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ANALYSIS OF SULINDAC AND METABOLITES IN PLASMA AND URINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A reversed-phase high-performance liquid chromatographic method with ultraviolet detection is described for the quantification of sulindac, sulindac sulfone and sulindac sulfide in plasma and sulindac, *trans*-sulindac, sulindac sulfone and sulindac sulfide in urine. Plasma samples are de-proteinized with acetonitrile and urine samples are injected directly following enzymatic hydrolysis of glucuronide metabolites. The resulting chromatograms are essentially free from endogenous interference and the limits of detection are 0.1 μg/ml for plasma and 0.2 μg/ml for urine for all of the above compounds.

INTRODUCTION

Sulindac (I, Fig. 1) is a non-steroidal anti-inflammatory indene derivative having analgesic and antipyretic activities. Following absorption sulindac undergoes two major biotransformations: reversible reduction to the sulfide metabolite (II) and irreversible oxidation to the sulfone metabolite (III) (Fig. 1). The biological activity has been attributed to inhibition of prostaglandin synthesis by the sulfide metabolite. Sulindac is approximately 90% absorbed after oral administration and the primary route of excretion is via the urine. Sulindac and the sulfone metabolite are excreted intact and as their glucuronic acid metabolites and account for approximately 50% of the administered dose. Less than 1% of the dose is excreted in the urine as the sulfide metabolite.

Several methods have been reported for the analysis of sulindac and its metabolites in biological fluids. Reversed-phase high-performance liquid chromatography (HPLC) with ultraviolet detection appears to be the method of choice for this analysis. Of the previously reported HPLC methods, two were limited to plasma analysis only and also involved tedious evaporation steps and, in one case, glassware silylation [1, 2]. One method applicable to urine and other biological fluids also required an evaporation step and a long HPLC analysis time (15 min).

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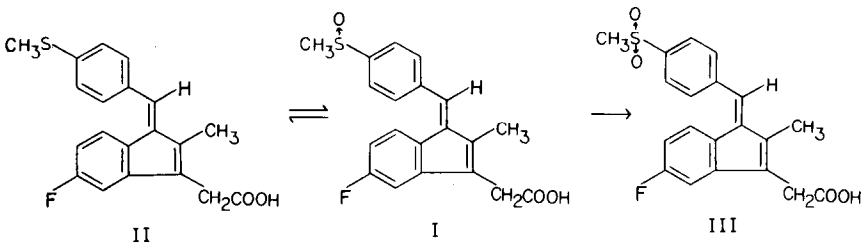

Fig. 1. Two major biotransformations of sulindac (I) following absorption: reversible reduction to the sulfide metabolite (II) and irreversible oxidation to the sulfone metabolite (III).

Also, for urine, the internal standard had to be added after a hydrolysis step with base [3]. An HPLC procedure was reported [4] for the analysis of sulindac and its metabolites in plasma and urine, but required solvent extraction of the biological fluids and gave no resolution of the drug isomers. The present method for the analysis of sulindac and its metabolites in plasma and urine is much simpler and faster than previously reported methods.

Sulindac exists as two geometric isomers, *cis* and *trans*, the drug being the *cis*-isomer. The present method resolves sulindac and *trans*-sulindac as well as sulindac sulfone and *trans*-sulindac sulfone. No evidence for resolution of sulindac sulfide and *trans*-sulindac sulfide has been found. Throughout this article all references to sulindac and its metabolites will assume the *cis*-configuration unless otherwise stated.

The sample preparation for plasma required protein precipitation and then injection of the supernatant. Sulindac, sulindac sulfone and sulindac sulfide were quantified in plasma. Urine samples were hydrolyzed enzymatically and then injected directly. No solvent extraction was found to be necessary. In urine sulindac, *trans*-sulindac and sulindac sulfone were resolved and quantified. Sulindac sulfide was not detected in any of the subjects' urine samples.

