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Note

High-performance liquid chromatography of sulindac and its sulphone and sulphide metabolites in plasma

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Sulindac (*cis*-5-fluoro-2-methyl-1-[*p*-(methylsulphinyl)benzylidene]-indene-3-acetic acid) is an anti-inflammatory drug used in the treatment of various arthritic conditions [1, 2]. Sulindac possesses little pharmacological activity, but its sulphide metabolite, which is formed by reversible reduction, is the active species and is about five times more potent than sulindac [3]. The sulphone metabolite, which is formed by irreversible oxidation, is pharmacologically inactive [3].

The technique most commonly used for the analysis of sulindac is high-performance liquid chromatography (HPLC) [4–7]. These methods all employ solvent programming to give good separation of sulindac and its metabolites from endogenous substances in plasma, and to reduce the assay time.

The isocratic HPLC method described here was developed to measure low concentrations of sulindac and its metabolites in a study that used low single oral doses (100 mg) of sulindac, and eliminated the need for more sophisticated equipment in a solvent programmer to give good sensitivity and assay time.

EXPERIMENTAL

Reagents

Dichloromethane, methanol, orthophosphoric acid, glacial acetic acid, sodium acetate and sodium hydroxide were analytical-reagent grade and supplied by Ajax Chemicals (Sydney, Australia). Sulindac and its sulphone and sulphide metabolites were supplied by Merck Sharp and Dohme (Rahway, NJ, U.S.A.). The internal standard, phenprocoumon, was supplied by Hoffman-La Roche.

Reagents required for the extraction and reconstitution steps were 2.5 *M* phos-

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phoric acid and 0.05 *M* sodium hydroxide; the latter was freshly prepared every week.

Standards

Stock solutions of sulindac and its sulphone and sulphide metabolites were prepared in methanol to give a concentration of 500 mg/l. These were stable for at least two months when stored at a temperature of 4°C. Combined working standards containing 30.0, 20.0, 10.0, 5.0, 1.0 and 0.5 mg/l of each compound were prepared every two weeks, and were diluted 1:9 with drug-free plasma daily to give standards containing 3.0, 2.0, 1.0, 0.5, 0.1 and 0.05 mg/l.

The internal standard, phenprocoumon, was prepared by dissolving 80 mg of this compound in 100 ml of methanol. A 0.125-ml aliquot of this stock solution was diluted to 1 l with dichloromethane to give a final concentration of 0.10 mg/l.

Equipment

A Waters HPLC system was used, which consisted of an M6000 pump, a Model 481 variable-wavelength detector set at 315 nm, a WISP (automatic sample injector) and a dual-pen recorder.

A Waters phenyl reversed-phase column (300 mm×3.9 mm I.D., 10 μm particle size) was used. The mobile phase consisted of 42% acetonitrile, 1% glacial acetic acid, and 57% distilled water, buffered with 10 m*M* sodium acetate. This solution had a pH of 4.2 and was filtered through a 0.47-μm Nylon 66 filter membrane under reduced pressure before use. All chromatography was performed at ambient temperature at a solvent flow-rate of 2.0 ml/min. The detector was set at 0.05 a.u.f.s. and the recorder at 2 and 10 mV for full scale deflection.

Method

To a labelled 15-ml culture tube fitted with a PTFE-lined screw cap were added 0.5 ml of plasma standard or sample, 0.25 ml of 2.5 *M* phosphoric acid and 6.0 ml of dichloromethane containing the internal standard. The tubes were vortex-mixed for 1 min then centrifuged at 1500 *g* for 10 min. The upper aqueous layer was aspirated and the lower organic layer transferred to a conical centrifuge tube. The dichloromethane was evaporated using a vortex evaporator and the residue reconstituted with 0.2 ml of 0.05 *M* sodium hydroxide. Complete dissolution of the residue was ensured by vortex mixing the tube contents for 1 min. Then 0.075–0.15 ml of the solution were injected onto the column.

RESULTS AND DISCUSSION

Fig. 1A shows the chromatogram of a plasma blank and was recorded using parameters that gave maximum sensitivity for the assay (injection volume 0.15 ml, chart recorder setting 2 mV and detector setting 0.05 a.u.f.s.). There is minimal interference for sulindac (peak 1), and no interference for the sulphone (peak 2), the internal standard (peak 3) and the sulphide (peak 4). Fig. 1B shows the chromatogram of a 1.0 mg/l standard and Fig. 1C the chromatogram of a sample from a patient. The chromatograms shown for Fig.1B and C were

