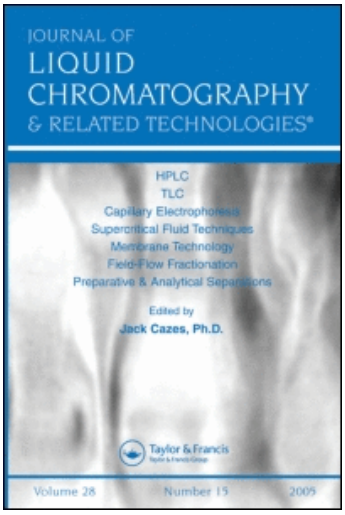

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Determination of Tenoxicam in Human Plasma using Solid-Phase Extraction and High-Performance Liquid Chromatography with Ultraviolet Detection

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DETERMINATION OF TENOXICAM IN HUMAN PLASMA USING SOLID-PHASE EXTRACTION AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

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ABSTRACT

A sensitive, specific and rapid liquid chromatographic procedure to selectively monitor tenoxicam in human plasma was developed and validated. Plasma samples were acidified and extracted using solid-phase exctration column. The procedure was linear from 0.1 to 10 μg/ml with a detection limit of 0.05μg/ml. The coefficient of variation for the procedure is 6.2% and 2.0% for the range of concentrations examinated. This method is suitable for pharmacological, toxicological and pharmacokinetic studies of tenoxicam.

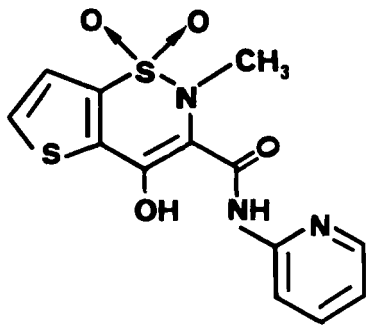

FIGURE 1: Chemical structure of tenoxicam.

INTRODUCTION

Tenoxicam, or. 4-hydroxy-2-methyl-N-2-pyridinyl -2H-thieno [2,3-e] - 1,2-thiazine - 3-carboxamide - 1,1-dioxide, Fig. 1, is a non steroidal anti-inflammatory and analgesic agent of the oxicam class. Tenoxicam in common with other NSAIDs, is an inhibitor of the prostaglandin synthesis by inhibiting cyclo-oxygenase, which catalyzes the formation of cyclic endoperoxidases from arachidonic acid [1,2]. In common with other NSAIDs, tenoxicam is also a potent inhibitor of collagen-induced platelet aggregation and exerts a very strong inhibitory action on leucocyte migration into inflamed sites. Interest in this group of compounds has prompted us to develop a selective and sensitive

high-performance liquid chromatographic method for the therapeutic monitoring of tenoxicam. Recently, Pickup et al. [3] and Dixon et al. [4] reported a high-performance liquid chromatographic method for the determination of tenoxicam in human plasma. These methods are based on liquid-liquid extraction. The aim of this work was to develop a rapid method of solid-phase extraction of tenoxicam in human plasma samples in preparation for their analysis by a new HPLC procedure developed with ultraviolet detection. This method is suitable for pharmacological, toxicological and pharmacokinetic studies of tenoxicam. The method yields accurate and reproducible results.

EXPERIMENTAL

Chemicals

Tenoxicam was supplied by Sigma-Tau (Rome, Italy), 1(2-hydroxyethyl)-3-hydroxy-7-chloro-1,3-dihydro-5-(o-fluorophenyl)-2H-1,4-benzodiazepine-2-one, the internal standard, was supplied by Schiapparelli Farmaceutici (Turin, Italy). HPLC-grade methanol, acetonitrile, citric acid, disodium hydrogen phosphate and all other analytical grade reagents were obtained from Farmitalia Carlo Erba (Milan,

Italy). HPLC-grade water was obtained by distillation in glass and purification through a Milli-Q water purification system (Millipore, Bedford, MA, U.S.A.). Water was filtered through Whatman 0.45μm WCN type filters, whilst methanol and acetonitrile were filtered through 0.5μm WTP type filters Whatman (Whatman, Maidstone, U.K.).

