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Note

Rapid and sensitive method for determination of piroxicam in human plasma by high-performance liquid chromatography

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Piroxicam, a non-steroidal anti-inflammatory drug, has been reported to be effective in the treatment of osteoarthrosis and rheumatic arthritis [1–3]. High-performance liquid chromatographic (HPLC) methods are recommended for the measurement of piroxicam plasma levels [4–8]. All these methods require liquid–liquid extraction with consecutive evaporation.

This report describes a rapid and sensitive method for the determination of piroxicam in human plasma. The sample preparation involves only protein precipitation and centrifugation; no extraction and evaporation step is required.

EXPERIMENTAL

Chemicals

Piroxicam [4-hydroxy-2-methyl-N-(2-pyridyl)-2*h*-1,2-benzothiazine-3-carboxamide 1,1-dioxide] was obtained from Pfizer (Karlsruhe, F.R.G.). All other chemicals were of analytical grade (Lachema, Brno, Czechoslovakia). The HPLC mobile phase was filtered through a 0.45-μm filter (Supelco, Bellefonte, PA, U.S.A.) and degassed under helium.

Chromatography

Chromatographic equipment was obtained from Spectra-Physics (San Jose, CA, U.S.A.). An SP-8100 liquid chromatograph equipped with an SP-8110 autosampler was connected with an SP-8440 variable-wavelength UV–VIS detector and an SP-4200 computing integrator. The volume of the injection loop was 50 μl.

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Analyses were performed at 35 °C on a glass column packed with cyanopropyl-bonded silica (Separon SIX CN, 15 cm × 3.3 mm I.D., 5 μm particle size, Laboratorní Přístroje, Prague, Czechoslovakia). The mobile phase was 0.05 *M* potassium dihydrogenphosphate (pH 2.2)–methanol (65:35, v/v), delivered at a flow-rate of 0.5 ml/min with pressure 10 MPa. Column effluent was monitored at 360 nm at a sensitivity of 0.04 a.u.f.s., and the time constant was 1 s.

Sample preparation

Methanol (0.3 ml) was added to 0.2 ml of plasma. The tube was vigorously shaken for 1 min, and after 10 min equilibration it was shaken again for 1 min. After centrifugation (10 min, 7000 *g*) the supernatant was transferred to an auto-sampler vial.

Calibration procedure

A stock solution of piroxicam was prepared in methanol at a concentration of 27.1 μg/ml. This solution was further diluted with methanol to the desired concentrations. A 0.3-ml volume of standard solution (concentrations 0.27–10.8 μl/ml) was added to 0.2 ml of blank plasma, and the sample was processed as described above.

Application to plasma samples

A 20-mg dose of piroxicam (Felden) was administered orally to eleven healthy volunteers. Blood samples were taken at 0, 2, 4, 6, 8, 12, 24 and 48 h following the dose. The K_2EDTA plasma was prepared from the blood by the standard method.

RESULTS AND DISCUSSION

Chromatography

The chromatogram of blank plasma is shown in Fig. 1A. All the endogenous compounds visible at 360 nm are eluted in the front peak and no later peaks appear on the chromatogram. This was impossible to achieve on a C_{18} column and therefore we chose a CN column. Piroxicam is well separated from the front peak; it has a retention time of 4.53 min (Fig. 1B). The low pH (2.2) of the mobile phase greatly improved the column efficiency.

Of the drugs tested for interference (Table I), none was found to interfere with piroxicam.

Sample preparation

It was necessary to inject 50 μl of the sample in order to obtain the desired sensitivity. For this reason it was impossible to perform protein precipitation with acetonitrile, as was suggested for the determination of isoxicam [9]. Although acetonitrile yielded a cleaner solution after protein precipitation, it impaired the peak shape substantially, perhaps because the column equilibration was disturbed. Nevertheless, the methanolic extract can be cleaned by centrifugation at 7000 *g* (10 min).

