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Short Communication

Determination of four metabolites of dothiepin in urine by high-performance liquid chromatography

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Introduction

Tricyclic antidepressants are extensively metabolised by microsomal oxidation enzymes of the liver [1–4], so that following oral administration the level of unchanged drug in plasma or urine is low [5–8]. The metabolism of dothiepin hydrochloride (DP) was investigated by Crampton *et al.* [9] and the structures of four of its metabolites are shown in Fig. 1. Northiaden (monodesmethyldothiepin, NOR), dothiepin sulphoxide (DP–SO) and northiaden sulphoxide (NOR–SO) were identified in rat, cat, dog and human urine, and the glucuronide of hydroxydothiepin in rat urine after oral administration of (^{14}C)-labelled-DP. Rees reported that the excretion of DP and NOR was small and that sulphoxidation was the main metabolic pathway of DP in man after an oral dose of ^{14}C-DP [10].

The authors have recently identified two new metabolites, dothiepin sulphone (DP–SO_2) and northiaden sulphone (NOR–SO_2) in rat urine using gas chromatography–mass spectrometry [11]. This report describes a reversed-phase high-performance liquid chromatographic (HPLC) method of analysis for DP–SO, DP–SO_2, NOR–SO and NOR–SO_2 in human urine following a single oral dose (25 mg) of dothiepin.

Experimental

Materials and reagents

Acetonitrile (HPLC grade) and diethyl ether (for pesticide residue analysis) were purchased from Nakarai Chemicals (Kyoto, Japan) and other reagents (analytical grade) from Wako Pure Chemical (Osaka, Japan).

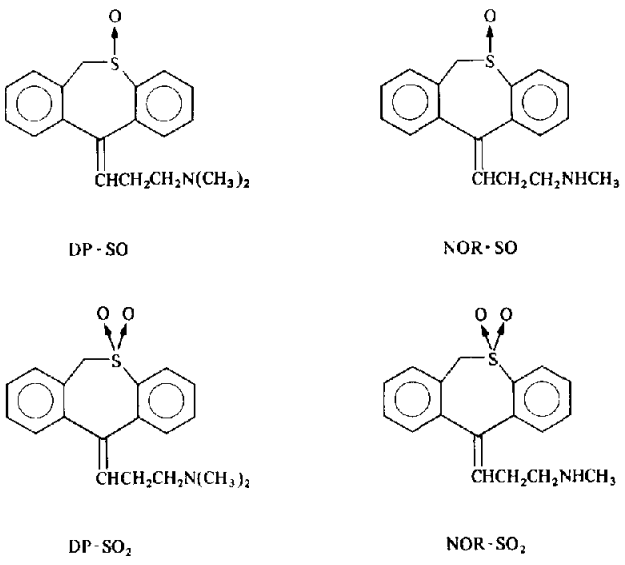

Figure 1
Chemical structures of four metabolites.

DP–SO (hydrochloride) and NOR–SO (hydrochloride) were obtained from SPOFA (Praha, Czechoslovakia). DP–SO_2 (hydrochloride), NOR–SO_2 (maleate) and nortriptyline (hydrochloride) were synthesized in this laboratory. Nortriptyline (2 μg ml^{-1}) was used as the internal standard, dissolved in an aqueous solution of 5% dimethylamine hydrochloride and stored at 4°C in the dark.

Extraction efficiency

The extraction efficiency for each metabolite and the internal standard were determined with equal volumes (10 ml) of aqueous solution (pH 1 and 13) and organic phase (diethyl ether and *n*-hexane). The tubes were mechanically shaken for 20 min. After centrifugation at 25°C for 5 min (swing bucket rotor, 1660 × g), the absorbances of the aqueous layer were measured at 240 nm.

Mobile phase composition

The effects of pH were investigated over a pH range of 5.0–7.5, employing 0.015 M phosphate buffer and a mobile phase composition of buffer–acetonitrile–1 M dimethylamine (50:50:1; v/v/v). With the (buffer + acetonitrile) to 1 M dimethylamine ratio maintained at 100:1; v/v, the effect of varying the acetonitrile concentration was studied over the range of 35–60% v/v.

Sample preparation

One ml of the internal standard solution and 0.2 ml of 3 N hydrochloric acid were added to 0.1–1 ml of urine in a 20 ml glass test tube. The aqueous mixture was washed twice with 5 ml of ether. After the addition of 1 ml of 5% sodium chloride solution and 1 ml of 2 N sodium hydroxide, the mixture was extracted with 10 ml of diethyl ether. The ether layer was evaporated to dryness under reduced pressure in a flask. The residue was

dissolved in 100 μl of the mobile phase and a 20-μl aliquot injected into the HPLC system.

Calibration graphs of the four metabolites (0.1–3 μg ml^{-1}) were prepared by spiking 1 ml of control urine.

