Journal of chromatography, 222 (1981) 399—408
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 752

SIMULTANEOUS MEASUREMENT OF DOTHIEPIN AND ITS MAJOR METABOLITES IN PLASMA AND WHOLE BLOOD BY GAS CHROMATOGRAPHY—MASS FRAGMENTOGRAPHY

KAY P. MAGUIRE*, TREVOR R. NORMAN and GRAHAM D. BURROWS

Department of Psychiatry, University of Melbourne, Parkville, Victoria (Australia)

and

BRUCE A. SCOGGINS

Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne, Parkville, Victoria (Australia)

(First received July 17th, 1980; revised manuscript received September 29th, 1980)

SUMMARY

A method for the simultaneous measurement of dothiepin and two of its major metabolites, northiaden and dothiepin S-oxide in both plasma and whole blood is described. The method involves the use of gas chromatography—mass fragmentography. It is selective, sensitive (1 μg/l) and reproducible.

It has been used to analyse both plasma and blood samples following single oral doses of 75 mg dothiepin in seven volunteers.

INTRODUCTION

Dothiepin [11-(3-dimethylaminopropylidene)-6,11-dihydrodibenz[*b*,*e*]-thiepin hydrochloride] is one of the tricyclic antidepressant drugs. It is metabolised in man to three active metabolites, northiaden, dothiepin S-oxide and northiaden S-oxide [1, 2]. One procedure has been published for the simultaneous measurement of dothiepin and northiaden in plasma [3]. The two drugs were analysed by high-performance liquid chromatography with a sensitivity limit of 20 μg/l. Although suitable for analysis of steady-state concentrations, this would not be adequate for measuring plasma levels following single oral doses of 75 mg. A more sensitive technique is gas chromatography—mass fragmentography (GC—MF). One GC—MF method has been investigated involving

0378-4347/81/0000—0000/$02.50

chemical ionization but was applied to dothiepin measurement only [4]. A GC—MF method using electron ionization has been developed for the simultaneous analysis of dothiepin and northiaden and dothiepin S-oxide in blood and plasma samples and will be described in this paper.

EXPERIMENTAL

Drug standards

Deuterodothiepin [11-(3-N-trideuteromethyl-N-methylaminopropylidene)-6, 11-dihydrodibenz[*b*,*e*]thiepin hydrochloride] was used as the internal standard for dothiepin and dothiepin S-oxide. This was synthesized and supplied by Boots (Nottingham, Great Britain).

Protriptyline hydrochloride was used as the internal standard for northiaden and was obtained from Merck Sharp & Dohme (Sydney, Australia).

Dothiepin, northiaden, dothiepin S-oxide, and northiaden S-oxide hydrochlorides were supplied by Boots. The structures of the compounds are shown in Fig. 1.

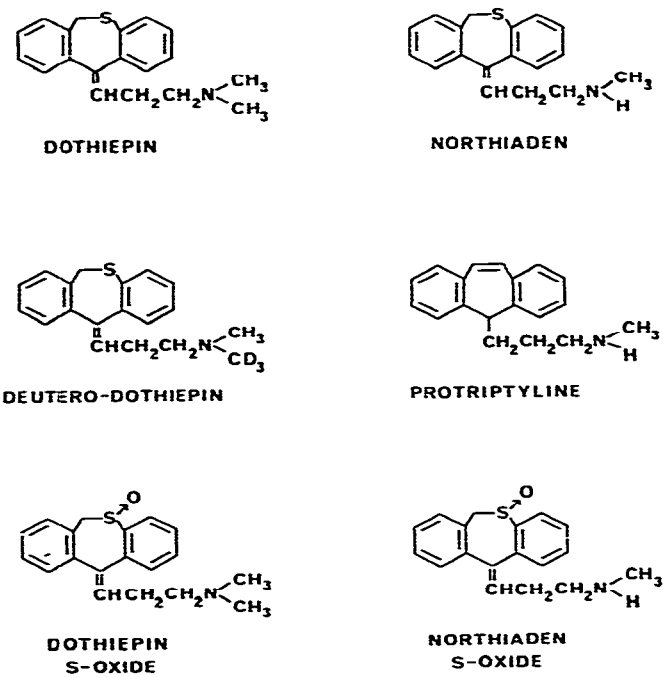

Fig. 1. The structures of dothiepin, deuterodothiepin, northiaden, protriptyline, dothiepin S-oxide and northiaden S-oxide.

