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GAS—LIQUID CHROMATOGRAPHIC DETERMINATION OF FREE DIMETHINDENE IN HUMAN SERUM AND URINE AT LOW CONCENTRATIONS

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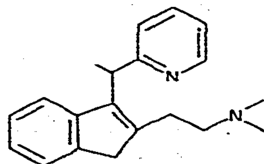
SUMMARY

A simple method for the determination of dimethindene down to a concentration of 10 ng/ml in human urine and serum is described. After the addition of an internal standard, the dimethindene is extracted as the free base with pentane. Measurements are made directly by gas—liquid chromatography using a flame ionization detector.

INTRODUCTION

Dimethindene maleate, N,N-dimethyl-3-[1-(2-pyridinyl)ethyl)]-1H-indene-2-ethanediamine maleate*, is an antihistaminic drug requiring daily doses of only 3—6 mg.



dimethindene

A sensitive method is needed for measuring nanogram quantities of dimethindene in human urine or serum. This paper describes a gas—liquid chromatographic (GLC) method for the determination of the free base, dimethindene, in urine and serum, at concentrations as low as 10 ng/ml, using docosane as an internal standard.

*Trademarks: Forhistal®, Fenistil®.

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EXPERIMENTAL

Reagents

The dimethindene maleate was of pharmaceutical grade. The tetradecane and docosane were of analytical grade (puriss; Fluka, Buchs, Switzerland). The extraction solvent, *n*-pentane (Rathburn Chemicals, Walkerburn, Great Britain) and other reagents, 25% ammonia solution and 32% hydrochloric acid (Merck, Darmstadt, G.F.R.), were used without further purification. The water used for the dilution of the urine samples and for the calibration curves was always freshly twice-distilled (Fontavapor 250; Büchi, Flawil, Switzerland).

Standard solutions

Solutions of 1 µg/ml dimethindene maleate, in about 0.1 *N* hydrochloric acid and of 0.5 µg/ml docosane in *n*-pentane were prepared just before use.

Glassware

Extraction tubes (15 ml) made wholly of Teflon were specially made for this work. The glassware used for concentration was cleaned with 32% hydrochloric acid, washed with distilled water and dried; the surface of the glass was deactivated with hexamethyldisilazane (Fluka).

Apparatus

The gas chromatograph (HP 5730A; Hewlett-Packard, Geneva, Switzerland) was equipped with a linear flame ionization detector. The operating temperatures were: column 240°C, injector 200°C and detector 250°C. The nitrogen flow-rate was 30 ml/min. The glass columns, 2 m × 2 mm I.D. (Supelco, Crans-près-Céligny, Switzerland), were washed with 32% hydrochloric acid, rinsed with distilled water, dried and finally deactivated with hexamethyldisilazane. After this treatment the columns were again washed with distilled water and dried at 120°C. The column packing was 10% Apiezon L—2% potassium hydroxide on 80—100 mesh Chromosorb W AW (Supelco). The filled columns were flushed with the carrier gas, gradually heated to 240°C and kept at this temperature for 3 days.

Extraction procedure

Five millilitres of urine or serum were pipetted into a 15-ml all-Teflon extraction tube; 5 µl of tetradecane, 0.5 ml of 25% ammonia solution and 5 ml of internal standard solution were then added. The tube was stoppered (with a Teflon stopper) and shaken manually for 30 sec and then centrifuged at ca. 800 *g* for 3—4 min. The organic layer was removed with a glass syringe and the aqueous phase was extracted with a fresh 5-ml portion of pentane. The combined organic layers were evaporated in a water bath at 45°C, under argon. The "almost dry" residue (the tetradecane still remained) was centrifuged and kept, still under argon, in a cold water bath until its injection as a 0.5-µl aliquot.

Collection of biological samples

Urine and serum were obtained from healthy volunteers who had been

instructed not to take any drugs for seven days before the experiment. The urine pH had been stabilized on the acid side, by the oral administration of ammonium chloride capsules, in order to prevent degradation or reabsorption of the excreted drug. Urine was collected during the following time intervals: 0—1, 1—2, 2—3, 3—4, 4—6, 6—8, 8—10, 10—12 and 12—24 h.

The volume was measured and the pH was determined with a pH-meter (Metrohm, Herisau, Switzerland); 20 ml were stored at —20°C until analyzed.

