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Note

Determination of cyclizine and norcyclizine in plasma and urine using gas—liquid chromatography with nitrogen selective detection

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Cyclizine is a clinically useful drug of the benzhydrylpiperazine series [1] and norcyclizine is its demethylated metabolite (Fig. 1). Previous methods for the estimation of these substances in biological material were complexing with methyl orange [2] and derivatisation with tritiated acetic anhydride [3]. The accuracy of these methods is limited at low concentrations owing to non-selective reactions with naturally occurring substances.

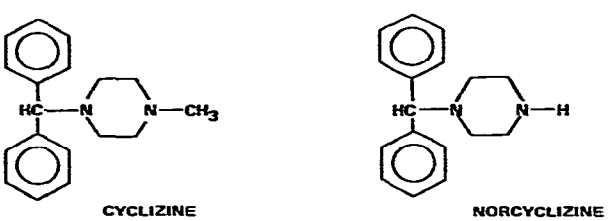

Fig. 1. Molecular structure of cyclizine and its demethylated metabolite, norcyclizine.

The method described below, which employs gas—liquid chromatography (GLC) with a nitrogen selective alkaline flame ionisation detector (AFID) can measure cyclizine in plasma and urine with adequate sensitivity for pharmacokinetic studies. The method can also measure norcyclizine in urine.

MATERIALS AND METHODS

Chemicals

Cyclizine, norcyclizine and chlorcyclizine were obtained from the Wellcome Foundation (Dartford, Great Britain) and recrystallised as hydrochlorides

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before use. Dowtherm A, an eutectic mixture of biphenyl and dibenzofuran, was obtained from Fluka (Fluorochem, Glossop, Great Britain); cyclohexane AR, which was glass distilled before use, and isopropanol (HPLC grade) were obtained from Fisons (Loughborough, Great Britain). All aqueous solutions were made up using glass distilled deionised water.

Glassware

Screw-capped tubes (20 ml Soveril, type 611-03; V.A. Howe, London, Great Britain) were used for the extractions. Microtubes of 3.5 ml maximum capacity [Du-Pont (U.K.) part No. 834078; Hitchin, Great Britain] were used for the solvent evaporation stages. The microtubes end in a fine point which was useful for manipulation of the concentrate. All glassware was washed with hydrochloric acid (2 *M*) and rinsed with deionised water before use. The microtubes were dried at 210°C in a vacuum oven before use.

Determination of plasma cyclizine

Standard solutions of cyclizine ranging from 0—500 ng/ml were prepared by dilution of the aqueous stock solution (1 mg/ml) with heparinised human plasma. An aliquot of the standard or sample (1 ml) was placed in an extraction tube followed by chlorcyclizine (internal standard, 50 μl; 10 μg/ml) sodium hydroxide (2 ml; 2 *M*) and cyclohexane (10 ml). Cyclizine and chlorcyclizine were then extracted into cyclohexane by rocking the tube for 20 min along its long axis at 25 oscillations per min (flat bed shaker) after which the liquid phases were separated by centrifugation (1200 *g* for 10 min). As much of the cyclohexane (top layer) as possible was removed and transferred to another extraction tube containing hydrochloric acid (2 ml; 2 *M*). The cyclizine and chlorcyclizine were back extracted into the acid layer and the organic layer was removed and discarded. The cyclizine and chlorcyclizine were re-extracted into cyclohexane by adding sodium hydroxide (2 ml; 4 *M*) and cyclohexane (4 ml). The cyclohexane (3 ml) was transferred to a microtube containing Dowtherm A (20 μl) and was then removed at room temperature under a stream of nitrogen. The alkaline solution was re-extracted with a further 3 ml of cyclohexane. The cyclohexane (3 ml) from this final extraction was added to the liquid residue in the microtube and the cyclohexane removed as above. Dowtherm A prevented the solutes from solidifying and also imparted selective solution of the cyclizines. The resultant oily residue was injected into the gas chromatograph (4 μl per injection). Standards and unknowns were analysed in duplicate.