Solvents

Nanograde quality *n*-hexane was obtained from Mallinckrodt (St. Louis, MO, U.S.A.). Trifluoroacetic anhydride (TFA) was purchased from Pierce (Rockford, IL, U.S.A.) and absolute ethanol was obtained from E. Merck (Darmstadt, G.F.R.).

Glassware

All glassware (tubes, stoppers, pipettes) was soaked overnight in chromic acid, thoroughly rinsed in tap water, followed by rinsing with glass-distilled water.

Extraction procedure

Samples of either 1 or 2 ml of plasma (or blood) were diluted to 5 ml with glass-distilled water in 20-ml glass tubes. Unknown samples, four plasma (or blood) standards and a quality control were included in each extraction. The internal standard solution, containing deuterodothiepin and protriptyline in ethanol, was added to give a concentration of 50 μg deuterodothiepin and 20 μg protriptyline per liter of sample. After mixing, the samples were alkalinized with 0.5 ml of 5 *M* sodium hydroxide, and 5 ml of *n*-hexane added. The samples were shaken for 10—15 min on a horizontal shaker and centrifuged for 5 min at 1400 *g*. A maximum aliquot of the solvent phase was transferred to clean tubes containing 1 ml 1 *M* hydrochloric acid. The plasma phase was re-extracted with a further 5 ml of *n*-hexane and a maximum aliquot of the solvent added to the first extract. The combined solvent phases were extracted with the acid by shaking for 10—15 min followed by centrifugation as before. The acid phase was removed to clean 5-ml glass tubes, alkalinized with 0.3 ml of 5 *M* sodium hydroxide, and extracted by gentle rotation (10—15 min) with 2 ml *n*-hexane. After centrifugation the solvent phase was transferred to 5-ml V-shaped glass tubes and evaporated under air at 37°C. A second extraction of the acid phase was carried out and the solvent added to the same V-shaped tubes. A 100-μl volume of TFA was added and the samples vortexed for 30 sec. The solvents were evaporated under air at 37°C and the samples were stored at −4°C. Prior to analysis, samples were reconstituted in 7 μl of ethanol. Aliquots of 4—5 μl were injected into the gas chromatograph—mass spectrometer.

Gas chromatography—mass fragmentography

The analysis was carried out on a Hewlett-Packard Model 5992A gas chromatograph—mass spectrometer. Gas chromatographic separation was carried out on a silanised glass column (2 m × 2 mm I.D.) packed with a 3% OV-101 on Gas-Chrom W HP (80—100 mesh). Helium was used as carrier gas at a flow-rate of 20 ml/min. The injection port and jet separator were maintained at 260°C and the oven was programmed from 210°C to 230°C at 4°C/min and kept at 230°C for the remainder of the run. The mass spectrometer was focused on *m/e* 58 (50 msec), 61 (50 msec), 191 (50 msec), and 217 (150 msec). The ionization energy was 70 eV, and the electron multiplier energy ranged from 2 to 3 kV.

Quantitation

Peak areas were integrated using the gas chromatograph—mass spectrometer controller unit (Hewlett-Packard 9825A desktop computer). The ratio of peak area of *m/e* 58 at t_R 3.6 min (dothiepin) to the area of *m/e* 61 at t_R 3.6 min (deuterodothiepin), the ratio of *m/e* 58 at t_R 4.0 min (dothiepin S-oxide) to the area of *m/e* 61 at t_R 3.6 min (deuterodothiepin), and the ratio of the area of 217 at t_R 5.8 min (northiaden TFA) to the area of *m/e* 191 at t_R 4.1 min

