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Journal of Chromatography B, 665 (1995) 410–415

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Short communication

Simple method for the analysis of tenoxicam in human plasma using high-performance liquid chromatography

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First received 14 September 1994; revised manuscript received 16 November 1994; accepted 16 November 1994

Abstract

A simple, rapid and cost-effective method for the determination of tenoxicam in human plasma is described, using ketorolac as the internal standard. The extraction procedure utilised 5% zinc sulphate and methanol. A nucleosil C_{18} column and 35:65 acetonitrile–water phosphate buffered mobile phase (pH 2.8) were used, with ultraviolet detection at 355 nm. The assay was linear in the range 40 ng/ml–10 μg/ml, with recovery of extraction ranging from 87 to 102%. The intra- and inter-assay reproducibility had coefficients of variation of 3.9–7.7 and 1.6% respectively. The limit of detection for this method was 40 ng/ml.

1. Introduction

Tenoxicam (4 - hydroxy - 2 - methyl - N - 2 - pyridinyl - 2H - thienol - [2,3 - e] - 1,2 - thiazine - 3 - carboxamide 1,2-dioxide) is a thienothiazine oxicam non-steroidal anti-inflammatory drug (NSAID) used in clinical medicine for the treatment of acute and chronic musculo-skeletal pain. NSAIDs inhibit prostaglandin biosynthesis by binding to cyclo-oxygenases [1]. This effect reduces the initiation of pain signals. Tenoxicam is 99% protein-bound in human plasma and has an elimination half life of 60–75 h [2]. Tenoxicam is almost entirely eliminated by the liver, the main metabolites in humans being the 5'-hydroxy- and the glucuronidated 6-O- metabolites, both of which are pharmacologically inactive [3]. Maximum plasma concentrations after one oral dose of 20 mg tenoxicam have been reported as 2.76 μg/ml with the corresponding concentration 24 h after dosing being 1.84 μg/ml [4]. Steady-state tenoxicam concentrations following multiple doses of 20 mg daily are reached between 10 and 14 days, mean concentrations after 14 days being 9.2 μg/ml [5].

Many of the HPLC methods reported for the measurement of tenoxicam are liquid–liquid extractions followed by evaporation [6–9]. The method described here requires no evaporation stage and therefore allows for a more rapid turnover of samples. Other methods not involving evaporation have been described [10,11]. One of these involves the use of solid-phase extraction cartridges, which increases the cost of analysis [11]. Also our procedure has a lower detection limit than either of these methods, 40 ng/ml compared to 200 and 100 ng/ml, respectively [10,11].

The range of linearity, precision and accuracy

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0378-4347/95/$09.50
SSDI 0378-4347(94)00534-6

of the described method make it ideal for the determination of tenoxicam in plasma following single or multiple doses.

2. Experimental

2.1. Materials

Zinc sulphate heptahydrate (ACS grade) was purchased from Sigma (Poole, UK); sodium lauryl sulphate (AR grade), sodium dihydrogen orthophosphate (AR grade), acetonitrile (HPLC grade), orthophosphoric acid (AR grade) and methanol (HPLC grade) were purchased from Fisons Scientific Equipment (Loughborough, UK). Tenoxicam was kindly provided by Roche Products (Welwyn Garden City, UK). Ketorolac trometamol was purchased from pharmacy.

2.2. Apparatus

The HPLC system consisted of an SP8800 pump, SP8780 autosampler, SpectraChrom 100 variable-wavelength detector and SP4400 Chromjet integrator (Thermo Separation Products, Stone, UK) and a C_{18} Nucleosil, 25 cm × 4.6 mm I.D., 5 μm particle size, reversed-phase column (Jones Chromatography, Hengoed, UK).

2.3. Chromatographic conditions

The mobile phase consisted of 10 m*M* sodium dihydrogen orthophosphate, 1 m*M* sodium lauryl sulphate and acetonitrile–water (35:65, v/v), the pH being adjusted to 2.8 with ortho-phosphoric acid. The mobile phase was filtered through a 0.45-μm Gelman Sciences membrane filter before use. The flow-rate was 1.5 ml/min and the temperature ambient (range 27–30°C). The UV detector was set at a wavelength of 355 nm and 0.01 AUFS. Ketorolac was used as the internal standard.

2.4. Calibration

All standards were made from stock solutions of tenoxicam and ketorolac (100 μg/ml in mobile phase and 1 mg/ml in water, respectively), which were stored in amber glassware at 4°C, fresh stocks being made up on a monthly basis. Freshly made calibration standards were prepared on a daily basis when required in the following concentrations, tenoxicam 40 ng/ml–10 μg/ml and ketorolac 10 μg/ml. Wherever possible, during daily use, stock solutions and spiked plasma samples were kept in darkened conditions.

