min intervals using an autosampler (Laboratory Data Control).

The mobile phase was degassed before being used at a flow-rate of 0.75 ml min^{-1}. The retention times of tenoxicam and piroxicam were about 2.3 and 4.5 min, respectively. If required, resolution could be improved, at the expense of increased retention times, by using buffer at pH 4 or by reducing the amount of acetonitrile to 25%.

Recovery and reproducibility

The percentage recovery of piroxicam was determined by comparing the extraction from plasma spiked with piroxicam (5 $\mu g\ ml^{-1}$) with the equivalent

concentration of standard solution in methanol blown down and redissolved directly into mobile phase. In both cases internal marker was added and blown down after removal of the final organic layer prior to addition of mobile phase. Reproducibility of the method could also be determined from the six duplicate results.

Application to plasma samples after a single oral dose of piroxicam (20 mg)

Three normal male volunteers received an oral dose of piroxicam (20 mg) at 09.00 a.m. after fasting from 22.00 hours the previous evening. Venous blood samples (10 ml) were taken via a standard BD cannula at 0, 1, 1.5, 2, 3, 4, 6, 8, 24, 48, 72, 96 and 168 h following the dose. The blood samples were placed in lithium heparin tubes, centrifuged (1800 *g* for 10 min) and the plasma transferred to a plain tube for storage at −20° C to await analysis.

Assay for plasma tenoxicam

When tenoxicam is measured and piroxicam is used as internal standard, then 400 μl of the 10 μg ml^{-1} aqueous solution of the latter are added to 1 ml of plasma. A calibration range of 0.5—6.0 μg ml^{-1} for single-dose studies and 2—30 μg ml^{-1} for multiple-dose studies are recommended. Recovery of tenoxicam was determined using the same approach as that used to measure piroxicam recovery.

RESULTS

Extraction of plasma blanks demonstrated that neither piroxicam nor the internal marker were subject to interference from co-extracted endogenous substances. The two peaks were well separated as illustrated in Fig. 1.

A reproducible, linear calibration was obtained for plasma piroxicam concentrations ranging from 0.2 to 20 μg ml^{-1}. Peak height ratios for a 0.2—2.5 μg ml^{-1} calibration curve carried out on five separate occasions by three different analysts are shown in Table I. Mean recovery of piroxicam was found to be 81 ± 3.0% S.D. Reproducibility of the assay was acceptable giving a coefficient of variation of 3.6%. Accuracy of determined piroxicam concentrations > 0.6 μg ml^{-1} was $\leqslant \pm 2.0\%$ but deteriorated at lower concentrations, with a probable limit of quantitation around 0.1 μg ml^{-1}.

Application of the assay to plasma samples obtained from a single-dose kinetic profile demonstrated the successful use of the assay, and confirmed that metabolites of piroxicam did not interfere. The mean data from the three subjects are shown in Fig. 2. The plasma half-life of piroxicam for each subject was calculated using linear least-squares regression analysis and the mean result was 46.7 h. These profiles also demonstrated that this analytical method is sufficiently sensitive to measure piroxicam levels in plasma for at least 96 h after a single dose. It would therefore be suitable for monitoring pharmacokinetics in individual patients after single or multiple doses.

When the method was used for the measurement of tenoxicam a limit of quantitation similar to that of piroxicam was found. Mean recovery of tenoxicam was found to be 81 ± 7.9% S.D.

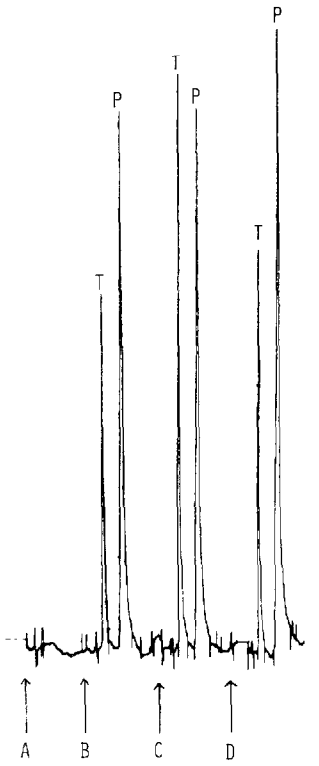

Fig. 1. Chromatograms showing: (A) blank plasma extract; (B) tenoxicam (1 μg ml^{-1}) and piroxicam (1 μg ml^{-1}) added to blank plasma; (C) sample from patient 4 h after receiving a single 20-mg oral dose of piroxicam, tenoxicam being used as internal marker; (D) sample from patient 3 h after receiving a single 20-mg oral dose of tenoxicam, piroxicam being used as internal marker. Peaks: T = tenoxicam, retention time 165 sec; P = piroxicam, retention time 370 sec.