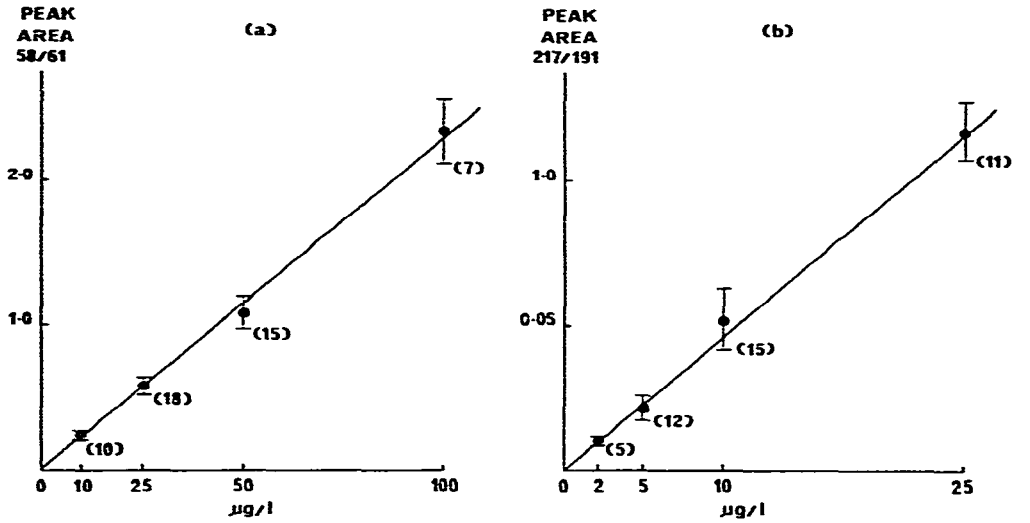

Fig. 2. Plasma standard curves for (a) dothiepin and (b) northiaden. Calculated linear regression lines were (a) $y = 0.0231x + 0.0010$, $r = 0.9992$; (b) $y = 0.0047x + 0.0009$, $r = 0.9978$. The error bars represent ± 1 S.D., the number in parentheses equals the number of determinations at each concentration.

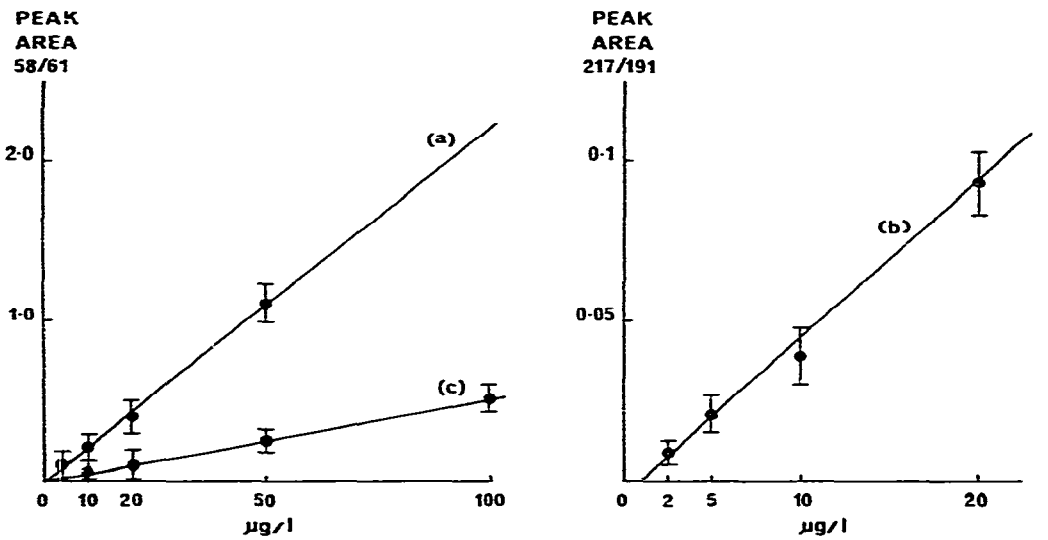

Fig. 3. Blood standard curves for (a) dothiepin, (b) northiaden and (c) dothiepin S-oxide. Calculated linear regression lines were (a) $y = 0.0226x - 0.0078$, $r = 0.9997$; (b) $y = 0.0049x - 0.0042$, $r = 0.9939$; and (c) $y = 0.0053x + 0.0047$, $r = 0.9999$. The error bars represent ± 1 S.D., the number of determinations at each point was six.

