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Short Communication

Determination of tenoxicam in plasma by high-performance liquid chromatography

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ABSTRACT

A high-performance liquid chromatographic technique with a lower detection limit for measuring tenoxicam plasma levels was standardized. Tenoxicam was extracted with dichloromethane from acidified plasma and the evaporated extracts were analysed on a reversed-phase column using a methanol–phosphate buffer mobile phase and setting ultraviolet detection at 355 nm. A reproducible calibration curve in the 5–2000 ng/ml range was obtained. The mean recovery of extraction was 98.99 ± 4.3% and the detection limit was 5 ng/ml.

INTRODUCTION

Tenoxicam is one of the newest non-steroidal anti-inflammatory drugs derived from thienothiazine; it belongs to the enolic acid group, along with oxyphenbutazone, phenylbutazone, piroxicam, isoxicam and sudoxicam [1–3]. It is 99% bound to plasma proteins and presents an extended plasma half-life, usually 72 ± 28 h. Clearance is carried out mainly through metabolic processes, and only 0.5% is eliminated unchanged in urine [3]. Several HPLC methods have been reported for the detection of tenoxicam levels in plasma and urine [4–6]. These methods have varying detection limits, ranging from 20 to 200 ng/ml; nevertheless, pharmacokinetic analyses of single doses require a very low detection limit [4]. In this paper we report a modified technique that allows a lower limit for the detection of tenoxicam plasma levels.

EXPERIMENTAL

Reagents and solvents

The reagents used were HPLC grade methanol and dichloromethane (J. T. Baker, Phillipsburg, NJ, USA), analytical grade potassium dihydrogenphosphate and disodium hydrogenphosphate dihydrate (Merck, Darmstadt, Germany), 37% hydrochloric acid (Carlo Erba, Milan, Italy) and deionized water. Tenoxicam and the internal standard (piroxicam) (Sigma, St. Louis, MO, USA) were used as reference compounds.

Stock and standard solutions

Tenoxicam and piroxicam stock solutions were prepared weekly in methanol at a 10 mg/100 ml

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concentration. Tenoxicam standard solutions were prepared daily in concentrations ranging from 5 to 2000 ng/100 μl. The piroxicam (internal standard) solution was prepared at a 200 ng/100 μl concentration. All solutions were stored in the dark at 4°C.

Extraction procedure

We used a modified version of the technique described by Dixon *et al.* [6]: 1 ml of 1 *M* hydrochloric acid and 1 ml of deionized water were added to 1 ml of drug-free plasma. Each mixture was then spiked with 100 μl of internal standard and 100 μl of tenoxicam following the different concentrations of the calibration curve or, in the case of blank samples, with 100 μl of methanol. Afterwards, the samples were extracted with 5 ml of dichloromethane and the whole mixed for 5 min and then centrifuged for 10 min at 1800 *g*. The organic phase was evaporated to dryness under nitrogen at 30°C. The dry residue was resuspended in 100 μl of mobile phase (0.1 *M* phosphate buffer pH 7.4–methanol, 3:2) following the technique described by Dell *et al.* [5]. Finally, 40 μl per vial were injected into the chromatograph.

Chromatographic conditions

We used a Beckman System Gold Model 126 liquid chromatograph (Beckman, San Ramon, CA, USA) equipped with a Spectra Physics 4270 integrator (Spectra Physics, San Jose, CA, USA) and a UV detector that was set at 355 nm. Samples were injected with a 210A sample injection valve (Beckman). A LiChrospher 100 RP-18 (5 μm), 125 mm × 4 mm I.D. column (Merck) was employed. The elution conditions were as follows: 3:2 (v/v) mixture of phosphate buffer (0.1 *M*, pH 7.4)–methanol. The flow-rate was set at 1.1 ml/min and column temperature was 20°C.

Recovery and reproducibility

The recovery of extraction of tenoxicam was determined by comparing the extraction from plasma spiked with tenoxicam with the equivalent concentration of standard solution in methanol blown down and redissolved in mobile phase.

RESULTS AND DISCUSSION

The extraction of tenoxicam and its internal standard from plasma was carried out without interferences due to the extraction procedure. Under the afore-mentioned chromatographic conditions, the mean retention times for tenoxicam and the internal standard were 3.4 and 4.5 min, respectively (Fig. 1). A reproducible linear calibration was obtained for plasma tenoxicam concentrations ranging from 5 to 2000 ng/ml. The correlation coefficient, slope and intercept for one typical calibration curve were, respectively, 0.9997, 1.002 and −2.313. The mean recovery of extraction of different tenoxicam concentrations was 98.99 ± 4.3. Table I shows the mean recovery of extraction, standard deviation (S.D.) and coefficient of variation (C.V.) for each concentration batch. Intra-assay precision (calculated from repeated analysis during one working day) was 5.3 ± 3.1% within the range 10–200 ng/ml. Inter-assay precision (calculated from repeated analyse on different days) was 4.3 ± 3.1% within the range 5–1000 ng/ml.

