Journal of Chromatography, 183 (1980) 104—108
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 564

Note

Analysis of piroxicam in plasma by high-performance liquid chromatography

THOMAS M. TWOMEY*, S. RICHARD BARTOLUCCI and DONALD C. HOBBS

Pfizer Central Research, Groton, CT 06340 (U.S.A.)

(First received October 30th, 1979; revised manuscript received February 5th, 1980)

Piroxicam, a non-steroidal anti-inflammatory agent of the benzothiazine family, has demonstrated efficacy in man in the treatment of rheumatoid arthritis and other inflammatory disorders [1]. During pharmacokinetic studies in man, mean peak piroxicam plasma concentrations of 1.5 μg/ml were measured after the administration of single 20-mg doses. The mean steady-state level after chronic 20-mg daily doses for 14 days was reported to be 4.5 μg/ml. Alteration of the dose level resulted in a near linear relationship between the amount of drug administered and plasma concentration [2].

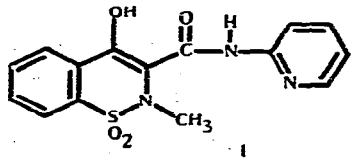
The quantitative determination of piroxicam in plasma during these pharmacokinetic studies was accomplished by degradative fluorometric wet chemistry methods involving strong acid hydrolysis of drug to generate 2-aminopyridine and measurement of this species after various purification steps. Concentrations were calculated from the fluorescence measured at specific excitation and emission wavelengths as compared to externally standardized samples. The high-performance liquid chromatographic (HPLC) method reported here and recommended for future measurement of piroxicam plasma levels has a number of analytical advantages. It is exceptionally reproducible and accurate, does not require chemical modification of the drug, uses small amounts of plasma (0.1 ml), is simple and rapid with assay steps performed in disposable glassware, obviates the need for quantitative transfers by the the incorporation of an internal standard, and is compatible with automated analytical equipment.

0378-4347/80/0000—0000/$02.25

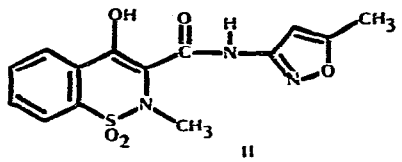
EXPERIMENTAL

Reagents and materials

Piroxicam [4-hydroxy-2-methyl-N-(2-pyridyl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide] (I) and the internal standard, isoxicam [4-hydroxy-2-methyl-N-(5-methyl-3-oxazolyl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide] (II), were prepared as aqueous solutions in 0.1 *N* NaOH at a concentration



I



II

of 1.0 mg/ml and stored in the dark at 4°C. No degradation was noted for at least one month. Appropriate dilutions of these solutions were made weekly with water to produce working standards containing 50 μg/ml and 5 μg/ml for piroxicam and 100 μg/ml for isoxicam. Acetonitrile was glass-distilled (Burdick and Jackson, Muskegon, MI, U.S.A.) as was the water used in preparing the reagents and the chromatographic mobile phase. Diethyl ether, acetic acid, sulfuric acid, and tris(hydroxymethyl)aminomethane were of analytical reagent grade.

Sample preparation

In disposable 16 mm × 100 mm culture tubes, 0.1 ml of plasma was fortified with 10 μg of internal standard, mixed with 0.5 ml of 0.1 *N* sulfuric acid, extracted with 4 ml of diethyl ether on a vortex mixer for 30 sec and centrifuged. The solvent layer was transferred to another tube and evaporated to dryness using a vortex evaporator. The residue was reconstituted in 1 ml of 0.05 *M* tris(hydroxymethyl)aminomethane and aliquots subjected to HPLC. Samples at this stage could be held for at least seven days at room temperature as demonstrated by the same peak area count ratio of piroxicam to the internal standard measured on days 1, 3, and 8.

Chromatography

A Waters Assoc. (Milford, MA, U.S.A.) analytical liquid chromatograph equipped with a 300 mm × 3.9 mm I.D. 10-μm μBondapak cyano column (Waters Assoc., part No. 84042) was fitted with a Waters Model 440 UV detector using low-dead-volume hardware. A mobile phase of acetonitrile–water–acetic acid (25:70:5) was filtered, degassed, and used at a flow-rate of 1.2 ml/min. The effluent stream was monitored while employing a 365-nm

filter on the detector. The range setting was fixed at 0.02 a.u.f.s., with the signal monitored by a 10-mV strip chart recorder (0.5 cm/min) interfaced with a Spectra Physics System IV electronic integrator. Samples were automatically injected at 11-min intervals using an HPLC autosampler (Micromeritics Model 725) equipped with a 100-μl sample loop. Column effluent was recycled back into the mobile phase reservoir (800 ml). The mobile phase was replaced every three days (approximately 350 samples).

Calibration and specificity

Calibration curves were constructed by determining the response from known amounts of piroxicam and the internal standard added to control plasma. Assay linearity was demonstrated over the range 0.5—20.0 μg/ml; the amount of the internal standard was held constant at 10 μg/sample. For daily validation, six samples fortified at mid-range (5.0 μg/ml) were processed with each group of test samples, two at the beginning of the run, two at mid-run, and two at the end of the run. The mean ratio of piroxicam to the internal standard (IS) integrator area counts was determined and the piroxicam concentration in test samples calculated from the expression

$$\text{Concentration } (\mu g/ml) = \frac{\text{area counts drug}}{\text{area counts IS}} \times \frac{C}{\text{mean ratio}}$$

where C represents the concentration (μg/ml) of piroxicam in the fortified samples.