(protriptyline TFA) were calculated for each sample. Standard curves were constructed by linear regression analysis of the calculated ratios versus amount of drug added (Figs. 2 and 3). Unknown samples were calculated using the regression equations.

Precision studies

Drug-free whole blood or plasma (Blood Bank) was used to prepare standards to which known amounts of both dothiepin and northiaden were added. Dothiepin S-oxide was also added to the blood standards. These were used each run to prepare standard curves and to evaluate the day-to-day precision of the assay.

Plasma and erythrocyte distribution of dothiepin

Blood samples were taken by venipuncture from six healthy volunteers. Dothiepin (100 ng/0.1 ml in isotonic saline, pH 7.4) was added to 2 ml of whole blood which was then incubated at 37°C for 15—20 min. One duplicate of each blood sample was centrifuged to obtain the plasma fraction. Both blood and plasma were analysed for dothiepin content.

Single-dose experiments

Seven healthy volunteers were given 75 mg of dothiepin in the form of three 25-mg capsules. Samples were taken over the first 8 h via an indwelling heparinized cannula, and by venipuncture over the following 2 days. Half of each blood sample was frozen immediately, the other half centrifuged to obtain plasma. The plasma was then separated and stored frozen till analysed.

RESULTS AND DISCUSSION

Deuterodothiepin provides an ideal internal standard for dothiepin as the recovery of both compounds through the procedure is identical. Protriptyline was chosen as the internal standard for northiaden as it is also a secondary amine tricyclic antidepressant and has similar extraction and derivatization characteristics to northiaden. Derivatization was necessary to prevent the secondary amines adsorbing to the column packing. TFA was found to be the most satisfactory derivatizing reagent compared to acetic anhydride and heptafluorobutyric anhydride, in terms of ease of derivatization and chromatographic characteristics of the derivatives.

The mass spectra of dothiepin, deuterodothiepin, northiaden-TFA and protriptyline-TFA are shown in Figs. 4—7. The most abundant ions were *m/e* 58,

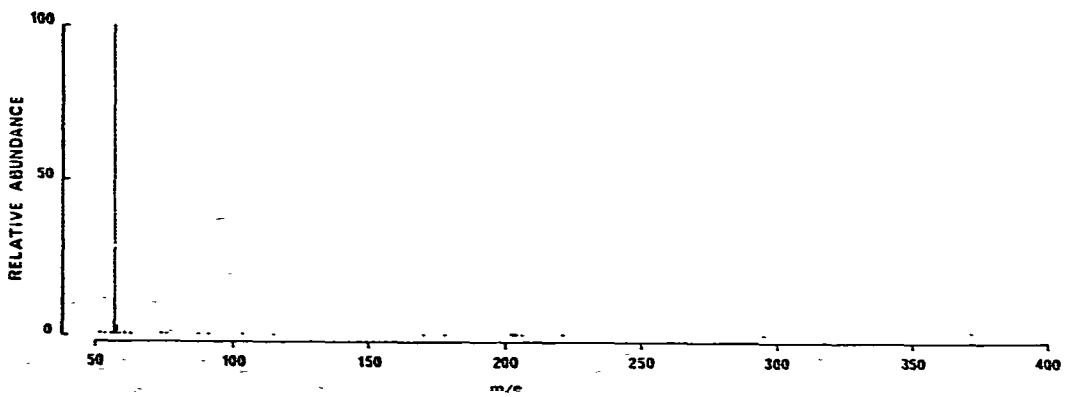

Fig. 4. Mass spectrum of dothiepin, base peak *m/e* 58.

