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A GAS–LIQUID CHROMATOGRAPHIC METHOD FOR THE QUANTITATIVE DETERMINATION OF TOLMETIN IN PLASMA AND TOLMETIN AND ITS MAJOR METABOLITE IN URINE

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SUMMARY

A sensitive and specific gas chromatographic method for the determination of the non-steroidal anti-inflammatory drug tolmetin in human plasma and tolmetin and its major metabolite in human urine is described. The compounds were extracted as acids into diethyl ether and after a clean-up procedure converted to their methyl esters and separated on a gas chromatograph equipped with a flame ionization detector.

The method has been applied to samples of plasma and urine from subjects receiving tolmetin under experimental and therapeutic conditions.

INTRODUCTION

Tolmetin (I) has been synthesized[1] and pharmacologically evaluated[2] as a non-steroidal anti-inflammatory agent. Studies concerned with its metabolic fate have identified the carboxylic acid (II) in human urine (Fig. 1).

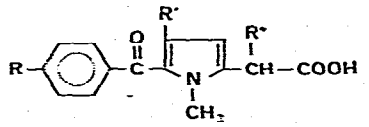
An investigation of the pharmacokinetics of tolmetin in arthritic patients required a method for the determination of I in plasma and I and II, together with their respective glucuronide conjugates, in urine. The study was undertaken to develop a sensitive and specific gas chromatographic procedure suitable for this purpose.

EXPERIMENTAL

Materials

A chloro analogue of tolmetin (III) was used as an internal standard (Fig. 1). Tolmetin was supplied by McNeil Labs. (Fort Washington, Pa., U.S.A.) as the sodium salt dihydrate and compounds II and III as the free acids. β-Glucuronidase, bacterial type II, was obtained from Sigma (St. Louis, Mo., U.S.A.). All reagents and solvents were analytical reagent grade except diethyl ether, which was anaesthetic grade (BP). The diethyl ether was distilled immediately before use.

Diazomethane in diethyl ether. Diazomethane was generated from N-methyl-N-nitroso-*p*-toluenesulphonamide[3]. The ethereal solution was kept in dry ice–acetone when in use and stored over potassium hydroxide pellets at −20°.



I R = CH_3, R' = H, R'' = H

II R = COOH, R' = H, R'' = H

III R = Cl, R' = CH_3, R'' = CH_3

Fig. 1. Structural formulae of tolmetin (I), its major metabolite (II) and the internal standard (III).

Stock solutions. Aqueous solutions of I, II and III at concentrations of 0.5 mg/ml, 0.4 mg/ml and 0.05 mg/ml, respectively, were prepared and stored at 4 . Compounds II and III were dissolved in the minimum quantity of either 0.1 *M* NaOH (II) or 0.5 *M* NaOH (III) prior to dilution.

Methods

Gas–liquid chromatography. A Hewlett-Packard Model 5755 gas chromatograph equipped with a flame ionization detector was used. The column was glass (5 ft. × 1/4 in O.D.) packed with 3% OV-17 on 100–120 mesh Gas-Chrom Q (Applied Science Labs., State College, Pa., U.S.A.), conditioned for 12 h at 250 (no nitrogen flow) followed by 24 h at 250 (40 ml/min nitrogen flow).

The column was silylated by the injection of 10 μl of Silyl 8 at 230 before each day's analysis. The operating conditions were: injection port temperature, 270 ; column temperature, 230 ; detector temperature, 260 ; carrier gas (nitrogen) flow-rate, 40 ml/min (50 p.s.i.g.); hydrogen flow-rate, 40 ml/min and air flow-rate, 400 ml/min. The retention times for the methyl esters of I, II and III under these conditions were 5, 6.25 and 13.5 min, respectively.