Blood samples were withdrawn at the beginning of the experiment (at about 8 a.m.) and at 5, 10, 15, 30, 45 and 60 min later. The blood samples, of about 12 ml, were allowed to coagulate at room temperature and were then centrifuged for 30 min at ca. 500 *g*. The separated serum was removed and stored at —20°C until analyzed.

RESULTS AND DISCUSSION

Evaporation

When the conventional evaporation-to-dryness techniques were applied, reproducible results were not obtained. Considerable loss of material was found to have occurred during the evaporation—redissolution operations. Reliable values were, however, obtained when the organic phase was "almost dried". The addition of a small amount of a high-boiling solvent, such as tetradecane, to the pentane layer prevented complete drying of the residue and the necessity to redissolve it. Evaporation of the pentane in a current of argon, followed by rapid, but effective, centrifugation (ca. 1000 *g*) successfully completed the sample preparation.

Urine or serum interference

Fig. 1 shows chromatograms of a blank urine sample extract and that of a urine sample to which had been added 50 ng/ml internal standard and 40 ng/ml dimethindene maleate. No interference with the usual urine components was recorded. Similarly, Fig. 2 shows a blank serum extract and an extract of a serum to which had been added 100 ng/ml internal standard and dimethindene maleate.

Sensitivity and accuracy

Table I gives the results obtained with urine samples containing the additions in the concentration range 10—200 ng/ml. The 95% confidence intervals were calculated for 4—11 replicate analyses on each sample. The lower concentration may be taken as the limit of sensitivity of the estimation.

A typical calibration curve for the estimation of dimethindene concentration in urine, as well as in plasma, between 0—200 ng/ml, as a function of peak height ratio gives the equation (peak height of dimethindene)/(peak height of internal standard = (0.0145 × conc. dimethindene) — 0.061. The *y*-axis intercept of —0.061 demonstrates the impossibility of measuring quantities of the drugs lower than about 4 ng. The correlation coefficient of 0.998 indicates good linearity of the data.

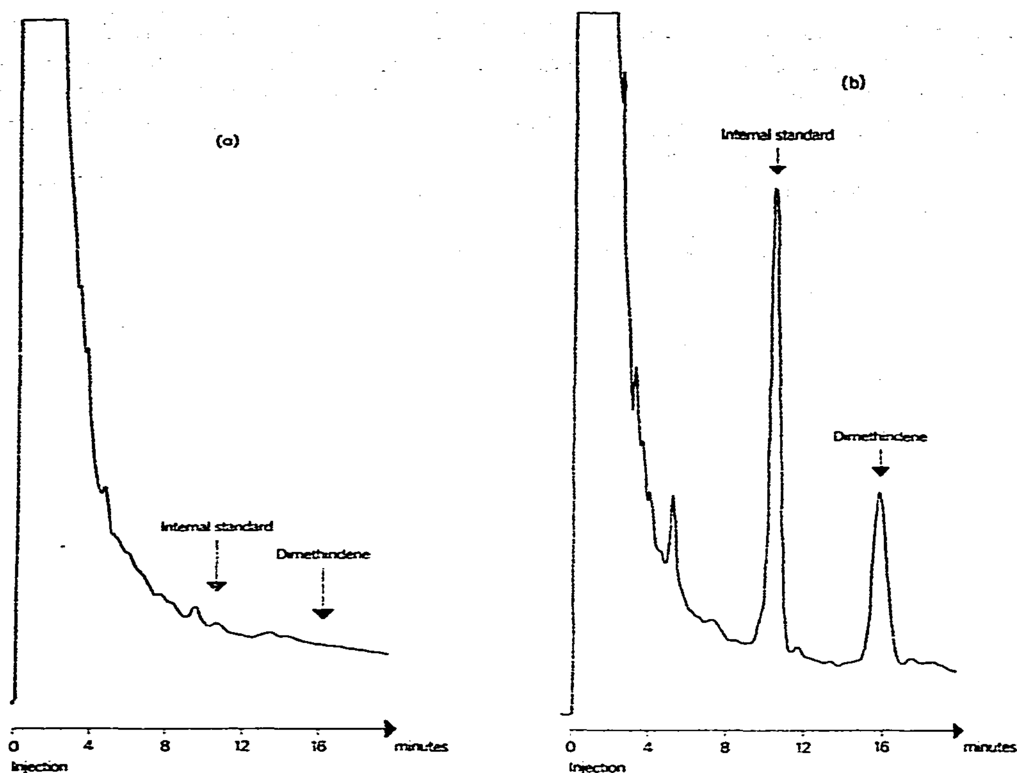

Fig. 1. Gas chromatograms of (a) a blank urine sample extract, and (b) a urine sample extract containing 50 ng/ml internal standard and 40 ng/ml dimethindene maleate.