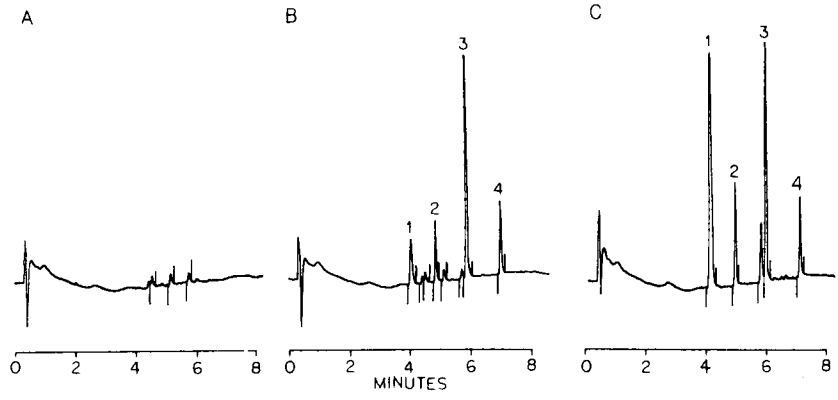

Fig. 2. Typical chromatograms for sulindac and metabolites in plasma. (A) Blank plasma; (B) blank plasma containing 1 μg/ml sulindac (1), 1 μg/ml sulindac sulfone (2), 8 μg/ml internal standard (3) and 1 μg/ml sulindac sulfide (4); (C) patient plasma containing 5.2 μg/ml sulindac (1), 1.6 μg/ml sulindac sulfone (2), 8 μg/ml internal standard (3) and 1.1 μg/ml sulindac sulfide (4).

TABLE I

INTER-DAY VARIATION OF SULINDAC AND METABOLITES IN PLASMA ($n=5$)

Compound	Concentration (μg/ml)	Mean area drug/internal standard	S.D.	R.S.D. (%)
Sulindac	0.25	0.080	0.00563	7.0
	0.5	0.153	0.00351	2.3
	1.0	0.308	0.00586	1.9
	2.5	0.807	0.01313	1.6
	5.0	1.610	0.01445	0.9
	7.5	2.408	0.02792	1.6
	10.0	3.202	0.06667	2.1
Sulindac sulfone	0.25	0.082	0.00589	7.2
	0.5	0.150	0.00716	4.8
	1.0	0.284	0.00723	2.6
	2.5	0.711	0.01203	1.7
	5.0	1.412	0.01420	1.0
	7.5	2.106	0.02219	1.1
	10.0	2.788	0.05548	2.0
Sulindac sulfide	0.25	0.097	0.00239	2.5
	0.5	0.184	0.00879	4.8
	1.0	0.382	0.00492	1.3
	2.5	0.969	0.01372	1.4
	5.0	1.924	0.02822	1.5
	7.5	2.862	0.03344	1.2
	10.0	3.786	0.09131	2.4

This method uses a fast HPLC column with gradient elution. Sharp peaks were obtained for all compounds being measured and the total run time was 9 min. The internal standard, indomethacin, was added at the beginning of the sample preparation for both plasma and urine. The sample preparation is simple and produces chromatograms that are essentially free from endogenous interference.

TABLE II

ANALYSIS OF SPIKED QUALITY-CONTROL STANDARDS IN PLASMA

Compound	Nominal concentration (μg/ml)	n	Measured concentration (mean $\pm$S.D.) (μg/ml)	R.S.D. (%)
Sulindac	0.5	17	0.53 ± 0.03	5.7
Sulindac sulfone	0.5	14	0.49 ± 0.04	8.2
Sulindac sulfide	0.5	17	0.48 ± 0.03	6.0
Sulindac	7.5	16	7.44 ± 0.29	3.9
Sulindac sulfone	7.5	16	7.30 ± 0.24	3.3
Sulindac sulfide	7.5	16	6.95 ± 0.23	3.4