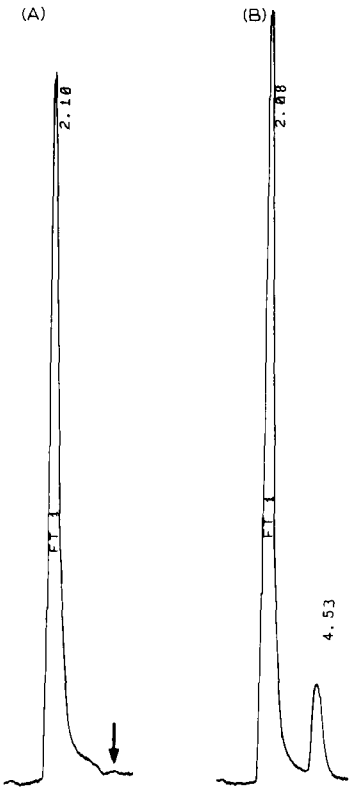

Fig. 1. (A) Chromatogram of blank plasma extract. (B) Chromatogram of plasma extract from a sample taken 8 h after a volunteer received 20 mg of piroxicam. The concentration of piroxicam in plasma was 1.98 μg/ml.

Our sample preparation compares favourably with previous methods because of its simplicity.

Linearity, sensitivity, precision and accuracy

The calibration curve was obtained by linear regression and was linear in the range 0.27–10.8 μg/ml. The detection limit was ca. 0.15 μg/ml (signal-to-noise

TABLE I

RETENTION TIMES OF POSSIBLE INTERFERING DRUGS

Drug tested	Retention time (min)
Sulfadimidine	2.29
Antipyrine	2.97
Ibuprofen	1.76
Plaquenil	2.53
Diclofenac	2.41
Isoxicam	7.13

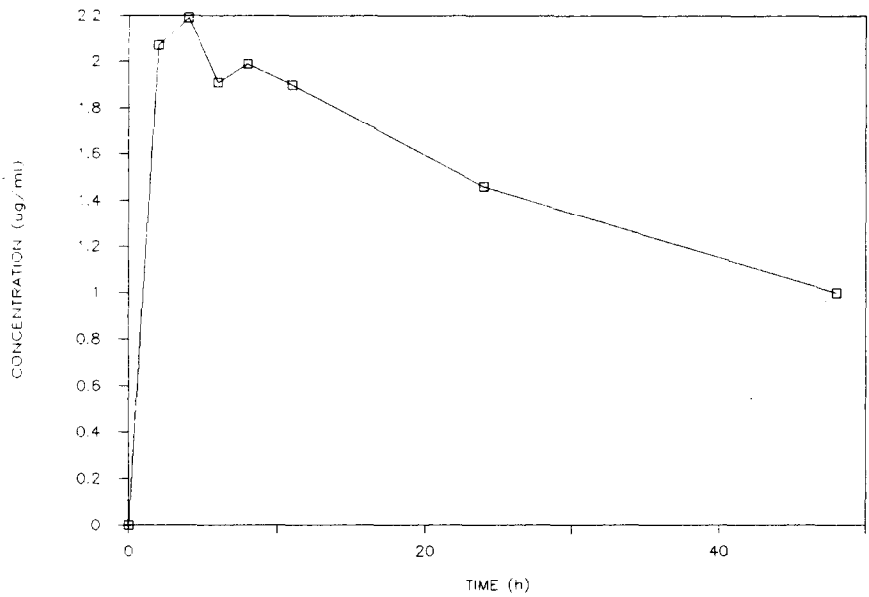

Fig. 2. Plasma concentrations of piroxicam after a 20-mg single oral dose (mean data for eleven subjects).

ratio 4:1). The precision expressed as relative standard deviation was 2.9% at 2.71 μg/ml ($n=5$). The accuracy of the assay was between 0.1 and 5.6% within the calibration range, with a mean value of 2.0% ($n=6$).

Application to plasma samples

The pharmacokinetic profile is shown in Fig. 2. Two maxima on the curve are caused by enterohepatal recirculation of the drug [10]. The method is sufficiently sensitive to monitor the concentration of piroxicam in plasma after a single oral dose.

CONCLUSION

The HPLC method described here is selective, sensitive and rapid. With the help of an autosampler up to 100 samples can be analysed in a day. The sensitivity, reproducibility and accuracy of the assay are sufficient for pharmacokinetic studies, results of which will be published elsewhere.

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