Applications

The method has been widely used for the estimation of the half-life of dimethindene in urine. Different dosage forms and modes of administration have been investigated; the results are discussed elsewhere [1]. Unfortunately, in a preliminary study, in which 1 mg of dimethindene maleate was given as an intravenous bolus injection to a healthy volunteer, detectable concentrations of unchanged drug were present for only 15 min after the injection. The same was true in the case of six volunteers who received a 2-mg injection. This very rapid disappearance of measurable concentrations probably reflects the distribution of the drug. Accordingly, it was decided to limit this study to elimination in the urine and to calculate the pharmacokinetic parameters from these data. A typical range of measurements for one volunteer who had taken 3 mg of dimethindene maleate orally, in the form of tablets, is reported in Table II.

A semi-logarithmic plot of the rate of excretion in the urine against time ($\Delta A_e/\Delta t$) allows the calculation, by linear regression of the slope of the curve and of the half-life (Fig. 3). From the value of the slope, the amount of dimethindene eliminated in the urine up to infinite time ($A_{e(\infty)}$) can be estimated. The ARE plot [2] allows another estimation of the elimination rate and of the half-life of the drug (Fig. 4).

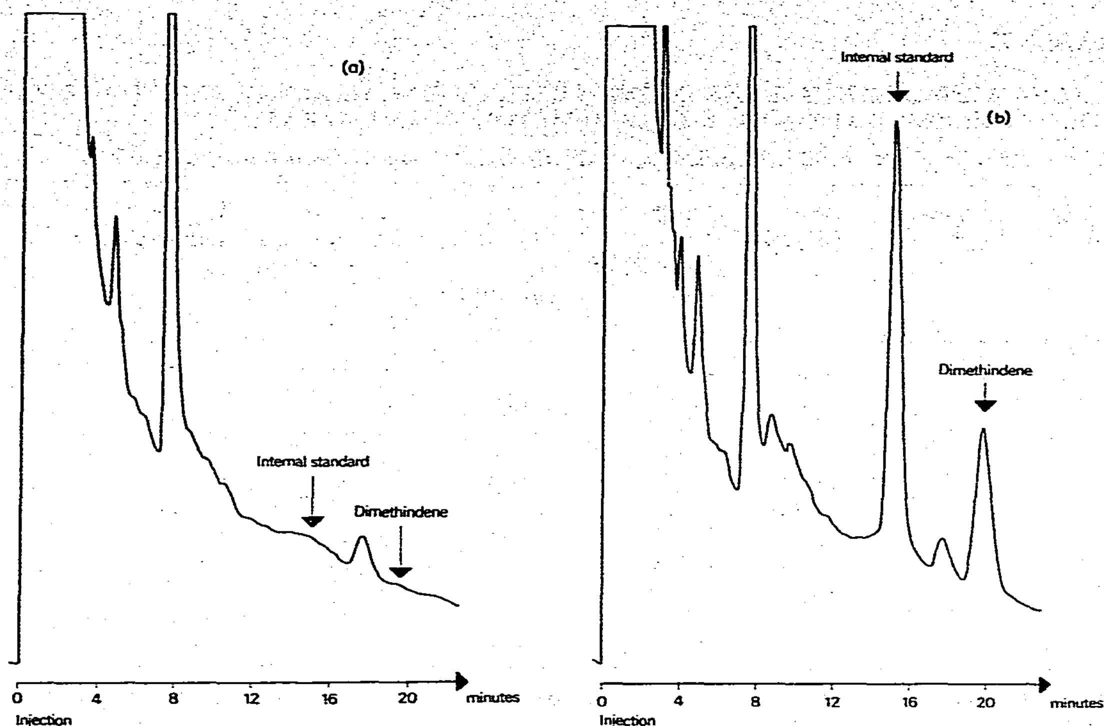

Fig. 2. Gas chromatograms of (a) a blank serum sample extract, and (b) a serum sample extract containing 100 ng/ml internal standard and 100 ng/ml dimethindene maleate.