2.5. Sample extraction and preparation

Blood samples were collected in 5-ml lithium heparin tubes and centrifuged at 2000 *g*, 27°C for 10 min. The plasma was then decanted off and either used immediately or stored at −70°C until use. Disposable polypropylene test tubes or wherever possible brown glassware was used for all subsequent work.

Plasma (1 ml) was spiked with internal standard (ketorolac, 10 μg/ml). To this was added 100 μl of 5% zinc sulphate (w/v in water). This was then vortex-mixed for 2 min. Methanol (3 ml) was added before vortex-mixing for a further 2 min. Aqueous buffer (0.44 ml) was then added. This buffer consisted of 100 m*M* sodium dihydrogen orthophosphate and 10 m*M* sodium lauryl sulphate. The pH was adjusted to 2.8 with orthophosphoric acid and the solution filtered (0.45-μm filter) before use. The sample was then finally vortex-mixed for a further 1 min. The sample was centrifuged at 2000 *g*, 27°C for 10 min and the supernatant decanted off. Brown autosampler vials were filled with supernatant and the autosampler set to inject 100 μl onto the HPLC column.

2.6. Extraction efficiency

Drug-free plasma was spiked with three different concentrations of tenoxicam (0.1, 1 or 2 μg/ml) and ketorolac 10 μg/ml ($n = 6$ for each

concentration) and then taken through the extraction procedure. The results were compared to a series of non-extracted aqueous standards to determine the extraction efficiency.

2.7. Reproducibility

Intra-assay reproducibility was investigated using three concentrations of tenoxicam, 0.05, 0.2 and 1 μg/ml ($n = 10$ for each concentration) and ketorolac 10 μg/ml, which were analysed during one working day. Inter-assay reproducibility ($n = 10$) was investigated using pooled plasma (tenoxicam 1 μg/ml, ketorolac 10 μg/ml) as a quality control. This was stored at −70°C prior to use, one aliquot being used per sample run.

2.8. Light sensitivity

Aliquots of drug-free plasma (1 ml) were dispensed into colourless polypropylene test tubes and then spiked with tenoxicam 1 μg/ml and ketorolac 10 μg/ml. These were left under normal laboratory conditions (ambient temperature, in daylight but out of direct sunlight) for 15 min to 6 h. These were then analysed to determine the tenoxicam and ketorolac content and thus the effect of light upon the stability of these drugs within plasma.

2.9. Stability of extracted samples

Stability of the extract, i.e. after the plasma sample has been taken through the extraction procedure, was investigated over a 24-h period at ambient temperature using brown glassware. Three concentrations of tenoxicam were used, 0.05, 0.2 and 1 μg/ml. Plasma was spiked with the required concentration of tenoxicam and 10 μg/ml ketorolac. The plasma samples were then taken through the extraction procedure and the resulting extract pooled. This was then divided equally between 24 brown autosampler vials, which were placed in the autosampler. This was then set to sample every hour for 24 h. The outcome of this experiment would determine whether a batch of samples could be prepared simultaneously and then left in an autosampler to be analysed overnight.

2.10. Measurement of tenoxicam following 20-mg intravenous injection

After taking a baseline blood sample, a male volunteer received tenoxicam intravenous (20 mg) at 09:00 am following overnight fasting. Blood samples (5 ml) were collected via an intravenous cannula at the following times after injection: 1, 2, 4, 6, 10, 15, 20, 30, 45 min and then 1, 2, 3, 4, 5, 6, 24, 48, 72, 96 and 192 h. The blood samples were centrifuged and analysed immediately.

3. Results and discussion

3.1. Measurement of tenoxicam

Fig. 1 shows chromatograms of peaks for tenoxicam (I) and ketorolac (II) from (A) plasma blank, (B) aqueous standard, tenoxicam 1 μg/ml and ketorolac 10 μg/ml, (C) plasma sample spiked with tenoxicam 1 μg/ml and ketorolac 10 μg/ml, and (D) plasma 2 min after intravenous administration of tenoxicam (20 mg) plus ketorolac 10 μg/ml. Retention times were approximately 4.6 min for tenoxicam and 10.3 min for ketorolac.