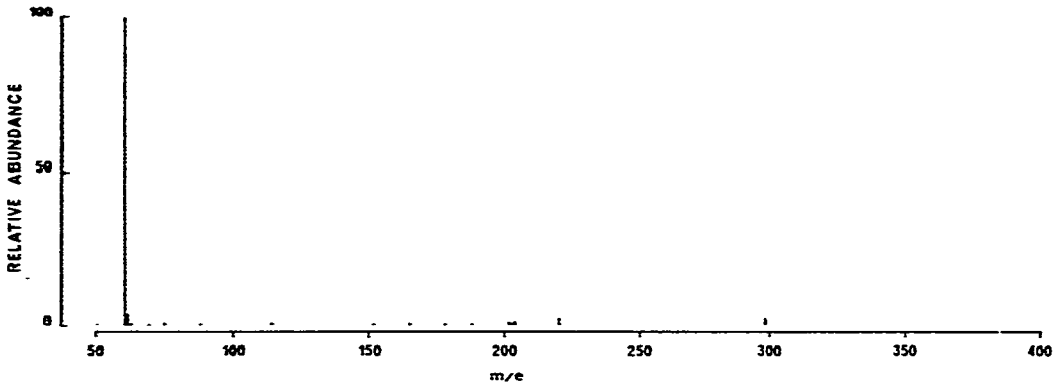

Fig. 5. Mass spectrum of deutero dothiepin, base peak *m/e* 61.

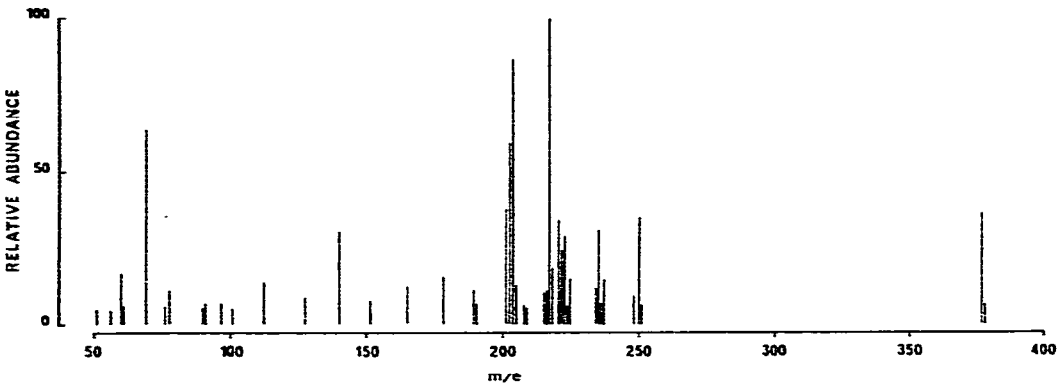

Fig. 6. Mass spectrum of northiaden-TFA, base peak *m/e* 217.

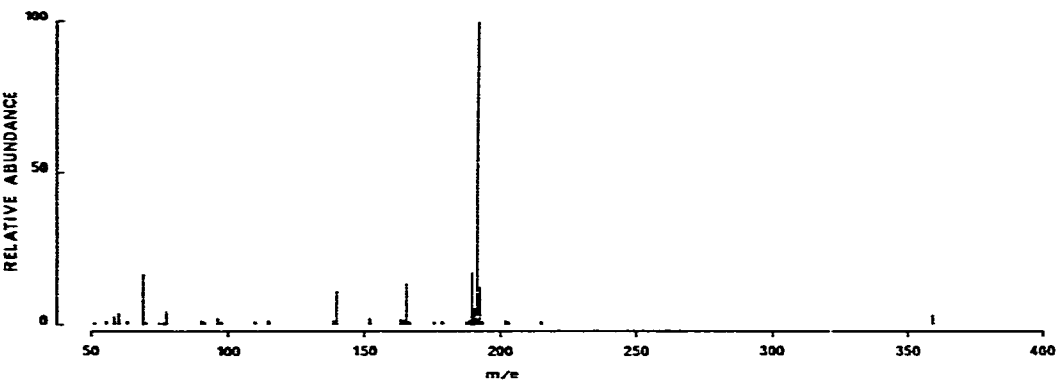

Fig. 7. Mass spectrum of protriptyline-TFA, base peak *m/e* 191.

61, 217, and 191 respectively and these were chosen for simultaneous ion monitoring. A longer dwell time was chosen for 217 due to the weaker abundance of this ion. The fragmentation of dothiepin (and deuterodothiepin) is similar to the other tricyclic antidepressants, amitriptyline and doxepin and *m/e* 58 (61) arises due to loss of part of the side-chain [5]. Similarly, the ion at *m/e* 191 of protriptyline-TFA arises from loss of the complete side-chain [5].