Determination of urinary cyclizine and norcyclizine

Combined standards of cyclizine and norcyclizine covering the range 0—200 ng/ml were prepared by dilution of the aqueous stock solution (1 mg/ml) in urine. A 1-ml aliquot of standard or unknown was placed in an extraction tube followed by chlorcyclizine (internal standard, 50 μl; 10 μg/ml), sodium hydroxide (2 ml; 2 *M*) and cyclohexane (4 ml). The contents were extracted twice as in the final alkaline extraction of the plasma analysis. The acid back extraction step and addition of Dowtherm A were unnecessary as cyclizine and norcyclizine were in high yield and well resolved from all the other urinary con-

stituents. However, the resultant dried residue in the microtube was redissolved in 50 µl of isopropanol just prior to injection into the gas chromatograph (4-µl injections). The urine samples and unknowns were analysed in duplicate.

Chromatographic conditions

The gas chromatograph used was a Model F30 Perkin-Elmer (Beaconsfield, Great Britain) with an AFID detector. A glass column (1.8 m × 4 mm I.D.) was hand packed with 5% OV-17 on Chromosorb W HP (100—120 mesh) and conditioned at 310°C with a helium carrier gas flow-rate of 50 ml/min for at least 24 h before use. The AFID detector was used in the nitrogen mode with hydrogen (8.5 ml/min), air (92 ml/min) and helium carrier gas (50 ml/min). Manifold, oven and injection port temperatures were 321, 246 and 310°C, respectively. With a fully conditioned column cyclizine, norcyclizine and chlorcyclizine were well resolved with retention times of 190, 242 and 329 sec, respectively. Tailing of the norcyclizine peak indicated poor column condition; cyclizine and chlorcyclizine were less affected. The limit of detection was approximately 200 pg for cyclizine and chlorcyclizine and 2 ng for norcyclizine injected on column.

RESULTS

Calculation of results

A data system (Hewlett-Packard Model 3352) was used to calculate the peak

TABLE I

ASSAY PRECISION FOR CYCLIZINE (PLASMA AND URINE) AND NORCYCLIZINE (URINE) ESTIMATIONS

n = 6 at each concentration.

Sample drug and concentration (ng/ml)	Mean drug to internal standard ratio	Mean standard deviation of ratios (± S.D.)	± % S.D. of mean value
Plasma cyclizine*			
50	0.1032	0.0104	10.1
100	0.1877	0.0184	9.8
200	0.3772	0.0142	3.8
500	0.9233	0.0387	4.2
Urine cyclizine**			
50	0.0747	0.0026	3.5
100	0.1635	0.0140	8.5
200	0.3302	0.0243	7.4
500	0.9378	0.0419	4.5
2000	3.9152	0.1953	5.0
Urine norcyclizine***			
50	0.051	0.010	19.2
100	0.110	0.014	12.6
200	0.256	0.020	7.7
500	0.645	0.050	7.7
2000	2.835	0.045	1.6

*$y = 0.001830\,x + 0.006$; $r = 0.9999$, where y = ratio of cyclizine or norcyclizine to chlorcyclizine (peak areas), and x = plasma concentrations of cyclizine or norcyclizine.
**$y = 0.001980\,x - 0.043$; $r = 0.9983$.
***$y = 0.001426\,x - 0.028$; $r = 0.9994$

areas of cyclizine, norcyclizine and their ratios to the internal standard peak area. The calibration curve was a plot of the cyclizine or norcyclizine concentration (abcissa) against its peak area ratio to that of the internal standard (ordinate). Since the same known mass of internal standard (500 ng of chlorcyclizine) was added to the unknown samples (plasma or urine) the amount of cyclizine or norcyclizine in the sample could be determined from its peak area ratio using the calculated (Texas T151 [III] calculator) least squares regression line derived from the calibration curve.

Validation

Methods were validated by adding known amounts of cyclizine to plasma and urine and known amounts of norcyclizine to urine; six determinations were made at each sample concentration. Table I shows the precision of cyclizine determinations at all concentrations tested in plasma and urine and for norcyclizine in urine. Recovery was linear relative to the drug standards (see Table I). Mean percentage deviations of duplicates were calculated and mean values for unknowns and standards compared using a *t*-test (with appropriate modifications if suggested by inequality of variance). No significant differences were seen.