3.2. Calibration

The standard curve for tenoxicam was linear between 40 ng/ml and 10 μg/ml ($n = 6$ for each of the 10 concentration points, analysed during one working day), with the regression equation $y = 0.673x - 0.0384$. The standard deviation for the slope and intercept were 0.0089 and 0.0337, respectively. The correlation coefficient was 0.999. The limit of detection was 40 ng/ml (signal-to-noise ratio of 3:1).

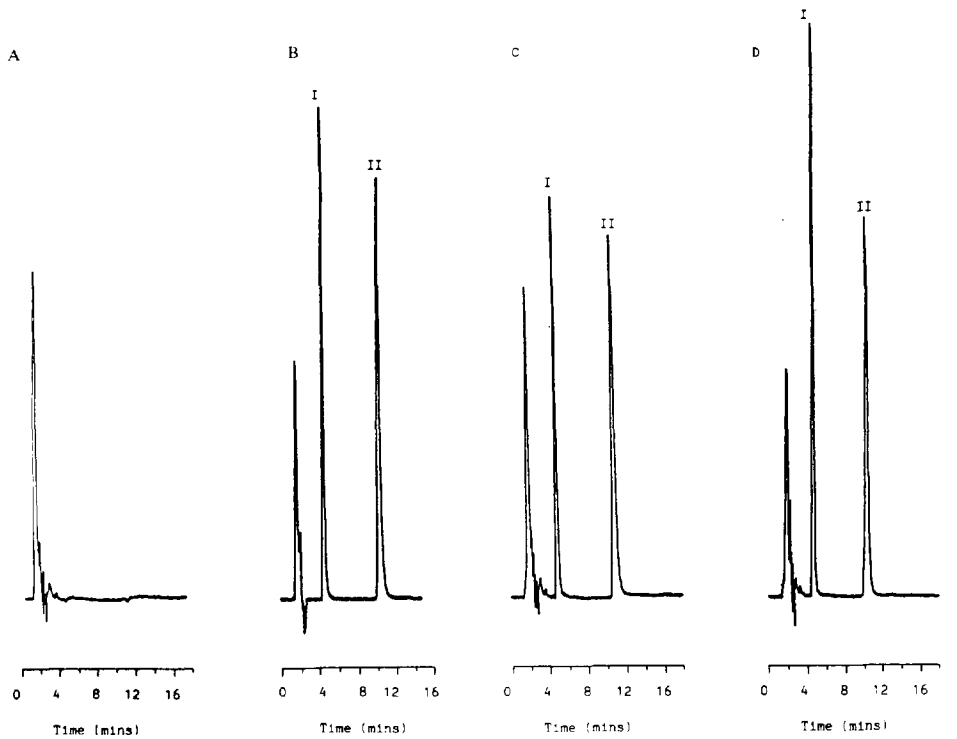

Fig. 1. Typical chromatograms of plasma blank (A), recovery of tenoxicam 1 μg/ml (I) and ketorolac 10 μg/ml (II) from aqueous standard (B) and spiked plasma (C), and plasma 2 min after intravenous 20-mg tenoxicam administration (D).

3.3. Extraction efficiency

Table 1 shows the mean percentage recoveries, standard deviations and coefficients of variation obtained from comparing the spiked plasma samples to the non-extracted standards. For all three concentration levels of tenoxicam studied, the coefficient of variation was less than 9%.

3.4. Reproducibility

Table 2 shows the intra-assay reproducibility for the three concentrations of tenoxicam investi-

Table 1
Recovery of extraction of tenoxicam and ketorolac (internal standard) ($n = 6$)

Tenoxicam concentration (μg/ml)	Tenoxicam		Ketorolac (10 μg/ml)	
	Recovery (mean ± S.D.) (%)	Coefficient of variation (%)	Recovery (mean ± S.D.) (%)	Coefficient of variation (%)
0.1	102.0 ± 8.9	8.7	103.2 ± 1.7	1.6
1.0	86.6 ± 2.9	3.3	87.4 ± 1.1	1.3
2.0	97.2 ± 1.9	2.0	99.4 ± 1.9	1.9

Table 2
Tenoxicam reproducibility and stability over 24-h period

Tenoxicam concentration (ng/ml)	Intra-assay reproducibility ($n = 10$) (Peak area ratio drug to I.S.)			Tenoxicam concentration of pooled extract over 24 h (ng/ml)	
	Mean	Standard deviation	Coefficient of variation (%)	Mean	Standard deviation
50	0.03	0.002	6.7	52	2.1
200	0.13	0.010	7.7	201	3.5
1000	0.61	0.024	3.9	994	11.2

gated ($n = 10$ for each concentration). Reproducibility ranged from 3.9 to 7.7. Over a one month period ($n = 10$), the quality control plasma samples (tenoxicam 1 μg/ml and ketorolac 10 μg/ml) had a coefficient of variation of 1.6%.