Sample preparation from plasma. Plasma (1 ml) and internal standard solution (1 ml) were placed in a 15-ml glass-stoppered centrifuge tube. To this mixture were added 0.1 ml 6 *M* HCl and 7 ml diethyl ether. The tube was mixed for 30 sec (vortex mixer) and centrifuged for 2 min at 3000 rpm. The tube was cooled in a dry ice–acetone bath for 2 min and the ether layer decanted into a second 15-ml glass-stoppered centrifuge tube containing 2 ml of a 0.1 *M* NaOH solution. The ether and NaOH mixture was mixed as described above and the ether phase discarded. The aqueous phase was acidified with 0.5 ml 6 *M* HCl and then extracted with 5 ml diethyl ether as described above. The separated ether phase was transferred to a 15-ml tube with a 50-μl elongated bubble at the base, a silica boiling chip was added and the solution concentrated to about 20 μl by immersion of the tube in a water-bath at 42 . The tube was then placed in a dry ice–acetone bath to condense the ether vapour and wash down the sides of the tube. An ethereal solution of diazomethane (0.1 ml) was added and after 5 min the ether solution was concentrated to approximately 5 μl and 2–5 μl were injected into the gas chromatograph.

Sample preparation from urine. Unconjugated I and II were determined by extracting 1-ml urine samples as described above for plasma. Samples with concentrations of I above 50 μg/ml and concentrations of II above 10 μg/ml were diluted prior to extraction. The glucuronide conjugates of I and II were determined by analysis of urine samples which had been subjected to enzymatic hydrolysis. To 0.5 ml of urine

was added 0.5 ml of 0.2 *M* phosphate buffer, pH 6.8, and 2500 units of β-glucuronidase. A few drops of chloroform were added and the mixture was incubated for 16 h at 37°. After the incubation period, the analysis was carried out as previously described. The amounts of I and II excreted as the glucuronides were calculated from the difference between the amounts of I and II present before and after enzymatic hydrolysis.

Calibration curves. Following chromatography, the heights of the peak corresponding to the methyl esters of I or II and the methyl ester of III were measured. Calibration curves of peak height ratio against amount of I or II were established by assaying drug-free plasma to which known amounts of I had been added or control urine to which known amounts of I and II had been added. The urinary calibration curves may be determined simultaneously or separately for each compound.

RESULTS AND DISCUSSION

Fig. 2 shows typical chromatograms for the analysis of I in plasma and I and II in urine from individuals receiving tolmetin. No interfering peaks were noted in a number of samples obtained from different patients and volunteers.

For plasma, the calibration curve for I in the range of 0.1–50 μg was given by the regression equation

$$y = 0.0014 + 0.0314\,x \quad (r = 0.9999,\ n = 9)$$

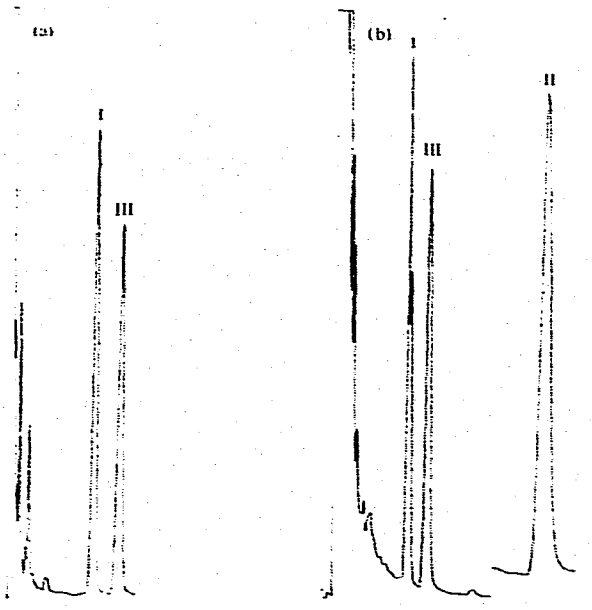


Fig. 2. Gas–liquid chromatograms of plasma and urine extracts assayed as described in the text. (a) Extract of plasma taken from arthritic patient D.L. 0.25 h after the oral administration of 300 mg of I as three 100-mg tablets. The concentration of I was 46.4 μg/ml. (b) Extract of urine from volunteer E.T. collected during the 24-h period following the oral administration of 300 mg of the sodium dihydrate salt of I. The concentration of I was 13.1 μg/ml and that of II 37.0 μg/ml.

For urine, the calibration curves for I and II in the range of 0.1–50 μg for I and 0.1–10 μg for II were given by the regression equations

$$y = 0.0251\ x\ (r = 0.9999,\ n = 9)$$

for I and

$$y = 0.0088\ x\ (r = 0.9999,\ n = 7)$$

for II.

