Journal of Chromatography, 374 (1986) 95—102
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2826

DETERMINATION OF TENOXICAM IN HUMAN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

P. HEIZMANN*, J. KÖRNER and K. ZINAPOLD

Biological and Pharmaceutical Research Department, F. Hoffmann-La Roche & Co., Ltd., Basle (Switzerland)

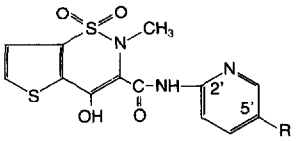
(First received March 12th, 1985; revised manuscript received August 18th, 1985)

SUMMARY

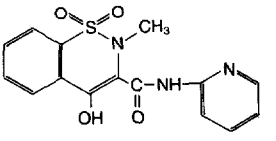
A high-performance liquid chromatographic method for the determination of tenoxicam in plasma has been developed. Tenoxicam was extracted from buffered plasma (pH 3 or 4, respectively) with dichloromethane and the evaporated extracts were analysed on a C_{18} reversed-phase column using a methanol—phosphate buffer mobile phase and with UV detection at 371 nm. The detection limit was 20 ng/ml using a 0.5-ml sample. The method is selective with respect to the 5'-hydroxy metabolite, which is present in plasma after multiple administration of tenoxicam; this metabolite may also be determined using this procedure.

INTRODUCTION

Tenoxicam, 4-hydroxy-2-methyl-N-2-pyridyl-2H-thieno[2,3-*e*]-1,2-thiazine-3-carboxamide-1,1-dioxide (I, Fig. 1), is a new representative of the oxicam class of non-steroidal anti-inflammatory drugs (NSAIDs). It shows analgesic, anti-inflammatory and antipyretic properties in animal models and is currently undergoing clinical trials [1, 2].



Tenoxicam (I) R = H
5'-Hydroxy metabolite (II) R = OH



Piroxicam (III), internal standard

Fig. 1. Structural formulae of tenoxicam (I), its metabolite (II) and the internal standard (III).

0378-4347/86/$03.50

In man, the drug is almost completely metabolized, the major urinary metabolite being the 5′-hydroxy derivative (II, Fig. 1) [3].

A high-performance liquid chromatographic (HPLC) method for the determination of tenoxicam and its major metabolite in urine samples has been reported [3]. A thin-layer chromatographic (TLC) method [4] and an HPLC method [5] for the determination of tenoxicam in plasma have been described. The sensitivity of the HPLC method [5] was 50 ng/ml, but the published chromatograms indicated significant interference at the 100 ng/ml level. The method is non-selective with respect to the 5′-hydroxy metabolite. A second HPLC method has also been published recently [6].

For single-dose pharmacokinetic studies, a lower detection limit was desired. The HPLC method described here is capable of quantifying 20 ng/ml tenoxicam in plasma. The 5′-hydroxy metabolite, which may be present after repetitive administration of tenoxicam, is separated from the parent drug and can be determined with the same procedure.

EXPERIMENTAL

Reagents and solvents

Methanol, dichloromethane, Titrisol buffers (pH 3 and 4), 0.2 *M* sodium hydroxide and potassium dihydrogen phosphate were all p.a. grade from Merck (Darmstadt, F.R.G.). Double-distilled water was used for the preparation of all aqueous standards and buffer solutions. Tenoxicam (Ro 12-0068, I) and its 5′-hydroxy metabolite (Ro 17-6661, II) are compounds from Hoffmann-La Roche (Basle, Switzerland).

Internal standard

Piroxicam, 4-hydroxy-2-methyl-N-2-pyridyl-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide, was used as internal standard (III, Fig. 1).

Chromatography

The HPLC system consisted of the following components: an Altex 110 pump (Kontron, Switzerland), an autosampler ISS 100 (Perkin-Elmer, F.R.G.),

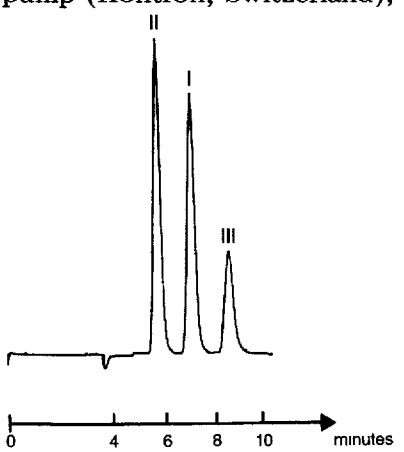


Fig. 2. Chromatogram of a mixture of I, II and III.

a variable-wavelength detector Pye LC-UV (Philips, F.R.G.) coupled to a computing integrator SP 4100 (Spectra-Physics, F.R.G.). The column was a C_{18} μBondapak, particle size 10 μm, 30 cm × 3.9 mm I.D. (Waters Assoc., F.R.G.). The mobile phase consisted of a methanol—0.1 *M* phosphate buffer at pH 5.6 (50:50), at a flow-rate of 0.8 ml/min (60—90 bar, room temperature). The column effluent was monitored at 371 nm. Under these conditions, the retention times were 5.5 min for II, 7.1 min for I and 8.7 min for III (see Fig. 2).

