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Note

Determination of isoxepac in plasma by high-performance liquid chromatography

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Isoxepac (6,11-dihydro-11-oxodibenz[*b*,*e*]oxepin-2-acetic acid) is a non-steroidal anti-inflammatory agent under investigation for the treatment of rheumatoid arthritis. The metabolism [1] and the gas—liquid chromatographic (GLC) determination [2] of isoxepac have previously been described. A high-performance liquid chromatographic (HPLC) analysis was developed to meet the need for a facile and rapid method to determine the pharmacokinetics of isoxepac in patients with rheumatoid arthritis.

EXPERIMENTAL

Materials

Isoxepac and the propionic acid analog (6,11-dihydro-11-oxodibenz[*b*,*e*]-oxepin-2-propionic acid) [2] were obtained from Hoechst UK, Pharmaceuticals Division (Hounslow, Great Britain) and naproxen from Syntex (Palo Alto, CA, U.S.A.). Diethyl ether was glass distilled and methanol was HPLC grade. All other chemicals were AR grade and were used without further treatment.

Extraction

Plasma samples were stored at −20°C till required. In order to minimise the photodecomposition of isoxepac [2] plasma samples were allowed to thaw in the dark and care was taken to avoid direct sunlight during the extraction procedure. A 20-μl aliquot of the internal standard (isoxepac analog 500 μg ml^{-1} in methanol), was added to a 10-ml test tube followed by 0.5 ml plasma and 0.15 ml 2 *N* hydrochloric acid. Diethyl ether (4 ml) was added, the contents mixed by vortexing and the layers separated by centrifugation (5 min at 1000 *g*). A 3.5-ml aliquot of the organic layer was removed and evaporated to

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dryness using a stream of nitrogen. The residue was dissolved in 0.3 ml methanol and stored in the dark at 4°C till analysed.

A calibration curve was constructed over the range 1—32 $\mu g\ ml^{-1}$ by addition of isoxepac in methanol to test tubes and evaporating to dryness with a stream of nitrogen. The procedure was then as described above.

If naproxen was utilised as the internal standard 20 μl of a 250-$\mu g\ ml^{-1}$ methanol solution were added.

Chromatography

A 100 × 5 mm Shandon column (Shandon Southern Products, Runcorn, Great Britain) was slurry packed with LiChrosorb RP-18, particle size 5 μm (BDH Chemicals, Poole, Great Britain) using a constant-pressure pump (Haskel MCP-71). The isocratic mobile phase consisted of methanol—5% glacial acetic acid in water (6:4) and was pumped at a constant flow-rate of 1.3 ml min^{-1} using a Waters 6000A solvent delivery system (Waters Assoc., Northwich, Great Britain). A Rheodyne 7120 valve injector (HPLC Technology, Wilmslow, Great Britain) was fitted with a 10-μl loop and detection was at 254 nm using a Waters 440 absorbance detector.

RESULTS AND DISCUSSION

Isoxepac was quantified by HPLC following diethyl ether extraction of acidified plasma. The extraction efficiencies of various solvents for isoxepac have previously been discussed [2]. Calibration curves were linear over the range 1—32 $\mu g\ ml^{-1}$ with correlation coefficients greater than 0.993 and linear regression analysis gave $y = 0.047x + 0.089$. The coefficient of variation for replicate extractions ($n = 7$) was 1.6% and for replicate injections ($n = 7$) was less than 1%. The capacity factors for isoxepac, isoxepac propionic acid analog and naproxen were 3.0, 4.9 and 5.5 respectively. Typical chromatograms are given in Fig. 1.

Plasma samples were taken from 15 patients undergoing oral isoxepac therapy, 150 mg twice daily and 300 mg at night, 2—6 h following the morning dose. The mean concentration was found to be 24 $\mu g\ ml^{-1}$ (S.D. = 15 $\mu g\ ml^{-1}$) and the range 7—61 $\mu g\ ml^{-1}$. When the concentration exceeded 32 $\mu g\ ml^{-1}$ the plasma samples were suitably diluted and reanalysed. The sensitivity of the method was 1 $\mu g\ ml^{-1}$ of isoxepac in plasma.

The use of the isoxepac analog as an internal standard is preferable as it exhibits similar extraction properties to isoxepac [2] but it has the disadvantage of limited availability. Naproxen is readily available and has a similar capacity factor to the isoxepac analog but care must be taken that patients have not been treated with naproxen for a period of less than two weeks prior to study.

The possibility exists that rheumatoid arthritis patients undergoing isoxepac therapy might also be taking other non-steroidal anti-inflammatory drugs which could interfere in the analysis of isoxepac. Table I gives the retention data for 18 other non-steroidal anti-inflammatory drugs and 5 of these, i.e. indomethacin, ketoprofen, salsalate, sulindac and tolmetin, would interfere. The only identified metabolite of isoxepac is the glucuronide ester [1] and this would not interfere in the assay for the parent drug.

