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Note

Liquid chromatographic assay of isoxicam in human plasma and urine

R.W. BURY

Department of Clinical Pharmacology and Therapeutics, The Royal Melbourne Hospital, Parkville, Victoria 3052 (Australia)

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Isoxicam is a long-action non-steroidal anti-inflammatory agent which has been reported to be effective in the treatment of osteo-arthritis and rheumatoid arthritis [1—3]. It is a member of the oxicam class of anti-inflammatory compounds, a group that differs structurally from other types of non-steroidal anti-inflammatory drugs [4].

A method for estimation of isoxicam in plasma and urine using high-performance liquid chromatography (HPLC) has been reported recently [5]. The present paper describes an alternative reversed-phase liquid chromatographic assay for isoxicam in plasma and urine which can be more rapidly and simply performed. The procedures have been employed successfully in multiple-dose pharmacokinetic studies of isoxicam in man [6].

MATERIALS AND METHODS

Chemicals and solutions

With the exception of acetonitrile and tetrahydrofuran which were HPLC grade (Ajax Chemicals, Melbourne, Australia), all chemicals used were of analytical quality. Water was distilled. Isoxicam was supplied as pure powder by Parke-Davis (Australia). Piroxicam (Pfizer, Australia) and diazepam (Roche Products, Australia) were used as internal standards for the assays.

Stock solutions of isoxicam were prepared in acetonitrile containing 10% tetrahydrofuran, at concentrations ranging from 2 to 100 μg/ml. Stock solutions of the internal standards, piroxicam (100 μg/ml) and diazepam (40 μg/ml) were prepared in acetonitrile. These solutions were stable for at least six months when stored at 4°C.

The following aqueous solutions were used: (a) 0.05 mol/l potassium

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dihydrogen orthophosphate, pH adjusted to 3.0 with orthophosphoric acid; and (b) saturated potassium dihydrogen orthophosphate, pH adjusted to 2.4 with orthophosphoric acid.

Sample preparation

Plasma samples (100 μl) were pipetted into disposable polypropylene Wasserman tubes containing 50 μl of a 100 μg/ml stock solution of piroxicam, the internal standard. After addition of 300 μl acetonitrile, the tubes were vortex-mixed vigorously for 1 min, then subjected to centrifugation at 2000 *g* for 2 min. A 20-μl volume of the supernatant was injected into the chromatograph.

Urine specimens (1.0 ml) containing 50 μl of a 40 μg/ml stock solution of diazepam as the internal standard and 100 μl of saturated phosphate buffer (pH 2.4) were extracted for 2 min with 3 ml dichloromethane using a multi-tube vortex-mixer (Model 2601, Scientific Manufacturing Industries, Emeryville, CA, U.S.A.). After centrifugation at 2000 *g* for 2 min, the organic phase was transferred to a tapered glass tube and evaporated to dryness at 50°C under a gentle stream of nitrogen. The residue was reconstituted with 50 μl of acetonitrile of which 20 μl were injected into the chromatograph.

Assay calibration

The assays were calibrated by addition of stock solutions of isoxicam and the internal standard piroxicam, to pooled blood blank plasma, and by addition of stock solutions of isoxicam and the internal standard, diazepam, to drug-free urine specimens. Calibration curves were established for concentrations of isoxicam up to 100 μg/ml in plasma, and up to 2 μg/ml in urine. Peak area ratios of isoxicam to the appropriate internal standard were used in quantitation of the assays.

Chromatographic analyses

The liquid chromatograph consisted of a Varian Model 5000 high-pressure pumping system (Palo Alto, CA, U.S.A.), a Rheodyne Model 7120 injection valve with a 20-μl loop (Berkeley, CA, U.S.A.), a Varian Vari-chrom ultraviolet absorbance detector and a Hewlett-Packard Model 3380A integrator (Palo Alto, CA, U.S.A.).