The major ion *m/e* 217 of northiaden-TFA is not so easily explained as loss of part of the side-chain gives rise to *m/e* 250. Subsequent rearrangement and elimination may be the mechanism by which *m/e* 217 occurs. The fragmentation pattern of northiaden-TFA appears quite complex as judged by the number of relatively abundant ions.

The three-step extraction procedure was found necessary to provide clean extracts for analysis. A single solvent extraction of blank plasma gave rise to interfering peaks coinciding with both dothiepin and northiaden. Even with the rigorous extraction procedure as described, occasional interference with the dothiepin peak was observed. This limited the lowest measurable concentration to 1 μg/l. The sensitivity of the method for northiaden was similar, due mainly to the relatively low abundance of *m/e* 217. Extracts of drug-free whole blood were similar to those of plasma.

After completion of the evaluation of the assay for dothiepin and northiaden in plasma, and on analysis of the single-dose samples, a major peak was observed in the single ion trace *m/e* 58 at t_R 4.0 min. A sample of dothiepin S-oxide was then obtained which was found to account for this peak. Dothiepin S-oxide had a slightly longer retention time than dothiepin itself, but the major ion fragment was the same for both compounds (*m/e* 58). The use of deuterodothiepin as the internal standard for measuring dothiepin S-oxide is not ideal since the S-oxide does not extract as well. However, since reproducible results were obtained (see Table I) it was decided to use deuterodothiepin rather than adding a third internal standard which would then necessitate re-evaluating the

TABLE I

REPRODUCIBILITY OF THE ASSAY FOR DOTHIEPIN AND NORTHIADEN LEVELS IN PLASMA AND BLOOD, AND DOTHIEPIN S-OXIDE IN BLOOD

$n = 10$ at all concentrations.

Drug	Expected concentration (μg/l)	Plasma		Blood	
		Found (μg/l)	C.V. (%)	Found (μg/l)	C.V. (%)
Dothiepin	5	4.9	± 10	4.8	± 11
	10	10.3	± 6	10.6	± 7
	25/20*	25.8	± 5	20.1	± 7
	50	50.7	± 6	51.1	± 5
Northiaden	2	2.1	± 16	2.5	± 20
	5	5.0	± 14	4.9	± 10
	10	10.1	± 8	10.1	± 13
	25/20*	23.4	± 7	20.9	± 4
Dothiepin S-oxide	10			10.7	± 10
	20			22.8	± 13
	50			50.5	± 13
	100			105.0	± 9

*25 μg/l for plasma standards, 20 μg/l for blood standards.

complete procedure. Consequently, evaluation of the precision of the assay for whole blood concentrations was done for dothiepin S-oxide in addition to dothiepin and northiaden.

One other metabolite of dothiepin, northiaden S-oxide was available for testing the specificity of the assay. This metabolite on derivatization gave a compound identical in retention time and major ion fragment to that of

TABLE II

DISTRIBUTION OF DOTHIEPIN

No.	Sex	Age	Blood level (μg/l)	Plasma level (μg/l)	Ratio blood to plasma
1	F	28	109.4	110.0	0.99
2	M	26	109.4	110.6	0.99
3	M	23	113.2	107.7	1.05
4	M	24	109.6	121.4	0.90
5	F	38	107.8	86.2	1.25
6	M	33	110.3	93.1	1.18

northiaden-TFA. However, when added to blank plasma in concentrations of up to 250 μg/l, a response equal to 1—2 μg/l of northiaden resulted. It appears that the S-oxide does not extract under the conditions used.

The precision of the assay has been investigated over a six-month period. The results obtained for day-to-day reproducibility over the concentration ranges 5—50 μg/l for dothiepin and 2—25 μg/l for northiaden in plasma are presented in Table I. Acceptable precision was achieved even at the lowest concentration. The day-to-day reproducibility for dothiepin, northiaden and dothiepin S-oxide in whole blood is also shown in Table I. Again, acceptable precision was obtained, even for the S-oxide.

In general, there was little difference between the extracts obtained from blood or plasma with the exception that the recovery from blood was slightly lower than that from plasma.