Solutions and calibration standards

Tenoxicam stock solution. Tenoxicam (10 mg) was weighed into a 10-ml flask. A 0.5-ml volume of 0.2 *M* sodium hydroxide and 0.5 ml of water were added. After dissolution, the volume was made up to 10 ml with water.

The following calibration standards were prepared from drug-free plasma: 20, 10, 5, 2, 1, 0.5, 0.2, 0.1, 0.05 and 0.020 μg/ml. The 20 μg/ml standard was prepared by adding 200 μl of the above stock solution to 10 ml of drug-free plasma. The other standards were then prepared by stepwise dilution with drug-free plasma and stored deep-frozen (−18°C) in small portions (2.5 ml) until needed for analysis. The calibration standards > 5 μg/ml were needed only for the analysis of samples from multiple-dose studies.

Internal standard. A 2-mg quantity of the internal standard was dissolved in 10 ml of methanol. From this stock solution, suitable dilutions in Titrisol buffer (pH 3 or 4) were made, which were then used for internal standardization.

Sample preparation

Plasma (0.5 ml) was transferred into a conical glass tube (15 ml volume) and mixed with 0.5 ml of Titrisol buffer (pH 3 for concentrations ≥ 0.5 μg/ml, pH 4 for concentrations ≤ 0.5 μg/ml) containing the internal standard. After the addition of 7 ml of dichloromethane, the sample was extracted on a rotary extractor (5 min, 20 rpm) and then centrifuged to break any emulsion (5 min, 1000 *g*, 10°C). The upper, aqueous phase was discarded and the organic extract (6 ml) was transferred to another glass tube and evaporated to dryness under a gentle stream of pure nitrogen at 35°C. The dry residue was taken up in the mobile phase (300 μl for concentrations < 0.5 μg/ml, 500 μl for concentrations > 0.5 μg/ml). The mixture was whirlimixed for a short time, allowed to stand for 3 min, then whirlimixed again for 45 s and injected onto the HPLC column (100 μl for concentrations < 0.5 μg/ml, 50 μl for concentrations > 0.5 μg/ml and 25 μl for concentrations > 5 μg/ml).

Calibration

Five calibration standards, covering the appropriate concentration range of the unknown samples, were processed as described above and analysed together with the unknowns. Calibration curves were obtained by least-squares linear regression (weighting $1/y^2$) of the peak-height ratios tenoxicam/piroxicam versus the respective concentrations. These curves were then used to calculate the concentration of tenoxicam in the unknown samples. The following calibration ranges were used: 0.02—0.5 and 0.5—5 μg/ml for samples from single-dose studies (20 mg) and 2—20 μg/ml for samples from multiple-dose studies.

RESULTS AND DISCUSSION

Stability of tenoxicam

Plasma samples fortified with tenoxicam and stored in colourless-glass vessels under laboratory lighting conditions (sunny outside, sample kept out of direct sunlight) suffered a 45% loss of tenoxicam in 3 h (Fig. 3). In a darkened room, however, no degradation was found over the same time period. Aqueous solutions of tenoxicam were also unstable to light: a 75% loss occurred in direct sunlight within 1 h (in colourless vessels). Out of direct sunlight, the degradation was 40% over this time period. In a darkened room, no loss of tenoxicam was detected for up to 20 h, at least.

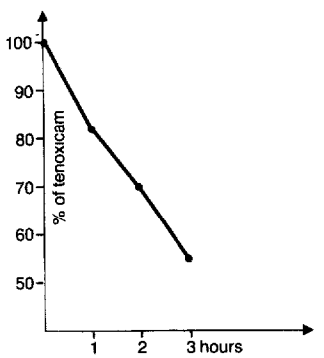


Fig. 3. Stability of tenoxicam in plasma under sunny daylight conditions.