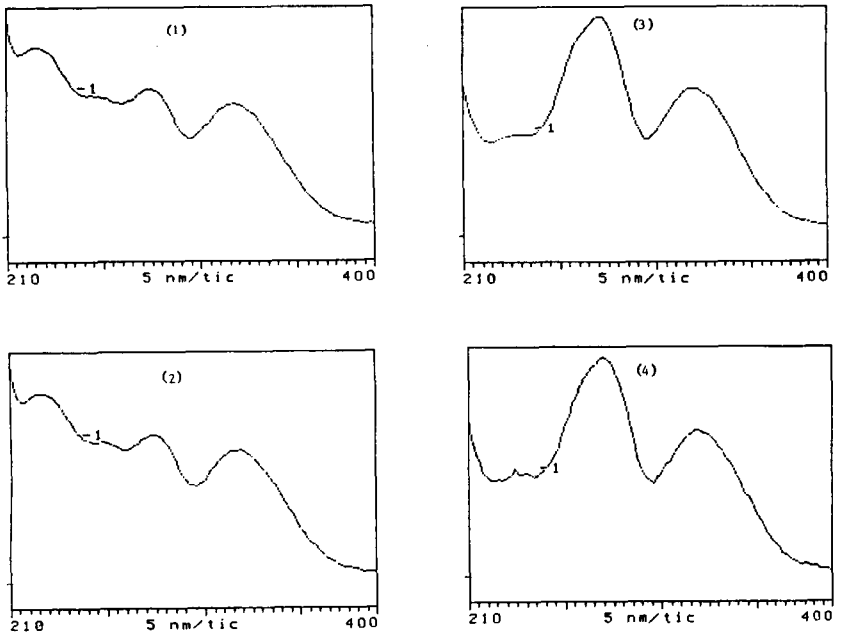

Fig. 3. Comparison of ultraviolet spectra collected by the photodiode array spectrophotometer of reference *cis*-isomer (1), *cis*-isomer in urine (2), reference *trans*-isomer (3) and *trans*-isomer in urine (4).

EXPERIMENTAL

Reagents

Water was purified in-house via filtration through a Millipore Milli-Q system. Blank human plasma was obtained from Sera-Tec Biologicals (North Brunswick, NJ, U.S.A.) Ammonium phosphate buffer (0.05 *M*, pH 3) was prepared from certified ACS-grade ammonium dihydrogen phosphate (Fisher, Pittsburgh, PA, U.S.A.) and HPLC-grade phosphoric acid (Fisher). Acetonitrile was HPLC grade from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.) and sodium acetate was obtained from Fisher. Potassium phosphate buffer (0.1 *M*) was prepared from certified ACS-grade monobasic potassium phosphate (Fisher Scientific, Fair Lawn, NJ, U.S.A.). Sodium hydroxide (1 *M*) and hydrochloric acid (1 *M*) were certified grade from Fisher Scientific and were used as received. The pH meter was a Beckman Research Model 1019 (Fullerton, CA, U.S.A.). Glusulase® was obtained from DuPont (Wilmington, DE, U.S.A.). A working solution was prepared using Glusulase (250 μl) and sodium acetate (1 *M*, pH 5.2, 500 μl) in water (25 ml). Sulindac, its metabolites and indomethacin were obtained from Merck Sharp & Dohme Research Labs. (Rahway, NJ, U.S.A.).

Standard preparation

Sulindac, *trans*-sulindac, sulindac sulfone, sulindac sulfide and indomethacin, the internal standard, were prepared as stock solutions in acetonitrile–water (80:20, v/v; 1 mg/ml).