The distribution of dothiepin between plasma and erythrocytes was examined under in vitro conditions (Table II). Dothiepin was equally distributed between plasma and erythrocytes, thus whole blood and plasma levels are more or less identical, and either could be used for pharmacokinetic studies. To check this in vivo both plasma and blood samples were analysed from the single-dose studies. Dothiepin blood concentrations were again more or less identical to that found in plasma whereas northiaden plasma concentrations were greater than blood concentrations. Dothiepin S-oxide was only measured in blood.

The concentrations of the three compounds following the single oral doses are shown in Fig. 8. The mean dothiepin peak level was 43 μg/l at 3 h, the mean northiaden peak level was 9 μg/l at 8 h, and dothiepin S-oxide reached the highest peak concentration (78 μg/l) at 4 h post-dose. A full description of the study and the pharmacokinetic parameters calculated from it will be published separately [6].

In six out of the seven volunteers, dothiepin S-oxide reached higher concen-

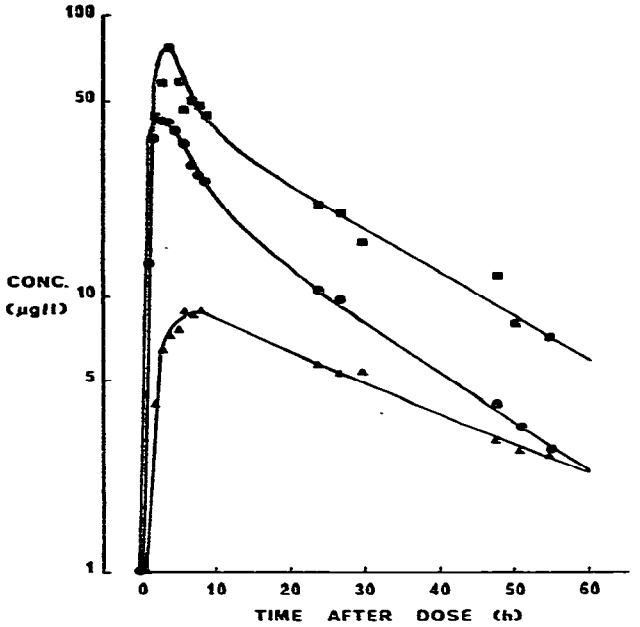

Fig. 8. Mean plasma concentrations of dothiepin and northiaden and the mean blood concentration of dothiepin S-oxide following a single oral dose of 75 mg dothiepin in seven healthy volunteers. ■, Dothiepin S-oxide; ●, dothiepin; ▲, northiaden.

trations than dothiepin itself. Recent studies from our laboratory have shown that both dothiepin and northiaden S-oxides inhibit platelet uptake of [^{14}C]-serotonin [2]. These metabolites should thus be analysed in addition to dothiepin and northiaden in studies of clinical response and drug concentrations. A different extraction procedure would be required to enable quantitation of northiaden S-oxide but the derivatization, chromatography and mass spectrometric conditions would not require alteration.

In conclusion, the GC—MF method as described has been shown to be specific, sensitive and reproducible for dothiepin and two of its major metabolites. It appears more than satisfactory for pharmacokinetic studies of single oral doses of dothiepin.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the support of the National Health and Medical Research Council of Australia and Boots (Australia). We also thank Ms. Pauline Gorban for technical assistance in extracting the samples.

REFERENCES

1 E.L. Crampton, W. Dickinson, G. Haran, B. Marchant and P.C. Risdall, Br. J. Pharmacol., 64 (1978) 405P.
2 T.R. Norman and H. Cheng, in E. Usdin, S. Dahl, L.F. Gram and O. Lingjaerde (Editors), Proceedings of the 2nd International meeting on Clinical Pharmacology in Psychiatry, Macmillan, New York, London, in press.

3 R.R. Brodie, L.F. Chasseud, E.L. Crampton, D.R. Hawkins and P.C. Ridsall, J. Int. Med. Res., 5 (1977) 387.
4 E.L. Crampton, R.C. Glass, B. Marchant and J.A. Rees, J. Chromatogr., 183 (1980) 141.
5 J.T. Biggs, W.H. Holland, S. Chang, P.P. Hipps and W.R. Sherman, J. Pharm. Sci., 65 (1976) 261.
6 K.P. Maguire, G.D. Burrows, T.R. Norman and B.A. Scoggins, Br. J. Clin. Pharmacol., submitted for publication.