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**Letter to the Editor**

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# Simultaneous determination of serum trimethadione and its metabolite by gas chromatography

Sir,

We have previously reported a sensitive and selective method for the determination of low concentrations of trimethadione (TMO) and its only metabolite, dimethadione (DMO), by using gas chromatography (GC) with hydrogen flame ionization detection (FID) [1] or flame thermionic detection (FTD) [2]. A pharmacokinetic study using this sensitive method was carried out on rats with chemically induced liver injury [1,2]. When 5% polyethylene glycol (PEG) 6000 was used as the stationary phase, however, occasional tailing of the DMO peak occurred, suggesting some quantitative unreliability of this method in the determination of TMO and DMO in blood. In this work we have improved the analytical reliability of the method and carried out a pharmacokinetic study in rats and humans by using the improved method. FID was used for samples from rats and FTD for samples from humans.

TMO was purified from commercially available powder containing 66.7% Mino-Aleviatin (Dainippon Seiyaku, Osaka, Japan). DMO and maleinimide were purchased from Tokyo Kasei (Tokyo, Japan). All other chemicals were of analytical-reagent grade.

To 100 μl of serum in a 2.5-ml tube were added 200 μl of 5 *M* monobasic sodium phosphate, minimum amounts (about 50 mg) of sodium sulphate and magnesium sulphate and 100 μl of ethyl acetate containing 2.5 or 25 μg/ml maleinimide as internal standard. The tube was shaken for 2 min and centrifuged at 1800 *g* for 5 min. A 2-μl aliquot of the organic phase was then analysed by GC.

Analysis of serum TMO and DMO was carried out with a Shimadzu-9A instrument with FID or FTD. The glass column (50 cm × 2.6 mm I.D.) was packed with Tenax GC (60–80 mesh) (Wako, Osaka, Japan). The column oven temperature was raised from 130 to 190°C at 15°C/min and held at 190°C for 5 min. The injection port and detector temperatures were 230°C. Helium, hydrogen and air flow-rates were 50, 30 and 145 ml/min, respectively.

Drug standards were added to drug-free serum in amounts equivalent to 1–150 μg/ml (FID) or 0.05–10 μg/ml (FTD). The recovery of the drug from serum after

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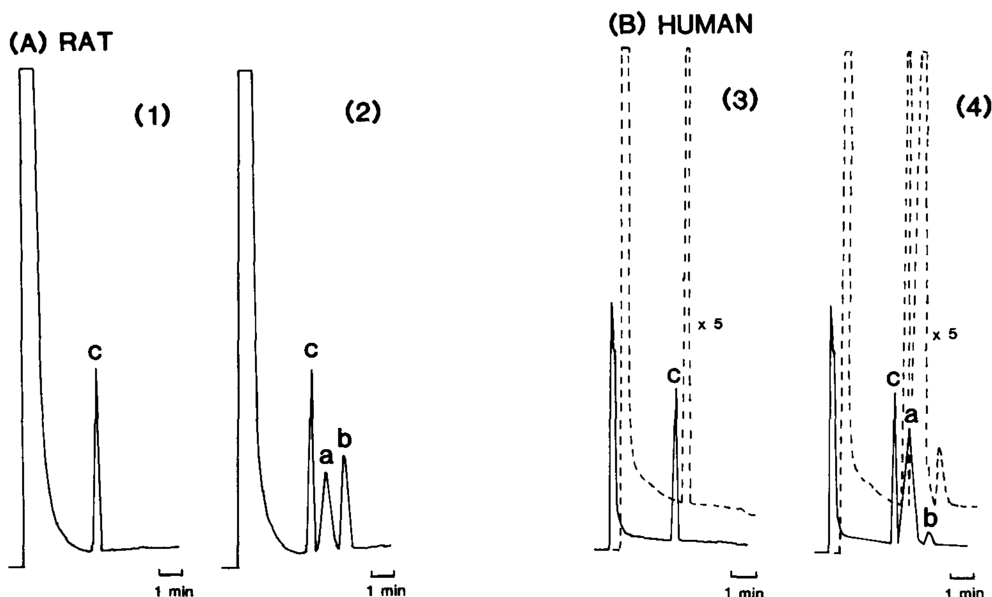