By comparing peak areas with those given by injection of the cyclizine and

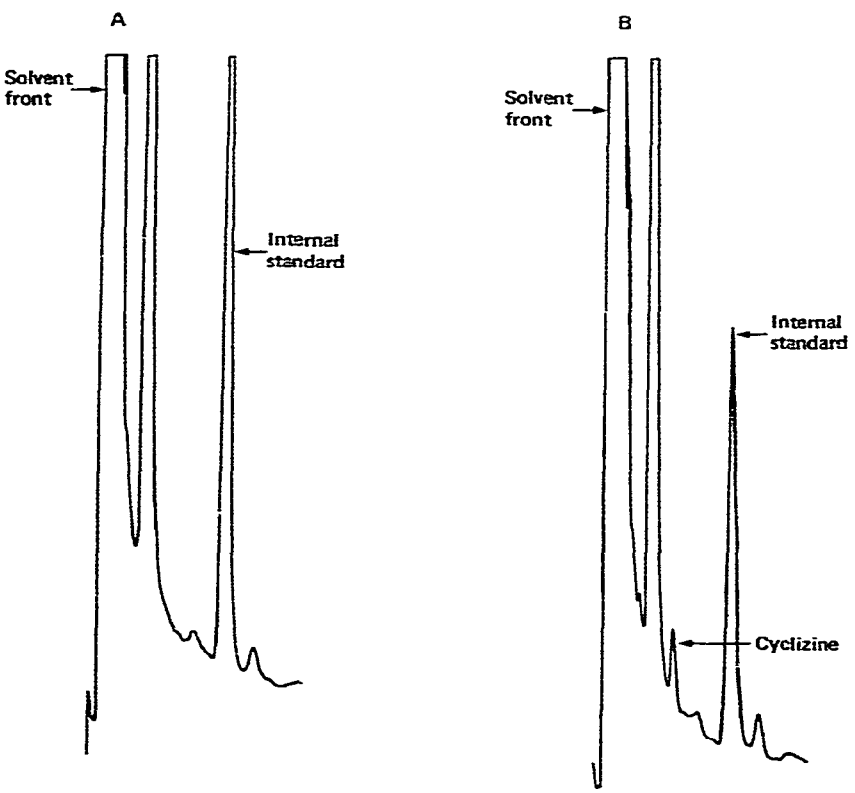

Fig. 2. Chromatograms from the analysis of (A) blank plasma and (B) plasma to which cyclizine (50 ng/ml) was added. Internal standard, chlorcyclizine, 10 μg/ml.

norcyclizine bases (in isopropranol) the total extraction efficiencies were about 45% for cyclizine in plasma and greater than 80% for cyclizine and norcyclizine in urine. This reflects the greater number of transfer steps and the final selective solution into Dowtherm A required for the plasma determinations. A small contaminant in the plasma extracts elutes near norcyclizine and as norcyclizine is poorly recovered from plasma, quantitation below 500 ng/ml in plasma is unreliable.

Chromatograms of the plasma and urine extracts are shown in Figs. 2 and 3. An example of the plasma concentration curve in a healthy adult male who was given 50 mg cyclizine hydrochloride intravenously is shown in Fig. 4. The delay in excretion seen in the plasma profile around 30 min is well known for basic drugs (e.g. amphetamine [4]).