The following measurements were carried out according to our normal standard procedure for stability determinations [7]: spiked plasma samples were prepared at different concentrations. One set of samples was stored in colourless-glass tubes at room temperature and under normal laboratory lighting conditions. After 24 h of standing (day and night), about 70% of the tenoxicam content in the plasma had disappeared. Compared to this, storage in brown glass over a 96-h period resulted in a much lower loss (ca. 15%). Another set of spiked samples was frozen, stored at −18°C in darkness for three months and then analysed. In addition to this, biological samples have been reanalysed after a three-month period. From both experiments, no relevant instability of tenoxicam could be found.

These results indicate that tenoxicam is unstable in plasma and in solution under the influence of light, but stable for at least three months when stored at −18°C in the dark. Any handling of blood, plasma or urine samples should therefore be carried out in a darkened room. All solutions should be kept in brown-glass vessels and stored in the dark.

Extraction

In order to minimize co-extraction of endogenous interfering compounds, it was decided to extract at less acidic conditions than those reported [5].

Tenoxicam (pK_a = 5.3) could be extracted over the pH range 1–5. The metabolite showed a maximum recovery within the pH range 1–4 and a fall-off in extractability above pH 5 (Fig. 4). From these results, pH 3 was chosen for

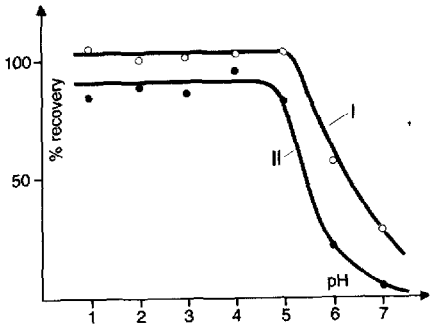


Fig. 4. Recovery of tenoxicam (I) and its metabolite (II) from plasma over the pH range 1—7.

extraction. For the extraction of tenoxicam samples at low concentrations ($\leq$ 0.1 μg/ml), pH 4 was preferred because of small interferences which were encountered at pH 3. On the other hand, the use of pH 4 buffer sometimes gave rise to unacceptable reproducibility in the high concentration range ($\geq$ 5 μg/ml). Therefore, pH 4 was used routinely for concentrations $\leq$ 0.5 μg/ml and pH 3 for concentrations $\geq$ 0.5 μg/ml.

These extraction conditions, together with modified HPLC conditions, resulted in much cleaner extracts than those previously reported [5]. The recovery of tenoxicam was from 95 to 102% in the range 0.025—20 μg/ml (Table II).

Linearity

Linear correlations between peak-height ratio and concentration of tenoxicam in plasma were obtained over the concentration ranges 20–2, 5–0.5 (coefficient of correlation > 0.99) and 0.5—0.02 μg/ml (coefficient of correlation > 0.999).

Selectivity

The major metabolite (5'-hydroxytenoxicam) was co-extracted under the conditions described but could be separated chromatographically from the parent drug (Fig. 2).

Precision

Intra-assay precision (calculated from repeated analysis during one working day) was ± 1.8% within the range 0.025—20 μg/ml (Table I). Inter-assay precision (calculated from repeated analysis of quality control samples on different days) was ± 5.5% (Table II).

Limit of determination

Starting with 0.5 ml of plasma and injecting 100 μl of the final extract (300 μl), the limit of determination at a signal-to-noise ratio of 10:1 was 0.02 μg/ml.

Application of the method

Plasma from volunteers and patients receiving single and multiple doses of

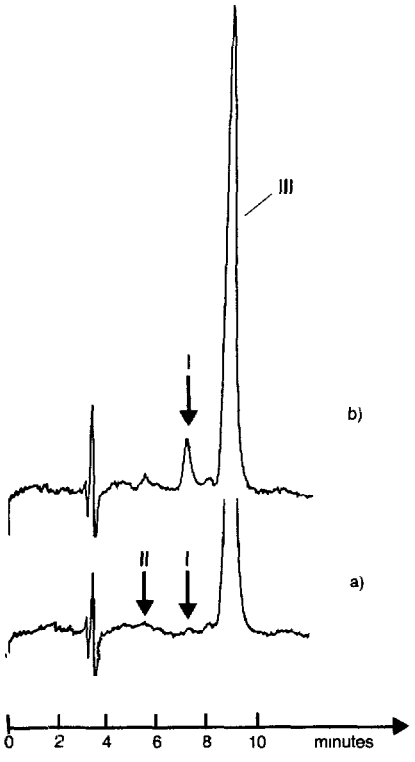


Fig. 5. Chromatograms of plasma extracts. (a) Drug-free plasma; (b) volunteer's plasma, containing 50 ng/ml of tenoxicam. Conditions: detector 0.02 a.u.f.s., integrator AT 8, injected volume 125 μl. The arrows indicate the retention of the compounds from Fig. 1.

