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Note

Determination of sulindac and its metabolites in serum by high-performance liquid chromatography

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Sulindac (Clinoril; (2)-5-fluoro-2-methyl-1-{[*p*-(methyl-sulphinyl)phenyl]-methylene}-1H-indene-3-acetic acid) is a new non-steroid anti-inflammatory drug. It is reported to possess anti-inflammatory properties without causing the normal side effects usually encountered in such treatment[1,2]. Following absorption, sulindac undergoes two major biotransformations—reversible reduction to the sulphide metabolite, and irreversible oxidation to the sulphone metabolite[3].

The sulphide has been proposed to be a pharmacologically active species[2], and hence it is necessary to have a method which would separate and measure both sulindac and its sulphide. Hare *et al.*[4] has published a method for the determination of sulindac and its metabolites using radio-immuno assay. However the method is complex and too long for routine application. Gas–liquid chromatography has been tried unsuccessfully as the compounds cannot be adequately separated and a derivatisation step must be employed to convert the corresponding acids into more volatile compounds.

We have used high-performance liquid chromatography (HPLC) with a μBondapak column to separate and quantitate sulindac and its metabolites. The method is sensitive down to a level of 0.2 μg/ml and a protein precipitation with acetonitrile on 200 μl of serum is used to extract the drugs. The method is rapid and suitable for routine determinations of sulindac and its metabolites.

EXPERIMENTAL AND RESULTS

Reagents

All glassware was silylated using a 2% solution of Dri-film SC-87 (Pierce, Rockford, Ill., U.S.A.) in hexane and then rinsed with hexane and air dried. Acetonitrile (Nanograde) was obtained from Mallinckrodt (St. Louis, Mo., U.S.A.), and sulindac sulphone and sulindac sulphide from Merck Sharp & Dohme (Rahway, N.J., U.S.A.). The internal standard was an 0.1 μg/μl solution of indomethacin in ethanol.

High-performance liquid chromatography

A Waters Assoc. (Milford, Mass., U.S.A.) high-performance liquid chromatograph (Model 6000A) was used. The detector was a Waters Assoc. 400 fixed

wavelength UV at 254 nm. The column was a 30 cm × 3.9 mm I.D. tube packed with μBondapak C_{18} (Waters Assoc.). Samples were introduced by a syringe into a Waters Assoc. Model U6K variable loop injector. The elution solvent was 60% acetonitrile in 45 m*M* KH_2PO_4 adjusted to pH 3.0 with phosphoric acid and used at a flow-rate of 1.5 ml/min. Under these conditions the retention times of sulindac, sulindac sulphone, indomethacin and sulindac sulphide were 2.3, 3.3, 4.7 and 7.6 min, respectively.

Extraction procedure

To a 200-μl sample of serum in a pointed glass tube was added 500 ng of the internal standard and 1.0 ml of acetonitrile. The tube was shaken vigorously by hand for 2 min and then centrifuged. A 1.0-ml volume of the supernatant was transferred to another tube and taken to dryness at 50° under a stream of nitrogen. The residue was redissolved in 100 μl of the eluting solvent and an aliquot (10–20 μl) injected into the high-performance liquid chromatograph.

Quantitation

Amounts of the drugs ranging from 100–2000 ng were added to 500 ng of the internal standard solution. Each solution was taken to dryness reconstituted in 100 μl of the eluting solvent and an aliquot injected into the high-performance liquid chromatograph. Over this range, the ratio of the peak heights of sulindac, the sulphone and the sulphide to that of the internal standard was linear (Fig. 1). A series of standards was injected before each run.