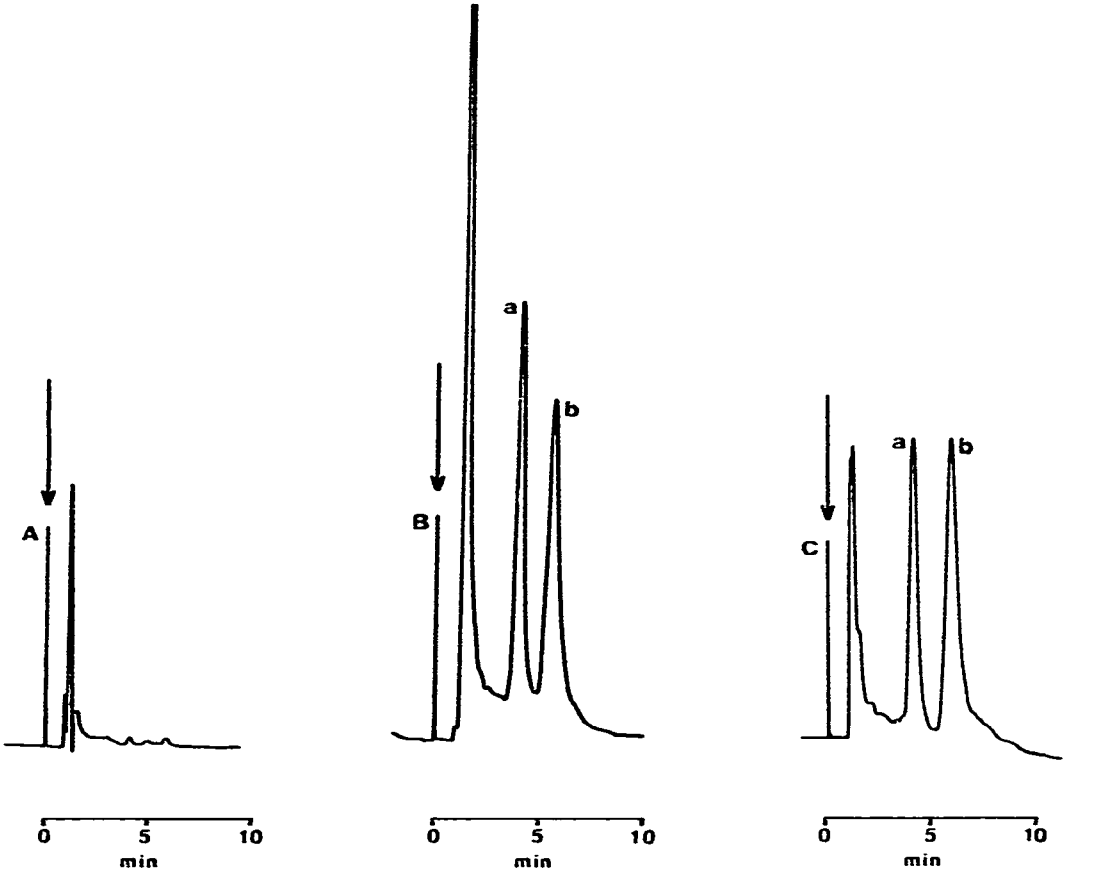

Fig. 1. Chromatograms of extracts of (A) blank plasma; (B) plasma spiked with isoxepac (a) and internal standard (b); (C) plasma from patient undergoing therapy with isoxepac (150 mg twice daily and 300 mg at night); this sample corresponds to an isoxepac concentration of 19 $\mu g\ ml^{-1}$.

TABLE I

HPLC RETENTION DATA FOR 19 NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

HPLC conditions: column: 100 × 5 mm packed with 5-μm RP-18; mobile phase:methanol—5% acetic acid in water (6:4).

Drug	Capacity factor	Drug	Capacity factor
Diclofenac	12.1	Naproxen	5.5
Fenbufen	5.0	Oxyphenylbutazone	2.3
Fenclofenac	14.1	Paracetamol	0.2
Fenoprofen	7.7	Penicillamine	0.4
Feprazone	7.4	Phenylbutazone	7.2
Flurbiprofen	9.7	Piroxicam	2.3
Ibuprofen	14.3	Salicylic acid	1.4
Indomethacin*	3.2	Salsalate*	3.8
Isoxepac	3.0	Sulindac*	3.4
Ketoprofen*	3.4	Tolmetin*	3.2

*Drugs which may interfere in the analysis of isoxepac.

HPLC was preferable to GLC [2] because no derivatisation was required and the analysis time was not prolonged by the elution of cholesterol. The use of a diethyl ether extract gave prolonged column life and the method should also be suitable for the quantitative analysis of the glucuronide conjugate in urine [1] following hydrolysis.

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