Biological Samples

Healthy volunteers, between 25 and 39 years of age who did not smoke cigarettes or consume alcohol chronically, entered the study after giving informed consent. The volunteers received a single 20mg tablet of tenoxicam orally after an overnight fast. Blood samples were collected from an antecubital vein and whole blood was anticoagulated with heparin. Blood samples were taken at the following times relative to the tenoxicam dose: 0.25, 0.50, 1, 2, 4, 6, 12, 24, 48, 72 and 96 h.

Chromatographic system and conditions

The Waters HPLC system (Waters Assoc., Milford, MA, U.S.A.) employed consisted of a model 510 FR solvent delivery system, a Lambda Max model 481 LC-variable spectrophotometric detector connected to a model 740

Data Module integrator. The injector was a model 7125 Rheodyne (Rheodyne, Cotati, CA, U.S.A.) with a 20 µl sample loop. The extraction apparatus was a Supelco solid phase extraction manifold equipped with a Drying Attachment (Supelco, Bellefonte, PA, U.S.A.). Bakerbond SPE octadecyl columns (1.0 ml capacity) were purchased from B. H. Schilling Società Chimica (Milan, Italy). The separation was performed on a reversed-phase Viosfer LC-18, 250 x 4.6 mm I.D., 10µm particle size, column (Violet, Rome, Italy) protected by a 2 cm Pelliguard column (40 µm, particle size) (Supelco). The separations were performed at room temperature and the detector set at 0.1 absorbance units full scale. The mobile phase consisted of a mixture of methanol - buffer solution (pH3) (45:55, v/v). The buffer solution was prepared by adding disodium hydrogen phosphate 0.2M to 0.1 M citric acid, until pH 3, followed by dilution to 1000 ml with water. The buffer solution was filtered and degassed prior to use. The mobile phase was delivered at a flow rate of 1.2 ml/min.

Sample preparation

Heparinized blood samples from various volunteers were centrifuged and plasma was collected and frozen

at -20°C. Samples were thawed just before the extraction procedure, thoroughly agitated and centrifuged at 800g for 10 min. The Bakerbond cartridges were placed in a luer that fitted the top of the Supelco vacuum manifold, which may be loaded with up to 12 cartridges. A vacuum of 250-500 Torr was applied to the manifold to carry out the various steps of the extraction. A 1.0 ml methanol rinse followed by 2 ml of HPLC-grade water served to desorb any organic impurities from the cartridge and to wet the silica packing before introducing of the plasma samples. 100 µl of internal standard (3.0µg/ml)added to 0.5 ml of plasma were passed through the cartridge, followed by 2 ml of water. The effluent was discarded. 2 ml of acetonitrile were then applied to the cartridge and the eluate collected. This fraction was finally centrifuged (1000 g for 10 min), transferred to a new tube and evaporated to dryness with a nitrogen stream under vacuum utilising the Supelco Drying Attachment. The samples were then reconstituted to 200 µl with the mobile phase and mixed on a vortex agitator. Aliquots of each sample (20µl) were chromatographed using the described mobile phase. Column eluate was monitored at 361 nm.

Pharmacokinetic data analysis

The concentration of tenoxicam in plasma versus time was plotted. A two-compartment open model with an absorption phase was used to calculate the pharmacokinetic parameters. The exponential equation $C=-(A+B)\ e^{-kat}+ Ae^{-\alpha t}+ Be^{-\beta t}$ was used to express the serum concentration versus time. The distribution volume of the central compartment (Vd) was calculated as Cl·t½/ln2, while distribution volume at steady-state (Vdss) as Cl·MRT (Mean Residence Time). Clearance was calculated as Dose/AUC (Area Under Curve). Half-lives for the absorption, and elimination phase of the serum concentration-time data were expressed as t½abs.=ln2/Kabs., and t½ß=ln2/ß respectively. The AUC was calculated using the trapezoidal rule.

Standard solutions

Stock solutions (1.0 mg/ml) of tenoxicam and the internal standard were prepared in methanol. Standard solutions containing from 0.1 to 10 µg/ml of tenoxicam were prepared by diluting the stock solution with methanol. The internal standard solution was diluted with methanol to a final concentration of 3 µg/ml.

TABLE 1:
Precision and accuracy of tenoxicam calibration standards in human plasma during a three-day period.