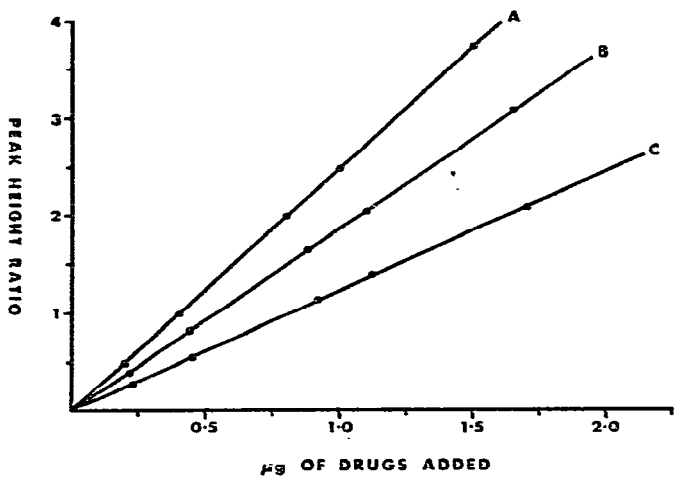

Fig. 1. Relationship of peak height ratios of (A) sulindac, (B) sulphone and (C) sulphide to the internal standard against the amount of each drug present.

Recovery studies

Amounts of sulindac, the sulphone and the sulphide over the range 0.2–5 μg/ml were added to blank serum to examine the efficiency of the extraction procedure (Fig. 2). The mean recovery of fifteen spiked samples was 93 ± 7% for sulindac, 96 ± 5% for the sulphone, and 97 ± 5% for the sulphide.

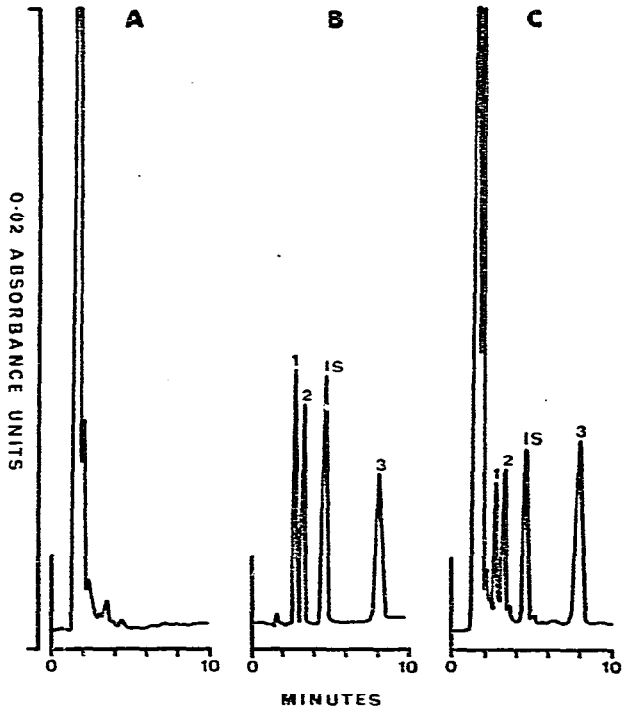

Fig. 2. (A) HPLC trace of a blank serum extract. (B) HPLC trace of a standard solution containing 40 ng of each drug and 50 ng of internal standard. (C) HPLC trace of a serum extract from a patient receiving 400 mg of sulindac daily. Peaks: 1 = sulindac; 2 = sulindac sulphone; IS = indomethacin (internal standard); 3 = sulindac sulphide.

Specificity

No interferences have been observed in the extracts of drug-free human serum. In addition none of the common anti-inflammatory drugs such as naproxen, oxyphenbutazone, phenylbutazone, salicylate and menfenamic acid co-chromatographed with sulindac or the sulphide. However oxyphenbutazone has a retention time similar to the sulphone and could interfere with its analysis. This does not present a problem since the sulphone has no reported biological activity and a level for this compound is therefore not imperative.

TABLE I

SERUM LEVELS OF SULINDAC, SULPHONE AND SULPHIDE IN PATIENTS RECEIVING SULINDAC

Patient	*Daily medication (mg)*	*Serum level (μg/ml)*		
		Sulindac	*Sulphone*	*Sulphide*
1	400	1.5	2.2	4.2
2	400	1.8	1.9	4.0
3	200	1.1	1.5	2.5
4	400	2.1	3.0	4.1

DISCUSSION

The method has been used to determine sulindac and its metabolites in the serum of patients receiving the drug (Table I). The levels found correlate with those published using the radio-immuno assay procedure[5].

The method outlined in this paper has proved satisfactory, reliable and rapid, five determinations being performed per hour. All the metabolites are well separated and the sensitivity is such that levels of 0.2 μg/ml can be achieved using only 200 μl of serum.

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