TABLE I

PRECISION AND ACCURACY OF THE ASSAY APPLIED TO SPIKED URINE SAMPLES

Amount added (ng/ml)	Amount found* (ng/ml)	Standard deviation (ng/ml)	Relative standard deviation (%)	95% confidence interval (ng/ml)
10	12.8	1.56	12.2	9.7—15.9
40	39.2	3.0	7.7	33—45
80	79.7	4.0	5.0	72—88
100	97.6**	3.4	3.5	91—104
120	121.3	3.4	2.8	114—128
160	158.3	3.4	5.0	151—165
200	201.5	6.3	3.1	180—214

*Average of five assays.
**Eleven values.

TABLE II

UNCHANGED DIMETHINDENE IN THE URINE OF ONE HEALTHY SUBJECT AFTER ORAL ADMINISTRATION OF 3 MG OF DIMETHINDENE MALEATE

Elimination half-life: 5.89 h. Elimination half-life by the sigma-minus method: 5.63 h.

Time (h)	Urinary pH	Conc. (ng/ml)	Elimination (percentage dose)	A_e (percentage dose)	$\Delta A_e/\Delta t$ (percentage dose per h)	$A_{e(\infty)}-A_{e(t)}$ (percentage dose)
0	4.9	0	—	—	—	5.917
0–1	4.9	16.4	0.118	0.118	0.118	5.799
1–2	4.7	99.5	0.608	0.726	0.608	5.191
2–3	4.9	179.5	0.554	1.280	0.554	4.637
3–4	4.7	196.0	0.568	1.848	0.568	4.069
4–6	4.7	213.9	1.063	2.911	0.532	3.006
6–8	5.2	93.9	0.465	3.376	0.233	2.541
8–10	5.0	129.8	0.642	4.018	0.321	1.899
10–12	4.8	140.1	0.332	4.350	0.166	1.567
12–24	5.1	63.8	1.224	5.574	0.102	0.343
	—	—	—	5.917*	—	

*The amount eliminated in the urine at infinity $A_{e(\infty)}$ can be calculated from amount eliminated at time $A_{e(t)}$ from equation

$$A_{e(\infty)} = A_{e(t)} \cdot \frac{1}{1-\exp(-k_e \cdot t)}$$

where k_e is the elimination rate constant determined from the plot $\Delta A_e/\Delta t$ versus time [2].

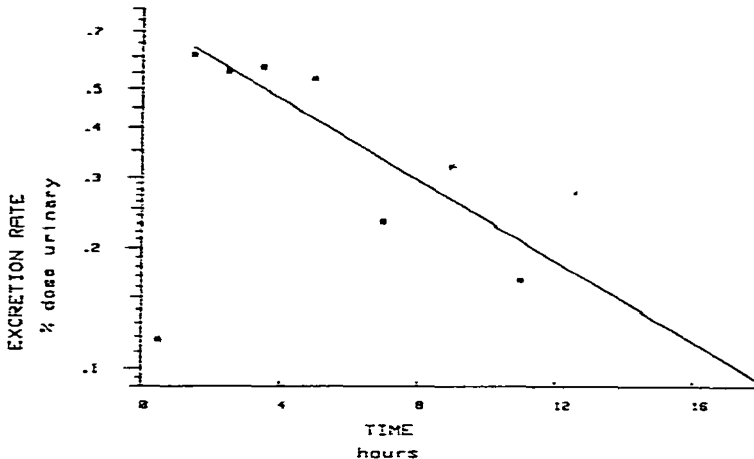

Fig. 3. Urinary excretion rate of dimethindene maleate after the administration of Fenistil®: typical measurements for one volunteer who took 3 mg orally in the form of coated tablets.