Conc. added (μg/ml)	Conc. found* (μg/ml)	CV (%)	RE (%)
0.1	0.097±0.005	5.1	3.0
0.2	0.192±0.010	5.2	4.1
0.3	0.290±0.018	6.2	3.4
0.5	0.489±0.022	4.4	2.2
1.0	0.970±0.048	4.9	3.0
2.0	1.920±0.067	3.5	4.1
5.0	4.830±0.150	3.1	3.5
10.0	9.750±0.196	2.0	2.5

*Mean of five assays ±SD; CV Coefficient of Variation; RE Relative Error.

RESULTS AND DISCUSSION

The assay was validated by analyzing eight tenoxicam plasma standards extracted in triplicate over three consecutive days. Peak-height ratios were proportional to tenoxicam concentration over the range 0.1 to 10 μg/ml. The best-fit line was determined daily by least squares regression analysis. The results of a typical regression were : peak-height ratio = 0.865 · tenoxicam concentration + 0.004 (r=0.999). The mean slope and intercept data from a three-days period are 0.865 (±0.021) and 0.0035 (±0.003), respectively. The accuracy and precision of the calibration curves were determined

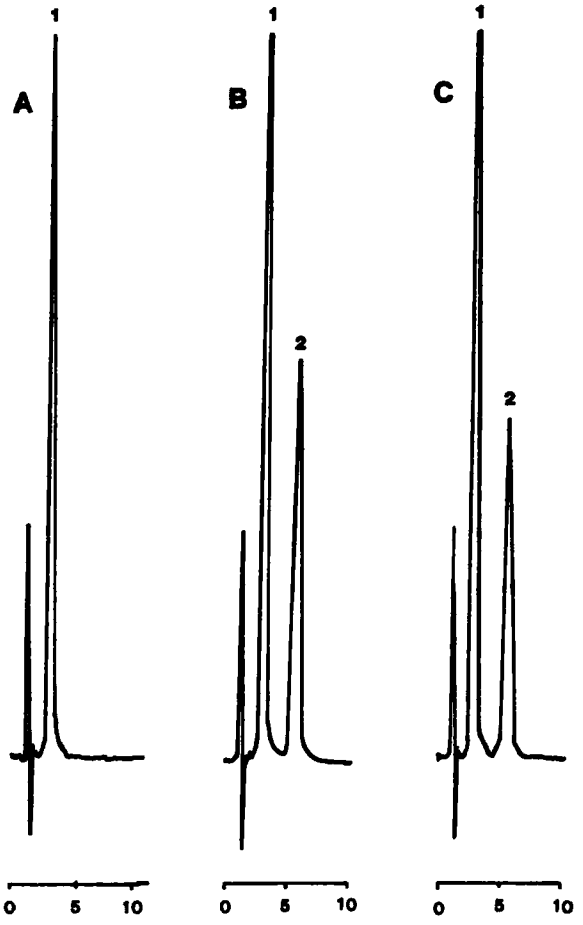

FIGURE 2: Chromatograms from human plasma extracts: (A) blank plasma spiked with 100µl of internal standard (3.0µg/ml); (B) blank plasma spiked with 100µl (3.0µg/ml) of internal standard (1) and 100µl(10 µg/ml) of tenoxicam (2); (C) plasma sample after a dose of 20mg of tenoxicam. Horizontal axis: retention time (min). Injection volume was 20µl.

by the variation of the standards from the regression line. Precision for the human plasma calibration standards ranged from 2.0% to 6.2% (CV) with relative errors of 2.2% to 4.1% Table 1. Based on these results the method is linear from 0.1 to 10μg/ml. The detection limit was 0.05μg/ml, at a signal to noise ratio of 3:1. Chromatograms of tenoxicam in human plasma are shown in Fig.2. These do not contain any interfering peak with a retention time similar to that of tenoxicam, for which the average retention time was 6.2 min. The retention time of the internal standard was 3.1 min. The recovery of tenoxicam from plasma was determined by comparing the peak-height obtained by analysing extracted, spiked plasma specimens to the peak-height obtained by direct injection of methanolic solutions of tenoxicam, containing amounts of tenoxicam equal to those in the spiked plasma specimens. The mean recovery of tenoxicam from plasma samples was c. 97% . The mean plasma tenoxicam concentration-time profiles after oral administration of 20 mg of drug are shown in Fig.3, and the pharmacokinetic parameters are summarized in Table 2. The parmacokinetic parameters calculated from the results obtained were comparable