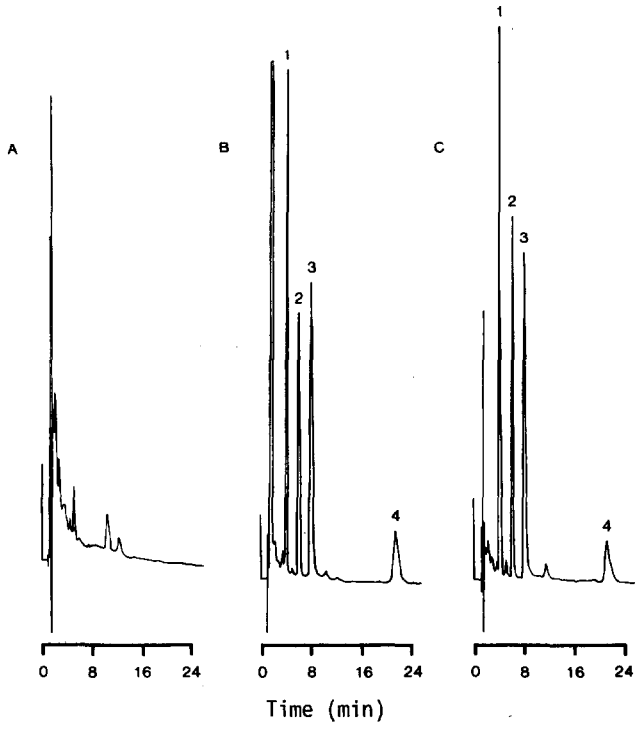

Fig. 1. Chromatograms obtained from extracted human plasma containing sulindac and the sulphone and sulphide metabolites. (A) Blank human plasma; (B) plasma standard containing 1.0 mg/l sulindac (peak 1), the sulphone (peak 2), the internal standard (peak 3) and the sulphide (peak 4); (C) plasma sample from a patient. Chromatogram A was recorded using conditions (greater injection volume and more sensitive settings on the chart recorder) that gave a 7.5-fold increase in overall sensitivity on the chromatograms recorded in B and C.

recorded at a less sensitive setting, i.e. chart recorder setting 10 mV, injection volume 0.1 ml and detector setting 0.05 a.u.f.s. The retention times for sulindac, the sulphone, the internal standard and the sulphide were 4.2, 6.4, 8.6 and 23.0 min, respectively.

The use of a phenyl reversed-phase column enabled sulindac and its two metabolites to be separated within a reasonable time (23 min) under isocratic conditions. Other workers have used C_{18} reversed-phase columns with solvent programming for the analysis of sulindac and its metabolites [4–7]. The use of a C_{18} reversed-phase column and isocratic solvent flow would not have permitted the adequate separation of sulindac, the sulphone and the internal standard from endogenous substances in the plasma without extending the assay time beyond that currently obtained with this method.

Although HPLC methods using solvent programming have slightly shorter assay times and shorter retention times for the sulphide metabolite, which elutes lasts, no significant loss of sensitivity is apparent with the present method as a consequence of the longer retention time. The present method has similar sensitivity to other extraction methods for sulindac and its metabolites, and does not require the more expensive solvent-programming HPLC system for analysis.

TABLE I

DATA FOR INTRA-ASSAY PRECISION AND ACCURACY ($n = 10$)

Known concentration (mg/l)	Analyte	Measured concentration (mean ± S.D.) (mg/l)	Coefficient of variation (%)
0.05	Sulindac	0.048 ± 0.002	4.17
	Sulphone	0.050 ± 0.004	8.00
	Sulphide	0.049 ± 0.003	6.12
0.50	Sulindac	0.491 ± 0.013	2.65
	Sulphone	0.489 ± 0.020	4.09
	Sulphide	0.479 ± 0.020	4.18
3.00	Sulindac	2.988 ± 0.102	3.41
	Sulphone	2.987 ± 0.115	3.85
	Sulphide	2.945 ± 0.132	4.48

Recoveries were performed in duplicate at each of the six calibration concentrations (0.05, 0.1, 0.5, 1.0, 2.0, 3.0 mg/l) for each analyte. The peak heights for sulindac and the metabolites at each standard concentration were compared with those of aqueous standards of the same concentration. The peak heights for sulindac and the metabolites from the extracted standards were divided by 2.5 to allow for sample concentrations due to extraction. The recoveries (mean ± S.D., $n = 12$) of sulindac, sulphone and the sulphide were 95.1 ± 5.24%, 90.7 ± 5.27% and 79.2 ± 6.19%, respectively. The recovery of the internal standard was performed in the same way and was calculated to be 93.2 ± 6.0% (mean ± S.D., $n = 6$).

To determine the intra-assay precision and accuracy of the method, plasma samples were prepared containing 0.05, 0.50 and 3.00 mg/l of each analyte. These samples were each analysed ten times, and the concentration (mean ± S.D.) and coefficients of variation (C.V.) of each analyte in each control were calculated (Table I). The accuracy and precision were good at all three concentrations and the C.V. was less than 8% in all cases.