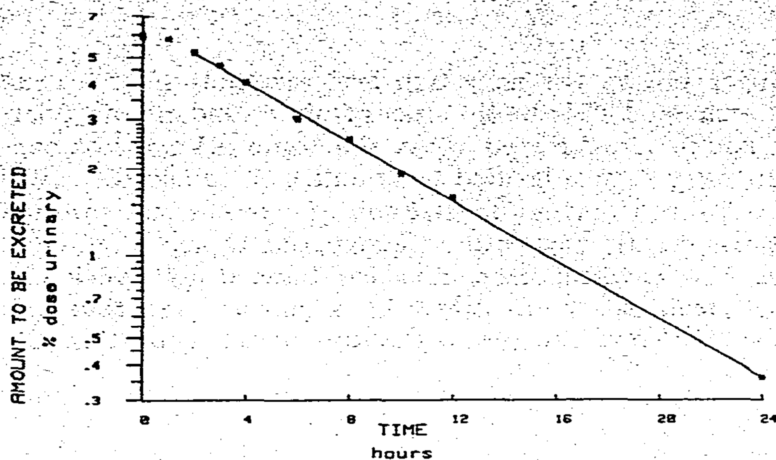

Fig. 4. ARE plot for dimethindene maleate after the administration of Fenistil®. Conditions as in Fig. 3.

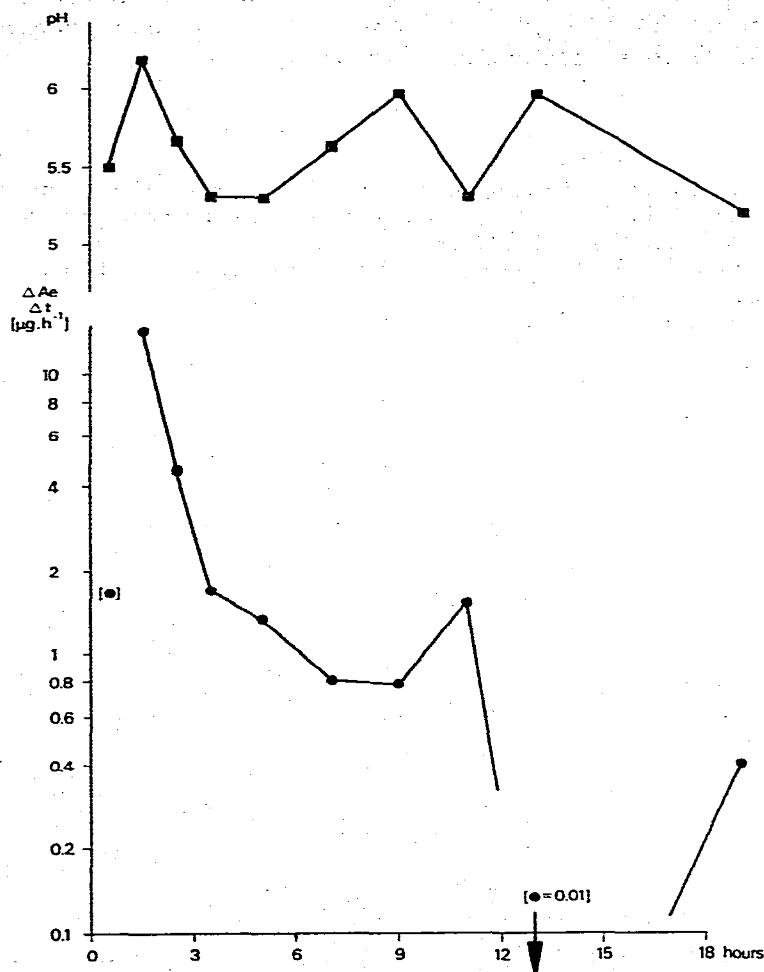

Fig. 5. Influence of poor control of the urinary pH (▪) on the urinary elimination rate of dimethindene maleate (•). (3 mg of Fenistil® were taken orally as a solution.)

Acidic urine conditions

The influence of urinary pH on the excretion and metabolism of a drug has already been widely examined [3]. In the case of dimethindene, no pharmacokinetic parameters could be estimated when the urine pH was not controlled (Fig. 5). Consequently, it was decided to conduct all the studies after acidification of the urine by the oral administration of ammonium chloride capsules: 400 mg, five times on the day before the experiment and on the day of the experiment. This is a classical procedure for basic drugs which can potentially be reabsorbed from the renal tubuli by a passive (and thus pH-dependent) process. Table II shows the low pH values obtained by this procedure.

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