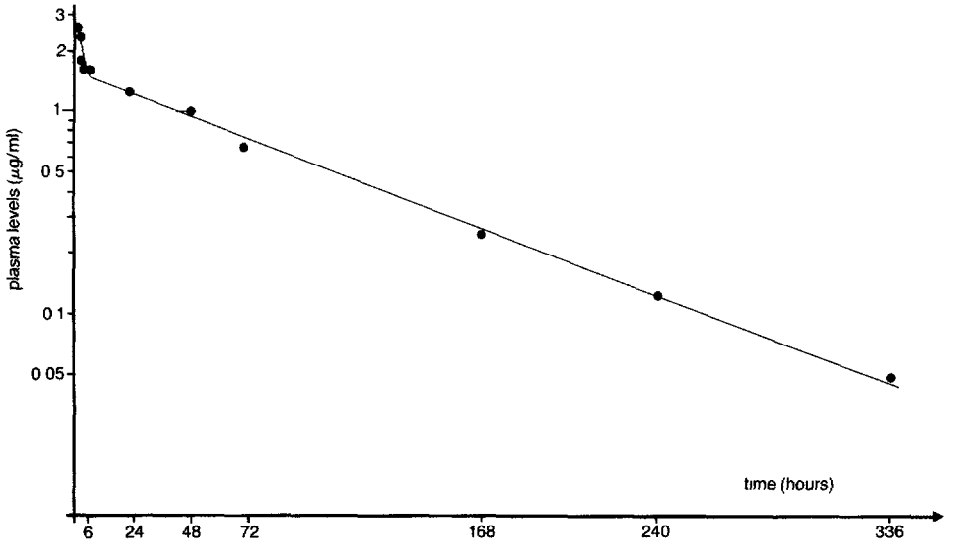


Fig. 6. Plasma levels of tenoxicam after oral administration of 20 mg of tenoxicam to a volunteer.

TABLE I

RECOVERY AND INTRA-ASSAY PRECISION OF TENOXICAM (n = 4)

Concentration (μg/ml)	Recovery (mean ± S.D.) (%)	Coefficient of variation (%)
0.025	95 ± 2.5	1.1
0.2	102 ± 1.6	0.9
1.0	95 ± 2.7	2.0
5.0	102 ± 2.7	0.4
20.0	97 ± 8.0	4.7

TABLE II

INTER-ASSAY PRECISION AND ACCURACY OF TENOXICAM

Added (μg/ml)	Found (μg/ml)	Coefficient of variation (%)	Accuracy* (%)	Replicates (n)	Period of time (weeks)
14.0	14.2	7.1	+1.5	8	4
6.0	5.9	4.9	−1.7	8	4
2.5	2.4	4.5	−4.2	23	8
1.0	0.97	5.1	−3.0	36	10
0.4	0.39	6.2	−2.5	18	4
0.2	0.191	4.7	−4.5	9	14
0.05	0.052	6.4	+4.0	9	14

*Deviation between added and found.

tenoxicam have been analysed by this method. Fig. 5 shows chromatograms of plasma extracts from a volunteer after oral administration of 20 mg of tenoxicam. Plasma levels of the parent drug after oral administration of 20 mg of tenoxicam are presented in Fig. 6.

These data demonstrate that the present method is sufficiently sensitive to measure plasma levels for 336 h after a single oral dose (four to five half-lives). It is therefore suitable for monitoring pharmacokinetics in individual patients after single or multiple doses.

In plasma, the 5′-hydroxy metabolite could only be found after repeated doses of tenoxicam.

ACKNOWLEDGEMENT

We would like to thank Dr. Dell for helpful discussions and for correcting the manuscript.

REFERENCES

1 E.F. Valdes, C.A. Barcley and C. Trabolli, Curr. Ther. Res., 31 (1982) 402.
2 Original monograph, Drugs Fut., 8 (1983) 658.
3 D. Dell, R. Joly, W. Meister, W. Arnold, C. Partos and B. Guldimann, J. Chromatogr., 317 (1984) 483.

4 R.C. Heintz and K. Tosch, personal communication.
5 M.E. Pickup, J.R. Lowe and D.B. Galloway, J. Chromatogr., 225 (1981) 493.
6 J.S. Dixon, J.R. Lowe and D.B. Galloway, J. Chromatogr., 310 (1984) 455.
7 U. Timm, M. Wall and D. Dell, J. Pharm. Sci., 74 (1985) 972.