Application of the GLC assay to biological samples

Four healthy volunteers each received an oral solution containing 100 mg of the sodium dihydrate salt of I following an overnight fast. A venous blood sample was taken immediately before drug administration to serve as a blank and sampling was continued for a period of up to 8 h following medication. Blood samples were collected in tubes containing heparin. Plasma was separated by centrifugation as soon after sampling as possible and was stored at −20° until assayed. Control experiments showed that I and II were stable under these conditions. The plasma levels of I obtained are presented in Fig. 3. These results show that the drug is rapidly absorbed

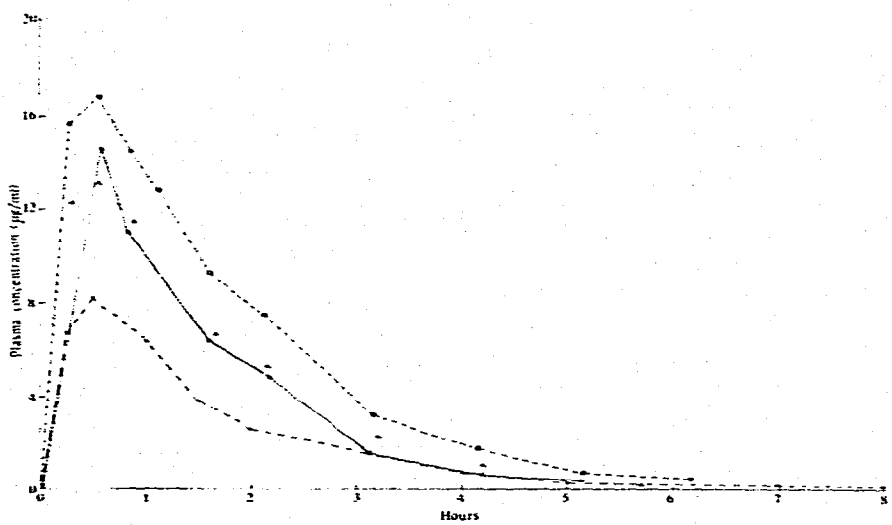


Fig. 3. Plasma levels of I in four healthy volunteers given 100 mg of the sodium dihydrate salt of I as an oral solution. Subjects: M.V. (●---●); P.E. (▲···▲); C.G. (■—■); M.S. (▲-·-·-▲).

from the gastrointestinal tract and rapidly disappears from the plasma. The data in Fig. 3 were plotted semilogarithmically and the half-life for the elimination of I from plasma calculated by the method of least squares. The half-lives obtained were: P.E., 0.90 h; C.G., 0.83 h; M.V., 1.08 h; and M.S., 0.99 h. Fig. 4 shows the plasma levels of I obtained in two arthritic patients following the ingestion of 300 mg of I as three 100-mg tablets. The half-life values were: D.L., 0.83 h; and V.W., 0.92 h.

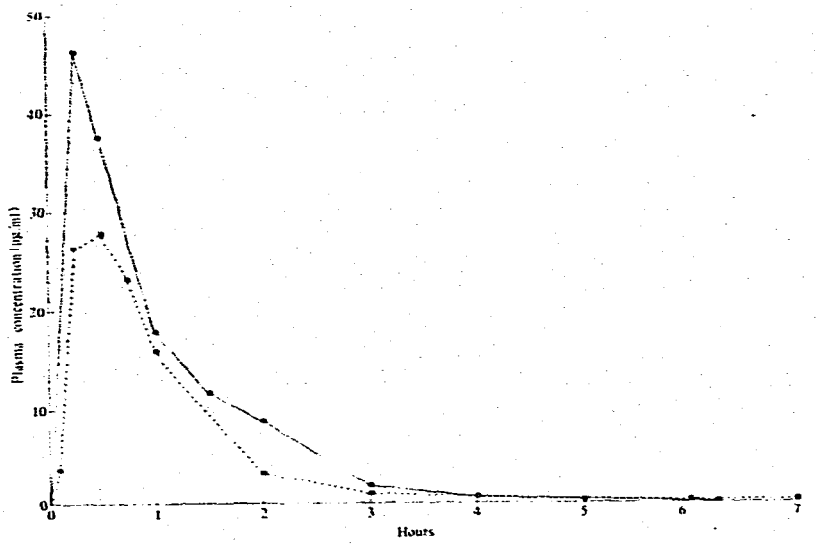


Fig. 4. Plasma levels of I in two arthritic patients following the ingestion of 300 mg of I as three 100-mg tablets. Subjects: D.L. (●—●); V.W. (■---■).