Fig. 1. Representative gas chromatograms of TMO and DMO 2 or 4 h after oral administration of TMO to (A) a rat (100 mg/kg) or (B) a human (4 mg/kg). (1) Rat blank serum; (2) serum from treated rat; (3) human blank serum; (4) serum from treated human. Peaks: a = TMO; b = DMO; c = internal standard (I.S.) (maleinimide). In B, the broken lines are the traces obtained with the sensitivity mode five times higher than the solid lines. The retention times of the I.S., TMO and DMO were 2.9, 3.5 and 4.4 min, respectively.

protein precipitation was determined at the initial concentrations of 1, 2, 5, 10, 50, 100 and 150 μg/ml (FID) or 0.05, 0.1, 0.5, 1, 5 and 10 μg/ml (FTD) serum by comparing their peak heights with those obtained from aqueous solutions containing known concentrations of TMO and DMO. The reproducibility was determined for the same concentration range by triplicate analyses of samples at each concentration. For statistical analysis, a paired Student's *t*-test was used.

Fig. 1 shows representative chromatograms of TMO and DMO after oral administration of TMO to rats (100 mg/kg) and humans (4 mg/kg). The results indicate that TMO, DMO and the internal standard (I.S.) were well separated. The retention times of the I.S., TMO and DMO were 2.9, 3.5 and 4.4 min, respectively. Table I gives the extraction recoveries of TMO and DMO from serum. For both compounds, the recovery from rat serum was 96–99% in the pre-extraction concentration range of 2–150 μg/ml and that from human serum was similarly 95–97% in the range 0.1–10 μg/ml. These results were reproducible.

Calibration graphs for FID showed a linear relationship between the peak-height ratios of TMO or DMO to the I.S. in the concentration range 2–150 μg/ml (TMO, $r = 0.980$; DMO, $r = 0.976$). In a similar fashion, the concentrations of TMO and DMO with FTD showed a linear relationship between the peak-height ratios of TMO or DMO to the I.S. in the concentration range 0.1–10 μg/ml (TMO, $r = 0.955$; DMO, $r = 0.969$).

No interfering peaks appeared in the presence of acetazolamide, carbamaze-

TABLE I

RECOVERY OF TMO AND DMO FROM RAT AND HUMAN SERUM

The values are means ± standard errors for three determinations.

| Sample | Amount added (μg/ml) | TMO | | DMO | |
|---|---|---|---|---|---|
| | | Amount found (μg/ml) | Recovery (%) | Amount found (μg/ml) | Recovery (%) |
| Rat serum | 2 | 1.98 ± 0.05 | 99 | 1.96 ± 0.09 | 98 |
| | 5 | 4.8 ± 0.21 | 96 | 4.8 ± 0.11 | 96 |
| | 10 | 9.7 ± 0.52 | 97 | 9.7 ± 0.28 | 97 |
| | 50 | 49.6 ± 0.82 | 99 | 48.6 ± 0.99 | 97 |
| | 100 | 97.6 ± 1.77 | 98 | 98.2 ± 1.96 | 98 |
| | 150 | 147.9 ± 2.01 | 99 | 148.6 ± 3.29 | 99 |
| Human serum | 0.1 | 0.094 ± 0.002 | 94 | 0.096 ± 0.004 | 96 |
| | 0.5 | 0.48 ± 0.02 | 96 | 0.48 ± 0.04 | 96 |
| | 1 | 0.97 ± 0.06 | 97 | 0.95 ± 0.08 | 95 |
| | 5 | 4.73 ± 0.11 | 95 | 4.82 ± 0.18 | 96 |
| | 10 | 9.33 ± 0.32 | 93 | 0.67 ± 0.55 | 97 |

pine, pentobarbital, phenobarbital, phenytoin or primidone, all of which are usually administered to patients in combination with TMO.

This method can also be applied to plasma and urine samples. These data suggest that this improved method is reliable in terms of sensitivity, selectivity and reproducibility for the simultaneous determination of TMO and DMO in either rat or human serum.

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