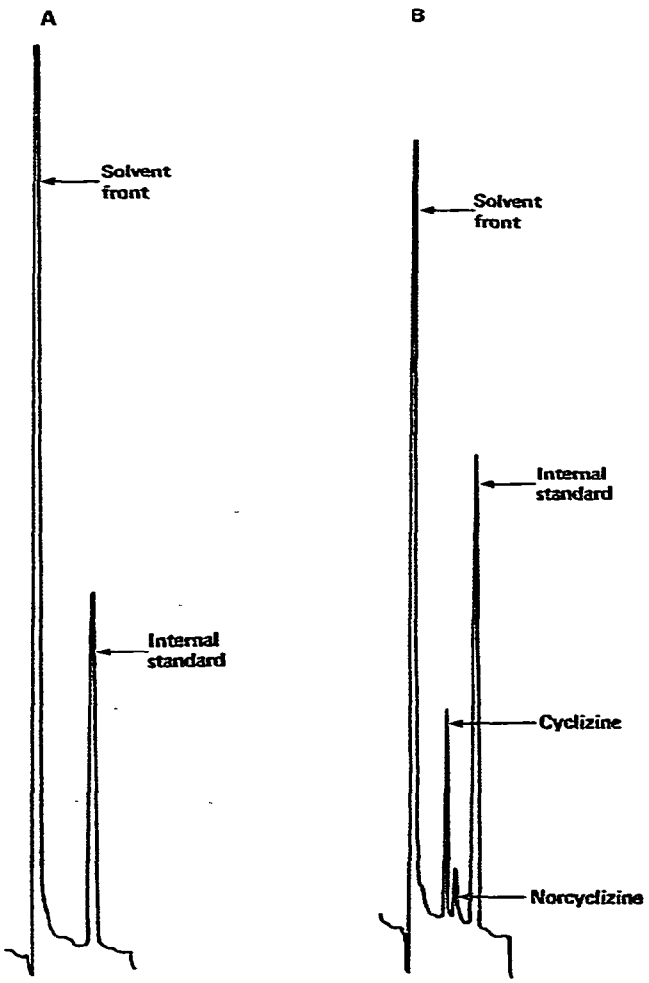

Fig. 3. Chromatograms from the analysis of (A) blank urine and (B) urine to which cyclizine (200 ng/ml) and norcyclizine (200 ng/ml) were added. Internal standard, chlorcyclizine, 10 μg/ml.

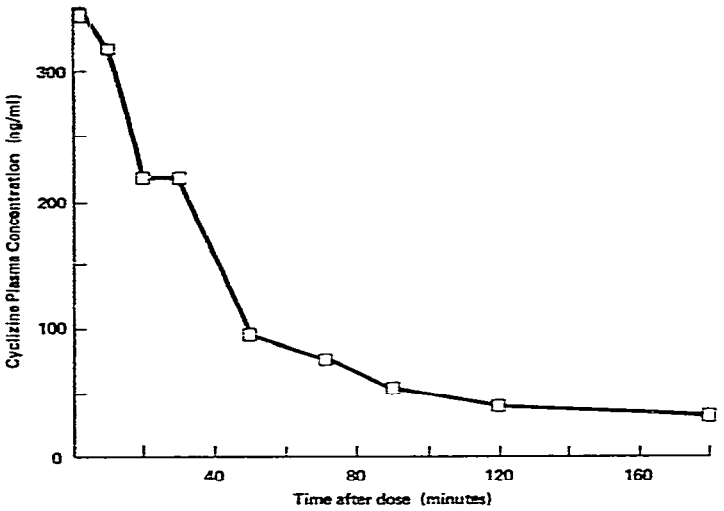

Fig. 4. Plasma cyclizine profile in healthy adult male subject after administration of 50 mg cyclizine intravenously.

DISCUSSION

The GLC technique described here was found to be specific for unchanged cyclizine in plasma and urine and for norcyclizine in urine. Dipipanone hydrochloride, morphine, ergotamine tartrate and caffeine are present in combination with cyclizine in some pharmaceutical preparations but were found not to interfere with cyclizine chromatography. Cyclizine determinations showed good precision at all concentrations tested in plasma and urine. For norcyclizine in urine good precision was achieved at the higher concentrations but at the lower concentrations (50 ng/ml) the precision was poor (% S.D. of mean was ±19%). Attempts to improve the extraction efficiences with solvents more polar than cyclohexane resulted in a loss of resolution of cyclizine from other substances eluting with the solvent front.

A chloroform syringe wash between injections minimised carryover caused by a low avidity adsorption of the compounds to glass. Without the wash low concentration samples could be erroneously high especially if they followed a high concentration sample. For the same reason all glassware was routinely acid washed. The use of an internal standard reduces the effects of error from transfer losses.

The methods described are suitable for the analysis of cyclizine in plasma and urine and norcyclizine in urine with sufficient sensitivity for human bioavailability and pharmacokinetic studies, the limit of sensitivity being 10 ng/ml for cyclizine in plasma and urine and 40 ng/ml for norcyclizine in urine.

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