The HPLC technique standardized for the detection of tenoxicam and its internal standard (piroxicam) in plasma was sensitive and reproducible. The detection limit and the limit of quantification (LOQ) attained in this study were 5 and 10 ng/ml, respectively. The coefficient of variation (C.V.) of the LOQ was 9.18%. The

TABLE I

RECOVERY OF EXTRACTION OF TENOXICAM

Concentration (ng/ml)	Recovery (mean ± S.D., n = 5) (%)	Coefficient of variation (%)
5	88.47 ± 19.88	22.47
10	101.25 ± 9.29	9.18
20	102.70 ± 4.70	4.58
50	96.44 ± 6.85	7.10
100	100.75 ± 8.14	8.07
200	99.92 ± 3.65	3.62
500	100.18 ± 3.57	3.56
1000	100.93 ± 3.62	3.59
2000	100.31 ± 3.98	3.97

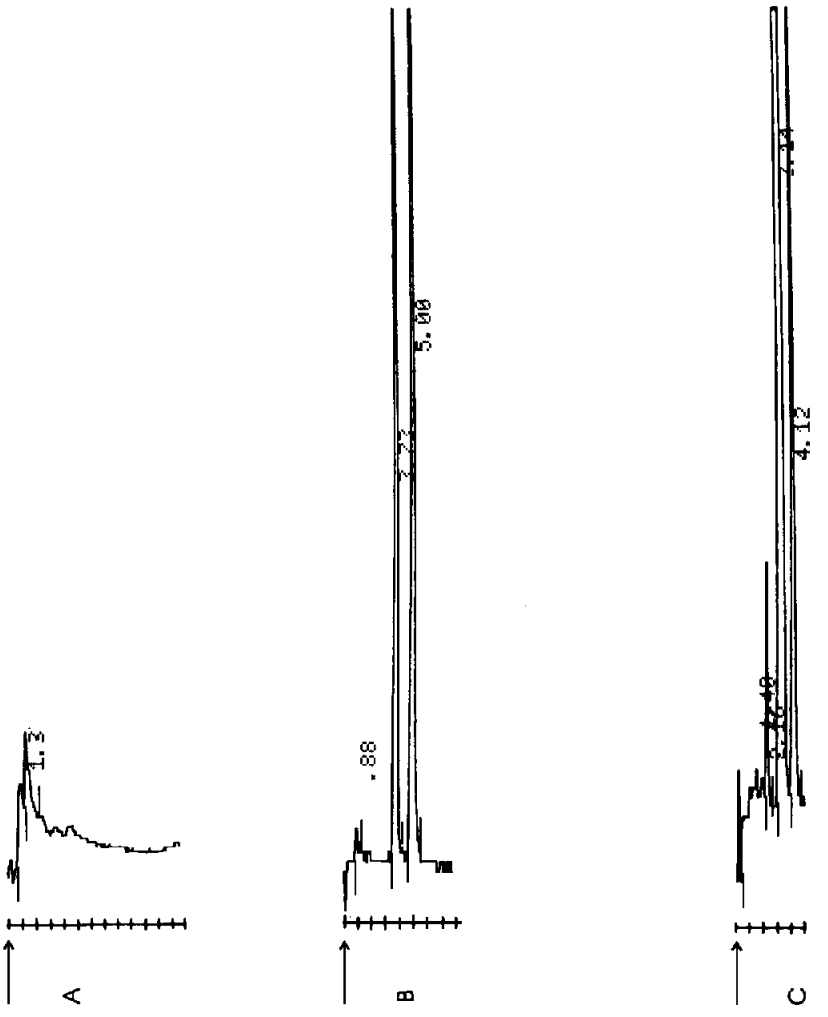

Fig. 1. Chromatograms of plasma extracts showing (A) blank human plasma, (B) tenoxicam (200 ng/ml, t_R = 3.73 min) plus internal standard (piroxicam) (200 ng/ml, t_R = 5.00 min) and (C) tenoxicam peak (t_R = 3.14 min) in a human volunteer 8 h after a single oral dose of 20 mg plus internal standard (piroxicam) (200 ng/ml, t_R = 4.1 min).

S.D. for the detection limit was ±0.99. The techniques described in previous works showed lower sensitivity: Heizmann *et al.* [4] obtained a detection limit of 20 ng/ml in plasma using a 0.5-ml sample; Dell *et al.* [5] determined tenoxicam and its hydroxy metabolite in urine and attained a detection limit of 50 ng/ml; Carlucci *et al.* [7] attained a detection limit of 50 ng/ml in human plasma using a solid-phase extraction column; finally, Dixon *et al.* [6] reported a detection limit of 200 ng/ml when measuring piroxicam and tenoxicam plasma levels.

The lower detection limit obtained with this technique allows the quantification of tenoxicam plasma levels after the administration of a single dose in pharmacokinetic analyses, especially in those cases where expected plasma and other body fluid concentrations (*i.e.* synovial fluid levels) are generally quite low [8].

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