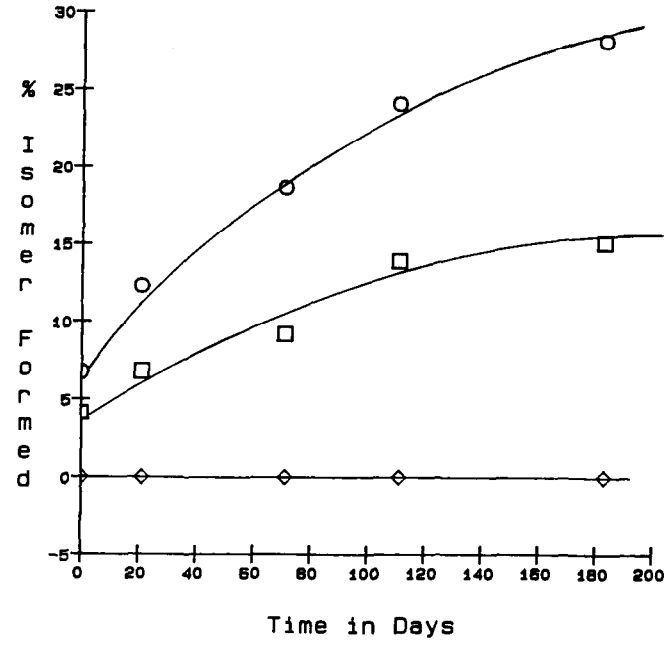

Fig. 4. Rate of isomerization of sulindac in urine (pH 5.6) and 0.1 *M* phosphate buffer (pH 5.6). (○) Percentage *cis* in urine; (□) percentage *trans* in urine; (◇) percentage *trans* in buffer.

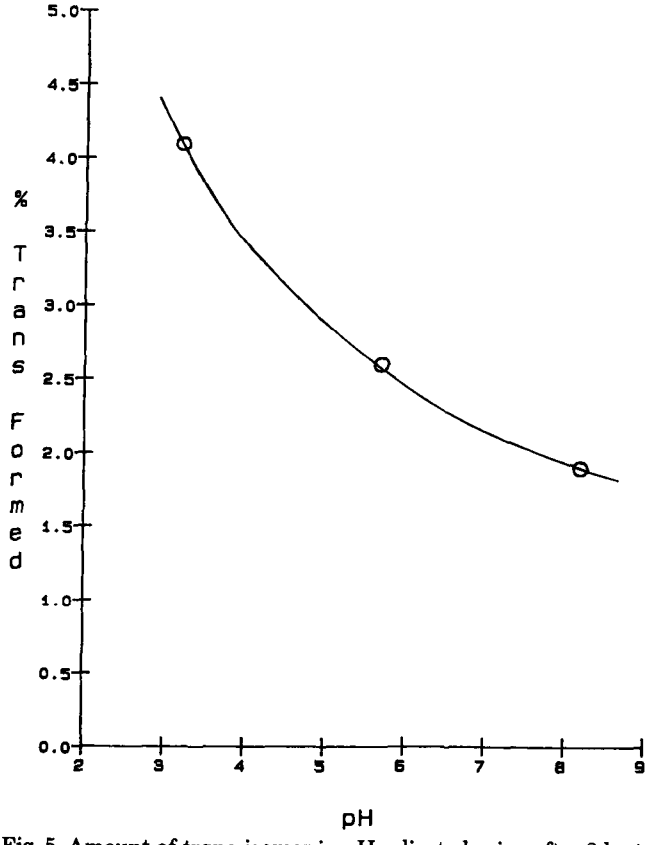

Fig. 5. Amount of *trans*-isomer in pH-adjusted urine after 2 h at room temperature.