TABLE I

PEAK HEIGHT RATIOS AND REGRESSION ANALYSIS FOR FIVE CALIBRATION CURVES

Calibration number	Analyst	Concentration of piroxicam (μg ml^{-1})								Correlation coefficient	Gradient	Intercept
		0.2	0.4	0.6	0.8	1.0	1.5	2.0	2.5			
1	A	0.095	0.241	0.379	0.563	—	1.014	1.415	1.720	0.999	0.711	0.038
2	A	—	0.241	0.322	0.478	0.632	0.978	1.369	1.697	0.999	0.709	−0.076
3	B	0.121	0.214	—	0.427	0.571	0.924	1.195	1.627	0.997	0.651	−0.055
4	C	0.137	0.273	0.436	0.584	0.678	1.070	1.421	1.830	0.999	0.728	−0.016
5	C	0.182	0.337	0.407	0.506	0.690	0.998	1.474	1.800	0.996	0.713	−0.003

DISCUSSION

This assay provides a rapid, sensitive and reproducible method for the quantitative analysis of either tenoxicam or piroxicam in plasma. The method allows for marginally more sensitive and more accurate measurement of piroxicam than the method of Riedel and Laufen [2]. The latter method involves sophisticated automation and hence rapid sample throughput, whereas our own laboratory uses readily available, standard HPLC equipment. Nevertheless seventy samples can readily be extracted and chromatographed in a 24-h

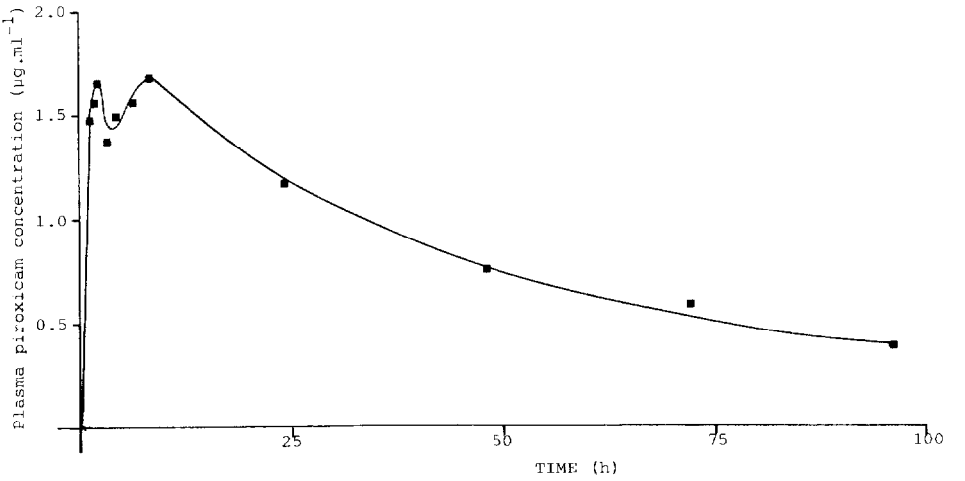

Fig. 2. Plasma concentrations of piroxicam after a 20-mg single oral dose (mean data for three subjects).

cycle. Our method also compares favourably with the previous HPLC method of Twomey et al. [1], though their lower limit of quantitation is 0.5 μg ml^{-1} compared with 0.2 μg ml^{-1}. A third HPLC method for the determination of piroxicam has been described [4], but full validation details were not given.

The principal advantage of this method is the use of tenoxicam as internal marker, readily allowing for the adaptation of the method for measuring either drug. The third oxicam non-steroidal anti-inflammatory drug undergoing clinical trials is isoxicam and this drug can also be detected by this assay technique, though the assay characteristics for this drug have not been fully validated. In conclusion this method provides an alternative assay for the determination of piroxicam and also illustrates the value of choosing an internal marker which is also of quantitative interest.

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