To assess assay specificity, control plasma samples were fortified at 100 μg/ml with all known animal and human metabolites of piroxicam [3, 4] and processed through the assay. Salicylate at concentrations of 100 μg/ml of plasma was also tested.

RESULTS AND DISCUSSION

Reproducibility was determined at each fortification level of the calibration curve (Table I). Relative standard deviations ($n = 4$) ranged from 0.6 to 5.6%. A

TABLE I

PIROXICAM CALIBRATION CURVE FROM HUMAN PLASMA

Piroxicam level (μg/ml)	Piroxicam/IS* mean ratio ($n = 4$)	S.D.	R.S.D.** (%)
0	0	—	—
0.5	0.0355	0.0019	5.4
1.0	0.0873	0.0029	3.3
2.0	0.175	0.0043	2.5
5.0	0.528	0.0034	0.6
10.0	1.098	0.061	5.6
20.0	2.242	0.022	1.0

*Internal standard concentration was 100 μg/ml. Ratio values were derived from electronic integrator area count units.

**Relative standard deviation.

regression analysis of data pairs in Table I (concentration vs. area count ratio) resulted in a regression coefficient of 0.999916, indicating exceptional linearity over the assay range. Assay recovery was determined by comparing the response from known amounts of drug and the internal standard with processed fortified plasma samples (n = 6). Recoveries for piroxicam and the internal standard were 73.6 and 77.3%, respectively. In experiments designed to assess assay specificity, salicylate at plasma concentrations of 100 μg/ml did not respond. Of all the known animal and human biotransformation products of piroxicam, only the 5′-hydroxylated metabolite yielded a chromatographic peak; retention times, however, were sufficiently different so as not to interfere with piroxicam measurements. Additionally, the extraction conditions specified result in a relatively poor recovery of this metabolite (<20%). Retention times in the system as described were: piroxicam, 4.8 min; 5′-hydroxypiroxicam, 5.3 min; and isoxicam (internal standard), 7.0 min.

The chromatograms in Fig. 1 demonstrate the lack of interference and the specificity of the assay procedure for the measurement of piroxicam in plasma.

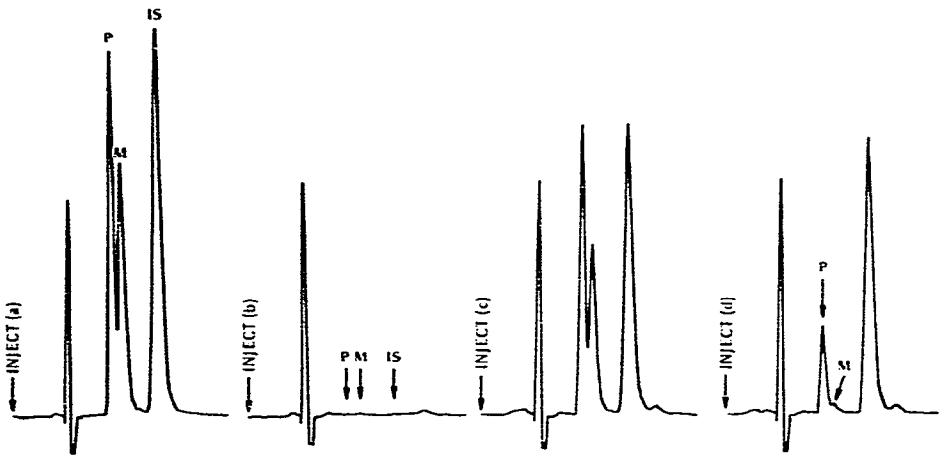


Fig. 1. Chromatograms of: (a) a standard mixture of 50 ng of piroxicam (P), 50 ng of 5′-hydroxypiroxicam (M), and 1 μg of isoxicam (IS); (b) control plasma extract; (c) extract from plasma fortified with P at 5 μg/ml, M at 20 μg/ml and IS at 100 μg/ml; (d) plasma extract from a sample taken 8 h after a volunteer received 20 mg of piroxicam.

Latitude to increase assay detection limits where necessary is evident and can be approached by using larger plasma aliquots, larger injection volumes, increased detector sensitivity settings, or any combination of these parameters; a ten-fold improvement of the lower detection limit (from 0.5 to 0.05 μg/ml) has been demonstrated in preliminary experiments.

ACKNOWLEDGEMENTS

The authors greatly appreciate the contributions and technical expertise of Ms. Diane Farrell and of Dr. Joseph G. Lombardino who provided samples of piroxicam, 5′-hydroxypiroxicam, and isoxicam.

REFERENCES

1 N.E. Pitts and R.R. Proctor, in W.M. O'Brien and E.H. Wiseman (Editors), Piroxicam, The Royal Society of Medicine, London, Academic Press, London, Grune & Stratton, New York, 1978, p. 97.
2 D.C. Hobbs and T.M. Twomey, J. Clin. Pharmacol., 19 (1979) 270.
3 D.C. Hobbs and T.M. Twomey, Pharmacologist, 18 (1976) 152.
4 T.M. Twomey and D.C. Hobbs, Fed. Proc., Fed. Amer. Soc. Exp. Biol., 37 (1978) 271.