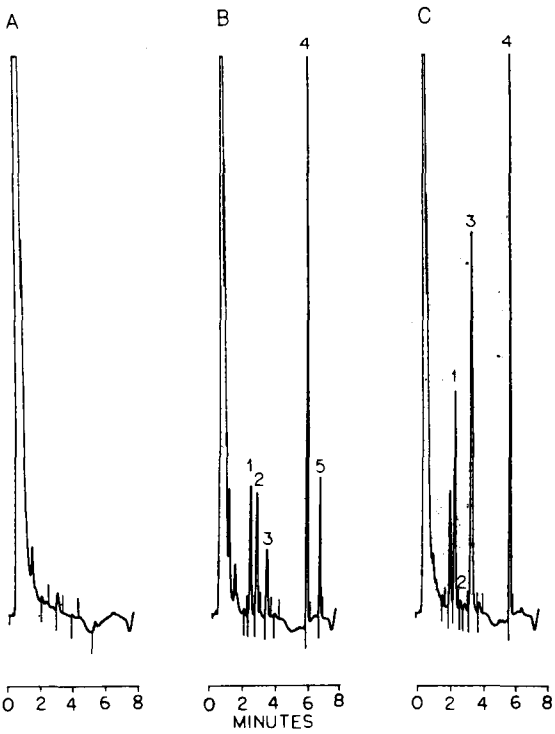

Fig. 6. Typical chromatograms for sulindac and metabolites in urine. (A) Blank urine; (B) blank urine containing 2.5 μg/ml sulindac (1), 2.5 μg/ml *trans*-sulindac (2), 2.5 μg/ml sulindac sulfone (3), 25 μg/ml internal standard (4) and 2.5 μg/ml sulindac sulfide (5); (C) patient urine containing 4.2 μg/ml sulindac (1), 0.2 μg/ml *trans*-sulindac (2), 6.8 μg/ml sulindac sulfone (3), and 25 μg/ml internal standard (4).

Sulindac, sulindac sulfone and sulindac sulfide were prepared as a series of mixed working standards covering the range 1.25–50 μg/ml for each compound. This produced equivalent plasma concentrations of 0.25–10 μg/ml for each compound. A working internal standard solution of indomethacin (40 μg/ml) was also prepared for plasma analysis.

Sulindac, *trans*-sulindac, sulindac sulfone and sulindac sulfide were similarly prepared to give mixed working standards covering the range 2.5–100 μg/ml for the analysis of urine samples. The equivalent urine concentrations were 0.5–20 μg/ml for each compound. A working internal standard solution of indomethacin (125 μg/ml) was used for urine analysis.

Analysis of plasma

Plasma standards were prepared by combining blank plasma (500 μl), mixed working standard solution (100 μl), working internal standard (100 μl) and acetonitrile (1 ml). The mixture was vortex-mixed (10 s) and centrifuged (10 min at 2000 *g*). The supernatant was decanted into an autosampler vial containing water (1 ml) and an aliquot (30 μl) of this solution injected for HPLC analysis.

Plasma samples were assayed using the same procedure, substituting patients'

TABLE III

INTER-DAY VARIATION OF SULINDAC AND METABOLITES IN URINE ($n=5$)

Compound	Concentration (μg/ml)	Mean area drug/internal standard	S.D.	R.S.D. (%)
Sulindac	0.5	0.068	0.0068	10.1
	1.0	0.127	0.0054	4.3
	2.5	0.310	0.0070	2.3
	5	0.610	0.0040	0.7
	10	1.219	0.0258	2.1
	15	1.765	0.0248	1.4
	20	2.471	0.0205	0.8
trans-Sulindac	0.5	0.069	0.0015	2.2
	1.0	0.136	0.0027	2.0
	2.5	0.358	0.0132	3.7
	5	0.719	0.0037	0.5
	10	1.462	0.0297	2.0
	15	2.137	0.0275	1.3
	20	3.055	0.0582	1.9
Sulindac sulfone	0.5	0.042	0.0019	4.5
	1.0	0.091	0.0026	2.8
	2.5	0.242	0.0057	2.3
	5	0.481	0.0017	0.3
	10	0.983	0.0187	1.9
	15	1.431	0.0180	1.3
	20	2.008	0.0196	1.0
Sulindac sulfide	0.5	0.069	0.0021	3.0
	1.0	0.142	0.0013	0.9
	2.5	0.377	0.0045	1.2
	5	0.749	0.0011	0.2
	10	1.506	0.0202	1.3
	15	2.190	0.0255	1.2
	20	3.072	0.0178	0.6

plasma (500 μl) and acetonitrile–water (80:20, v/v, 100 μl) for blank plasma and working standard solution, respectively.