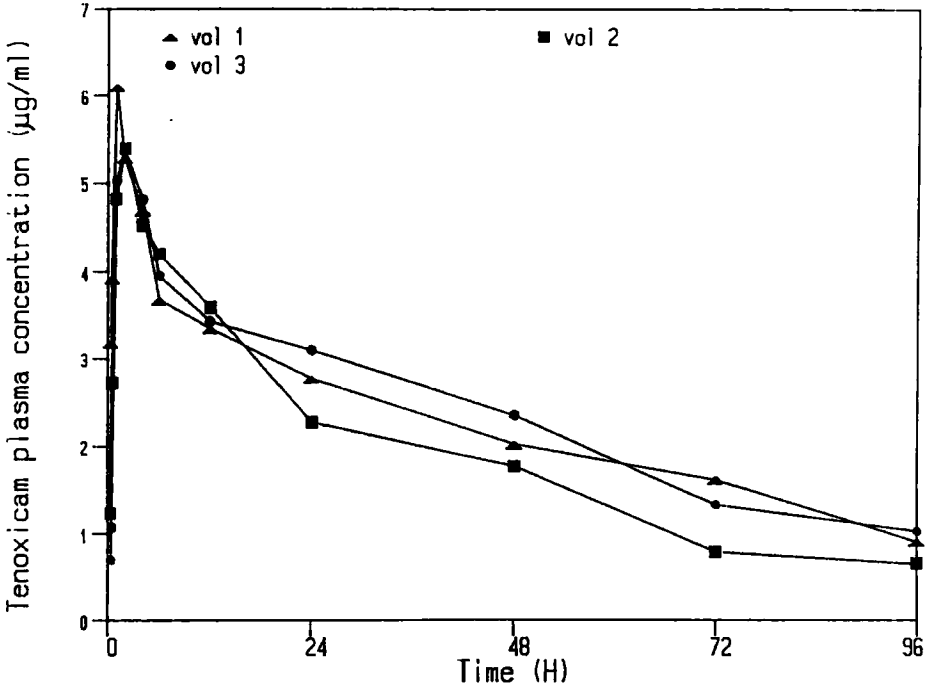

FIGURE 3: Concentration-time curves after oral administration of 20mg of tenoxicam to volunteers.

TABLE 2: Pharmacokinetic data for tenoxicam.

Parameter	volunteer 1	volunteer 2	volunteer 3
t½ el. (h)	47.30	32.81	46.01
t½ abs.(h)	0.31	0.44	0.40
AUC (µg·h/ml)	277.28	212.04	214.25
Cmax (µg/ml)	6.10	5.38	5.40
Tmax (h)	1.00	2.00	2.00
Cl (L/h)	0.07	0.09	0.07
Vd (L)	4.92	4.47	4.49
Vd_{ss}(L)	9.31	11.25	9.71

to those found in the literature [5]. The solid-phase extraction procedure described here is simple, does not require large amounts of organic solvents and is very rapid (twelve samples can be processed in c. 15 min). The simple HPLC method should be of value for monitoring the tenoxicam concentration in patient plasma, for assessing the patient compliance in assuming prescribed tenoxicam regimes and for examinig the relationship between tenoxicam concentration in plasma and anti-inflammatory effects.

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REFERENCES

1. J. Vane , The evolution of non- steroidal anti-inflammatory drugs and their mechanism of action, Drugs, 33 (1987) 18-27.

2. K.M.Strub, L. Aeppli and A.Daum, Anti-inflammatory and analgesic effects of tenoxicam (tilcotil^{R}) in rats. In Advances in inflammatory, Raven Press, New York,(1986) 387-390.

3. M.E.Pickup, J.R. Lowe and D.B. Galloway - Determination of Ro 12-0068, a new anti - inflammatory and analgesic compound, in plasma by means of high performance liquid chromatography, J. Chromatogr., 225 (1981) 493-497.

4. J.S. Dixon, J.R. Lowe and D.B. Galloway - Rapid method for the determination of either piroxicam or tenoxicam in plasma using high performance liquid chromatography, J. Chromatogr., 310 (1984) 455-459.

5. R.J. Francis, J.G. Allen, D. Looi, J.S. Dixon, H.A. Bird and V. Wright, Pharmacokinetics of tenoxicam after oral administration in healthy human subjects of various single doses, Eur. J. Drug Metab. Pharmacokinetics, 12 (1987) 59-63.