Apparatus

The experiments were performed on a Hitachi model 638-50 liquid chromatograph equipped with a Hitachi model 635M absorbance detector, operated at 210 nm. The mobile phase was a mixture of acetonitrile–0.015 M phosphate buffer (pH 7.0)–1 M dimethylamine hydrochloride (50:50:1; v/v/v) at a constant flow-rate of 2 ml min^{-1}. The column (15 cm × 0.4 cm i.d.) was packed with 10-μm C_{18}-microBondapak (Waters Association, Milford, USA).

Drug administration and urine collection

Five healthy male adults, 39–49 years of age and 57–72 kg in weight, each received 25 mg of dothiepin in capsule form after a 16-h fast. The urine specimens were collected just before and at 6, 12, 24, 48 and 72 h after administration.

Pharmacokinetics

The rate constants for metabolism and urinary excretion of DP–SO and NOR–SO were estimated using a one-compartment model by non-linear least-squares fit of the excretion time course data. The calculations were carried out on N 5200 computer (NEC, Tokyo, Japan) according to Multi [12].

Results and Discussion

Effects of mobile phase composition

The retention times of the four metabolites and internal standard were significantly increased by an increase in buffer pH (Fig. 2). Each metabolite and the internal standard were adequately separated at pHs 7.0 and 7.5. The elution order of DP–SO and NOR–SO_2 was reversed at pH 6.0.

Each metabolite and internal standard was adequately separated employing between 35–50% v/v. Figure 3 shows that an increase in acetonitrile concentration resulted in a decrease in the retention times of all compounds, without altering their order of elution. Dimethylamine was added to the mobile phase to decrease peak tailing, and this also had the effect of reducing the retention times of all the components.

Extraction efficiency

Table 1 shows the percentage extraction of the four metabolites and the internal standard. DP–SO_2 and the internal standard were completely extracted with *n*-hexane at pH 13, but DP–SO, NOR–SO and NOR–SO_2 only slightly. However, the four metabolites and the internal standard were satisfactorily extracted with diethyl ether at pH 13. None of the compounds in the aqueous layer at pH 1 could be extracted with either organic solvent. Therefore, diethyl ether was used as an extracting solvent for the metabolites and the internal standard.

Calibration graph, reproducibility and recovery

Calibration graphs were constructed by plotting the peak height ratio of each metabolite to the internal standard against the amount of each metabolite and were

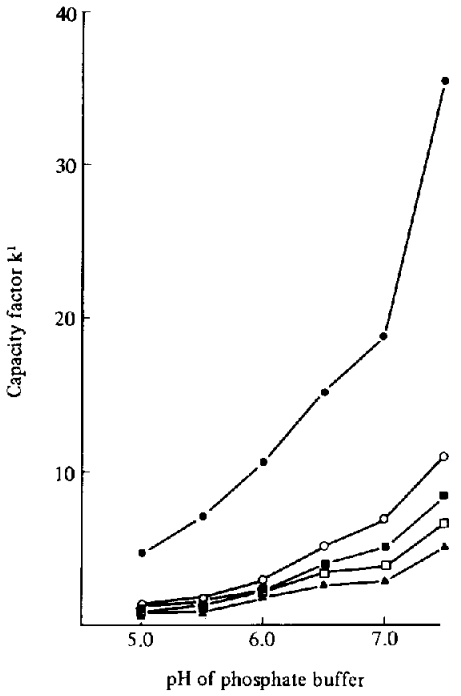

Figure 2
Effects of the pH of the buffer on the k' values of four metabolites and the internal standard. ▲, NOR–SO; □, NOR–SO_2; ■, DP–SO; ○, DP–SO_2; ●, IS.

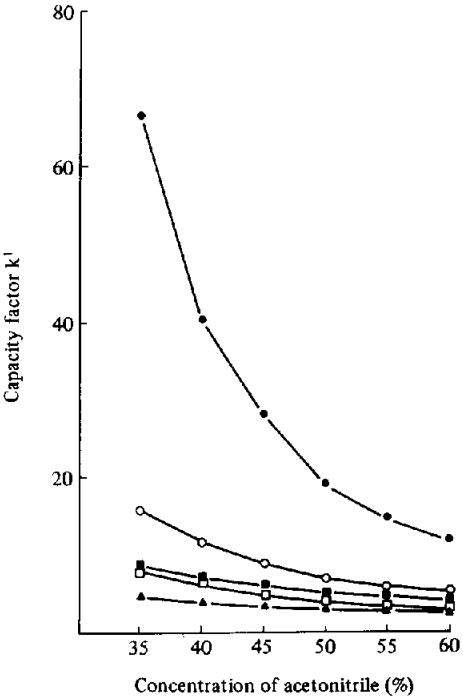

Figure 3
Effect of rate of buffer and acetonitrile on the k' values of four metabolites and the internal standard. ▲, NOR–SO; □, NOR–SO_2; ■, DP–SO; ○, DP–SO_2; ●, IS.