Analysis of urine

Urine standards were prepared by combining blank urine (500 μl), mixed working standard solution (100 μl) and working internal standard solution (100 μl). Glusulase working enzyme solution (250 μl) was added and the solutions incubated (1 h at 37°C). An aliquot of this solution (25 μl) was injected for HPLC analysis.

Urine samples were assayed using the same procedure, substituting patient urine and acetonitrile–water for blank urine and working standard solution, respectively.

TABLE IV

ANALYSIS OF SPIKED QUALITY-CONTROL STANDARDS IN URINE

Compound	Nominal concentration (μg/ml)	Measured concentration (mean ± S.D.) (μg/ml)	R.S.D. (%)
Sulindac	2.5	2.49 ± 0.18	7.3
trans-Sulindac	2.5	2.36 ± 0.11	4.8
Sulindac sulfone	2.5	2.32 ± 0.10	4.3
Sulindac	15	14.98 ± 0.14	0.9
trans-Sulindac	15	14.78 ± 0.55	3.7
Sulindac sulfone	15	14.31 ± 0.43	3.0

Chromatography

A Hewlett-Packard HP-1090 high-performance liquid chromatograph was used for this analysis. A Waters 480 variable-wavelength ultraviolet detector set at 340 nm was used to monitor the mobile phase. The detector was run at a sensitivity of 0.02 a.u.f.s. for plasma and 0.05 a.u.f.s. for urine with a 0.2-s time constant. A photodiode array spectrophotometric detector (HP 1040A) was used to collect ultraviolet spectra of the peaks of interest. Spectra were obtained at the upslope, apex and downslope. The wavelength range of the monitored peaks was 200–400 nm. A 5 cm × 4.6 mm, 3-μm Sepralyte C_{18} analytical column (Analytichem International, Harbor City, CA, U.S.A.) was used with a mobile phase of ammonium phosphate (0.05 *M*), phosphoric acid (0.01 *M*) and acetonitrile as the organic modifier. The analytical column was maintained at a temperature of 50°C, and a flow-rate of 1.5 ml/min was used. The solvent program was 66% buffer–34% acetonitrile held isocratically for 3 min and then a linear gradient to 70% acetonitrile in a further 2 min. 70% Acetonitrile was held for 1 min and the initial composition then re-established. The total run time was 9 min. The SP4270 integrator was run at an attenuation of 8 with a 1 cm/min chart speed for plasma analysis and a 0.5 cm/min chart speed for urine analysis, and was used to measure peak areas.

Identification of the isomer

The *trans*-isomer, present only in the urine, was identified by comparing retention time and ultraviolet spectrum of the peak in question to authentic reference standard.

Kinetic data at 5°C

The *cis*- and *trans*-isomers of sulindac were added to urine and to 0.1 *M* phosphate buffer adjusted to the same pH (5.6) as the urine; the concentration was ca. 14 μg/ml for each isomer. Samples stored in the refrigerator were withdrawn for analysis at prescribed intervals.

Formation of trans-isomer versus pH

Urine was pH-adjusted with hydrochloric acid (1 *M*) or sodium hydroxide (1 *M*). Sulindac was added and analyzed after storage for 2 h at room temperature.

RESULTS AND DISCUSSION

Plasma

Preparation of plasma samples for HPLC analysis involved precipitation of protein with acetonitrile and injection of the supernatant. Gradient elution gave baseline resolution of sulindac, sulindac sulfone, sulindac sulfide and indomethacin, the internal standard, in a 9-min analysis. There was no interference from endogenous material and typical chromatograms are shown in Fig. 2. The retention times were 4.0 min for sulindac, 4.9 min for sulindac sulfone, 5.9 min for indomethacin, the internal standard, and 7.0 min for sulindac sulfide. The limit of detection for each compound being measured was approximately 0.1 μg/ml (based on a signal-to-noise ratio of 5:1).