3.5. Light sensitivity

Under normal laboratory conditions, spiked plasma samples lost 43% of the tenoxicam content within 3 h (Fig. 2). This loss is similar to that reported elsewhere [7]. At 6 h this loss had increased to 100%. Therefore, all samples and solutions were kept and handled under darkened conditions and brown glassware used wherever possible to reduce the exposure to light.

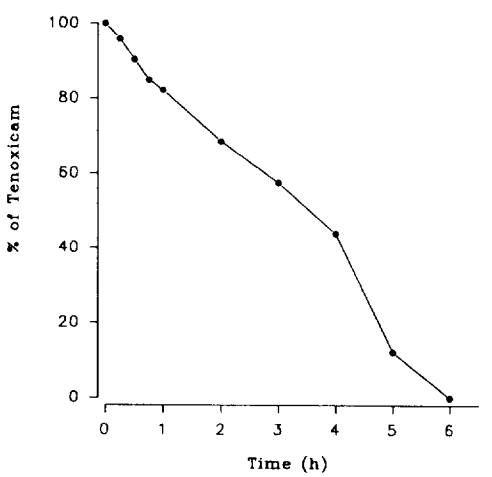

Fig. 2. Loss of tenoxicam in plasma under normal laboratory conditions. The x-axis denotes time (h) post-preparation, with storage in clear polypropylene test tubes at ambient temperature.

3.6. Stability

For the 24-h period investigated, the tenoxicam content of the pooled plasma, kept in darkened conditions, was stable. Table 2 shows the mean and standard deviation values of the three tenoxicam concentrations investigated.

3.7. Measurement of tenoxicam following 20-mg intravenous injection

Fig. 3 shows the plasma levels of tenoxicam after a 20-mg intravenous administration. Using the described method, plasma levels of tenoxicam are measurable for 192 h following administration (2–3 elimination half-lives).

4. Conclusions

Concentrations as low as 40 ng/ml (signal-to-noise ratio of 3:1) can be measured using the described procedure. Recovery using this technique was good over a range of tenoxicam concentrations, e.g. for 0.1 and 2 μg/ml recovery was 102 and 97%, respectively. This method was reproducible, intra- and inter-assay reproducibility had coefficients of variation be-

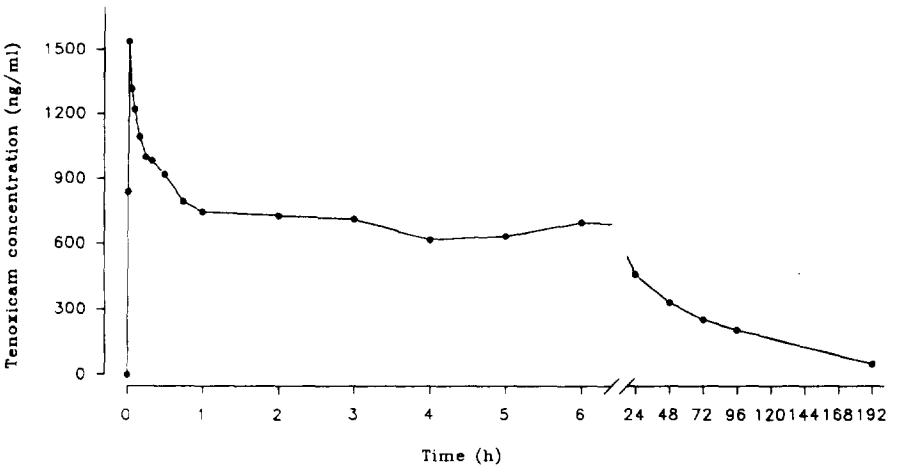

Fig. 3. Plasma levels of tenoxicam in a volunteer after intravenous administration of 20 mg tenoxicam.

tween 3.9–7.7 and 1.6%, respectively. This procedure provides chromatograms with no inherent background interference at or around the retention times of tenoxicam or ketorolac in blank plasma samples (Fig. 1). Handling and analysis of solutions and samples should be done in darkened conditions using brown glassware wherever possible due to the sensitivity of tenoxicam to light. Provided this is done, a batch of samples can be taken through the extraction procedure and analysed any time within 24 h, i.e. an autosampler can be utilised for overnight analysis.

Acknowledgements

We would like to thank Mr. Steve Ashmore for helpful discussions and Dr. Charlie Leeson-Payne for donating his blood.

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