The inter-assay accuracy and precision were evaluated by preparing plasma samples containing 0.25 and 2.50 mg/l of each analyte. These were used as quality-control samples throughout the course of a sulindac pharmacokinetic study to check the calibration data. The concentrations (mean ± S.D.) and C.V.s (Table II) indicate that the method has good reproducibility. The C.V. was less than 7.0% for all analytes in both controls.

The calibration data, when plotted as peak-height ratio (peak height of analyte divided by peak height of internal standard) versus concentration, gave a straight-line calibration curve for each analyte. Linear regression analysis was performed on each set of calibration data and the following linear equations are typical for a data set: sulindac, $y = 1.270x - 0.040$ ($r = 0.998$); sulphone, $y = 0.762x - 0.019$ ($r = 0.998$); sulphide, $y = 0.161x - 0.008$ ($r = 0.998$); y is the concentration in mg/l and x is the peak-height ratio.

The limit of sensitivity, defined here as the concentration where the ratio of peak height of analyte to background is 10:1, is 0.015 mg/l for sulindac, 0.020 mg/l for the sulphone and 0.030 mg/l for the sulphide.

TABLE II

DATA FOR INTER-ASSAY PRECISION AND ACCURACY ($n=36$)

Known concentration (mg/l)	Analyte	Measured concentration (mean ± S.D.) (mg/l)	Coefficient of variation (%)
0.25	Sulindac	0.240 ± 0.012	5.00
	Sulphone	0.241 ± 0.012	5.04
	Sulphide	0.241 ± 0.014	4.98
2.50	Sulindac	2.473 ± 0.141	5.70
	Sulphone	2.473 ± 0.164	6.61
	Sulphide	2.464 ± 0.143	5.80

Clinical results

The concentrations of sulindac, the sulphone and the sulphide were measured in plasma after a single oral dose of clinoril (100 mg). In addition to a subject blank, seventeen samples were collected at times as close as practicable to the following times over a 54-h collection period: 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 15.0, 26.0, 28.0, 32.0, 50.0, 52.0, 54.0 h. All analytes were measured to the 0.05 mg/l level, and from the plot of log concentration versus time (Fig. 2) it can be seen that sulindac and the sulphide disappear quickly and the sulphone can still be measured 54 h after the dose. This profile is consistent with those reported by other investigators [8].

CONCLUSION

The method reported here is as sensitive as other HPLC methods using solvent extraction and more sensitive and selective than HPLC methods using protein

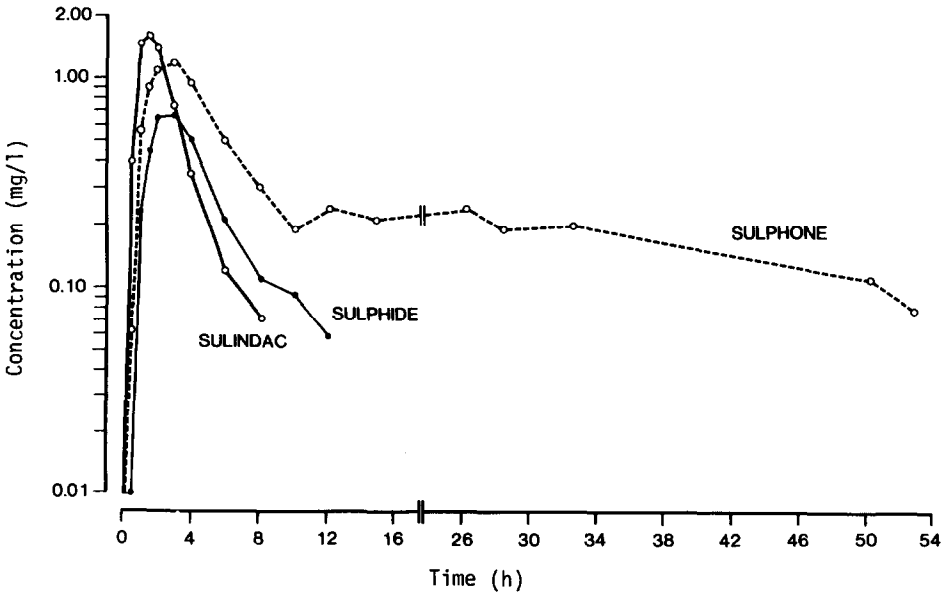

Fig. 2. Log-linear plot of concentration for sulindac, the sulphone and the sulphide against time.

precipitation. This method does not require solvent programming to achieve suitable separation and elution times, and has been shown to measure concentrations as low as 0.05 mg/l for sulindac and its two metabolites.

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