The reproducibility of the assay was assessed by calculating the percentage relative standard deviation (R.S.D.) for each point on the three standard lines for five separate days of analysis. The data are summarized in Table I. All points had R.S.D. values of 7.2% or less. The accuracy of the method was checked by preparing quality-control samples at low and high points on the standard lines. These samples were assayed with patients' samples throughout the analysis. The results are given in Table II. The measured concentrations were in good agreement with the target concentrations and the R.S.D. values were 8.4% or less throughout the analysis.

The linearity of each standard line was confirmed by plotting the drug concentration versus the ratio of drug area to internal standard area. Correlation coefficients of higher than 0.9995 were regularly obtained. The specificity of the assay was checked by running blank plasma and patient's pre-dose plasma. No significant endogenous interference was encountered.

Urine

Enzymatic hydrolysis of urinary conjugates was used in this assay procedure. Indomethacin, the internal standard, could then be added before this hydrolysis step. Base hydrolysis gave satisfactory hydrolysis of conjugated sulindac metabolites but also hydrolyzed the internal standard, and could not, therefore, be used. The *trans*-isomer of sulindac was baseline-resolved from the *cis*-isomer. A peak was also detected that eluted after sulindac sulfone with baseline resolution that was not present in pre-dose urine. It was suspected that this might be *trans*-sulindac sulfone, but no authentic reference standard was available to confirm its identity. Some isomerization of *cis* to *trans* occurred in urine for both sulindac and sulindac sulfone (up to 3% after 5 h at 37°C).

The two isomers in urine were confirmed by comparing retention time and ultraviolet spectrum of the peak of interest to authentic reference standard. Typical ultraviolet spectra are illustrated in Fig. 3.

Isomerization of the *cis*- to *trans*-isomer and vice versa occurred in the urine. Fig. 4 indicates the appearance of the other isomer when either the *cis*- or the *trans*-isomer were added to urine stored at 5°C. However, isomerization did not occur when sulindac was added to 0.1 *M* phosphate buffer. The pH of the buffer was adjusted to that of the urine.

The formation of the *trans*-isomer was faster at acidic pH. A representative profile for the amount of *trans*-sulindac formed from *cis*-sulindac in pH-adjusted urine is shown in Fig. 5. Sulindac in urine was incubated for 2 h at room temperature before analysis.

Some conversion could, therefore, occur in the bladder prior to excretion and in any time that urine was left at room temperature. The rate of formation of the isomer is partly dependent on the pH of the urine.

Typical chromatograms for urine samples are shown in Fig. 6. The retention times were 2.3 min for sulindac, 2.7 min for *trans*-sulindac, 3.3 min for sulindac sulfone, 5.7 min for indomethacin, the internal standard, and 6.5 min for sulindac sulfide. There was no interference from endogenous compounds. Sulindac sulfide standards were prepared, but none was detected in patients' urine. The limit of detection for each compound being measured was approximately 0.2 μg/ml (based on a signal-to-noise ratio of 5:1).

The reproducibility of the assay was determined in the same way as described for plasma. The data are summarized in Table III. All points had R.S.D. values of 10.1% or less. The accuracy of the method was checked by preparing quality-control samples as described for plasma. The results are given in Table IV. The measured concentrations were close to the target values and the R.S.D. values were 7.3% or less throughout the analysis.

The linearity of each standard line was confirmed and typical correlation coefficients were $\geqslant 0.9995$. The specificity of the assay was checked by running blank urine and patients' pre-dose urine. No significant interference was encountered.

The recovery of sulindac and its metabolites from plasma and urine was checked by spiking the appropriate range of working standards into corresponding volumes of water to give two direct standard lines. The results of plasma and urine standards were compared with those of the direct standards. The recoveries were $\geqslant 98\%$ for all compounds in both plasma and urine.

This method has been used to assay clinical samples from patients receiving therapeutic doses of sulindac. The assay has proved to be reliable and rugged, and the short analysis time has allowed large numbers of samples to be run in a comparatively short time.

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