Analyses were performed at ambient temperature on an octadecylsilane (ODS) reversed-phase column (Hibar LiChrosorb, 10 μm particle size, RP18, 250 mm × 4 mm, E. Merck, Darmstadt, F.R.G.). For analyses of isoxicam in plasma, the mobile phase was acetonitrile—0.05 mol/l potassium dihydrogen phosphate buffer, pH 3.0 (50:50) delivered at a flow-rate of 2 ml/min. Ultraviolet absorbance at 325 nm was monitored at a sensitivity of 0.05 a.u.f.s. For analyses of isoxicam in urine, the mobile phase was acetonitrile—0.05 mol/l potassium dihydrogen phosphate buffer, pH 3.0 (45:55). All other chromatographic conditions remained the same as for plasma analyses.

RESULTS AND DISCUSSION

Typical chromatograms of extracted plasma specimens (Fig. 1) show that control samples are free from interfering peaks. Retention times for isoxicam and the internal standard, piroxicam, were 3.1 and 2.4 min, respectively. Since

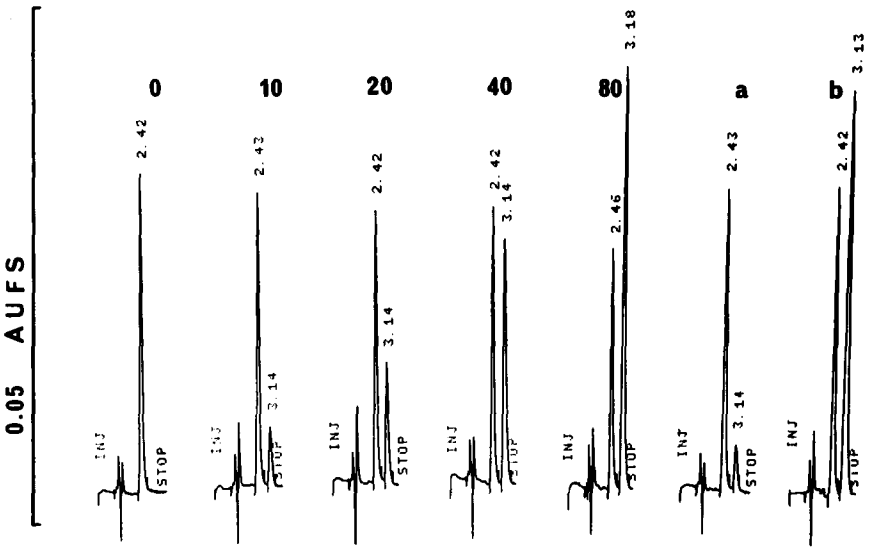

Fig. 1. Chromatograms of human plasma extracts: 0—80 μg/ml isoxicam in pooled blood bank plasma; (a) 6.5 μg/ml and (b) 60.3 μg/ml isoxicam in plasma from a subject who had received medication with isoxicam. Retention times for isoxicam and the internal standard, piroxicam, were 3.1 min and 2.4 min, respectively.