Table 1
Extraction of four metabolites and internal standard with organic solvent from aqueous solution at pH 13

	DP–SO	DP–SO_2	NOR–SO	NOR–SO_2	IS*
Diethyl ether	100	100	79.1	91.1	100
n-Hexane	59.2	100	21.6	31.8	100

*Internal standard.
Values show the rate of extraction (%).

found to be linear over the concentration range, 0.1–3 μg ml^{-1}, with correlation coefficients of 0.9997 (DP–SO), 0.9994 (DP–SO_2), 0.9994 (NOR–SO) and 0.9995 (NOR–SO_2). The relative standard deviations are shown in Table 2 and indicate satisfactory reproducibility. The minimum detectable concentration for each metabolite was 0.1 μg ml^{-1} when 1 ml of urine was used.

Figure 4 shows chromatograms of extracts from 1 ml of blank urine and spiked urine containing 1 μg ml^{-1} of each metabolite and 2 μg ml^{-1} of the internal standard. The retention times of NOR–SO, NOP–SO_2, DP–SO, DP–SO_2 and the internal standard were 4.4, 5.8, 7.9, 10.9 and 25.9 min, respectively. Under these HPLC conditions, the retention times of DP and NOR were 47.1 and 19.3 min, respectively, so that even if large amounts of DP and NOR were excreted in urine, neither compound would interfere with the analysis of the four metabolites.

Table 2
Accuracy and precision of the determination of four metabolites in urine

Compound	Amount added (μg)	Amount found (μg)	RSD (%)
DP–SO	0.1	0.10	6.9
	0.5	0.49	4.2
	1.0	1.01	4.4
	3.0	3.01	1.1
		Average =	4.1
DP–SO_2	0.1	0.10	5.7
	0.5	0.50	2.5
	1.0	1.01	3.4
	3.0	2.96	2.6
		Average =	3.6
NOR–SO	0.1	0.10	5.0
	0.5	0.51	1.0
	1.0	0.99	4.5
	3.0	2.99	2.8
		Average =	3.3
NOR–SO_2	0.1	0.10	6.2
	0.5	0.51	2.9
	1.0	0.99	3.0
	3.0	2.98	2.5
		Average =	3.7

Each value represents the mean of 3 experiments.

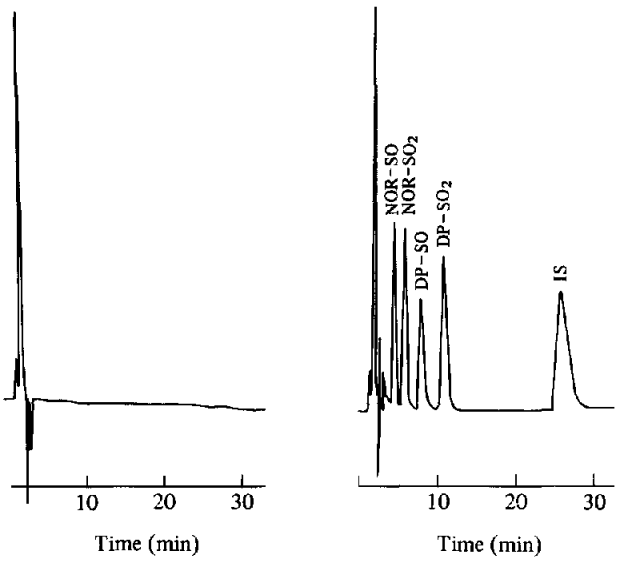

Figure 4
Chromatograms of extracts from (A) blank urine and (B) urine spiked with 1 μg of each metabolite and 2 μg of internal standard.

Clinical applications

The method described was applied to several urine samples from volunteers who had received 25 mg of DP. The excretion of the four metabolites in urine is shown in Table 3. DP–SO, NOR–SO, DP–SO_2 and NOR–SO_2 were excreted as 26.8, 17.7, 0.2 and 0.4% of the dose, respectively in urine after 72 h. These findings suggest that S-oxidation and N-demethylation are the major metabolic pathways of dothiepin.

The metabolic rate constant (K_1) and excretion rate constants (K_2) of NOR–SO were 0.633 h^{-1} and 0.0557 h^{-1} ("flip.flop" K_1 = 0.0557 h^{-1}, K_2 = 0.633 h^{-1}), respectively. Those of DP–SO were 0.602 h^{-1} and 0.0598 h^{-1} ("flip-flop" K_1 = 0.0598 h^{-1}, K_2 = 0.0602 h^{-1}), respectively. Those of DP–SO_2 and NOR–SO_2 were not estimated due to insufficient data.

Table 3
Cumulative excretion of four metabolites in urine (% of dose)

Metabolite	Time (h) 0–6	0–12	0–24	0–48	0–72
DP–SO	4.9	10.7	18.8	25.0	26.8
DP–SO_2	—*	0.1	0.2	0.2	0.2
NOR–SO	3.6	7.9	12.0	15.6	17.7
NOR–SO_2	—*	—*	0.2	0.4	0.4

* Below detection limit.
Each value represents the mean of 5 subjects.

Conclusion

The method described permits the specific and reproducible determination of four dothiepin metabolites in urine and is suitable for pharmacokinetic studies.

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