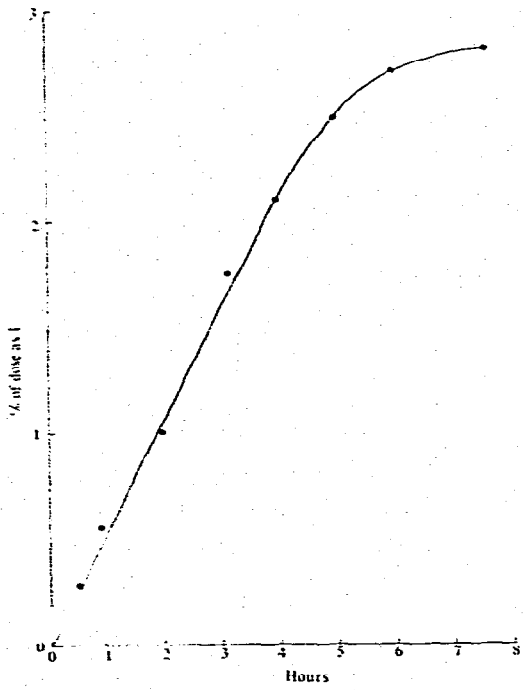


Fig. 5. Cumulative urinary excretion (% of dose) of I after the administration of 100 mg of the sodium dihydrate salt of I as an oral solution to volunteer M.V.

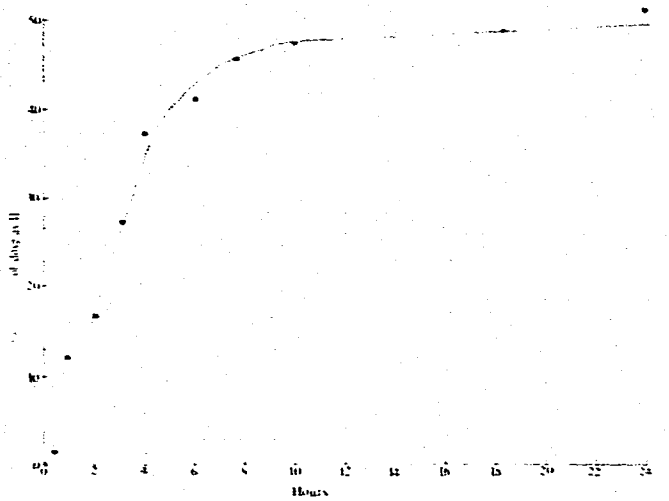


Fig. 6. Cumulative urinary excretion (% of dose) of II after the administration of 100 mg of the sodium dihydrate salt of I as an oral solution to volunteer M.V.

Urine was collected from three of the normal volunteers receiving 100 mg of the sodium dihydrate salt of I and analysed for unconjugated I and II. Cumulative urinary excretion curves indicated that I is substantially excreted in 8 h and II in 24 h following drug administration. Figs. 5 and 6 show the cumulative data obtained for volunteer M.V.

In a separate study three healthy volunteers received 100 mg of the sodium dihydrate salt of I on one occasion and 300 mg on another. The urinary excretion of I and II and their respective glucuronides is given in Table I. The glucuronide conjugate of II was measurably excreted in the urine only at the higher dose level.

TABLE I

EXCRETION OF I, I GLUCURONIDE, II AND II GLUCURONIDE FROM THREE HEALTHY MALE VOLUNTEERS FOLLOWING ADMINISTRATION OF AN ORAL SOLUTION OF THE SODIUM DIHYDRATE SALT OF I

Subject	*Dose (mg)*	*Percentage of dose in 24-h urine*			
		I	*I Glucuronide*	*II*	*II Glucuronide*
G.M.	100	6.8	5.1	35.9	—
	300	17.0	4.8	67.1	4.8
S.W.	100	2.3	41.0	3.8	
	300	10.7	57.9	7.8	8.4
E.T.	100	5.1	54.5	6.6	—
	300	8.8	52.72	3.8	3.4

CONCLUSION

The analytical procedure described has been found to be satisfactory for clinical application and is being used to develop a pharmacokinetic model for tolmetin in patients receiving the drug under clinical conditions.

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