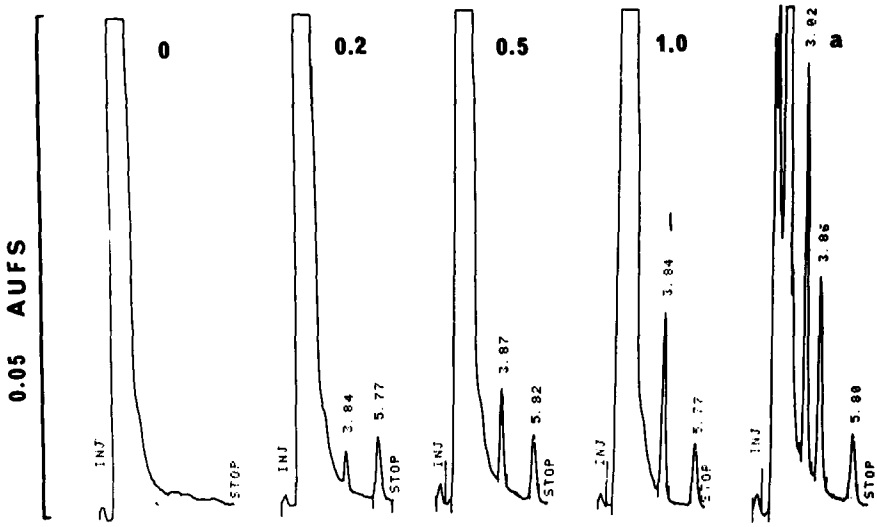

Fig. 2. Chromatograms of human urine extracts: 0—1.0 μg/ml isoxicam in drug-free urine; (a) 0.95 μg/ml isoxicam in urine from a subject who had received medication with isoxicam. Retention times for isoxicam and the internal standard, diazepam, were 3.8 and 5.8 min, respectively.

no endogenous substances with prolonged retention on-column were detected, injection of specimens could be repeated immediately after the elution of isoxicam.

Typical chromatograms of urinary extracts (Fig. 2) show that control samples are free from interfering peaks. Isoxicam eluted in 3.8 min and the internal standard, diazepam, in 5.8 min. Endogenous contaminants remaining in the extracts were eluted before isoxicam. Injections of urinary extracts could

therefore be repeated immediately after elution of the internal standard. Extracts of urine from subjects who had been receiving regular oral doses of isoxicam contained an additional substance which eluted at 3.0 min (Fig. 2). This was assumed to be a metabolite of isoxicam. Since it co-eluted with piroxicam, the latter was unsuitable as an internal standard in the urinary analyses.

For both plasma and urinary assays, calibration curves for isoxicam passed through the origin. They were linear up to 100 μg/ml in plasma and up to 2 μg/ml in urine, the maximum concentrations used. For a signal-to-noise ratio of 4, the minimum detectable concentration of isoxicam in plasma was 0.2 μg/ml and in urine was 0.02 μg/ml.

Recovery of isoxicam from plasma and urine following extraction was assessed by comparison of peak areas from the extracts with those arising from standard solutions of isoxicam in acetonitrile. At a plasma isoxicam concentration of 0.5 μg/ml, recovery was 96%. For isoxicam at plasma concentrations between 10 and 100 μg/ml, mean recovery was 94%. The mean difference beween results of duplicate analyses was 6.1% (n = 60). The inter-assay coefficient of variation was 10.0% (n = 10) at a plasma isoxicam concentration of 0.5 μg/ml, 4.1% (n = 16) at 20 μg/ml, and 2.6% (n = 9) at 80 μg/ml. For isoxicam in urine, recovery was 89% for concentrations between 0.2 and 2 μg/ml. The inter-assay coefficient of variation at an isoxicam concentration in urine of 0.2 μg/ml was 7.1% (n = 9).

The practical effectiveness of the assay was demonstrated by assaying blood and urine specimens obtained from twenty human subjects who had received 200 mg isoxicam daily for up to fifteen days. Steady-state plasma concentrations ranged from 12.2 to 85.9 μg/ml and urinary concentrations ranged from 0.10 to 1.76 μg/ml. No interference with the chromatographic measurement of isoxicam was observed despite concurrent medication of these subjects with a wide variety of drugs including furosemide, chlorothiazide, hydrochlorothiazide, propranolol, metoprolol, prazosin, methyldopa, hydralazine, labetolol, minoxidil, allopurinol, sulphinpyrazone, digoxin, naproxen, doxepin, imipramine, trifluoperazine, cotrimoxazole, cephalexin and mepenzolate.

The HPLC method described here permits selective determination of isoxicam in both plasma and urine. Since sample preparation and chromatographic run times are short, the procedure is suitable for use in clinical research involving large numbers of specimens — 60 plasma or 40 urine specimens may be assayed in duplicate per day. The sensitivity and reproducibility of the assay procedure have proven sufficient for pharmacokinetic studies [6].

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